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ASSESSMENT

Fourteenth external quality assessment for *Salmonella* typing in EU/EEA and EU enlargement countries, 2024

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Abbreviations

AD	Allelic distance
AMR	Antimicrobial resistance
bp	Base pair
cgMLST	Core genome multi-locus sequence typing
EEA	European Economic Area
EQA	External Quality Assessment
EU	European Union
EURL	European Union Reference Laboratory
FWD-Net	European Food-and Waterborne Diseases and Zoonosis Network
ID	Identification
IQR	Interquartile range
GC	Guanine-cytosine
MLST	Multi-locus sequence typing
MLVA	Multiple locus variable-number tandem repeat analysis
MST	Minimum Spanning Tree
NPHRL	National Public Health Reference Laboratory
NRL	National Reference Laboratory
PFGE	Pulsed-field gel electrophoresis
PT	Proficiency test
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment)
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
TESSy	The European Surveillance System
UPGMA	Unweighted pair group method with arithmetic mean
WGS	Whole genome sequencing

Executive summary

Infection with *Salmonella spp.* is the second most reported zoonotic disease in humans. In 2023,77 486 cases were reported in the European Union (EU). *Salmonella* is also associated with the highest number of foodborne outbreaks [2]. The overall EU trend in salmonellosis incidence between 2017 and 2023 has not significantly decreased or increased. To prevent foodborne diseases such as salmonellosis from spreading further it is essential to employ human surveillance systems at different levels to monitor the disease and ensure countries can detect outbreaks early and be able to respond to them.

To this end, ECDC set surveillance objectives to monitor trends and carry out multinational outbreak detection of salmonellosis and other pathogens. These objectives contribute to the evaluation and monitoring of prevention and control programmes; identify population groups at risk and in need of targeted prevention; contribute to the assessment of the burden of disease; generate hypotheses on sources and transmission modes, and identify needs for research projects.

The fulfilment of these surveillance objectives relies heavily on the data provided by the National Public Health Reference Laboratories (NPHRL) of the EU and European Economic Area (EEA) countries. ECDC commissions an annual External Quality Assessment (EQA) scheme for the serotyping and molecular-based cluster analysis of *Salmonella* to monitor the typing methods used, data quality and comparability, and the capability of laboratories to perform these methods.

This 14th EQA scheme for *Salmonella* typing (EQA-14) was sub-contracted to the National Institute for Public Health and the Environment (RIVM) and consisted of a serotyping part and a molecular typing-based cluster analysis part. Participants were expected to use routinely applied methods for both parts of the EQA and were assessed on their performance. Serotyping consisted of 12 isolates with different, carefully selected serovars. For cluster analysis, 10 *S.* Typhimurium monophasic isolates were selected, containing cluster and non-cluster isolates. These isolates mimicked a real outbreak situation, originating from a neighbourhood party scenario. In addition, raw reads of five isolates were made available to the participants that used whole genome sequencing (WGS) for cluster analysis. These isolates acted as food isolates and participants were asked which food product was most likely to have caused the outbreak.

For serotyping, 29 laboratories participated and 55% (16/29) used phenotypical serotyping, based on agglutination with antisera only, while 3% (1/29) used a combination of agglutination with antisera and molecular methods other than WGS. Twenty four percent% (7/29) of the laboratories used a combination of agglutination with antisera and prediction of serotype with WGS, and 17% (5/29) used prediction of serotype with WGS only. Performance was high for most laboratories, with 14 laboratories achieving performance scores of 100% and five of 92%. Of the 10 laboratories that had the lowest performance values (<92%), six only used phenotypic methods, two only used serotype prediction with WGS, and two used a combination of agglutination with antisera and prediction of serotype with WGS. Comparison between only using phenotypical serotyping with antisera, prediction of serotype with only WGS and a combination of the two revealed that there was no significant difference between the overall performance in serotyping when applying one of these methods ($p=0.5598$, χ^2).

Twenty-four laboratories took part in the molecular typing-based cluster analysis, which was comparable to EQA-13 (2023). This was an increase compared to EQA-12 (2022) and EQA-11 (2021), in which 20 laboratories participated in the molecular typing-based cluster analysis. In EQA-14, the proportion of participants that used WGS as a routine approach for their cluster analysis increased from 96% to 100% compared to EQA-13, while the number of participants that also applied multiple locus variable-number tandem repeat analysis (MLVA)-based cluster analysis decreased from 13% to 8%. All participants that used MLVA also used WGS for cluster analysis. While in EQA-13, pulsed-field gel electrophoresis (PFGE)-based cluster analysis was used by one participant, in EQA-14 this technique was not applied by any participant.

In the WGS-based cluster analysis, 83% of participants (20/24) applied a gene-by-gene approach, while the other 17% (4/24) applied SNP-typing. In total, 22 different combinations of platforms, approaches, kits, cluster analysis tools, typing schemes and cluster cut-offs were used. However, the methods used did not affect the high performance, with an overall performance score of 97% correct cluster assignment for the isolates and sequences provided. Twenty-one laboratories had 100% performance in assigning the provided isolates to clusters.

Two laboratories applied MLVA-based typing and produced identical MLVA profiles for all samples, except for EQA2419, for which no MLVA profile was provided by one laboratory and an incomplete profile was provided by the other laboratory. Overall, a high technical performance of 90% and 96% (45/50 and 48/50 alleles) was observed for the participating laboratories. Despite the incomplete MLVA profile for EQA2419, the sample was still correctly assessed as not part of the same cluster as the index isolate. Overall performance for cluster assignment of provided isolates was as high with MLVA typing as with WGS-based cluster analysis (100%). With the sample set used in EQA-14, participating laboratories had good capability in applying MLVA.

The use of PFGE-based cluster analysis is not recommended because of the inferior resolution, the poor portability and the limited usage which hampers its use in (inter)national outbreak assessments involving multiple institutes and therefore restricts the fulfilment of ECDC's surveillance objectives. This seems to be recognised by the participating laboratories, since in EQA-14, PFGE-based cluster analysis was not performed by any laboratory. Comparison with EQA-13, EQA-12 and EQA-11 also shows that the number of laboratories applying this technique consistently decreases.

Laboratories are recommended to use WGS-based cluster analysis, as a minimum in outbreak situations. Member States are asked to submit *Salmonella* WGS data in real-time to the European Surveillance System (TESSy) to be used for EU-wide WGS-enhanced salmonellosis surveillance. WGS data should be submitted whenever new data are available in laboratories or in relation to on-going multi-country outbreak investigations. Sharing of WGS data allows ECDC to perform regular multi-country cluster detections for *Salmonella* while supporting and improving the timeliness of multi-country outbreak investigations.

The EQA-14 assessed the typing methods used, their quality and comparability, and the capability of the performing laboratories. Results from a feedback survey showed that multiple laboratories took corrective action based on the results of EQA-14, proving the added value of this EQA for the typing capability of the NPHRLs in EU/EEA countries. Ensuring that the NPHRLs operate at maximum capability and capacity contributes to high standards of surveillance and outbreak detection at both regional and national level, and fulfils the international surveillance objectives of ECDC and the European Food- and Waterborne Diseases and Zoonosis Network (FWD-Net).

1. Introduction

1.1 Background

ECDC is an EU agency whose mission is to identify, assess and communicate current and emerging threats to human health from communicable diseases. ECDC's founding regulation outlines its mandate as fostering the development of sufficient capacity within EU/EEA dedicated surveillance networks for diagnosis, detection, identification and characterisation of infectious agents that may threaten public health. Under this mandate, ECDC supports the implementation of quality assurance schemes [1].

External quality assessments (EQAs) are an essential part of quality management. An external organiser is used to assess the performance of laboratories on test samples supplied specifically for quality assessment purposes.

The aim of EQAs is to identify areas of improvement in the laboratory diagnostic capacities relevant for epidemiological surveillance of communicable diseases, as in Decision No 1082/2013/EU [2], and to ensure the reliability and comparability of results generated by laboratories across all EU/EEA countries.

The main objectives of EQA schemes are to:

- assess the general standard of performance ('state of the art');
- assess the effects of analytical procedures (method principle, instruments, reagents, calibration);
- support method development;
- evaluate individual laboratory performance;
- identify problem areas;
- provide continuing education;
- identify needs for training activities for laboratory staff.

The provision of an annual EQA scheme for the serotyping and molecular-based cluster analysis of *Salmonella* in 2021–2025 has been subcontracted to RIVM by ECDC. This report presents the aggregated results of the EQA *Salmonella* serotyping and molecular-based cluster analysis of 2024 (EQA-14).

1.2 Impact of salmonellosis and surveillance objectives

Salmonellosis is caused by non-typhoidal *Salmonella* serovars and usually presents as a self-limiting mild diarrhoea, including cramping and fever, but it can also cause severe invasive infections [1]. Infection with *Salmonella spp.* is the second most reported zoonotic disease in humans, with 77 486 cases reported in the EU in 2023, and it accounts for the highest number of foodborne outbreaks [2]. In 2020, absolute case numbers for salmonellosis decreased compared to 2016–2019, mainly because of the withdrawal of the United Kingdom from the EU and the impact of COVID-19 control measures. Since 2020, absolute case numbers have steadily increased [2].

To control human salmonellosis, it is important to employ a 'One Health' approach and reduce *Salmonella* in animals and food items, as regulated by EU Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, in which salmonellosis is a priority [3]. To prevent foodborne diseases such as salmonellosis from spreading, it is essential to employ surveillance systems at different levels to monitor disease and ensure early outbreak detection and response [4]. International networks for human surveillance were set up following the implementation of EU Decision 1082/2013/EU3 'on serious cross-border threats to health' [5].

ECDC conducts indicator-based and event-based surveillance of communicable diseases [6]. For salmonellosis specifically, surveillance is conducted by FWD-Net [7].

ECDC has set surveillance objectives for specific food-and waterborne diseases and zoonoses such as salmonellosis [6, 7]. Firstly, trends in disease and antimicrobial resistance for *Salmonella* are monitored over time and across Member States. In each Member State, national public health reference laboratories (NPHRLs) perform surveillance at the national or regional level, based on data and/or submitted samples from clinical microbiology laboratories. The resulting surveillance data is disease-based and reported to ECDC under the EU mandate using The European Surveillance System (TESSy) [7]. Secondly, multinational outbreaks of salmonellosis are detected and monitored in terms of source, time, population and place to provide a rationale for public health action [8]. To improve early warning, NPHRLs, ECDC and other international health authorities can report potential international public health threats to the EpiPulse and the Early Warning and Response System portals [8, 9]. Data analysis for salmonellosis trends and outbreaks is performed by FWD-Net and summarised in annual epidemiological reports and EU One Health Zoonoses Reports [2, 10]. Using and analysing all the data collected, ECDC and FWD-Net, can pursue the surveillance objectives specific to salmonellosis. These objectives are to contribute to the evaluation and monitoring of prevention and control programmes; to identify population groups at risk and in need of targeted prevention; to contribute to the assessment of burden of disease, and to generate hypotheses on sources, transmission modes and identify needs for research projects [6, 7].

1.2 Microbiological methods and quality assessment

Microbiological surveillance for salmonellosis is undertaken in the EU/EEA and enlargement countries, mostly at NPHRLs. Serovar and antimicrobial resistance data for domestic and travel-associated cases are reported annually to ECDC through TESSy. Serovars are traditionally assessed by laboratories using classical phenotypical methods based on detection of O- and H-antigens using antisera agglutination [11]. However, some laboratories have replaced the traditional serotyping technique with in-silico serotyping, whereby the serovar is predicted from the presence or absence of O- and H- antigen synthesis genes, using data derived from WGS [12]. It is anticipated that an increasing number of laboratories will replace the traditional phenotypical serotyping with WGS-predictive serotyping methods.

In addition to establishing serovars, NPHRLs often assess the relatedness of encountered isolates using molecular-based clustering techniques. Traditionally, PFGE and MLVA were the most frequently used molecular subtyping methods. However, in recent years these have rapidly been replaced by WGS-based typing, due to its higher resolution and more accurate cluster assignment and microbial source tracing [13, 14, 15].

The fulfilment of ECDC and FWD-Net surveillance objectives relies heavily on the data provided by the NPHRLs in the Member States. Therefore, it is important to monitor the typing methods used, data quality and comparability, and the capability of the laboratories performing these methods. ECDC organises EQAs for NPHRLs in EU/EEA Member States to facilitate harmonisation and increase the quality of diagnostic laboratory methods. It supports the availability of high-quality and comparable laboratory surveillance data, thereby facilitating the detection of emerging threats at EU level [16]. In addition, EQAs are an important tool to support objectives in the ECDC public health microbiology strategy, such as facilitating a technology transition towards EU-wide use of WGS for surveillance purposes and strengthening public health microbiology capacity in general [17]. When implementing WGS for continuous surveillance and multi-country outbreak investigations, food and waterborne diseases and zoonoses, such as *Salmonella* enterica, were identified as a specific priority [18].

Since 2019, countries have been able to report WGS data to TESSy for *Salmonella*. The aims of integrating molecular typing data into EU-level surveillance are to:

- foster the rapid detection of dispersed international clusters/outbreaks;
- facilitate the detection and investigation of transmission chains and relatedness of strains across EU/EEA;
- contribute to global outbreak investigations;
- detect the emergence of new and/or evolving pathogenic strains;
- support investigations to trace the source of an outbreak and identify new risk factors;
- aid the study of a particular pathogen's characteristics and behaviour in a community of hosts.

Molecular typing-enhanced surveillance gives Member State users access to EU-wide molecular typing data for the pathogens included. It also provides users with the opportunity to perform cluster searches and cross-sector comparability of EU-level data to determine whether isolates characterised by molecular typing at the national level(s) are part of a multinational cluster that may require cross-border response collaboration.

1.3 Objectives of the EQA-14 on *Salmonella*

EQA schemes offer quality support to those NPHRLs that perform molecular typing-enhanced surveillance and those implementing it into their surveillance system at the national level.

1.3.1 Serotyping

The objective of the serotyping part of EQA-14 was to assess the capabilities for identifying *Salmonella* serovars within the NPHRLs of the EU/EEA and enlargement countries. Laboratories were asked to use their routinely applied method for serotyping on provided isolates. This made it possible to monitor the methods used and their performance in serotyping.

1.3.2 Molecular typing-based cluster analysis

The objective of the molecular typing-based cluster analysis part of EQA-14 was to assess the ability of NPHRLs in the EU/EEA and enlargement countries to designate clusters of *Salmonella* isolates. Laboratories were able to use WGS, MLVA and/or PFGE techniques to perform the cluster analysis on the isolates provided. This made it possible to monitor the methods used and their performance in relation to cluster assignments. In addition, for participants using WGS-based cluster analysis, an extra five sequences were provided. The EQA provider had manipulated some of the sequences to mimic inferior-quality genomes. The participants were expected to identify the inferior-quality sequences and perform cluster assignment of the good-quality sequences.

2 Study design and methods

2.1 Organisation and participants

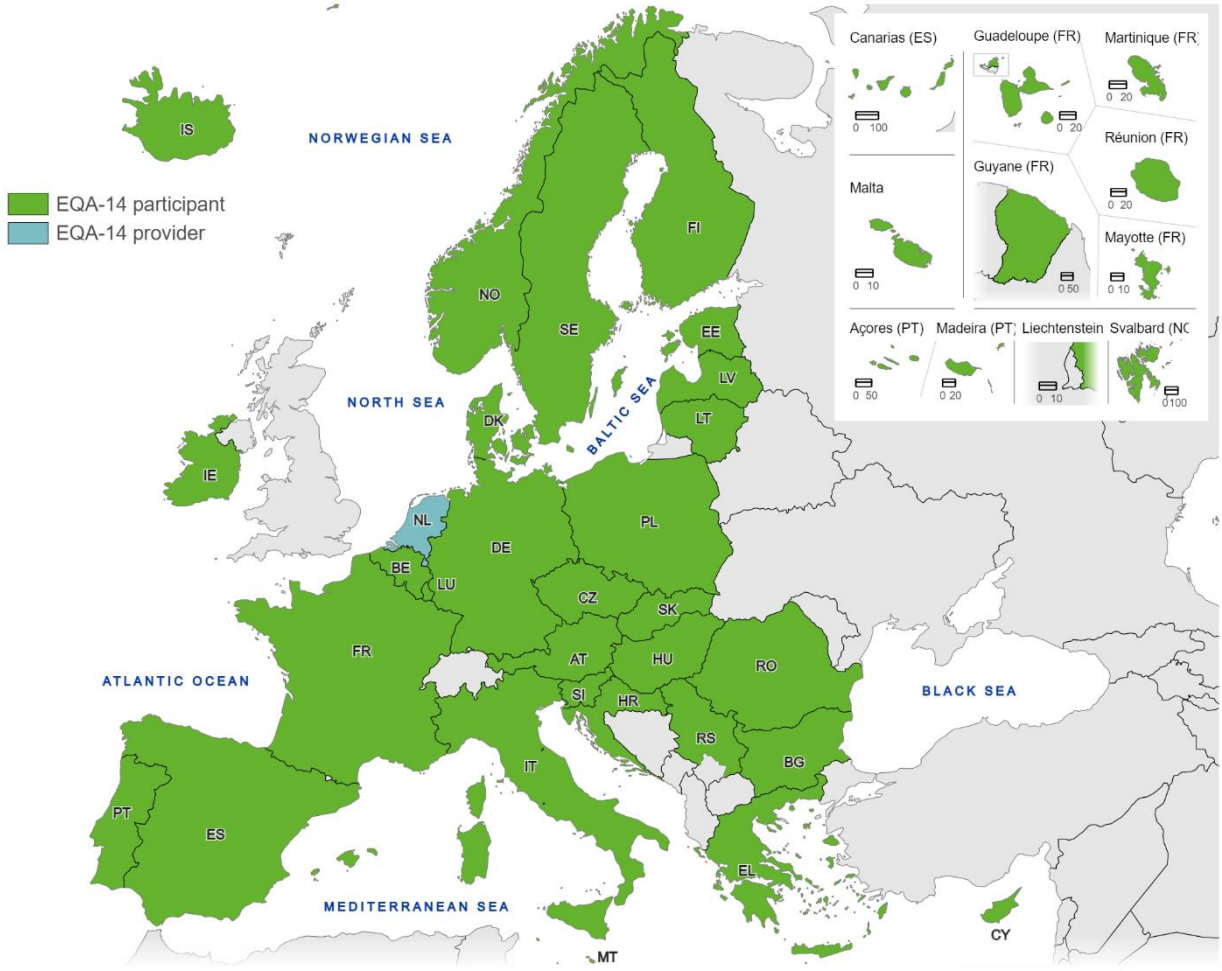
On behalf of ECDC, the EQA-14 was organised by RIVM under framework contract ECDC/2021/014-lot 1 for NPHRLs in the EU/EEA and enlargement countries. Participation of one laboratory per country was funded by ECDC.

Invitations for the EQA-14 were distributed by RIVM to the FWD-Net contact points for EU/EEA countries. In addition, invitations were sent to EU candidate countries by ECDC.

Participating laboratories were able to register for the EQA-14 via an online form, using a link in the invitation. The online form contained questions including contact person, shipping address, whether the participant would participate in both parts (serotyping and cluster analysis) and the main methods used (Annex 1).

The EQA-14 comprised of two parts, serotyping and molecular typing-based cluster analysis. Laboratories were encouraged to participate in both parts, but participation in one part was possible. In total, 30 countries were invited, 29 (97%) of them registered for participation in at least one part, and all 29 (100%) completed at least one part of the assessment (Figure 1, Annex 2).

Figure 1. Geographical overview of participants in EQA-14



2.1.1 Timeline

The invitation for the EQA-14 was sent on 15 April 2024, and the deadline for registration was 10 May 2024. A reminder was sent on 30 April, and the final participant list, drawn up on 13 May 2024, contained 29 participating laboratories.

The samples were distributed to 29 laboratories on 12 June 2024. A total of 28 laboratories (96.6%) received the parcel two days later and one laboratory (3.4%) received it three days after shipping.

The deadline for result reporting was 13 September 2024. Seven laboratories requested an extended deadline. The first results were completed on 10 July and the last on 3 October 2024, with a median of 92 days (range 28–114 days) from shipping to result completion. Individual evaluation reports were shared with participants on 14 November 2024, as scheduled.

2.2 Sample preparation

This EQA was prepared according to ISO standards 15189:2012 and ISO 17043:2010 and Chapter 11 from ISO 13528:2015 for the design and analysis of qualitative proficiency tests. The process of selection and preparation of specimens, confirmatory testing and shipment of the EQA are described in detail below.

2.2.1 Panel selection

For the serotyping part, serovars were selected based on a rationale depicted in Table 1. Three to five isolates of each serovar were cultured and assessed for their reaction in agglutination. For each serovar, the isolate with the most profound reactions was selected and given a number between EQA2401–EQA2412. The selected isolates were tested blindly using traditional agglutination by another team member to reach an expert consensus on the assigned values. All pure cultures were subjected to Illumina sequencing to assess contamination and assignment of serovar using WGS serotype prediction. Isolates were stored in agar slants at room temperature until bulk culturing.

Table 1. Selected panel for serotyping part of EQA-14, including selection rationale

EQA #	Subspecies[a]	Serovar	Formula	Rationale
EQA2401	*enterica*	London	3,10:l,v:1,6	Serotype shown to be difficult to type in the previous round of this EQA.
EQA2402	*enterica*	Typhimurium, monophasic	4,5:i:-	[b]
EQA2403	*enterica*	Fillmore	6,8:e,h:e,x	Challenging serotype when using phenotypic methods only.
EQA2404	*enterica*	Schwarzengrund	4:d:7	Serotype caused a multi-country outbreak in 2022.
EQA2405	*enterica*	Oslo	7:a:e,x	To have some diverse O- and H-types.
EQA2406	*diarizonae*	IIIb 47:k:z35	47:k:z_{35}	Different subspecies as bonus isolate; serotype shown to be difficult to type in the previous round of this EQA.
EQA2407	*enterica*	Enteritidis	9:g,m:-	[b]
EQA2408	*enterica*	Infantis	7:r:1,5	[b]
EQA2409	*enterica*	Derby	4:f,g:-	[b]
EQA2410	*enterica*	Typhimurium	4,5:i:1,2	[b]
EQA2411	*diarizonae*	Moroto	28:z_{10}:l,w	To have some diverse O- and H-types
EQA2412	*enterica*	Molade	8,20:z_{10}:z_6	Challenging serotype when using molecular methods only.

[a]All isolates were Salmonella enterica. [b]One of five most reported serotypes of human salmonellosis in Europe [42], of which some also caused multi-country outbreaks in 2020-2023.

For the cluster analysis, a mock outbreak situation was provided for the participants. The outbreak situation is outlined below:

> 'During a neighborhood party, a buffet-style dinner was served to 50 guests. All dishes were homemade or store-bought by participating residents. Two days after, 39 of the invitees fell ill with diarrhea. One of the guests was admitted to hospital. A *Salmonella* isolate was cultured from her feces, isolate EQA2413. After this, microbiological investigation in the remaining cases rendered nine isolates and nine more cases.'

To mimic this outbreak situation, nine *Salmonella* isolates with cluster and non-cluster isolates were selected from the Dutch national surveillance collection, based on cgMLST analysis (Table 2). One of the isolates was numbered twice, to include a technical duplicate (EQA2414 and EQA2416), resulting in 10 isolates for the cluster analysis

(EQA2413-EQA2422). Participants were requested to report the isolates that clustered with the index case (EQA2413) according to their own cluster cut-off. When using WGS techniques, four of the 10 *Salmonella* isolates clustered closely with the index. Therefore, a participant could report up to four isolates in the cluster, depending on the resulting allele distance and the cluster cut-off used. All 10 isolates were analysed with MLVA (Table 2). Using MLVA, four of the ten isolates clustered to the index EQA2413 because they exhibited exactly the same MLVA profile. Another team member analysed the same data to reach consensus on the assigned clustering using cgMLST and MLVA. All isolates were stored in agar slants at room temperature until bulk culturing.

Table 2. Selected panel for molecular-based cluster analysis part of EQA-14

# EQA	Serovar	Part of cluster[a]	Distance to index cgMLST(AD)	Distance to index cSNPs	MLVA profile
EQA2413	Monophasic Typhimurium	Index	0 (index)	0 (index)	03-15-11-00-211
EQA2414[b]	Monophasic Typhimurium	Yes	1	2	03-15-11-00-211
EQA2415	Monophasic Typhimurium	No	41	115	03-13-11-00-211
EQA2416[b]	Monophasic Typhimurium	Yes	1	2	03-15-11-00-211
EQA2417	Monophasic Typhimurium	No	38	81	03-14-14-00-211
EQA2418	Monophasic Typhimurium	No	14	41	03-13-12-00-211
EQA2419	Heidelberg	No	2276	33433	01-13-00-00-111
EQA2420	Monophasic Typhimurium	Yes	1	3	03-15-11-00-211
EQA2421	Monophasic Typhimurium	No	19	377	03-14-08-00-211
EQA2422	Monophasic Typhimurium	Yes	1	1	03-15-11-00-211

cSNPs = core single nucleotide polymorphism. [a]Cluster assignment of EQA provider based on cgMLST. [b]Technical duplicates.

The clustering of isolates using PFGE was not known beforehand, since RIVM no longer performs PFGE. In EQA-14, PFGE was not performed by any of the participants.

For the participants that used WGS-based cluster analysis, there was an additional exercise in the mock outbreak situation.

> 'The buffet at the neighborhood party consisted of different food products (meats, fruit skewers, mixed salads, charcuterie, ice cream) from which leftovers were saved in the refrigerator of the community center where the party was hosted. The leftovers were sampled by the food safety authorities and *Salmonella* isolates (coded EQA2423 – EQA2427) cultured from these food products were sequenced using Illumina WGS techniques. The raw reads are available for download.'

To mimic this additional outbreak investigation, raw reads of five additional isolates were selected or manipulated and made available to the participants using WGS for cluster-analysis. These isolates acted as food isolates and participants were asked which food product was most likely to be causing the outbreak. The characteristics of these reads are depicted in Table 3. All reads were analysed for quality and clustering with the index case EQA2413 by another team member to reach consensus.

Table 3. Additional raw reads provided for WGS analysis

# EQA	Serovar	Manipulation	Quality	Distance to index cgMLST(AD)	Distance to index SNPs
EQA2423	Monophasic Typhimurium	Contaminated with 20% *Salmonella bongori*	Bad quality, contaminated	NA	NA
EQA2424	Monophasic Typhimurium	Low coverage	Bad quality, too low coverage	NA	NA
EQA2425	Monophasic Typhimurium	Contaminated with human DNA	Bad quality, contaminated	NA	NA
EQA2426	Monophasic Typhimurium	None	Good quality	1	3
EQA2427	Monophasic Typhimurium	None	Good quality	86	151

2.2.2 Confirmatory testing and distribution

When the panels were definitive, homogeneity of the specimens was assessed and confirmatory testing for qualitative serotype data was performed for the serotype panel. The passing criterium for these specimens was that serovars should be 100% in agreement with previous testing. Homogeneity for the cluster analysis panel was assessed by confirmatory sequencing and the passing criterium for these samples was that they should not exceed cluster cut-off of five alleles.

After establishing sufficient homogeneity, panels were prepared by culturing and aliquoting each strain from the same pure culture over agar tubes for the number of participants, plus 10 extra tubes. To assess the stability of the samples, the results of the homogeneity testing served as a starting point for confirmatory testing. All samples were retested on the day of shipment, on the day the last participant received the parcel according to the shipper, and on the last day the results could be submitted. The specimens of the serotyping part were confirmed using phenotypical testing with antisera and all agglutination reactions were identical at all timepoints. In addition, the specimens of the serotyping part were also confirmed using WGS, and all results were identical at all timepoints, indicating a stable serotyping panel. The specimens of the molecular typing-based panel were sequenced at all time points and analysed using cgMLST and MLVA. All samples fell within the same sample clusters at all timepoints, indicating a stable cluster analysis panel. All samples fell within the same clusters at all time points, indicating a stable MLVA-cluster analysis panel.

All specimens were distributed on agar slants and packaged in biological safety bags per panel. Dispatch and shipping documents were prepared and safety instructions, storage instructions, EQA protocol and instructions for reporting results were sent to participants together with the panels, and separately by email. All parcels were shipped at ambient temperature as biological substance category B, according to UN Regulation 3373.

2.3 Typing methods used by provider

For serotyping, the EQA provider used phenotypical serotyping with antisera and serotype prediction using WGS data. Phenotypic serotyping was performed with slide agglutination according to the White-Kauffman-Le Minor scheme [11], using a combination of commercially acquired (Sifin and SSI Diagnostica) and in-house prepared antisera. Phase inversion was performed using the Sven Gard method using 5g/l heart infusion agar with 0.1% glucose in 50mm Petri dishes. Subspecies were determined with commercially acquired biochemical tests; fermentation of dulcitol, D-sorbitol and salicin, malonate utilisation and the ortho-Nitrophenyl-β-galactosidase (ONPG) test (BioTrading and Tritium) in 15 ml tubes and interpreted according to White-Kauffman-Le Minor [11].

For production of WGS data, DNA from pure isolates was automatically extracted on a Maxwell RSC instrument using the Maxwell RSC cultured cells DNA Kit. Library preparation was performed using the Illumina DNA Prep kit. Illumina sequencing was performed on a Nextseq 500 or 550 machine using an Illumina NextSeq 500/550 Mid Output or High Output kit v2.5, producing 2 x 150 bp paired-end reads. Reads were processed using the in-house developed quality control and assembly pipeline 'Juno-assembly' v3.0.6 [19] based on SPAdes 3.15.3 [20], consisting of FastQC v0.11.9 [21] to assess the quality of the raw reads, FastP v0.20.1 [22] to remove poor quality data and adapters, Picard v2.26.0 [23] for library fragment determination, QUAST v5.0.2 [24], Bbtools v38.86 [25] and MultiQC v1.11 [26] to assess and visualise the quality of uploaded assemblies, complemented by CheckM v1.1.3 [27] and Kraken2 v2.1.2/Bracken v2.6.1 [28, 29] to calculate scores for completeness and contamination. Sequences with a Phred quality score ≥30 and resulting de novo assemblies with a total length between 4.4−5.8 Mbp, N_{50} > 30 Kbp, GC% of 51.6−52.3%, number of contigs <300, average coverage (assembled) ≥30x, genome completeness >96%, and a contamination of <4% pass the provider's quality criteria.

On the basis of the filtered and trimmed reads output, the *Salmonella* serotype was predicted using the in-house developed pipeline 'Juno-typing' v0.8.6 [30], based on SeqSero2 v1.1.1 in micro-assembly mode.

For cluster analysis, de novo assemblies were used for cgMLST and imported into Ridom SeqSphere v9.0.8. in which the Enterobase *S. enterica* cgMLST V2 scheme (3,002 loci) was used. Hamming distances were calculated, ignoring pair-wise missing alleles, and distances were visualised with a Minimum Spanning Tree (MST). For single nucleotide polymorphism (SNP)-analysis, the in-house developed pipeline 'Juno-SNP' v1.2.0 (accessed on 7-10-2024) [32] was used to establish core SNP variants against reference EQA2413, based on Snippy v4.6.0 [33] and VCF-kit v0.2.8 [34] for distance calculation and visualisation.

MLVA analysis was performed using capillary fragment length analysis on five previously-identified loci [35]. The resulting profiles of the alleles STTR9, STTR5, STTR6, STTR10 and STTR3 were analysed using BioNumerics v7.6.3.

PFGE typing was not performed by the provider since none of the participants performed PFGE typing during EQA-14.

2.4 Results assessment and reporting

Participants were expected to use their routinely applied methods for both parts of the EQA. Information on their analytical methods and results was collected and compiled using an online form system (Annex 3). Individual performances of participants on both specific tasks (i.e. serotyping and molecular typing-based cluster analysis) were assessed as qualitative results and reported in individual evaluation reports. Participants were asked for more information via email if the reported results were not clear enough.

2.4.1 Assessment of results

For specimens in the serotyping part, participants were expected to report the species, subspecies, seroformula and serovar. The final assessed qualitative result is the serovar reported. A correct result is defined as concordance with the EQA provider, depending on the technique used (phenotypical or molecular serotyping). As a challenging isolate, a subspecies other than *enterica* was added (EQA2406). With serotype prediction using WGS, the result of this isolate was unambiguously subspecies *diarizonae*, and this was the only result considered correct if participants used WGS prediction.

Some laboratories did not use the correct notation for seroformulae, these were considered as incorrect results, and feedback was provided on the incorrect notation. Using this approach, percentages of correctly identified serovars were calculated per laboratory and per sample. In addition, all incorrectly assigned serovars were further analysed using the detection of subspecies, O-antigens and H-antigens to establish the type of errors that could have caused the incorrect serotyping. All participants who used WGS for serotyping were welcome to optionally report the 7-locus MLST type of all isolates provided for the serotyping panel. The correctness of the 7-locus MLST type was assessed by the EQA provider.

For participants that used WGS for cluster analysis, correct results were defined as concordance with the EQA provider for cluster designations based on cgMLST or SNP typing, depending on the technique used. Participants were expected to use their routine analysis pipelines to evaluate genetic relatedness, including the raw reads provided by the organiser. In addition, participants had to be able to assess the quality of the raw reads provided, including indicating the specific issues if quality was insufficient.

Participants were required to upload their raw reads (fastq or fastq.gz) to the Research Drive sharing platform. The quality and distances to index EQA2413 of the sequences generated by participants using Illumina techniques was assessed by the EQA provider, using the methods for quality assessment described in Section 2.3 of this report.

For participants that used MLVA for cluster analysis (n=2), correct results were defined as congruence with the EQA provider for MLVA profiles.

All descriptive analyses and comparison of groups, including visualisation, were performed using Microsoft Excel, R v4.2.1 and ggplot2 3.5.1 [38].

2.4.2 Reporting of results

For serotyping, all results were analysed for each participant and reported in the individual evaluation reports, including a percentage of correctly reported serovars. If serovars were incorrectly reported, specific comments were made by the EQA provider. In this way, participants were able to easily interpret their own performance.

For the molecular-based cluster analysis part, results were analysed for each participant and set out in the individual evaluation reports, including a percentage of correctly assigned cluster isolates. In addition, a detailed quality report of the WGS performance was provided for Illumina data. The individual reports included feedback about specific recommendations for improvements or, where necessary, troubleshooting advice.

In this comprehensive technical report, all results were aggregated to compare results for serotyping among all participants that used the same technique and with results from the year before. Results were used to assess which serovars were challenging to use as input for the next EQA. In addition, results from the molecular typing-based cluster analysis were aggregated to compare cluster designations made by all participants that used the same technique, and to monitor the variety in MLVA types. In this way, stakeholders could be informed of the capability and capacity for serotyping and molecular typing-based cluster analysis of *Salmonella*.

2.5 Feedback survey

On 5 December 2024, after distribution of the individual evaluation reports, a feedback survey was sent to participating laboratories that had completed EQA-14 (Annex 4). In this survey, participant experiences and practical use of the EQA results, including corrective measures, were collected to ensure maximum benefit and to prepare for the next EQA programme.

3 Results

3.1 Serotyping results

All of the 29 laboratories that completed at least one part of the assessment registered for serotyping and submitted results. Their results are described in this section.

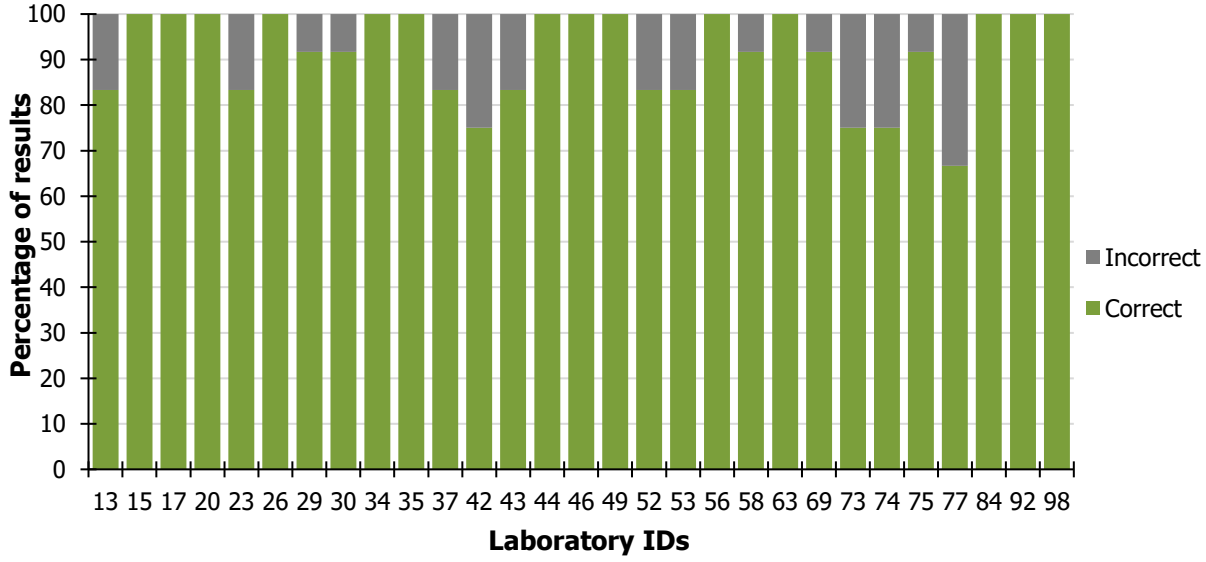
3.1.1 Methods used by participants

Of the 29 laboratories that had completed serotyping results, 16 (55%) used phenotypical serotyping based on agglutination with antisera only, five laboratories (17%) used prediction of serotype with WGS only, seven laboratories (24%) used a combination of agglutination with antisera and prediction of serotype with WGS, and one laboratory (3%) used a combination of molecular genetic serotyping with Luminex techniques combined with phenotypical serotyping based on agglutination. Details of methods for each participating laboratory can be found in Annex 5.

3.1.2 Results of participants

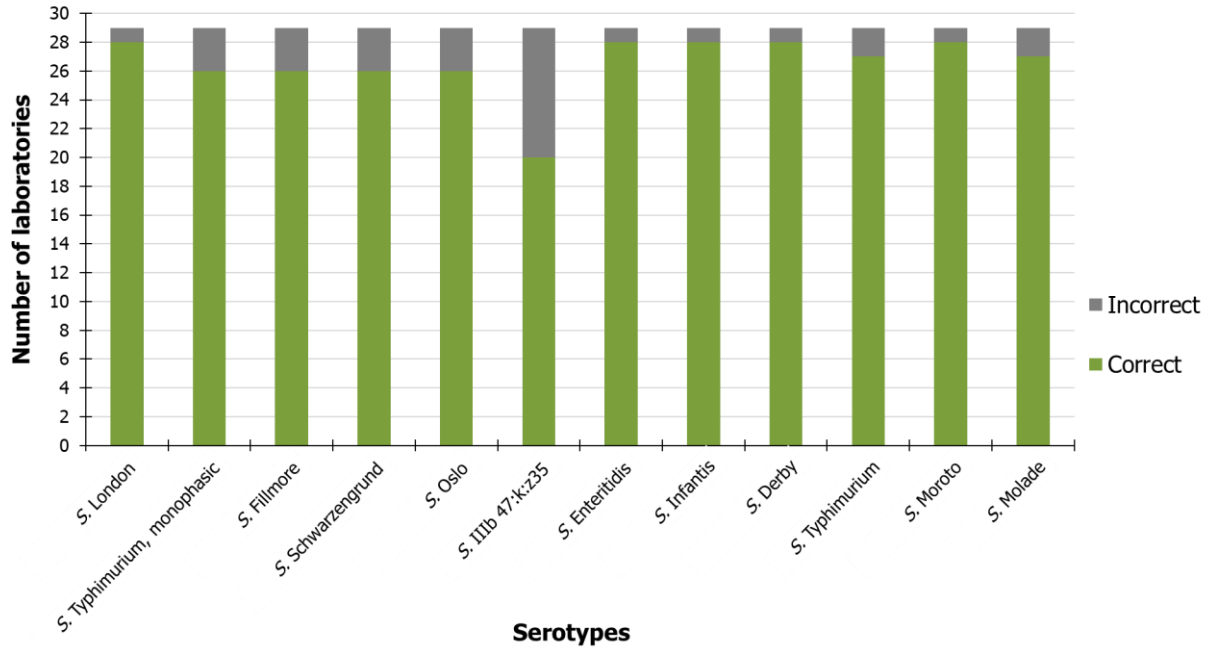
In total, 14 of 29 laboratories (48%) serotyped all isolates correctly, resulting in a performance score of 100%, five laboratories (17%) had a performance score of 11 out of 12 (92%), and six laboratories (21%) had a performance score of 10 out of 12 (83%) (Figure 2). Four laboratories serotyped between three and four isolates incorrectly, resulting in 75% and 67% as performance scores (Figure 2). A detailed description of all serotyping results for each participating laboratory is shown in Annexes 6 and 7.

Figure 2. Results of serotyping by participating laboratories



None of the 12 samples were correctly serotyped by all the 29 laboratories (Figure 3). *S.* London (EQA2401), *S.* Enteritidis (EQA2407), *S.* Infantis (EQA2308), *S.* Derby (EQA2409) and *S.* Moroto (EQA2411) were correctly serotyped by 28 of 29 laboratories. *S.* Typhimurium (EQA2410) and *S.* Molade (EQA2412) were correctly serotyped by 27 of 29 laboratories (Figure 3). *S.* Typhimurium monophasic variant (EQA2402), *S.* Fillmore (EQA2403), *S.* Schwarzengrund (EQA2404) and *S.* Oslo (EQA2405), were correctly serotyped by 26 of 29 laboratories, and *S.* IIIb 47:k:z35 (EQA2406) was correctly serotyped by 20 laboratories (Figure 3).

Figure 3. Results of serotyping by serotype



When all results were combined from all 29 laboratories that performed serotyping, 318 of 348 isolates (91%) were correctly assigned to the serotype (Annex 7). For the 30 incorrectly assigned serotypes, 32 different types of errors in the detection of subspecies, O-antigens and H-antigens in first and second phase formed the foundation. Most of these errors (84%, 27/32) were made only once, and 16% (5/32) of the errors were made twice or more by different laboratories. A detailed description of concordance and error type per sample is shown in Annex 8.

After excluding the laboratory that used a method other than phenotypic, WGS or a combination of both (n=1) the methods used by 28 laboratories were compared to assess whether the use of phenotypic (n=16), WGS predictive (n=5) or a combination of phenotypic and WGS predictive (n=7) methods effects overall performance. There was no difference observed in the performance score of the samples based on the method used (p-values ranging from 0.0921 to 0.6884, χ^2). When all samples serotyped by laboratories using only phenotypic methods were combined, 173 of 192 serovars were correctly assigned (90%). For the samples serotyped by laboratories using WGS predictive typing, 55 of 60 serovars were correctly assigned (92%). For the samples serotyped by laboratories using a combination of phenotypic methods and WGS predictive typing, 79 of 84 serovars were correctly assigned (94%). Comparison between the use of phenotypical serotyping with antisera only, prediction of serotype with WGS only and a combination of the two showed that there was no significant difference between the overall performance in serotyping when applying one of these methods (p=0.5598, χ^2).

Laboratories that performed serotyping were also given the option to report the MLST sequence types of the 12 isolates provided. Twelve of 29 participants (41%) provided MLST sequence types which were all in concordance with those found by the EQA provider, except for EQA2412, for which no MLST type could be determined by one laboratory (Annex 9).

3.2 Molecular-based cluster analysis

Of the 29 laboratories that completed at least one part of the assessment, 25 had registered for the molecular-based cluster analysis, 24 of which submitted results. Four laboratories registered for the serotyping, but did not register for the cluster analysis. The reasons for this were a lack of resources (n=1), no performance of cluster analysis (n=2) and only bi-annual participation in cluster analysis PT due to accreditation requirements (n=1).

In this section, the results of the 24 laboratories that completed the molecular-based cluster analysis part are described for each technique used.

3.2.1 Methods used by participants

All 24 laboratories that completed molecular-based cluster analysis results used WGS techniques (100%), with two of these laboratories also submitting MLVA results. Details of methods for each participating laboratory can be found in Annex 11.

Of all 24 laboratories that used WGS for their cluster analysis, 22 (92%) used Illumina as a platform, one used Nanopore andone used Ion Torrent. A total of 20 laboratories (83%) used a gene-by-gene-approach and four (17%) used SNP-typing (Annex 11). Of the laboratories that used Illumina sequencing (n=22), 10 (45%) used the Nextera XT DNA Library kit, 10 used the Illumina DNA Prep kit (45%), one used KAPA HyperPlus and one used the MiSeq Reagent Kit v3 as a library preparation kit (Annex 11).

Of the 20 laboratories that used a gene-by-gene approach, 10 (50%) used SeqSphere as MLST tool. ChewBBACA was used by five laboratories (25%). Enterobase and Bionumerics were used by two laboratories each (10% each) and one laboratory (5%) used an in-house pipeline as MLST tool (Annex 10). The Enterobase MLST scheme was the most frequently used (13/20, 65%), while other schemes used were cgMLST.org (n=2), INNUENDO (n=2), an in-house developed scheme (n=1), and Applied Maths/Enterobase (n=1). One laboratory used the unknown scheme 'Core Genome' (n=1). A median of five allelic distances was used as cluster cut-off (range: 3–20). One laboratory did not specify the used cluster cut-off (Annex 10).

Of the four laboratories that used SNP-typing, three (75%) used the index EQA2413 as reference genome in their analysis and one laboratory (25%) used Kmer to find the closest match. For SNP-typing, the laboratories used ND tree (n=1), CFSAN SNP pipeline v2.2.1 (n=1), CSI phylogeny (n=1) and an in-house SNP tool (n=1). Three laboratories used a cluster cut-off of five SNPs and one laboratory used a cut-off of 10 SNPs (Annex 11).

Species confirmation of the resulting WGS data was performed by all 24 (100%) laboratories, mainly (50%) using kraken/kraken2, either alone or in combination with another tool (Annex 11). Other tools used were Mash/Mash Screen (n=6), KmerFinder (n=3), SeqSero (n=2), ConFindr (n=1), rMLST (n=3), Enterobase (n=1), gtdbtk v2.3.2 (n=1), and BLAST against an in-house database (n=1).

In conclusion, 22 different combinations of platforms, approaches, kits, tools, schemes and cluster cut-off were used by the 24 participating laboratories for WGS-typing, showing very diverse methods and combinations of methods for WGS-based cluster analysis (Annex 11).

Laboratories were asked to report their routinely used parameters and thresholds for quality control of WGS data. Genome size was the most frequently assessed parameter (20/24, 83%). Coverage was assessed by 19 laboratories, and thresholds varied from 15 to 50 (median 30x). Coverage of contigs and unassembled reads were both assessed but not always defined as such. Eighteen laboratories (75%) determined the number of contigs, 14 laboratories determined the N50 value (58%) and 14 laboratories (58%) assessed the contamination of the sample, mainly through percentage of species alignment, but also through assessment of guanine-cytosine content (GC-content). Twelve laboratories (50%) assessed the percentage of good targets in the MLST scheme used. More details on parameters used and the threshold assigned by the participants can be found in Annex 12.

Laboratories that performed WGS-based cluster analysis were also given the option to report the MLST sequence types of the 10 isolates provided. All 24 participants (100%) provided MLST sequence types. MLST sequence type results of 21 of the 24 participants (88%) were all in concordance with those found by the EQA provider (Annex 13). Two laboratories (8%) determined a different MLST sequence type for EQA2419, and one laboratory (4%) reported different MLST sequence types for all ten isolates provided.

Two laboratories performed MLVA-based cluster analysis in addition to their WGS-based cluster analysis (Annex 10). As MLVA cluster cut-off, one locus difference was used by one laboratory, while 0 locus difference was used by the other laboratory.

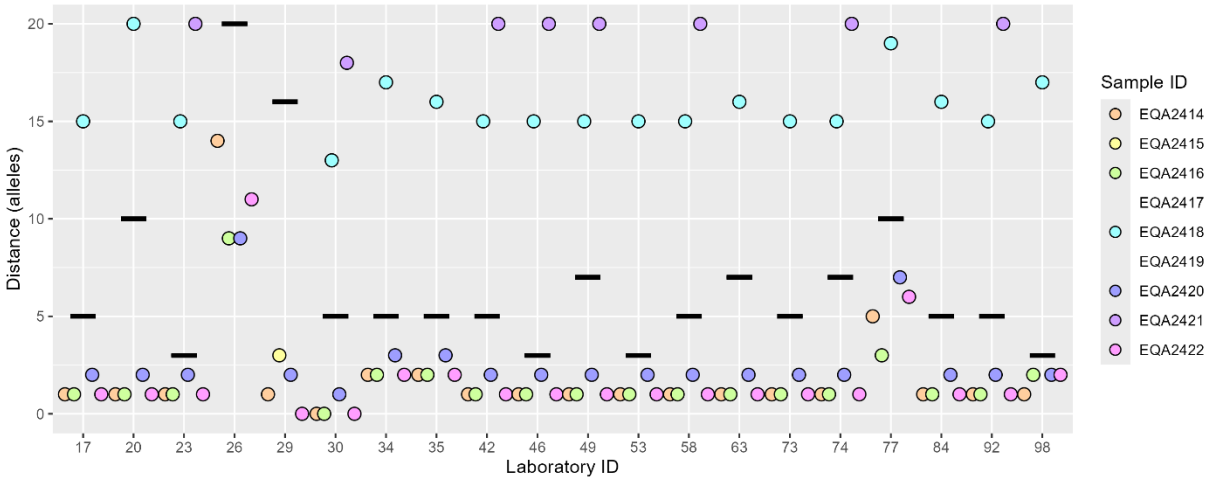
3.2.2 Results WGS-based cluster analysis

Using WGS-based cluster analysis, almost all isolates (256/264, 97%) were assigned correctly to the cluster of index EQA2413 or as singleton, despite the variety of methods used (Annex 11). This performance calculation was based on cluster or singleton assignment of provided isolates EQA2414-EQA2422 and provided good quality raw reads EQA2426 and EQA2427 (Annex 14).

Assessing the reported distances in alleles in relation to the cluster cut-off for laboratories that have used a gene-by-gene approach showed that the cluster isolates EQA2414, EQA2416, EQA2420 and EQA2422 have no or very few distances to reference EQA2413. The exceptions were laboratory 29, where an allelic distance of three was reported for EQA2415 which resulted in an incorrect inclusion of the isolate. The same laboratory also reported an allelic distance of 61 for EQA2416, which resulted in an incorrect exclusion of the isolate. Laboratory 26 reported overall bigger allelic distances compared to the provider, yet all samples were correctly assigned to the cluster due to the chosen cluster cut-off of 20 AD. Assignment to the cluster is dependent on the measured AD and cut-off used by the participants (Figure 4, Annex 15).

Assessing the reported distance by laboratories that used SNP-typing also showed no or close to no SNP difference to the index EQA2413 for EQA2414, EQA2416, EQA2420, and EQA2422 (Figure 5, Annex 15).

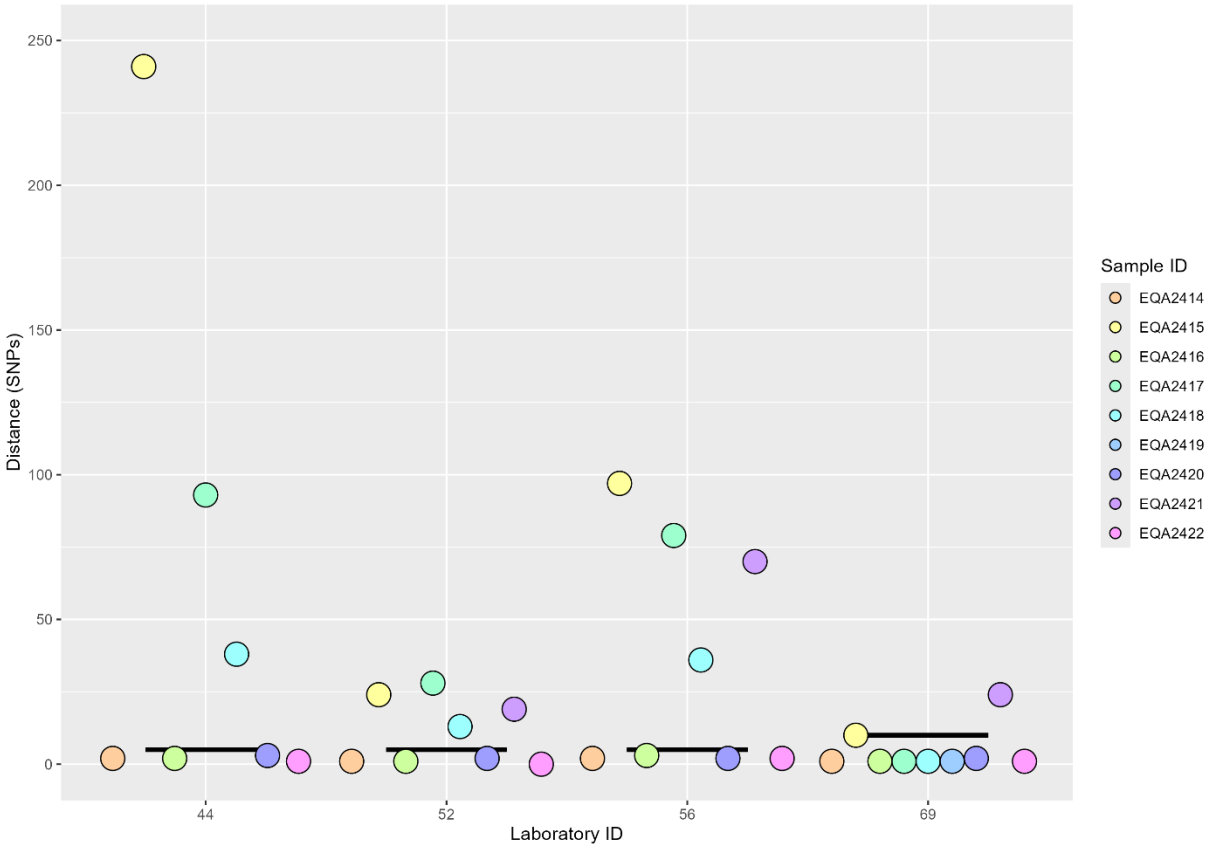
Figure 4. Distance from index EQA2413 in alleles for distances <20 AD, per laboratory



Black lines = cluster cut-offs set by participating laboratories themselves. Data points for EQA2417 and EQA2419 are above 20 AD from the index EQA2413.

To assess the differences of the sequences that participants produced from the isolates EQA2413-EQA2422, without taking all the different analysis methods into account, all the raw reads submitted by the participants were analysed using the provider's cgMLST methods, as described in Section 2.3. A minimum spanning tree (MST) was produced using Ridom SeqSphere v. 9.0.1 (Figure 6).

Figure 5. Distance from index EQA2413 in SNPs for distances <250 SNPs, per laboratory



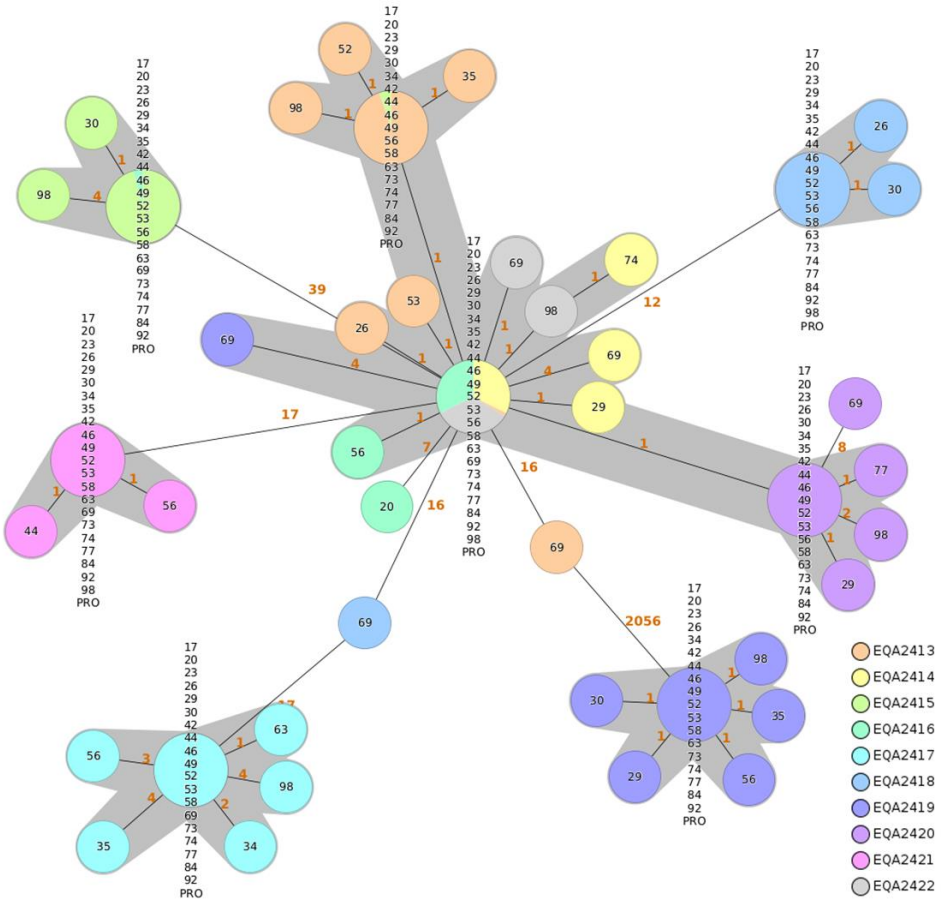
All sequences of the same samples clustered together using the cluster cut-off from the provider (≤5 AD), except EQA2414, EQA2416, EQA2420 and EQA2422 from laboratory 26, and EQA2420 and EQA2422 from laboratory 77. Sequences of laboratory 26 generally had a greater AD (9-14 AD) and the two mentioned sequences of laboratory 77 were still within close range (≤10 AD) of the cluster and would be considered as probably related, prompting further investigation in an outbreak situation (Figure 4).

Laboratory 29 seemed to have swapped isolates EQA2415 and EQA2416, as they assigned the former to the cluster instead of the latter. The position of those isolates in comparison to those of the provider shows that EQA2416 of laboratory 29 clusters closely (0 AD) with EQA2415 of the provider, and EQA2415 of laboratory 29 is at 1 AD to EQA2416 of the provider (Figure 6, Annex 14).

Of the four laboratories that used SNP-typing, three laboratories (75%) used a cluster cut-off of 5 SNPs, and one laboratory (laboratory 69) applied a cluster-cut off of 10 SNPs. Samples EQA2414, EQA2416, EQA2420 and EQA2422 were correctly assigned by all four laboratories, however, laboratory 69 also incorrectly assigned samples EQA2415, EQA2417, EQA2418 and EQA2419 to the cluster with reported SNP distances of 1-10 SNPs to the index as assessed by the EQA provider (Table 2, Figure 5). Overall, a relatively low coverage was observed for most isolates by laboratory 69, which might contribute to the cluster assignment of the participant.

Twenty-two out of 24 laboratories (92%) detected the inferior quality of EQA2423 that had a higher percentage of contamination, indicating the presence of multiple *Salmonella* species. The two laboratories that did not detect the inferior quality due to contamination with *Salmonella bongori* both did not perform quality control on parameters that assess contamination directly through percentage of contamination, but did assess contamination indirectly via percentage of species assignment (Annex 12). Three of the 24 laboratories (13%) did not detect the inferior quality of EQA2324 that had a low coverage. Two out of those three laboratories did employ quality control parameters that assess coverage at similar thresholds to that of the provider, which should have enabled the low coverage to be detected. Ten out of 24 laboratories (42%) did not detect the inferior quality of EQA2425 that was artificially contaminated with human DNA by the provider (Annexes 14 and 15). Three out of those ten laboratories did employ quality control parameters that assess contamination directly through the percentage of contamination and indirectly via percentage of species assignment, while of the remaining seven laboratories, six assessed contamination only indirectly. One laboratory did not state whether quality control parameters assessing contamination were employed. Laboratory 52 did not assess the quality of the five additional sequences EQA2423-EQA2427 and assessed all five to be of inferior quality.

Figure 6. Minimum spanning tree of cgMLST by provider for EQA2413-EQA2422



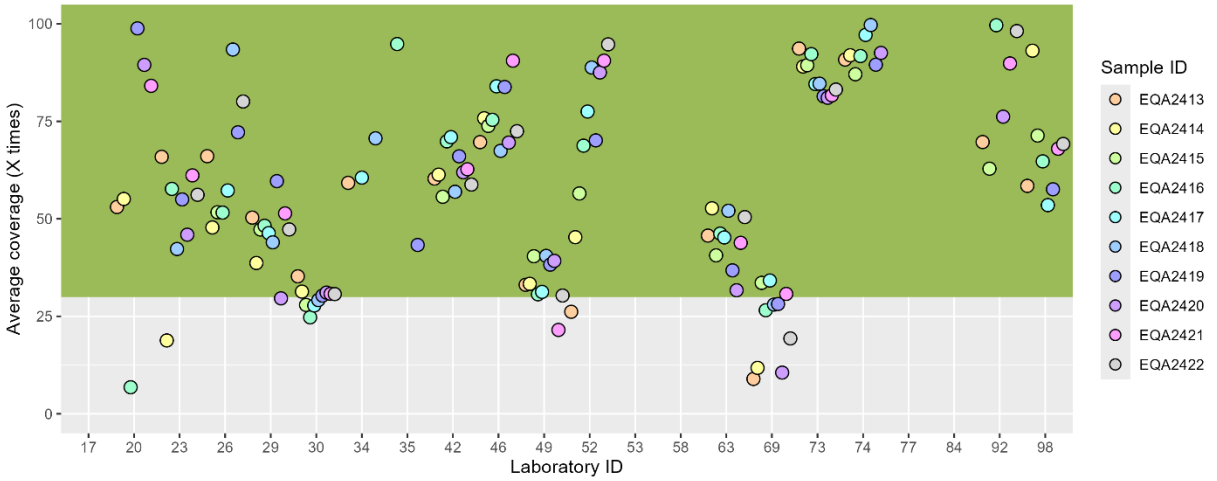
MST for 240 samples, distances based on Enterobase S. enterica V2 cgMLST scheme, pair-wise ignoring missing alleles. Nodes coloured by sample IDs, numbers are laboratories IDs. PRO = provider. Orange numbers = allelic distances. Grey halo = clusters based on ≤5 AD.

3.2.3 Quality assessment of submitted WGS data

All submitted reads resulting from Illumina sequencing were assessed for their quality by the provider with methods and quality criteria described in Section 2.3. This section describes the results of these 22 laboratories.

Average coverage varied strongly among laboratories, with 16 samples from seven different laboratories below the threshold set by the provider (≥30x, Figure 7). Eight laboratories had a very high coverage for most samples; laboratories 17, 34, 35, 77 and 84 had an average coverage between 100−200x and laboratories 53 and 58 had an average coverage with a median (IQR) of 210x (116-246) and 271x (197-374), respectively.

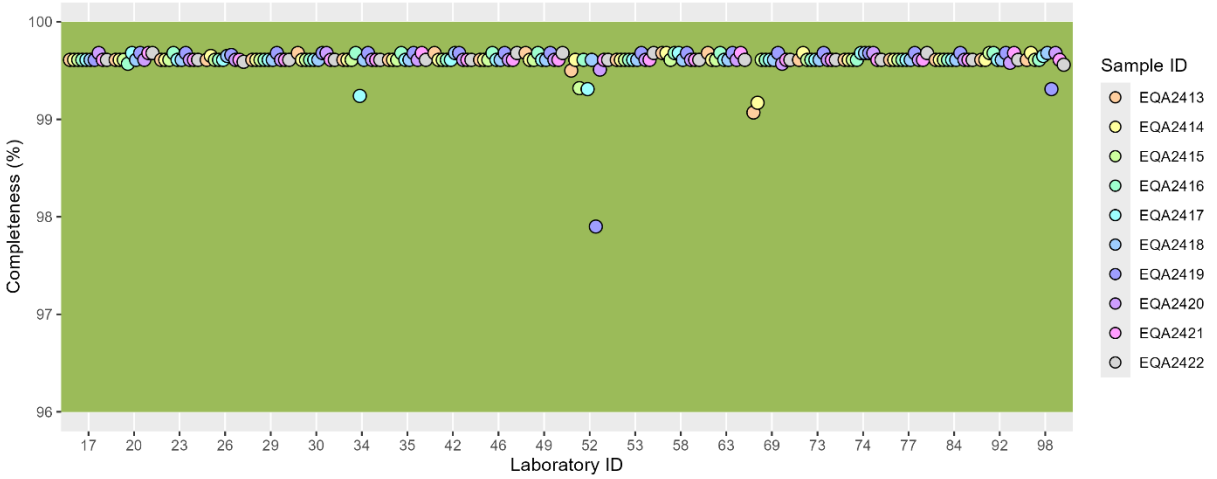
Figure 7. Average coverage of sequenced samples by participating laboratories, <100x



Green range = within quality threshold of provider (≥30x).

The completeness and contamination of all sequences was within the provider's thresholds for all samples and all laboratories (Figures 8 and 9).

Figure 8. Completeness of sequenced genomes by participating laboratories

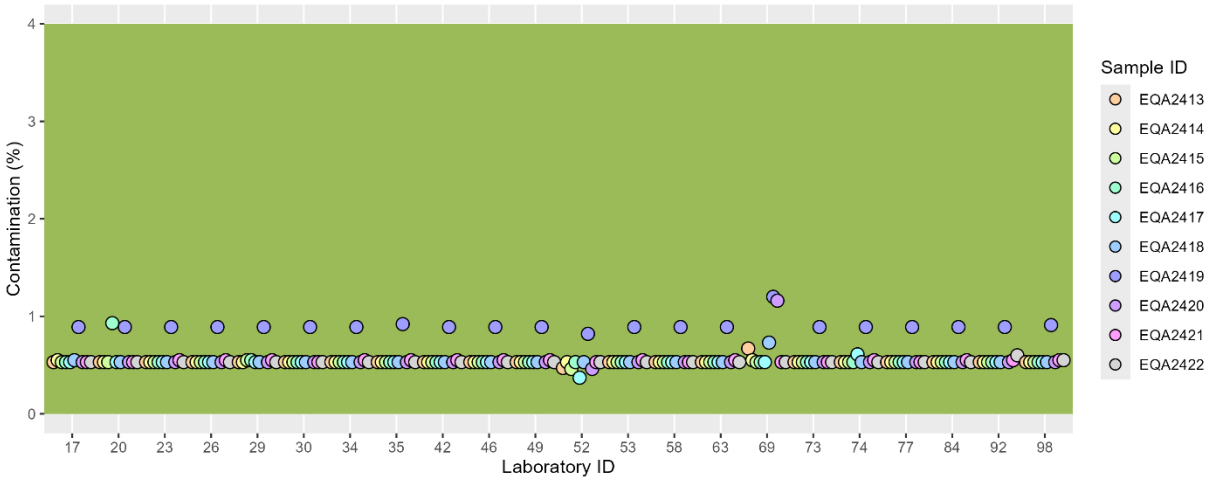


Green range = within quality threshold of provider (>96%).

The GC content of all laboratories' sequences were within the quality threshold of 51.6−52.3%, with a mean content of 52.15% (95% CI; ±0.0099), except for samples EQA2413 and EQA2417 from laboratories 69 and 98, which were slightly above the upper threshold with a GC content of 52.37 and 52.32, respectively.

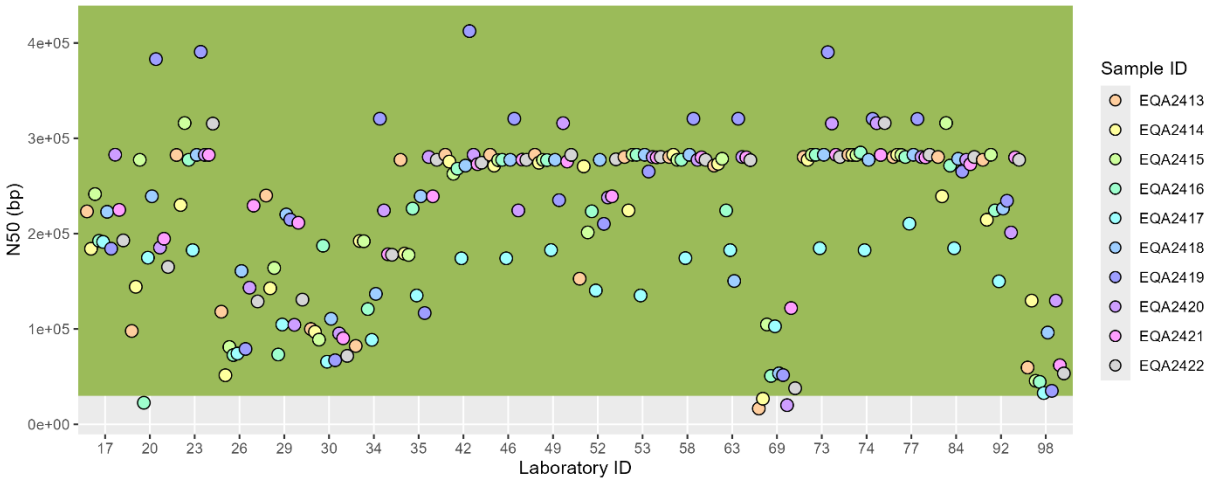
N50 values varied from 17 kbp to 412 kbp, but all but four were above the threshold set (>30 kbp) by the provider (Figure 10). Samples EQA2413, EQA2414, and EQA2420 from laboratory 69 had a N50 of 16.7, 26.9 and 20.2 kbp, respectively, and EQA2416 of laboratory 20 had a N50 of 22.7 kbp, which was below the provider's threshold. Total genome lengths (assembled) were all within the quality threshold and varied from 4.83 Mbp to 5.08 Mbp with a mean of 4 918 520 bp (95% CI; ± 6649). For detailed results of quality assessment of provider from raw sequences submitted by participants, see Annex 16.

Figure 9. Contamination in sequenced samples by participating laboratories



Green range = within quality threshold of provider (<4%).

Figure 10. N50 values in sequenced samples by participating laboratories



Green range = within quality threshold of provider (> 30 kbp).

3.2.4 Results MLVA-based cluster analysis

MLVA was performed by two laboratories, which both performed MLVA in combination with WGS (laboratories 63 and 74). In nine out of 10 isolates (90%) MLVA profiles could be determined and were in concordance with that of the provider (Table 2). For the remaining isolate (EQA2419), no MLVA profile was provided by the laboratory 63 sample. For cluster analysis, the strain set consisted of nine monophasic *S.* Typhimurium isolates and one *S.* Heidelberg isolate (EQA2419). MLVA is not suited for cluster analysis of this serovar. Nonetheless, both laboratories correctly assigned this sample as not part of the same cluster as index EQA2413. The isolates EQA2414, EQA2416, EQA2420 and EQA2422 were considered to be part of the same cluster as index EQA2413 by laboratories 26 and 74, who used a cluster cut-off of zero and one allele difference, respectively (Annex 17).

Overall, both laboratories displayed high technical skill, resulting in 100% (4545 and 48/50) of MLVA alleles being correctly identified, considering all nine isolates that could be typed with MLVA. The inability for typing of the *S.* Heidelberg isolate (EQA2419) was due to the limited applicability of the method, and not due to the capabilities of the participating laboratories. The incomplete typing of EQA2419 did not lead to erroneous inclusion of the sample.

3.2.5 Results PFGE-based cluster analysis

In EQA-14, none of the participating laboratories performed PFGE.

3.3 Results feedback survey

In total, nine out of 29 laboratories (31%) responded to the feedback survey. All used the results of this EQA as documentation for accreditation and/or licensing purposes for the methods used in their laboratory. All the laboratories were satisfied with their individual EQA reports. Seven of nine (78%) laboratories indicated that all their analytical test results conformed to the expected results, and two (22%) took corrective action based on the results of this EQA. One specified the corrective action, which involved review and adjustment of standard operating procedures and verification of reagents. Laboratories were asked to give suggestions for specific serotypes or cluster-analysis issues they would like to see included in future EQAs. Responses did not identify specific serotypes or issues that were not yet addressed in this or previous EQAs for *Salmonella* typing. Two laboratories made comments or suggestions for improving the organisation of the EQA. One suggested that provision of a scheme for the selection of cluster cut-off values in different epidemiological situations would be helpful (n=1). The other laboratory commented that the provision of rare serotypes for typing has added value since it enables participation in international collaboration regarding cross-border infections.

4 Discussion

Thirty countries were invited to participate in the EQA Twenty-nine laboratories (97%) registered for at least one part, received the specimen panel(s) and completed at least one part of EQA-14. Twenty-nine laboratories also completed the serotyping part and 24 completed the molecular typing-based cluster analysis part, which is a similar percentage of participation compared to EQA-13 and higher than for EQA-12 and EQA-11) [39,40].

4.1 Serotyping

Twenty-eight laboratories participated in the serotyping of 12 isolates. This resulted in 16 laboratories (55%) using phenotypic typing with antisera only, five laboratories using WGS-predictive serotyping (17%) only, seven laboratories using a combination of agglutination with antisera and prediction of serotype with WGS (24%), and one laboratory used a combination of genetic serotyping with Luminex combined with phenotypic typing with antisera (3%).

A total of 14 laboratories (48%) had a performance score of 100%. Five laboratories (17%) had a performance sore of 92%, six (21%) had a performance score of 83%, three had performance scores of 75% and one laboratory had a performance score of 67%.

When corrected for sample size, there was no difference observed in total performance scores per sample, with p-values between 0.0921 (for EQA2411; *S.* Moroto) and 0.6884 (for EQA2404; *S.* Schwarzengrund). Differences in overall performance scores were observed between the group of laboratories that used phenotypic methods only (90%, n=14), the group that used WGS-predictive methods only (92%, n=5), and the group that used a combination of agglutination with antisera and prediction of serotype with WGS (94%, n=7). However, statistical analysis showed that differences in typing outcomes using the three approaches were not significant. In EQA-12, comparison in the performance scores of laboratories that used phenotypic methods only, and laboratories that used WGS predictive techniques only showed that the latter performed significantly better ($p=0.0090$, χ^2). In the coming EQA rounds, performance differences in serotyping based on method of choice will be continuously assessed, to establish if this is a trend or a point finding.

In total, 32 different error types were made in the serotyping part, the majority (84%) being made only once. Most error types were false-positive detection, false-negative detection or misclassifications of H-antigens in both phases (n=17, 53%). At least 13 error types point towards the use of less specific antisera and three to incorrect prediction using WGS data, where a first and/or second H-phase was not detected. Additional error types were false-negative, false-positive detection or misclassification of O-antigens in five cases (16%), at least four of which point towards incorrect prediction using WGS data. In total, indication of the use of less specific antisera was observed for six laboratories (13, 29, 37, 43, 53, 73, 74, and 77). Participants that applied phenotypic typing with antisera for their serotyping approach (n=23) were specifically asked to provide information regarding the antisera manufacturer. One laboratory reported that reagents were used but did not specify any further, and one reported that antisera were prepared by the laboratory itself. The other 21 laboratories (91%) used antisera manufactured by Sifin SSI Diagnostica. However, no data on antisera types were provided, meaning that no definitive conclusions could be drawn on the causes of error. The remaining two error types were use of non-standard nomenclature (n=9) and type misclassifications of subspecies (n=1).

Overall, the number of laboratories that participated in serotyping for EQA-14 was slightly higher (4%) than for EQA-13, but 21% higher than for EQA-12. The performance of laboratories which completed the serotyping part in bothEQA-14 and EQA-13 were compared (n=22). Assessment of the serovars tested showed that in EQA-13 a rare serotype *S. IIIb 47:k:z:35* appeared to be challenging, since a wrong or incomplete seroformula was reported by several laboratories (17 of 22 laboratories (77%)). It was therefore included again in the serotyping panel of EQA-14 and results were comparable; 16 of 22 laboratories (73%) were able to report the correct seroformula. In both rounds of EQA, the correct O- and H-types were typed, but the wrong seroformula was reported by several laboratories. Two laboratories reported the incorrect seroformula in EQA-13, but subsequently reported the correct seroformula in EQA-14, which shows that there is a learning effect of participating in these EQAs. On the other hand, four laboratories reported the seroformula correctly in EQA-13, but subsequently reported the seroformula incorrectly in EQA-14. In two cases, the O- and H-antigens were typed correctly but the wrong seroformula was reported. In the other two cases, mistakes were made in determining one or several antigens (Annex 18 and 19). These results indicate that laboratories lack experience not only in typing but also in reporting rare serovars.

In 2022, for the first time in 10 years, FWD-Net and ECDC commissioned an EQA for *Salmonella* which included assessing serotyping and this assessment was continued in EQA-13 and EQA-14. These EQAs represented a starting point for assessing the capability of NPHRLs in serotyping *Salmonella* to monitor trends in methods used and performance for the coming years. More laboratories participated in each EQA compared to the previous ones (29, 27 and 22 in EQA-14, EQA-13 and EQA-12, respectively). Comparing results with previous years, laboratories were able to take successful corrective measures for errors in serotyping and achieve better performance scores. In addition, by re-testing challenging serovars in following EQAs, the capabilities of laboratories can be monitored. and results produced in the coming years will show whether this trend continues.

4.2 Molecular-typing based cluster analysis

In EQA-14, 24 laboratories participated in the molecular-typing based cluster. This resulted in all 24 laboratories (100%) using WGS-based cluster analysis, two of which also applied MLVA-based cluster analysis. PFGE-based cluster analysis was not performed by any of the participants in EQA-14.

A higher proportion of participants (100%, 24/24) applied WGS-based cluster analysis in this EQA than in EQA-13 (96%, 23/24), EQA-12 (85%, 17/20) or EQA-11 (70%, 14/20). The proportion of participants that applied MLVA-based cluster analysis decreased from 40% (8/20, in EQA-11) to 15% (3/20 in EQA-12) to 13% (3/24 in EQA-13 and EQA-14) of laboratories. The proportion of participants that used PFGE-based cluster analysis decreased from 30% (6/20, in EQA-11) to 10% (2/20, in EQA-12) to 4% (1/24) in EQA-13 to 0% (0/24) in EQA-14 [39, 40, 45].

4.2.1 WGS-based cluster analysis

A total of 24 laboratories used an WGS-based cluster analysis, 22 of them (92%) used Illumina sequencing, one (4%) used Ion Torrent and one (4%) used Nanopore sequencing. A gene-by-gene approach was performed by 83% (20/24) of laboratories, while 17% (4/24) performed SNP-typing. In EQA-13, a gene-by-gene approach was applied by 94% (23/24) of laboratories and 6% performed SNP-typing.

A very diverse set of platforms, approaches, kits, cluster analysis tools, typing schemes and cluster cut-offs was used, in which 22 different combinations of methods were employed by the 24 laboratories.

Overall, performance of cluster assignment using WGS-based methods was high, 88% of laboratories (21/24) correctly assigned all the cluster isolates provided, irrespective of whether they used a gene-by-gene or an SNP-approach and despite the variety of methods and cluster cut-offs used. Two laboratories assigned 9 of the 11 isolates correctly. Laboratory 29 appeared to have swapped two samples, which resulted in incorrect assignment of the samples to the cluster. Laboratory 52 did not assess the quality of the five provided sequences and reported all five to be of inferior quality, which resulted in two incorrect assessments. One laboratory assigned 7 of the 11 isolates correctly. Analysis of the uploaded sequence data of this laboratory showed that for most of the sequences, the average coverage was below the threshold of the provider, which could be the cause for the incorrect cluster assignments.

For all four laboratories, the reported distance in alleles or SNPs for the cluster isolates EQA2414, EQA22416, EQA2420 and EQA2422 to index EQA2413 was well below their reported cluster cut-off.

This confirmed the results of the provider's stability tests – that the selected monophasic *S.* Typhimurium and *S.* Heidelberg genomes were very stable – since after storing, transport, culturing procedures and sequencing, it was still possible for the laboratories to infer identity by comparison analysis. It also indicates that WGS-based cluster analysis supports early threat detection capacity for multi-country outbreaks with *Salmonella*, since at least monophasic *S.* Typhimurium and *S.* Heidelberg (in EQA-14), as well as *S.* Dublin (in EQA-13) sequences can be easily shared and meaningful results produced when used in analyses by another laboratory.

A quality assessment was performed on the submitted Illumina reads using the provider's methods and thresholds. For 16 of 22 (73%) laboratories, all sequences passed all the provider's quality criteria. Five laboratories had one sample with a lower coverage just below the providers' threshold, observed in different samples. Two laboratories submitted data that produced a low average coverage in provider's assembly pipeline and as a result, most of the isolates were below the provider's threshold. Two laboratories had very high average coverage. While this is not harmful, they can potentially reduce sequencing costs per sample by including more samples in each sequence run. Two laboratories had one sample with a N50 below the providers' threshold, observed in different samples and two laboratories had one sample with a GC% above the providers' threshold, observed in different samples.

An additional five sequences were made available to participants. One of these isolates was a cluster isolate, which was assigned correctly as a cluster isolate by all laboratories. The remaining four isolates were non-cluster sequences, of which three were manipulated by the provider. The non-manipulated sequence (EQA2427) was correctly assigned as a non-cluster isolate by all laboratories. Reads of EQA2423 were artificially contaminated with *S. bongori*, mimicking inter-species contamination. Reads of EQA2424 were down-sampled to 20% (10X coverage) to mimic a low read count. Reads of EQA2425 were down-sampled to 42% (18X coverage) and supplemented with 58% of reads from human DNA, mimicking contamination.

Of the 24 laboratories that performed WGS-based cluster analysis, 50% (12/24) identified the poor quality of all three isolates. Nine laboratories (38%) failed to identify poor quality in one of the samples, with the contamination with human DNA not being identified in most cases (8/9 laboratories)

Overall, the number of laboratories that participated in WGS-based cluster analysis was slightly (4%) higher than for EQA-13, but 41% higher than for EQA-12. Performance of laboratories which completed the cluster analysis part of this EQA-14 and EQA-13 were compared (n=21). In EQA-13, 16 of those laboratories (76%) had a performance score of 100% and 96% (221/231) of isolates were correctly assigned to the cluster in EQA-13. When considering only the results for the laboratories that also participated in EQA-13, 19 of 21 laboratories (90%) had a performance score of 100% and 97% (225/231) of isolates were correctly assigned to the cluster in EQA-14. Overall, the performance of laboratories that participated in EQA-14 and previous rounds of EQA increased, which shows that capabilities of participating laboratories for cluster analysis are gradually increasing. This is a positive trend which increases confidence in detecting national and international *Salmonella* outbreaks.

4.2.2 MLVA-based cluster analysis

Two laboratories participated in MLVA-based cluster analysis. Both laboratories achieved a 100% performance score in determination of MLVA profiles, only considering the nine isolates that were monophasic *S.* Typhimurium and therefore suitable for MLVA typing. Isolate EQA2419 was of serovar *S.* Heidelberg, and logically, neither laboratory nor the provider was able to determine the complete MLVA profile. Laboratories used an MLVA cluster cut-off of one and zero alleles difference. Although the MLVA profile for this sample could not be determined completely by either laboratory, both still correctly assigned the isolate as not part of the same cluster as index isolate EQA2413. The results show that MLVA is sufficiently specific to discriminate between monophasic *S.* Typhimurium and other serovars. The performance for cluster analysis using WGS and MLVA was compared for the two laboratories that used both methods and they both had a performance score of 100% for cluster analysis using WGS and MLVA. In the previous EQA-13, a set of *S.* Dublin isolates was chosen for cluster analysis for participants that used WGS (or PFGE) for cluster analysis and due to the restrictions of MLVA, an additional strain set was provided for participants using MLVA. This technique should only be applied for outbreaks associated with *S.* Typhimurium and *S.* Enteritidis. Although outbreaks in the EU/EEA are most commonly caused by these serovars, in recent years, other serovars such as *S.* Infantis, *S.* Derby and *S.* Newport have been associated with outbreaks in the EU/EEA [10]. This highlights the limited applicability of MLVA and a transition towards more suitable and reliable methods such as WGS should be considered.

The number of laboratories that participated in MLVA-based cluster analysis was lower (two laboratories) compared to EQA-13 and EQA-12 (three laboratories, respectively) [40, 45].

The material costs of WGS are higher than for MLVA. However, epidemiological outbreak investigation is less efficient when using lower resolution typing techniques such as MLVA, because cluster assignment is less accurate and renders more false-positively identified cluster isolates [15]. This leads to inefficiencies during outbreak investigation as more cases need to be interviewed. In addition, data from cases interviewed that do not actually belong to the outbreak dilute the source tracing data, prompting a need to interview more cases to have a statistically sound foundation for epidemiological analyses [15].

More and more laboratories are transitioning to WGS typing, at least in outbreak situations. Therefore, MLVA will be less used in communication regarding international outbreaks – e.g. regarding case definitions. The use of typing techniques such as MLVA hampers FWD-Net surveillance objectives being reached, such as improving the harmonisation of typing methods or early threat detection in the countries that use those techniques because there is limited backwards compatibility of WGS to MLVA [41].

4.2.3 PFGE-based cluster analysis

In EQA-14, PFGE-based cluster analysis was not performed by any participant. The number of laboratories participating in PFGE-based cluster analysis has steadily decreased in recent years. In EQA-13 and EQA-12, one and two laboratories performed PFGE-based cluster analysis, respectively [40, 45].

In line with the use of MLVA, the use of PFGE hampers improvement of international surveillance. Moreover, the poor portability and comparability of PFGE results between laboratories, combined with the fact that only one of the participating laboratories performed it, makes this method unsuitable for multi-country outbreak investigations.

4.3 Feedback from participants

A total of 31% (9/29) of participants that completed at least one of part of EQA-14 filled in the feedback survey. All used the results as documentation for accreditation and/or licensing purposes, showing the added value of this EQA to laboratory quality systems.

Seven out of nine (78%) laboratories indicated that all their analytical results conformed to the expected results, and two laboratories (22%) reported that they had taken a range of corrective actions based on their individual results. One specified the corrective action, which involved review and adjustment of SOPs and verification of reagents. This proves that an EQA can be used to identify previously unknown gaps in laboratory workflows and can therefore improve capability of serotyping and molecular-based cluster analysis in the EU/EEA and the enlargement countries.

Laboratories were asked to give suggestions for specific serotypes or issues that were not yet addressed in this or previous EQAs for *Salmonella* typing, and which would be of added value to increase the capabilities of network laboratories in the future. Results indicated that guidance regarding the selection of cluster cut-off values in different epidemiological situations would be desired (n=1) and that the continuous provision of rare serotypes for typing has added value (n=1).

5 Conclusions

5.1 Methods and capability of serotyping

For serotyping, 55% of participating laboratories in the EU/EEA and enlargement countries routinely apply phenotyping serotyping based on slide agglutination with O- and H-antisera. A total of 17% only use WGS-based methods in which the serotype is inferred from genetic characteristics. Four laboratories (24%) routinely apply WGS-based methods alongside phenotypic serotyping, and one laboratory (3%) routinely applies a combination of genetic serotyping using Luminex technique, supplemented with phenotypic methods.

The main methods (exclusively phenotypic serotyping, exclusively WGS-predictive serotyping and a combination of WGS-based methods alongside to phenotypic serotyping) did not influence the overall capability of the laboratories that apply them, nor the ability to type particular serovars. For all samples serotyped by laboratories using a combination of WGS predictive typing and phenotypic serotyping using antisera, 94% (79/84 serovars) were correctly assigned, resulting in a higher overall performance for assigning the correct serotype compared to typing using phenotypic serotyping or WGS-predictive serotyping exclusively (90% and 92%, respectively). However, differences in serotyping performance using the different methods were not significant. The typing of the non-enterica subspecies was challenging for laboratories, regardless of the typing method applied by laboratories. This serovar was incorrectly typed by eight laboratories, of which six used phenotypic serotyping only, one used WGS-predictive serotyping only and one used a combination of the two approaches. Statistically, there was no difference, probably due to the low sample size.

Performance was high for most laboratories, with 14 (48%) achieving performance scores of 100%, five 92% and six 83%. Another four laboratories with the lowest performance values (<83%) used phenotypic methods (n=2), WGS-predictive serotyping (n=1) and a combination of WGS-based methods alongside to phenotypic serotyping (n=1). Overall, the results and the type of errors indicated that less specific antisera were used. However, this cannot be definitively concluded based on the information requested in the results form.

5.2 Methods and capability of molecular typing-based cluster analysis

All participating laboratories (24/24) used WGS-based cluster analysis. It is not known from the information requested whether WGS-based cluster analysis is applied routinely or only in outbreak situations. Two laboratories conducted MLVA-based typing next to WGS. PFGE-based cluster analysis was not conducted by any laboratory in EQA-14.

Performance of WGS-based cluster analysis was high, with an overall performance score of 88%. In all, 21 of 24 laboratories had 100% performance in assigning the isolates provided to clusters, and another two laboratories had a performance score of 82% (9/11 isolates assigned correctly to the cluster). Overall, a large variety of combinations of platforms, approaches, kits, cluster analysis tools, typing schemes and cluster cut-offs was used, but this did not influence performance of cluster assignment or data quality.

Technical performance in laboratories that used MLVA-based cluster analysis (n=2) was 100%, as both participating laboratories were able to determine correct MLVA profiles for the nine monophasic *S.* Typhimurium isolates. For the remaining isolate in the strain set, no MLVA profile could be determined by any participating laboratory or the provider due to it being of the serovar *S.* Heidelberg, for which, MLVA-based typing is not possible. Both participants were able to correctly place all samples in relation to the index isolate. Therefore, it can be concluded that participating laboratories had good capabilities in the application of MLVA, even though the limited applicability of the technique itself did not allow the determination of the MLVA profile for one isolate. Transition towards more suitable and reliable methods such as WGS should be considered in order to perform cluster analysis for outbreaks associated with serovars other than *S.* Typhimurium or *S.* Enteritidis.

PFGE-based cluster analysis was not performed by any participant in EQA-14.

In the feedback survey, participants of EQA-14 were asked to give suggestions for specific serotypes or issues that were not yet addressed in this or previous EQAs for *Salmonella* typing and which would be of added value to increase the capabilities of network laboratories in the future. Results indicated that guidance regarding the selection of cluster cut-off values in different epidemiological situations would be desired and that the continuous provision of rare serotypes for typing has added value.

5.3 Evaluation of EQA-14

The participation rate in the cluster analysis part was relatively high, 97% of the 30 laboratories invited provided results, which is a higher participation rate than that for the three previous rounds of EQA (93%, 56% and 56% for EQA-13, EQA-12 and EQA-11, respectively). Participation in WGS-based cluster analysis has increased, while participation in MLVA- and PFGE-based cluster analysis has decreased. For the third time in a decade, serotyping capability was assessed, and participation rate was higher (97% of the laboratories invited) compared to EQA-13 (93% of the laboratories invited). Overall, participation in serotyping was higher than for the molecular typing-based cluster analysis part.

The EQA design was approved by ECDC, and prepared according to ISO standards: ISO 15189, ISO 17043 and Chapter 11 from ISO 13528. The difficulty level was evaluated in the feedback survey and assessed as suitable by all participating laboratories. The number of samples was appropriate to draw conclusions on performance.

Although essential conclusions can be drawn from the analysis of results, there is room for improvement in the design of the results form to be able to perform more in-depth analyses. However, individual reporting was evaluated as satisfactory by all laboratories that responded to the feedback survey.

Multiple laboratories took corrective action based on the results of EQA-14, proving the added value of this EQA for the typing capability of the NPHRLs in the EU/EEA and enlargement countries. Having NPHRLs working to maximum capability contributes to surveillance and outbreak detection at regional and national level in EU/EEA countries and fulfils the international surveillance objectives of ECDC and FWD-Net.

6 Recommendations

6.1 Recommendations for NPHRLs

Fulfilment of the EU-level surveillance objectives starts with strong monitoring of trends and efficient cluster and outbreak detection in the EU/EEA and enlargement countries at national level. For EU/EEA and enlargement countries to be able to monitor these trends and outbreaks requires good performance in typing – both serotyping of *Salmonella* and molecular typing for cluster analysis.

Most laboratories performed well on serotyping, but a few scored below 83%. Those laboratories used phenotypic methods exclusively, WGS-predictive serotyping exclusively or a combination of WGS-based methods alongside to phenotypic serotyping. Overall, the results and the type of errors indicated that less specific antisera were used. If desired, NPHRLs can contact the EQA provider for assistance and to receive recommendations tailored to their specific needs and resources.

Technical performance was very strong for both methods used for the molecular typing-based cluster analysis. Performance in cluster assignment was 88% for WGS and 100% for MLVA. Although performance using MLVA was high, the method has limited applicability. It is recommended that as a minimum, laboratories use WGS-based cluster analysis for outbreak situations. PFGE-based cluster analysis should be avoided because the inferior resolution and non-portability hampers its use for (inter)national outbreak assessments involving multiple institutes. In addition, because many laboratories have transitioned to WGS-based typing, the use of PFGE- and MLVA-based cluster analysis is less suitable in multi-country outbreak investigations because of the limited backwards compatibility. ECDC no longer collects MLVA data and MLVA data reporting has been replaced by WGS real-time reporting.

As EQAs can help identify opportunities for improving the quality of typing methods, NPHRLs are encouraged to participate in the EQAs organised and funded by ECDC.

6.2 Recommendations for FWD-Net and ECDC

ECDC will continue to encourage NPHRLs to participate in EQAs to maximise typing capabilities and harmonisation in order to fulfil surveillance objectives. In addition, ECDC is working with the FWD-Net to encourage and enable the transition to WGS-based typing techniques in laboratories to ensure better quality cluster analysis and outbreak detection. This can be achieved by emphasising the superiority of WGS-based techniques over other typing techniques and by promoting the submission of high-quality data to TESSy or its successive application EpiPulse Cases that will be implemented in the course of 2025.

6.3 Recommendations for EQA organisation and provider

The third assessment of *Salmonella* serotyping in a decade was successful and prompted laboratories to take action for improvement. The inclusion of rare serovars will continue as this challenges the laboratories and was specifically mentioned by laboratories to be of added value. In the EQA, cluster analysis could be performed using three different methods (WGS, MLVA and PFGE). In this EQA, PFGE was not applied by any participant and MLVA was used by two laboratories next to WGS. As most laboratories use WGS-based cluster assignment, the next EQA could provide strain sets for cluster analysis containing serovars other than *S.* Enteritidis, *S.* Typhimurium and monophasic *S.* Typhimurium to train laboratories for cluster assessment with different serovars which have caused outbreaks in the past. In EQA-14, the set of monophasic *S.* Typhimurium isolates that were chosen for cluster analysis consisted of isolates that clustered within only a few AD from the index isolate and non-cluster isolates, with the closest match having an distance of 14 AD. The majority of participants used a gene-by-gene approach for cluster analysis and applied a cluster cut-off equal to or less than 5 AD to the index isolate. In the next EQA, ECDC will consider providing a set of strains with isolates that are close to cluster cut-off values that are frequently applied by participants to map out how participants assess isolates that are close to their chosen cluster cut-off values. To improve the identification and analysis of error types even further, ECDC will include more questions on the methods and materials routinely used for phenotypical serotyping, WGS-predictive serotyping and SNP typing-based cluster analysis and its applications.

Comments from participants on the design and organisation of the EQA *Salmonella* 2024 will be taken into consideration by ECDC during the design of future EQAs for *Salmonella*.

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Annex 1. Online registration form

EQA Salmonella 2024

You are hereby invited to participate in the EQA *Salmonella* 2024

Please answer the questions below to register

*Fields marked with a * are mandatory*

* Would you like to participate in the *Salmonella* EQA 2024?

☐ Yes

☐ No

If participation is not desired

Please mention the reason not to participate:

* Name of contact person:

* Country:

* E-mail address of contact person:

* Name of Institution or Organisation:

* Phone number *(please add the prefix for your country)*:

Do you have additional comments *(if any)*?

☐ Yes

☐ No

If participation is desired

* Name of contact person:

* Country:

* E-mail address of contact person:

* Name of Institution or Organisation:

* Phone number *(please add the prefix for your country)*:

* Will you participate in the serotyping part?

☐ Yes

☐ No, because:

* Will you participate in the molecular typing-based cluster analysis part?

☐ Yes

☐ No, because

If participating in serotyping part

It is expected that you will participate in the serotyping part by using your regularly used methods for serotype reporting. If you want to use multiple techniques, please contact us at SalmonellaEQA@rivm.nl

* What method will you use for serotyping?

☐ Exclusively phenotypic (using antisera)

☐ Exclusively WGS

☐ A combination of WGS and phenotypic

☐ Other, please elaborate:

If using exclusively WGS for serotyping part

* What sequencing platform will you use?

- ☐ Illumina
- ☐ Nanopore
- ☐ PacBio
- ☐ IonTorrent
- ☐ Other, please elaborate:

If using a combination of WGS and phenotypic for serotyping part

* Please describe your regular workflow

* What sequencing platform will you use?

- ☐ Illumina
- ☐ Nanopore
- ☐ PacBio
- ☐ IonTorrent
- ☐ Other, please elaborate:

If participating in cluster analysis part

It is expected that you will participate in the molecular typing-cluster based part with your regularly used method for cluster detection.

* What kind of method will you use for molecular typing-based cluster analysis?

- ☐ WGS
- ☐ MLVA
- ☐ PFGE *

* PFGE will not be performed by the organizing laboratory, performance will be assessed by comparing PFGE and resulting clusters from other participants.

If using WGS-based cluster analysis

*What sequencing platform will you use?

- ☐ Illumina
- ☐ Nanopore
- ☐ PacBio
- ☐ IonTorrent
- ☐ Other

*What kind of approach do you have?

- ☐ Gene-by-gene approach (MLST)
- ☐ SNP typing

Details about shipping address for the parcels with isolate sets

* For the attention of (contact person):
* Country:
* Name of laboratory – in full:
* Name of institution or organisation – in full:
* Shipping address *(please include street name and number, postal code, city and country)* :
* Email address contact person:
* Email address second contact person *(optional)* :
* Phone number *(please add the prefix for your country)* :
* Do you have a different postal address for correspondence (the certificate)?

☐ Yes

☐ No

If different postal address

* For the attention of (contact person):
* Country:
* Name of Laboratory – in full:
* Name of Laboratory – acronym:
* Name of Institution or Organisation – in full:
* Name of Institution – acronym:
* Postal address (*Please include street name and number, postal code and city*):If registered

*Do you have any additional comments?

☐ Yes *

☐ No

* If yes: please enter your comments here:

Your submitted personal data is used only for the purpose of the execution of the EQA *Salmonella* 2024 and is handled with care. Original data is only accessible for RIVM and ECDC personnel involved in the project.

Annex 2. Participants

Country	Registered laboratory	Institution
Austria	National Reference Centre for Salmonella Austria	AGES - Austrian Agency for Health and Food Safety – Institute for Medical Microbiology and Hygiene Graz
Belgium	NRC Salmonella	Sciensano
Bulgaria	NRL for Enteric diseases, pathogenic cocci and dyphteria	National Center for Infectious and Parasitic Diseases
Croatia	Division for microbiology; National Reference Laboratory for Salmonellae	Croatian Institute of Public Health
Cyprus	Microbiology Department of Nicosia General Hospital	General hospital of Nicosia
Czechia	National Reference Laboratory for Salmonella	National Institute of Public Health
Denmark	Laboratory of Gastrointestinal Bacteria	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board
Finland	Bacteriology Laboratory	Finnish Institute for Health and Welfare
France	Centre National de Reference des E. coli, Shigella et Salmonella	Institut Pasteur
Germany	National Reference Center for Salmonella and other bacterial enteric pathogens	Robert Koch Institute
Greece	National Reference Laboratory for Salmonella	UNIVERSITY OF WEST ATTICA
Hungary	Food and Waterborne Diseases – National Reference Laboratory	National Public Health Center
Iceland	Department of Microbiology	Landspitali University Hospital
Ireland	Galway Reference Laboratory Services	Galway University Hospital
Italy	Department of Infectious Diseases	Istituto Superiore di Sanità
Latvia	National Microbiology Reference Laboratory	Riga East University Hospital, Latvian Centre of Infectious Diseases
Lithuania	National Public Health Surveillance Laboratory	National Public Health Surveillance Laboratory
Luxembourg	Pathogen Sequencing	Laboratoire National de Santé
Malta	Bacteriology Laboratory	Mater Dei Hospital
Norway	National Reference Laboratory Enteropathogenic Bacteria	Norwegian Institute of Public Health
Poland	Laboratory of Department of Bacteriology and Biocontamination Control	National Institute of Public Health NIH - NRI
Portugal	Laboratório Nacional de Referência de Infeções Gastrintestinais	Instituto Nacional de Saúde Doutor Ricardo Jorge
Romania	Molecular Epidemiology for Communicable Diseases	Cantacuzino National Military Medical Institute for Research and Development
Serbia	Reference Laboratory for Salmonella, Shigella, Vibrio cholerae, Yersinia enterocolitica	Institute of Public Health of Serbia
Slovakia	National Reference Center for Salmonelloses	Public Health Authority of the Slovak Republic
Slovenia	Department for Public Health Microbiology	National Laboratory of Health, Environment and Food
Spain	Laboratorio de Referencia e Investigación en Enfermedades bacterianas Transmitidas por Agua y Alimentos	Centro Nacional de Microbiología. Instituto de Salud Carlos III
Sweden	Unit for laboratory surveillance of bacterial pathogens	Public Health Agency of Sweden

Annex 3. Online results form

Fields marked with a * are mandatory

* Name of contact person:

* E-mail address contact person:

* Name of Institution or Organisation:

* Name of your laboratory:

* Country:

* Phone number (please add the prefix for your country):

* Date of arrival of the parcel with the isolates:

* Would you like to submit results for the serotyping part?

If you submit results for serotyping

* Which method did you use for serotyping?

* If exclusively phenotypic: From which manufacturer did you obtain the antisera you used?

Please report the species, subspecies, O- and H-antigens and the serovar name according to the White-Kauffmann-LeMinor scheme of 2007. If the serovar name is not present in this scheme, please report the complete seroformula.

Isolate	Species	Subspecies	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name or seroformula
EQA2401						
EQA2402						
EQA2403						
EQA2404						
EQA2405						
EQA2406						
EQA2407						
EQA2408						
EQA2409						
EQA2410						
EQA2411						
EQA2412						

* If exclusively WGS: Which sequencing platform did you use?

* If exclusively WGS: Which tool(s) did you use to determine the serovar?

* If a combination of WGS and phenotypic: From which manufacturer did you obtain the antisera you used?

* If a combination of WGS and phenotypic: Which sequencing platform did you use?

* If a combination of WGS and phenotypic: Which tool(s) did you use to determine the serovar?

Please report the species, subspecies, O- and H-antigens and the serovar name according to the White-Kauffmann-LeMinor scheme of 2007. If the serovar name is not present in this scheme, please report the complete seroformula.

Isolate	Species	Subspecies	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name or seroformula
EQA2401						
EQA2402						
EQA2403						
EQA2404						

Isolate	Species	Subspecies	O-antigens	H-antigens (phase 1)	H-antigens (phase 2)	Serovar name or seroformula
EQA2405						
EQA2406						
EQA2407						
EQA2408						
EQA2409						
EQA2410						
EQA2411						
EQA2412						

* Do you want to report the MLST Sequence Types of the isolates from the serotyping panel?

* If yes:

Isolate	Sequence Type (number)
EQA2401	
EQA2402	
EQA2403	
EQA2404	
EQA2405	
EQA2406	
EQA2407	
EQA2408	
EQA2409	
EQA2410	
EQA2411	
EQA2412	

Do you have any comments on the serotyping part?

* Would you like to submit results for the molecular typing-based cluster analysis part?

If you submit results for cluster analysis

Which kind of method did you use for molecular typing-based cluster analysis?

If you use WGS for cluster analysis

Please submit your raw reads (.fastq or fastq.gz) and your assemblies or variant call formats (.fasta or .vcf) to our sharing platform Research Drive.

* Which sequencing platform did you use?

* If Illumina: which library prep was used?

* If Illumina: For which purposes do you use WGS based cluster analysis?

* If Illumina: What kind of approach did you use for cluster analysis?

If you use a gene-by-gene approach for cluster analysis

* Which tool did you use for the allele analysis?

* Which scheme did you use for the allele analysis?

If you use a SNP typing approach for cluster analysis

* Which reference did you use for SNP analysis?

* Which reference did you use for SNP analysis?

For either gene-by-gene approach or SNP typing

* What distance (allelic or SNPs) do you use as cut-off for cluster analysis?

Please report the distance (allelic or SNPs) of the isolates to the index case (EQA2413) and whether you would consider the isolate part of the cruise ship outbreak:

Isolate	Distance to index case	Part of the outbreak?
EQA2414		
EQA2415		
EQA2416		
EQA2417		
EQA2418		
EQA2419		
EQA2420		
EQA2421		
EQA2422		

* Does your WGS analysis include a confirmation of species?

* If yes: Which method do you use to confirm the species?

* Did you determine the serovar of the cluster isolates?

* If yes: Which method or tool did you use to determine the serovar?

* Do you want to report the MLST Sequence Type of the cluster isolates?

* If yes:

Isolate	Sequence Type (number)
EQA2413	
EQA2414	
EQA2415	
EQA2416	
EQA2417	
EQA2418	
EQA2419	
EQA2420	
EQA2421	
EQA2422	

* Which criteria and thresholds do you use to assess the quality of your WGS reads or assemblies? (possibility to fill in up to 10 criteria)

Criterium 1:

Threshold:

Criterium 2:

Threshold:

Criterium 3:

Threshold:

Criterium 4:

Threshold:

Criterium 5:

Threshold:

Criterium 6:

Threshold:

Criterium 7:

Threshold:

Criterium 8:

Threshold:

Criterium 9:

Threshold:

Criterium 10:

Threshold:

Please download the zip-file with five genomes from our sharing platform Research Drive and assess if you would consider these possible sources of the neighbourhood party outbreak (part of a cluster with the index case EQA2413)

* What is your assessment of provided genome EQA2423 from an isolate obtained from meat?

* If 'possible source of outbreak' is selected: What is the distance of this genome to the index case?

* If 'insufficient quality' is selected: What is the reason why you would consider the quality of this genome insufficient?

* What is your assessment of provided genome EQA2424 from an isolate obtained from fruit skewer?

* If 'possible source of outbreak' is selected: What is the distance of this genome to the index case?

* If 'insufficient quality' is selected: What is the reason why you would consider the quality of this genome insufficient?

* What is your assessment of provided genome EQA2425 from an isolate obtained from beef mixed salad?

* If 'possible source of outbreak' is selected: What is the distance of this genome to the index case?

* If 'insufficient quality' is selected: What is the reason why you would consider the quality of this genome insufficient?

* What is your assessment of provided genome EQA2426 from an isolate obtained from charcuterie?

* If 'possible source of outbreak' is selected: What is the distance of this genome to the index case?

* If 'insufficient quality' is selected: What is the reason why you would consider the quality of this genome insufficient?

* What is your assessment of provided genome EQA2427 from an isolate obtained from ice cream?

* If 'possible source of outbreak' is selected: What is the distance of this genome to the index case?

* If 'insufficient quality' is selected: What is the reason why you would consider the quality of this genome insufficient?

Do you have any comments on the WGS part?

If you used MLVA typing for cluster analysis

Please submit your curve files (.fsa) to our sharing platform Research Drive. Instructions to do this are sent on June 19th.

* What sizemarker did you use for MLVA?

* What cut-off do you use for cluster analysis with MLVA (number)?

Please report the MLVA profile (STTR9-STTR5-STTR6-STTR10-STTR3) of the isolates and whether you would consider the isolate part of the neighbourhood party outbreak with index case EQA2413:

Isolate	MLVA profile	Part of the outbreak?
EQA2413		
EQA2414		
EQA2415		
EQA2416		
EQA2417		
EQA2418		
EQA2419		
EQA2420		
EQA2421		
EQA2422		

* Did you determine the serovar of the MLVA isolates?

If yes: How did you determine the serovar?

Isolate	Serovar
EQA2413	
EQA2414	
EQA2415	
EQA2416	
EQA2417	
EQA2418	
EQA2419	
EQA2420	
EQA2421	
EQA2422	

Do you have any comments on the MLVA part?

If you used PFGE typing for cluster analysis

PFGE is not performed by the organising laboratory, performance will be assessed by comparing PFGE profiles and resulting clusters from other participants. Please submit the resulting PFGE fingerprints as a .TIFF to our sharing platform Research Drive.

* Which restriction enzyme did you use for PFGE?

Please report which of the isolates you would consider to be part of the neighbourbood party outbreak with index case EQA2413, based on PFGE:

Isolate	Part of the outbreak?
EQA2413	
EQA2414	
EQA2415	
EQA2416	
EQA2417	
EQA2418	
EQA2419	
EQA2420	
EQA2421	

Do you have any comments on the PFGE part?

For all participants

Do you have any other comments on this EQA?

Annex 4. Feedback survey

External Quality Assessment *Salmonella* 2024

Fields marked with a * are mandatory

Dear Participant,

Recently you have participated in an ECDC external quality assessment exercise. To ensure maximum benefit we hereby invite you to answer this short survey. Please note ECDC will receive all your responses anonymised.

* **Question 1:** Were you satisfied with the EQA report of results specific to your laboratory?

- ○ Yes
- ○ No

* **Question 2:** Are results of this EQA exercise to be used as documentation for accreditation and/or licensing purposes for the method(s) used in your laboratory?

- ○ Yes
- ○ No
- ○ Not applicable

Please elaborate:

* **Question 3:** If any of your analytical test results were not conform with the expected results, can you specify which corrective actions were taken (e.g. review and adjust SOPs, verify reagents)?

- ◉ Not applicable: all our EQA analytical test results conformed to expected results.
- ○ No corrective actions for non-conformities were taken.
- ○ Yes, corrective actions were taken.

* **Question 4:** Are there any specific serotypes or cluster-analysis issues you would like to see included in a future EQA?

Question 5: Do you have any suggestions that would make the EQA scheme more useful?

Annex 5. Methods used for serotyping

Lab ID	Registered serotyping	Participated in serotyping	Method used	Sequencing platform
13	Yes	Yes	Exclusively phenotypic	
15	Yes	Yes	Combination of WGS and phenotypic	Illumina
17	Yes	Yes	Exclusively phenotypic	
20	Yes	Yes	Combination of WGS and phenotypic	Illumina
23	Yes	Yes	Combination of WGS and phenotypic	Illumina
26	Yes	Yes	Exclusively phenotypic	
29	Yes	Yes	Exclusively phenotypic	
30	Yes	Yes	Exclusively phenotypic	
34	Yes	Yes	Exclusively WGS	Illumina
35	Yes	Yes	Combination of WGS and phenotypic	Illumina
37	Yes	Yes	Exclusively phenotypic	
42	Yes	Yes	Exclusively WGS	Illumina
43	Yes	Yes	Exclusively phenotypic	
44	Yes	Yes	Combination of WGS and phenotypic	Ion Torrent
46	Yes	Yes	Exclusively phenotypic	
49	Yes	Yes	Exclusively WGS	Illumina
52	Yes	Yes	Exclusively WGS	Illumina
53	Yes	Yes	Exclusively phenotypic	
56	Yes	Yes	Exclusively phenotypic	
58	Yes	Yes	Exclusively phenotypic	
63[a]	Yes	Yes	Other	
69	Yes	Yes	Combination of WGS and phenotypic	Illumina
73	Yes	Yes	Exclusively phenotypic	
74	Yes	Yes	Exclusively phenotypic	
75	Yes	Yes	Exclusively phenotypic	
77	Yes	Yes	Exclusively WGS	Illumina
84	Yes	Yes	Exclusively phenotypic	
92	Yes	Yes	Combination of WGS and phenotypic	Illumina
98	Yes	Yes	Exclusively phenotypic	

[a] *Laboratory used molecular genetic serotyping with Luminex techniques alongside phenotypic method for serotyping.*

Annex 6. Serotyping results reported per laboratory

Laboratory ID 13

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	1,4,5,12	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4,12,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,z15	Denver
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	g,m	-	Essen
EQA2410	enterica	enterica	1,4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Grey = incorrect results

Laboratory ID 15

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	1,4,5,12	i	-	1,4,5,12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4,12,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2409	enterica	enterica	1,4,5,12	f,g	-	Derby
EQA2410	enterica	enterica	1,4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 17

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 20

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10,15	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	I 4,[5],12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 23

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb:47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,5	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	1,2	Typhiurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation

Laboratory ID 26

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	Monophasic Salmonella Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 29

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	Typhimurium, monophasic
EQA2403	enterica	enterica	6,8	e,h	e,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8	d	z6	Labadi

Grey = incorrect results

Laboratory ID 30

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10,15	l,v	1,6	London
EQA2402	enterica	enterica	1,4,5,12	i	-	Typhimurium monophasic
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4,12,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7,14	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	Lyon III b
EQA2407	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2409	enterica	enterica	1,4,5,12	f,g	1,2	Derby
EQA2410	enterica	enterica	1,4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation

Laboratory ID 34

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10,15	l,v	1,6	London
EQA2402	enterica	enterica	4,5	i	-	I 4,5:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 35

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5	i	-	Typhimurium, monophasisch
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 37

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	S. London
EQA2402	enterica	enterica	4,5,12	i	-	S. Typhimurium monophasic variant
EQA2403	enterica	enterica	6,8	e,h	e,n,x	S. Fillmore
EQA2404	enterica	enterica	4,12,27	d	1,7	S. Schwarzengrund
EQA2405	enterica	enterica	6,7	e,h	e,n,z15	S. Braendenderup
EQA2406	enterica	diarizonae	47	k	z35	S. subspecies IIIb
EQA2407	enterica	enterica	9	g,m	-	S. Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	S. Infantis
EQA2409	enterica	enterica	4,12	f,g	-	S. Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	S. Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	S. Moroto
EQA2412	enterica	enterica	8,20	z10	z6	S. Molade

Green = incorrect notation; Grey = incorrect results

Laboratory ID 42

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,{10},{15}	l,v	1,6	London
EQA2402	enterica	enterica	1,4,[5],12	i	-	I 1,4,[5],12:i:-
EQA2403	enterica	enterica	-	e,h	e,n,x	Fillmore | Tshiongwe | Kottbus
EQA2404	enterica	enterica	1,4,12,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7,14	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	51	k	z35	IIIb 51:k:z35
EQA2407	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2409	enterica	enterica	1,4,[5],12	f,g	-	Derby
EQA2410	enterica	enterica	1,4,[5],12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto | Telaviv
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation; Grey = incorrect results; Orange = Inconclusive result

Laboratory ID 43

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	6	London
EQA2402	enterica	enterica	4,5,12	i	-	II 1,4,[5],12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12,27	d	7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	-	-	-	Salmonella enterica subspecies diarizonae
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	5	Infantis
EQA2409	enterica	enterica	4,27	f,g	2	Derby
EQA2410	enterica	enterica	4,5	i	2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation; Grey = incorrect results

Laboratory ID 44

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 46

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	I 4,5,12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 49

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8	z10	z6	Molade

Laboratory ID 52

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	Potential monophasic variant of Typhimurium
EQA2403	enterica	enterica	8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4	i	-	Potential monophasic variant of Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	9	-	-	Gallinarum

Grey = incorrect results

Laboratory ID 53

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	1,4,5,12	i	-	Typhimurium monophasic
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4,12	d	e,n,z15	Duisburg
EQA2405	enterica	enterica	6,7	e,h	e,n,z15	Braenderup
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	1,4,12	f,g	-	Derby
EQA2410	enterica	enterica	1,4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Grey = incorrect results

Laboratory ID 56

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8	z10	z6	Molade

Laboratory ID 58

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Filmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,5,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation

Laboratory ID 63

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,[5],12	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,[5],12	f,g	-	Derby
EQA2410	enterica	enterica	4,[5],12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Laboratory ID 69

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	6	London
EQA2402	enterica	enterica	4,5	i	-	Typhimurium monophasic
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	1,4	d	7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation

Laboratory ID 73

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12,27	d	l,w	Mons
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m,q	-	Blegdam
EQA2408	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2409	enterica	enterica	4,12	f,g	1,2	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Grey = incorrect results

Laboratory ID 74

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	Salmonella London
EQA2402	enterica	enterica	4,5,12	i	e,n,z15	Salmonella Tsevie
EQA2403	enterica	enterica	6,8	?	e,n,x	Salmonella enterica ssp. enterica ser. 6,8:?:e,n,x
EQA2404	enterica	enterica	4,12	d	1,7	Salmonella Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Salmonella Oslo
EQA2406	enterica	diarizonae	?	k	?	Salmonella subsp. diarizonae ser. ?:k:?
EQA2407	enterica	enterica	1,9,12	g,m	-	Salmonella Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Salmonella Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Salmonella Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Salmonella Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Salmonella Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Salmonella Molade

Grey = incorrect results

Laboratory ID 75

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5	i	-	Monophasic Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	7,14	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	-
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Green = incorrect notation

Laboratory ID 77

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10,15	l,v	e	Ruzizi
EQA2402	enterica	enterica	4	i	2	Typhimurium
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	e,n,z15	Duisburg
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	enterica	47	k	e,n,z15	Lyon
EQA2407	enterica	enterica	9	m	-	Enteritidis
EQA2408	enterica	enterica	7	r	5	Infantis
EQA2409	enterica	enterica	4	f	-	Derby
EQA2410	enterica	enterica	4	i	2	Typhimurium
EQA2411	enterica	enterica	28	w	z10	Moroto
EQA2412	enterica	enterica	8	z6	z10	Molade

Grey = incorrect results

Laboratory ID 84

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4	i	-	I 4,[5],12:i:-
EQA2403	enterica	enterica	8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9	g,m	-	Enteritidis
EQA2408	enterica	enterica	7	r	1,5	Infantis
EQA2409	enterica	enterica	4	f,g	-	Derby
EQA2410	enterica	enterica	4	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8	z10	z6	Molade

Laboratory ID 92

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10,15	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	4,5,12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x	Fillmore
EQA2404	enterica	enterica	4,12,27	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k;z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8	z10	z6	Molade

Laboratory ID 98

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2401	enterica	enterica	3,10	l,v	1,6	London
EQA2402	enterica	enterica	4,5,12	i	-	4,5,12:i:-
EQA2403	enterica	enterica	6,8	e,h	e,n,x,z16	Fillmore
EQA2404	enterica	enterica	4,12	d	1,7	Schwarzengrund
EQA2405	enterica	enterica	6,7	a	e,n,x,z16	Oslo
EQA2406	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2407	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2408	enterica	enterica	6,7	r	1,5	Infantis
EQA2409	enterica	enterica	4,5,12	f,g	-	Derby
EQA2410	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2411	enterica	enterica	28	z10	l,w	Moroto
EQA2412	enterica	enterica	8,20	z10	z6	Molade

Annex 7. Assigned serovar per sample

Lab ID	EQA2401	EQA2402[a]	EQA2403	EQA2404	EQA2405	EQA2406[a]
Provider	**London**	**Typhimurium, monophasic**	**Fillmore**	**Schwarzengrund**	**Oslo**	**IIIb 47:k:z35**
13	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Denver	IIIb 47:k:z35
15	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
17	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
20	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
23	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb:47:k:z35
26	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
29	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
30	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	Lyon III b
34	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
35	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
37	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Braendenderup	S. Subsp. IIIb
42	London	Typhimurium, monophasic	Fillmore | Tshiongwe | Kottbus	Schwarzengrund	Oslo	IIIb 51:k:z35
43	London	II 1,4,[5],12:i:-	Fillmore	Schwarzengrund	Oslo	S. enterica subsp. diarizonae
44	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
46	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
49	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
52	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
53	London	Typhimurium, monophasic	Fillmore	Duisburg	Braenderup	IIIb 47:k:z35
56	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
58	London	Typhimurium, monophasic	Filmore	Schwarzengrund	Oslo	IIIb 47:k:z35
63	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
69	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb
73	London	Typhimurium, monophasic	Fillmore	Mons	Oslo	IIIb 47:k:z35
74	London	Tsevie	S. enterica subsp. enterica ser. 6,8:?:e,n,x	Schwarzengrund	Oslo	S. enterica subsp. diarizonae ser. ?:k:?
75	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	-
77	Ruzizi	Typhimurium	Fillmore	Duisburg	Oslo	Lyon
84	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
92	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35
98	London	Typhimurium, monophasic	Fillmore	Schwarzengrund	Oslo	IIIb 47:k:z35

[a]*Notation of serovars equalised. Grey=incorrect serovar.*

Lab ID	EQA2407	EQA2408	EQA2409	EQA2410	EQA2411	EQA2412
Provider	**Enteritidis**	**Infantis**	**Derby**	**Typhimurium**	**Moroto**	**Molade**
13	Enteritidis	Infantis	Essen	Typhimurium	Moroto	Molade
15	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
17	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
20	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
23	Enteritidis	Infantis	Derby	Typhiurium	Moroto	Molade
26	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
29	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Labadi
30	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
34	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
35	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
37	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
42	Enteritidis	Infantis	Derby	Typhimurium	Moroto | Telaviv	Molade
43	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
44	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
46	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
49	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
52	Enteritidis	Infantis	Derby	Potential monophasic variant of Typhimurium	Moroto	Gallinarum
53	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
56	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
58	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
63	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
69	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
73	Blegdam	Virchow	Derby	Typhimurium	Moroto	Molade
74	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
75	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
77	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
84	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
92	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade
98	Enteritidis	Infantis	Derby	Typhimurium	Moroto	Molade

[a]*Notation of serovars equalised.*
Grey=incorrect serovar.

Annex 8. Concordance and errors per sample

EQA #	Intended serovar name or seroformula	Concordance (%)	Type of errors
EQA2401	London	97	Type misclassification of He instead of H1,6 in 2nd phase (n=1, lab 77)
EQA2402	Typhimurium, monophasic	90	Non-standard nomenclature, serovar name incorrectly assigned as II 1,4,[5],12:- (n=1, lab 43) False-positive He,n,z15 detection in 2nd phase (n=1, lab 74) False-positive H2 detection in 2nd phase (n=1, lab 77)
EQA2403	Fillmore	93	Non-standard nomenclature, serovar name incorrectly assigned as Filmore (n=1, lab 58) False-negative O-antigen (n=1, lab 42) False-negative H-antigen in 1st phase (n=1, lab 74)
EQA2404	Schwarzengrund	86	Type misclassification of He,n,z15 instead of H1,7 in 2nd phase (n=2, lab 53 and 77) Type misclassification of Hl,w instead of H1,7 in 2nd phase (n=1, lab 73)
EQA2405	Oslo	90	Type misclassification of He,h instead of Ha in 1st phase (n=1, lab 53) Type misclassification of He,n,z15 instead of He,n,x in 2nd phase (n=3, lab 13, 37 and 53)
EQA2406	IIIb 47:k:z35	69	Non-standard nomenclature, seroformula name incorrectly assigned as IIIb:47:k:z35 (n=1, lab 23) Non-standard nomenclature, seroformula name incorrectly assigned as Lyon IIIb (n=1, lab 30) Non-standard nomenclature, seroformula name incorrectly assigned as IIIb (n=2, lab 37 and 69) Non-standard nomenclature, seroformula name incorrectly assigned as Salmonella enterica subspecies diarizonae (n=1, lab 43) Non-standard nomenclature, seroformula name incorrectly assigned as - (n=1, lab 75) Type misclassification of O51 instead of O47 (n=1, lab 42) Type misclassification of He,n,z15 instead of Hz35 in 2nd phase (n=1, lab 77) Type misclassification of subspecies *enterica* instead of *diarizonae* (n=1, lab 77) False-negative O-antigen (n=2, lab 43 and 74) False-negative H-antigen in 1st phase (n=1, lab 43) False-negative H-antigen in 2nd phase (n=2, lab 43 and 74)
EQA2407	Enteritidis	97	False-positive Oq detection (n=1, lab 73)
EQA2408	Infantis	97	Type misclassification of H1,2 instead of H1,5 in 2nd phase (n=1, lab 73)
EQA2409	Derby	97	Type misclassification of Hg,m instead of Hf,g in 1st phase (n=1, lab 13)
EQA2410	Typhimurium	93	Non-standard nomenclature, seroformula name incorrectly assigned as Typhiurium (n=1, lab 23) False-negative H-antigen 2nd phase (n=1, lab 52)
EQA2411	Moroto	97	Non-standard nomenclature, seroformula name incorrectly assigned as Moroto | Telaviv (n=1, lab 42)
EQA2412	Molade	93	Type misclassification of O9 instead of O8,20 (n=1, lab 52) Type misclassification of Hd instead of Hz10 in 2nd phase (n=1, lab 29) False-negative H-antigen in 1st phase (n=1, lab 52) False-negative H-antigen in 2nd phase (n=1, lab 52)

Annex 9. Reported MLST sequence types of serotyping strain panel

Lab ID	MLST reported	EQA2401	EQA2402	EQA2403	EQA2404	EQA2405	EQA2406	EQA2407	EQA2408	EQA2409	EQA2410	EQA2411	EQA2412
Provider	**Yes**	**155**	**34**	**9824**	**2250**	**1370**	**430**	**11**	**32**	**40**	**19**	**4909**	**544**
13	No												
15	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
17	No												
20	No												
23	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	unknown
26	No												
29	No												
30	No												
34	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
35	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
37	No												
42	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
43	No												
44	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
46	No												
49	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
52	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
53	No												
56	No												
58	No												
63	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
69	No												
73[a]	Yes			9824		1370						4909	
74	No												
75	No												
77	No												
84	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544
92	No												
98	Yes	155	34	9824	2250	1370	430	11	32	40	19	4909	544

[a] *Participant provided MLST results of sequenced isolates.*
Green=No reporting of MLST results.
Grey=incorrect MLST type.

Annex 10. Techniques used for molecular typing-based cluster analysis

Lab ID	Registered cluster analysis	Participated in cluster analysis	PFGE	MLVA	WGS	
					Gene-by-gene	SNP-typing
13	No	No				
15	Yes	No				
17	Yes	Yes			X	
20	Yes	Yes			X	
23	Yes	Yes			X	
26	Yes	Yes			X	
29	Yes	Yes			X	
30	Yes	Yes			X	
34	Yes	Yes			X	
35	Yes	Yes			X	
37	No	No				
42	Yes	Yes			X	
43	No	No				
44	Yes	Yes				X
46	Yes	Yes			X	
49	Yes	Yes			X	
52	Yes	Yes				X
53	Yes	Yes			X	
56	Yes	Yes				X
58	Yes	Yes			X	
63	Yes	Yes		X	X	
69	Yes	Yes				X
73	Yes	Yes			X	
74	Yes	Yes		X	X	
75	No	No				
77	Yes	Yes			X	
84	Yes	Yes			X	
92	Yes	Yes			X	
98	Yes	Yes			X	

Green = Did not participate in molecular typing-based cluster analysis.

Annex 11. Reported methods used for WGS-based cluster analysis

Lab ID	Platform	Approach	Library prep	MLST Tool	MLST scheme	Cluster cut-off[a]
17	Illumina	MLST	Nextera XT	ChewBBACA 3.3.3	Enterobase	5
20	Illumina	MLST	Nextera XT	Ridom SeqSphere	cgMLST	10
23	Illumina	MLST	Illumina DNA Prep	Ridom SeqSphere	Enterobase	3
26	Illumina	MLST	Nextera XT	Enterobase	Enterobase	20
29[c]	Illumina	MLST	Nextera XT	ChewBBACA 3.3.4	wgMLST schema for S. enterica from INNUENDO project	16
30	Illumina	MLST	Nextera XT	Ridom SeqSphere	K. pneumoniae cgMLST scheme from cgmlst.org	5
34	Illumina	MLST	Nextera XT	Enterobase	Enterobase	5
35[d]	Illumina	MLST	Nextera XT	Ridom SeqSphere	Enterobase	5
42[e]	Illumina	MLST	KAPA HyperPlus	Ridom SeqSphere	Enterobase	5
44[a,f]	IonTorrent	SNP				5
46	Illumina	MLST	Illumina Library kit	Ridom SeqSphere	Enterobase	3
49	Illumina	MLST	Illumina DNA Prep	Ridom SeqSphere	Enterobase	7
52[a,g]	Illumina	SNP	Illumina DNA Prep			5
53	Illumina	MLST	Illumina DNA Prep	ChewBBACA	Enterobase	3
56[a]	Nanopore	SNP				5
58	Illumina	MLST	Illumina DNA Prep	Ridom SeqSphere	Enterobase	5
63	Illumina	MLST	MiSeq Reagent Kit v3 - 500 cycles	In-house pipeline	Enterobase	
69[b]	Illumina	SNP	Nextera XT			10
73[h]	Illumina	MLST	Illumina DNA Prep	ChewBBACA	cgMLST schema for S. enterica from INNUENDO project	5
74	Illumina	MLST	Illumina DNA Prep	Ridom SeqSphere	Enterobase	
77	Illumina	MLST	Illumina DNA Prep	ChewBBACA 3.1.2	S. enterica cgMLST v2 scheme from cgmlst.org obtained on 2023-05-15	10
84	Illumina	MLST	Illumina DNA Prep	Bionumerics	Core Genome (Enterobase)	5
92	Illumina	MLST	Nextera XT	Ridom SeqSphere	Enterobase	5
98	Illumina	MLST	Nextera XT	Bionumerics 8.1.1	Applied Maths/Enterobase	3

[a]Laboratory used EQA2413 as reference genome for SNP analysis.
[b]Laboratory used NA ND tree which uses Kmer to find closest template.

Comments made by participants about allele calling and cluster cut-off:
[c]Reads quality control, species confirmation and bacterial de novo assembly were performed using the INNUca v4.2.2 pipeline (Llarena et al., 2018) (available at https://github.com/B-UMMI/INNUca), which consists of integrated modules for reads QA/QC, de novo assembly and post-assembly optimization steps. Briefly, after readsâ€™ quality analysis (FastQC v0.11.52) and cleaning (Trimmomatic v0.38), genomes were assembled with SPAdes v3.14 and subsequently improved using Pilon v1.23. Species confirmation and contamination screening were assessed using Kraken v.2.0.7 for both raw reads and final polished assemblies, using the standard 8G database. MLST prediction was determined using mlst v2.18.1 software. Serotype was predicted with SeqSero2 v1.2.1. ;
Cluster analysis: This gene-by-gene analysis was performed with chewBBACA v3.3.4 using the wgMLST Schema for S. enterica from the INNUENDO project, enrolling 8558 loci (available at chewie-NS: https://chewbbaca.online/species/8). A dynamic approach was performed with ReporTree v2.5.1 using both GrapeTree and single-linkage clustering algorithms, defining the core cgMLST loci using a threshold of 90%. Samples with less than 95% loci called were excluded from the analyses. Outbreak-related samples were determined using a 0.43% cut-off of the final core (i.e. 16 allele differences over 3757 cgMLST loci), as suggested by the INNUENDO project (Llarena et al., 2018).
[d]Comment to the question "What distance (allelic or SNPs) do you use as cut-off for cluster analysis?": We differentiate between WGS clusters and (suspected) outbreak clusters. For WGS clusters we apply 5 AD (for S. Enteritidis 3 AD). But we would not consider an isolate with e.g. 5 AD from the main node as part of a particular outbreak, unless there is clear epidemiological evidence.
Comment to the question "For which purpose do you use WGS based cluster analysis?":
a) for general surveillance purposes --> due to limited (personnel) resources and lack of automated workflows currently only for S. Enteritidis, S. Typhimurium and S. Typhi
b) for outbreak situations only --> for all other serovars
Comment to the question "Which criteria and thresholds do you use to assess the quality of your WGS reads or assemblies?": The number of contigs and their N50 might also be considered but here we have no defined thresholds.
According to our qc criteria the avg. coverage for EQA2425 is too low though the number of good targets is still ok - in case of doubtful cluster affiliation we would probably repeat WGS for this strain

[e]Genome EQA2425 has lower coverage (21) than the set threshold of >30. However, all other quality control parameters are good, and we would accept these results. In case this genome would cluster with the outbreak to indicate a possible source of the outbreak, the isolate would be re-sequenced.
[f]The distance, 5 SNPs cut-off, for cluster analysis is not fixed. Epidemiological information and cluster/outbreak characteristics often need to be considered as well.
[g]We combine SNP analysis with cgMLST typing for further verification if possible (cgMLSTFinder-1.2 from DTU or chewBBACA). All samples considered to be part of the outbreak had a cgST of 72367.
[h]The isolate EQA2419 was not included in the cgMLST analysis because it belongs to a different serotype, so a random number (i.e. 1111111) was inserted in the section where the allelic distance of the isolates to the index case was required.

Annex 12. Reported quality criteria used for assessment of WGS data

Lab ID	Species confirmation	Q score (Phred)	Coverage	% Good targets MLST	#contigs
17[a]	GTDB-Tk v2.3.2	>20	>20x		<2000
20	SeqSero2 and Kraken2		>29x	>90%	No threshold provided
23	Kraken2	20	>30x		500
26[b]	Kraken				<600
29	Confindr and Kraken with the standard 8G database		30x (as implemented in INNUca v4.2.2)	≥95% (as done by ReporTree during clustering analyses)	According to default criteria with standard 8G database
30	Mash		≥30x	≥95%	<300
34	Kraken		>45x		<250
35	Mash (implemented in SeqSphere) and Kraken		30x	98% (95% for some rare serovars or higher subspecies)	
42	Kraken and Mash Distance		>30x	>90%	
44	BLAST towards an in-house database with reference sequences		≥20x		
46[c]	Kmer finder		30x		<500
49	Mash Distance	>28	≥30x	>95%	<300
52[d]	Kraken2	>28			
53[e]	KmerFinder		>30x		<300
56[f]	Kraken2		20x		In correlation to N50
58[g]	K-mer			>99%	<500
63	Kraken2 + rMLST	>30	30x	>95%	
69	Enterobase				<300
73[h]	Mash				<300 bp
74	Mash Screen (included in SeqSphere)	>30	>50x	>95%	<200
77	Kraken2 and rMLST			>90%	<200
84[i]		≥30	≥30x	>97%	
92[j]	rMLST and Mash Distance		>40x	>95%	<300
98[k]	Kraken and Bracken analysis		≥50x	≥95%	No threshold provided
Median (range)[l]	**NA**	**28 (20-30)**	**30 (20-50)**	**95 (90-99)**	**<300 (199-1999)**

Green = Not reported.

[a] *Laboratory 17 also assessed the proportion of ambiguous bases with a cut-off of >800 and the read length with a cut-off of >50 nt.*
[b] *Laboratory 26 also assessed the proportion of scaffolding placeholders with a cut-off of <3%.*
[c] *Laboratory 46 also assessed the read length, which was set at 250-300 bp and the number of reads with threshold >600k.*
[d] *Laboratory 52 also assessed the N content, which as set to >3%, the sequence length, for which the majority of reads was expected to be full length. The laboratory also assessed sequence duplication, the amount of overrepresented sequences, the number of reads, and the outcomes of MLST and AMR typing.*
[e] *Laboratory 53 also assessed the average read length with a cut-off of 150 bp.*
[f] *Laboratory 56 also assessed the quality of reads with threshold >10.*
[g] *Laboratory 58 also assessed the average read length with threshold of 150 bp and the number of reads, which should be as high as possible.*
[h] *Laboratory 73 also assessed the Q30 rate with threshold >80, and contamination, for which the number of absent genes should be <2, the number of multiple alleles <1, and the amount of SNVs should be <7.*
[i] *Laboratory 84 also assessed the NrBAFperfect with threshold >4000 and the NrBAFMultiple with threshold <20.*
[j] *Laboratory 92 also assessed number of reads with threshold >1000000.*
[k] *Laboratory 98 also assessed number of unidentified bases (N) or ambiguous sites, but uses no threshold and also assessed multiple consensus calls with threshold of max. 30 loci with multiple consensus.*
[l] *Calculated by laboratories that reported numerical values.*

Lab ID	Genome size	N50	Species assignment	Contamination	% GC
17[a]				<10%	
20					
23	5 MB +/- 10%				
26[b]	4-5.8 Mbp	>20 kb	>70% contigs assigned		
29	Approx. 5.0 MB	Default parameters of INNUca v4.2.2		Default parameters of INNUca v4.2.2	
30	4.5-5.8 Mbp	≥30000	≥95%		
34	4.0-5.2 Mbp			<5%	
35					
42	4.9+1.2 Mbp	>50000			
44	Approx. 3.6-5.4 Mbp		No set threshold		
46[c]	~5 Mbp	>50000		<5%	~52%
49	4.9+/-1.2 Mbp			Number of SNVs <3	
52[d]					Expected range for species
53[e]	4.5-5.15 Mbp	>30000 bp		<5%	52%
56[f]	4.5-5.15 Mbp				~52.2% (+/- 1)
58[g]	4.4-5.8 Mb	>30000 bp		0%	
63	10% deviation allowed			No obvious contamination (other than plasmid, etc.)	
69	4.4-5.8 Mb	>30000 bp			
73[h]	4.3-5.3 Mbp	>30000		<2%	
74	5 Mpb +/- 5%	>100 kb			
77	4627000 < x ≤ 5006000	>50000			51.8 <x ≤52.3
84[i]	4.5-5.5 Mb	>100000			
92[j]	4.3-5.8 Mb	>30000		Genome size out of range, no. of contigs out of range, second species >5%	52.1-52.2%
98[k]	4510000-5300000 bp	No threshold provided		<5%	
Median (range)[l]	**4.5-5.3 Mb (3.6-4.9 – 5.0-6.1)**	**30 kb (20-100)**	**>82.5% (70-95)**	**5% (0-10)**	**52.0-52.2 (51.8-52.2 – 52.0-52.3)**

Green = Not reported.

[a]*Laboratory 17 also assessed the proportion of ambiguous bases with a cut-off of >800 and the read length with a cut-off of >50 nt.*
[b]*Laboratory 26 also assessed the proportion of scaffolding placeholders with a cut-off of <3%.*
[c]*Laboratory 46 also assessed the read length, which was set at 250-300 bp and the number of reads with threshold >600k.*
[d]*Laboratory 52 also assessed the N content, which as set to >3%, the sequence length, for which the majority of reads was expected to be full length. The laboratory also assessed sequence duplication, the amount of overrepresented sequences, the number of reads, and the outcomes of MLST and AMR typing.*
[e]*Laboratory 53 also assessed the average read length with a cut-off of 150 bp.*
[f]*Laboratory 56 also assessed the quality of reads with threshold >10.*
[g]*Laboratory 58 also assessed the average read length with threshold of 150 bp and the number of reads, which should be as high as possible.*
[h]*Laboratory 73 also assessed the Q30 rate with threshold >80, and contamination, for which the number of absent genes should be <2, the number of multiple alleles <1, and the amount of SNVs should be <7.*
[i]*Laboratory 84 also assessed the NrBAFperfect with threshold >4000 and the NrBAFMultiple with threshold <20.*
[j]*Laboratory 92 also assessed number of reads with threshold >1000000.*
[k]*Laboratory 98 also assessed number of unidentified bases (N) or ambiguous sites, but uses no threshold and also assessed multiple consensus calls with threshold of max. 30 loci with multiple consensus.*
[l]*Calculated by laboratories that reported numerical values.*

Annex 13. Reported MLST sequence types of cluster analysis strain panel

Lab ID	MLST reported	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Provider	**Yes**	**34**	**34**	**34**	**34**	**34**	**34**	**15**	**34**	**34**	**34**
17	Yes	34	34	34	34	34	34	15	34	34	34
20	Yes	34	34	34	34	34	34	15	34	34	34
23	Yes	34	34	34	34	34	34	15	34	34	34
26	Yes	34	34	34	34	34	34	15	34	34	34
29	Yes	34	34	34	34	34	34	15	34	34	34
30	Yes	34	34	34	34	34	34	15	34	34	34
34	Yes	34	34	34	34	34	34	15	34	34	34
35	Yes	34	34	34	34	34	34	15	34	34	34
42	Yes	34	34	34	34	34	34	15	34	34	34
44	Yes	34	34	34	34	34	34	15	34	34	34
46	Yes	34	34	34	34	34	34	15	34	34	34
49	Yes	34	34	34	34	34	34	15	34	34	34
52	Yes	34	34	34	34	34	34	15	34	34	34
53	Yes	34	34	34	34	34	34	15	34	34	34
56	Yes	72367	72367	1222719	72367	41182	29445	12643	72367	222903	72367
58	Yes	34	34	34	34	34	34	15	34	34	34
63	Yes	34	34	34	34	34	34	15	34	34	34
69	Yes	34	34	34	34	34	34	34	34	34	34
73	Yes	34	34	34	34	34	34	15	34	34	34
74	Yes	34	34	34	34	34	34	10	34	34	34
77	Yes	34	34	34	34	34	34	15	34	34	34
84	Yes	34	34	34	34	34	34	15	34	34	34
92	Yes	34	34	34	34	34	34	15	34	34	34
98	Yes	34	34	34	34	34	34	15	34	34	34

Grey=incorrect MLST type.

Annex 14. Results reported for WGS-based cluster assignments based on index EQA2413

Belonging to cluster yes/no

Lab ID	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422	EQA2423	EQA2424	EQA2425	EQA2426	EQA2427	% correctly assigned per lab[a]
Provider	**Yes**	**No**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**Yes**	**IQ**	**IQ**	**IQ**	**Yes**	**No**	**NA**
17	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
20	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
23	Yes	No	Yes	No	No	No	Yes	No	Yes	Yes	No	No	Yes	No	100
26	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
29	Yes	Yes	No	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	82
30	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
34	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
35	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
42	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
44	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
46	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
49	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
52[b]	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	IQ	IQ	82
53	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	No	IQ	Yes	No	100
56	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
58	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
63	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
69	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	Yes	No	64
73	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
74	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
77	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
84	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
92	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	No	Yes	No	100
98	Yes	No	Yes	No	No	No	Yes	No	Yes	IQ	IQ	IQ	Yes	No	100
% Correctly assigned per sample	100	92	96	96	96	96	100	100	100	NA	NA	NA	96	96	

IQ = insufficient quality. Orange = insufficient quality not detected. Grey = incorrectly assigned.

[a]Calculation based on cluster or singleton assignment of provided isolates EQA2414-EQA2422 and provided good quality raw reads EQA2426 and EQA2427.

[b]Laboratory did not assess the additional provided sequences EQA2423-EQA2427.

Annex 15. Distances reported based on index EQA2413

Gene-by-gene approach, allelic distances

Lab ID	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422	EQA2423	EQA2424	EQA2425	EQA2426	EQA2427
Provider	**1**	**41**	**1**	**38**	**14**	**2276**	**1**	**19**	**1**	**NA**	**NA**	**NA**	**1**	**86**
17[a]	1	42	1	45	15	2182	2	21	1	IQ	IQ	57	1	86
20[b]	1	49	1	41	20	2387	2	31	1	IQ	IQ	IQ	2	82
23	1	50	1	50	15	2236	2	20	1	18	141	55	1	78
26	14	55	9	59	23	2269	9	33	11	IQ	IQ	IQ	17	101
29[c]	1	3	61	57	22	2743	2	34	0	IQ	IQ	IQ	4	118
30	0	40	0	39	13	2217	1	18	0	IQ	IQ	IQ	1	78
34	2	44	2	50	17	2263	3	24	2	IQ	IQ	IQ	2	97
35[d]	2	43	2	46	16	2237	3	21	2	IQ	IQ	56	2	79
42[e]	1	42	1	41	15	2236	2	20	1	IQ	IQ	55	1	78
46	1	42	1	41	15	2239	2	20	1	IQ	IQ	IQ	1	78
49	1	42	1	41	15	2237	2	20	1	IQ	IQ	IQ	1	78
53[f]	1	42	1	46	15	2208	2	21	1	IQ	246	IQ	1	85
58	1	42	1	41	15	2282	2	20	1	IQ	IQ	58	1	78
63	1	41	1	51	16	2223	2	22	1	IQ	IQ	IQ	3	95
73[g]	1	48	1	49	15	111111	2	28	1	IQ	IQ	IQ	1	99
74	1	42	1	41	15	2239	2	20	1	IQ	IQ	55	1	78
77	5	44	3	47	19	2203	7	24	6	IQ	IQ	60	7	90
84	1	43	1	42	16	2257	2	22	1	IQ	IQ	58	1	79
92[h]	1	42	1	41	15	2238	2	20	1	IQ	IQ	55	1	78
98[i]	1	43	2	44	17	2194	2	22	2	IQ	IQ	IQ	1	77
Median	**1**	**42**	**1**	**45**	**15**	**2238**	**2**	**21**	**1**	**NA**	**NA**	**NA**	**1**	**79**

IQ = insufficient quality. Orange = insufficient quality not detected.

Comments made by participants about allele calling and cluster cut-off

[a]Sample EQA2425 was contaminated by human DNA ~50%. We routinely discard samples like this, but after cleanup of human DNA there was genome coverage 19.7x (which is slightly below hard threshold of 20 mean coverage). We decided to follow up with the analysis although we do not routinely process such samples.

[b]EQA2423 was not part of the out break. I was able to analyse the sequence however because sequence data was mixed; the answer was insufficient quality.

[c]Reads quality control, species confirmation and bacterial de novo assembly were performed using the INNUca v4.2.2 pipeline (Llarena et al., 2018) (available at https://github.com/B-UMMI/INNUca), which consists of integrated modules for reads QA/QC, de novo assembly and post-assembly optimization steps. Briefly, after read quality analysis (FastQC v0.11.52) and cleaning (Trimmomatic v0.38), genomes were assembled with SPAdes v3.14 and subsequently improved using Pilon v1.23. Species confirmation and contamination screening were assessed using Kraken v.2.0.7 for both raw reads and final polished assemblies, using the standard 8G database. MLST prediction was determined using mlst v2.18.1 software. Serotype was predicted with SeqSero2 v1.2.1.

- Cluster analysis: This gene-by-gene analysis was performed with chewBBACA v3.3.4 using the wgMLST Schema for S. enterica from the INNUENDO project, enrolling 8558 loci (available at chewie-NS: https://chewbbaca.online/species/8). A dynamic approach was performed with ReporTree v2.5.1 using both GrapeTree and single-linkage clustering algorithms, defining the core cgMLST loci using a threshold of 90%. Samples with less than 95% loci called were excluded from the analyses. Outbreak-related samples were determined using a 0.43% cut-off of the final core (i.e. 16 allele differences over 3757 cgMLST loci), as suggested by the INNUENDO project (Llarena et al., 2018).

[d]Comment to the question "What distance (allelic or SNPs) do you use as cut-off for cluster analysis?": We differentiate between WGS clusters and (suspected) outbreak clusters. For WGS clusters we apply 5 AD (for S. Enteritidis 3 AD). But we would not consider an isolate with e.g. 5 AD from the main node as part of a particular outbreak, unless there is clear epidemiological evidence.

Comment to the question "For which purpose do you use WGS based cluster analysis?":

a) for general surveillance purposes --> due to limited (personnel) resources and lack of automated workflows currently only for S. Enteritidis, S. Typhimurium and S. Typhi

b) for outbreak situations only --> for all other serovars

Comment to the question "Which criteria and thresholds do you use to assess the quality of your WGS reads or assemblies?": The number of contigs and their N50 might also be considered but here we have no defined thresholds.

According to our qc criteria the avg. coverage for EQA2425 is too low though the number of good targets is still ok - in case of doubtful cluster affiliation we would probably repeat WGS for this strain
[e]Genome EQA2425 has lower coverage (21) than the set threshold of >30. However, all other quality control parameters are good, and we would accept these results. In case this genome would cluster with the outbreak to indicate a possible source of the outbreak, the isolate would be re-sequenced.
[f] When done with SeqSero-1.2 (CGE) I 4,[5],12:i:- serovar was reported as potentiol variant of Typhimurium monophasic.
[g]The isolate EQA2419 was not included in the cgMLST analysis because it belongs to a different serotype, so a random number (i.e. 1111111) was inserted in the section where the allelic distance of the isolates to the index case was required.
[h]EQA2425 fastq file contained a large proportion of Homo sapiens reads which lowered the average coverage to 21x. However, considering the context of an outbreak investigation and the fact that the assembly displayed 98.5% good cgMLST targets, we decided to include it for the cluster detection analysis.
The cut-off for S enterica serovar I 1,4,[5],12:i:- (monophasic S. Typhimurium) was set to <= 5 allele difference (European Centre for Disease Prevention and Control, European Food Safety Authority, 2022. Multi-country outbreak of monophasic Salmonella Typhimurium sequence type 34 infections linked to chocolate products, first update - 18 May 2022).
[i]Genome EQA2426 could be accepted for analysis in case of an outbreak situation, since the core% and number of multiple consensus calls in the cgMLST analysis is not indicating contamination with other Salmonella. However, we would always also restreak for pure culture and resquence for confirmation.

SNP-typing, SNP distances

Lab ID	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422	EQA2423	EQA2424	EQA2425	EQA2426	EQA2427
Provider	**2**	**115**	**2**	**81**	**41**	**33433**	**3**	**377**	**1**	**NA**	**NA**	**NA**	**4**	**153**
44[a]	2	241	2	93	38	36156	3	477	1	IQ	IQ	IQ	4	153
52[b]	1	24	1	28	13	271	2	19	0					
56[c]	2	97	3	79	36	212241	2	70	2	IQ	IQ	IQ	6	147
69	1	10	1	1	1	1	2	24	1	27	289	76	1	104
Median	**2**	**97**	**2**	**79**	**36**	**21241**	**2**	**70**	**1**	**NA**	**NA**	**NA**	**4**	**150**

IQ = insufficient quality. Grey = Not assessed. Orange = insufficient quality not detected.
Comments made by participants about SNP calling and cluster cut-off
[a]The distance, 5 SNPs cut-off, for cluster analysis is not fixed. Epidemiological information and cluster/outbreak characteristics often need to be considered as well.
[b]We could not download or upload from the Research Drive in time so we have to skip that portion.
If SNP analysis reveals significant outliers like S24019 we might redo it without outliers to prevent overfiltering of SNPs. We combine SNP analysis with cgMLST typing for further verification if possible (cgMLSTFinder-1.2 from DTU or chewBBACA). All samples considered to be part of the outbreak had a cgST of 72367.
[c]MLST (7 loci) was ST34 for all S. monophasic Typhimurium isolates. S. Heidelberg was ST15

Annex 16. Quality assessment of submitted Illumina WGS data per laboratory

Laboratory ID 17

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.68	99.61	99.61
Contamination	0.53	0.55	0.53	0.53	0.53	0.55	0.89	0.53	0.53	0.53
Avg coverage	216.569	178.539	201.393	216.602	203.761	190.165	181.844	213.683	202.564	164.363
N50	223319	184140	241535	192481	191379	222923	184260	282794	224921	192925
GC%	52.17	52.17	52.13	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4903577	4905042	4922713	4903807	4902901	4911458	5071548	4904263	4907810	4903291
# reads	11118524	9165710	10376758	11140960	10470984	9758986	9670716	11024920	10411656	8432894
Mean read length	95	95	95	95	95	95.5	95	95	95	95.5

Laboratory ID 20

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.57	99.68	99.61	99.68	99.61	99.68	99.68
Contamination	0.53	0.53	0.53	0.93	0.53	0.53	0.89	0.53	0.53	0.53
Avg coverage	53.027	55.092	113.161	6.811	135.668	109.443	98.836	89.478	84.101	125.573
N50	97974	144283	277410	22686	174817	239211	383030	185409	194668	165126
GC%	52.19	52.17	52.14	52.25	52.16	52.16	51.92	52.17	52.18	52.17
Total length	4899217	4904219	4925485	4867214	4906959	4910232	5074380	4906422	4903551	4906781
# reads	1905284	1983102	4000510	286304	4777782	3812810	3527654	3103052	3071382	4356924
Mean read length	136	136	139	115.5	139	140.5	141.5	141	134	141

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 23

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.68	99.61	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	65.896	18.814	188.540	57.667	247.495	42.258	54.977	45.924	61.137	56.162
N50	282493	230057	315989	277410	182716	282607	390648	282607	282607	315398
GC%	52.16	52.16	52.13	52.17	52.16	52.16	51.92	52.16	52.17	52.16
Total length	4901978	4901077	4923863	4904019	4905751	4904783	5074465	4902891	4905411	4902403
# reads	2565136	734760	7261038	2191584	9420838	1603762	2243568	1813646	2410144	2214024
Mean read length	125.5	125	127.5	129	128.5	128.5	124	123.5	124	124

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 26

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.65	99.61	99.61	99.61	99.65	99.66	99.61	99.61	99.59
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	66.059	47.781	51.682	51.604	57.266	93.411	72.181	100.759	121.302	80.083
N50	118203	51533	81154	72700	74269	160798	79089	143365	229395	128937
GC%	52.19	52.3	52.24	52.23	52.2	52.2	51.99	52.18	52.17	52.2
Total length	4897934	4869707	4892012	4887411	4892088	4904649	5056095	4902315	4913879	4894808
# reads	1546242	1104322	1244646	1198998	1346468	2183126	1736496	2322134	2873188	1873242
Mean read length	209	210.5	203	210	208	209.5	210	212.5	207.5	209

Laboratory ID 29

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.55	0.55	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	50.307	38.668	47.251	48.222	46.333	43.967	59.660	29.607	51.393	47.273
N50	239978	142676	164012	73270	104720	220133	214755	104422	211430	130840
GC%	52.16	52.17	52.16	52.15	52.15	52.16	51.92	52.17	52.17	52.17
Total length	4898967	4898275	4897415	4906793	4893823	4898479	5067495	4898172	4900104	4898089
# reads	2216870	1725294	2092324	2131590	2042050	1939680	2722198	1348174	2278360	2107626
Mean read length	111	109.5	110	110.5	110.5	110.5	110.5	107.5	110	110

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 30

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.68	99.61	99.61	99.61	99.61	99.61	99.68	99.68	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.53	0.53
Avg coverage	35.253	31.331	27.919	24.737	27.768	29.156	30.305	31.077	30.760	30.696
N50	100001	97094	88867	187374	65729	110738	67281	95332	90312	71844
GC%	52.17	52.18	52.15	52.17	52.18	52.17	51.94	52.18	52.2	52.18
Total length	4901642	4903600	4914096	4901901	4898799	4905782	5067722	4900914	4900014	4899420
# reads	890606	769592	709418	677460	672466	727854	783820	803012	754904	750896
Mean read length	193.5	199.5	193.5	178.5	202.5	196.5	196	189.5	199.5	200

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 34

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.68	99.24	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	59.222	125.568	138.976	110.410	60.561	70.641	128.064	112.877	108.075	127.431
N50	82240	192287	192044	120940	88648	136874	320519	224333	178367	177878
GC%	52.22	52.17	52.16	52.19	52.2	52.2	51.93	52.19	52.19	52.19
Total length	4892482	4904661	4920382	4901511	4894295	4905939	5071939	4900556	4907331	4899352
# reads	2069638	4421986	4931760	3912662	2133150	2489706	4671682	3965634	3814200	4480378
Mean read length	140	139	138.5	138	139	139	138.5	139	139	139

Laboratory ID 35

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.68	99.61	99.61	99.68	99.61	99.68	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.92	0.53	0.55	0.53
Avg coverage	120.204	141.893	115.871	94.822	103.508	132.156	43.297	103.644	117.735	112.819
N50	277410	179074	177687	226330	135172	239208	116750	280410	239181	277410
GC%	52.17	52.18	52.15	52.17	52.16	52.17	51.96	52.17	52.17	52.17
Total length	4906616	4905807	4920564	4906016	4905761	4910720	5061252	4906511	4908315	4906795
# reads	4331288	5113024	4213520	3410916	3723312	4778652	1971168	3733606	4253056	4074574
Mean read length	135.5	135.5	135.5	136	136	135	110.5	136	135.5	135

Laboratory ID 42

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.68	99.61	99.61	99.61	99.61	99.68	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	60.311	61.329	55.650	69.864	70.950	56.931	66.026	61.928	62.714	58.777
N50	282725	275616	262799	268155	174161	271352	412368	282725	272878	274413
GC%	52.17	52.16	52.13	52.16	52.15	52.16	51.92	52.17	52.17	52.16
Total length	4901344	4902128	4918001	4903826	4900202	4904019	5068824	4901990	4903159	4901376
# reads	2472422	2504542	2290178	2898742	2942244	2318852	2799958	2552918	2582218	2394370
Mean read length	119	120	119	118	118	120	119.5	119	119	120

Laboratory ID 46

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.68	99.61	99.61	99.68	99.61	99.61	99.68
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	69.666	75.781	73.824	75.378	83.979	67.426	83.799	69.553	90.548	72.464
N50	282725	271352	277124	277410	174161	277410	320519	224333	277583	277410
GC%	52.17	52.17	52.14	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4904032	4903772	4920948	4905193	4903995	4906460	5073501	4905072	4907594	4904864
# reads	1421006	1621654	1538198	1517634	1729228	1401068	1821154	1401468	1884152	1489184
Mean read length	240	229.5	236.5	243	238	236	233.5	243.5	236	238.5

Laboratory ID 49

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.68	99.61	99.61	99.68	99.61	99.61	99.68	99.61	99.61	99.68
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	33.080	33.328	40.402	30.646	31.260	40.490	38.245	39.218	21.508	30.325
N50	282725	274347	277338	277178	182825	277410	235154	315879	275643	282607
GC%	52.16	52.17	52.13	52.16	52.15	52.16	51.92	52.16	52.17	52.16
Total length	4899685	4900880	4916692	4900349	4897110	4903936	5068588	4901796	4903087	4900915
# reads	1184770	1196310	1459848	1102398	1151000	1456588	1414434	1395410	779812	1091024
Mean read length	136.5	136	136	135.5	132.5	136	136.5	137	135	135.5

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 52

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.5	99.61	99.32	99.61	99.31	99.61	97.9	99.51	99.61	99.61
Contamination	0.47	0.53	0.46	0.53	0.37	0.53	0.82	0.46	0.53	0.53
Avg coverage	26.159	45.291	56.504	68.757	77.508	88.800	70.113	87.527	90.553	94.736
N50	152773	270535	201354	223412	140467	277410	210258	237911	239181	277956
GC%	52.23	52.17	52.19	52.17	52.19	52.16	52.11	52.17	52.17	52.17
Total length	4840537	4903738	4847951	4903335	4831896	4911746	4839585	4884769	4905834	4903070
# reads	875340	1524170	1878950	2306574	2640346	3199554	2505018	3255388	3390966	3523352
Mean read length	144	145.5	146	146	141.5	136	136	131	131	131

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 53

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.68	99.61	99.61	99.68
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	183.562	231.469	218.353	184.356	233.078	245.589	245.163	116.400	200.002	201.249
N50	280410	224333	282725	282725	135172	282725	265150	280410	279976	280410
GC%	52.17	52.17	52.14	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4906838	4907159	4927159	4906414	4906533	4911103	5076349	4906640	4910620	4906467
# reads	6164304	7776122	7372830	6198036	7851598	8287654	8527952	3900196	6744758	6758228
Mean read length	146	146	146	145.5	145	145	145.5	146	145	145.5

Laboratory ID 58

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.68	99.68	99.61	99.68	99.68	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.53	0.53
Avg coverage	373.637	300.456	344.484	348.681	230.029	288.955	248.147	196.630	253.599	202.301
N50	280410	282725	277410	277410	174368	282725	320519	277410	279976	277410
GC%	52.17	52.17	52.14	52.17	52.16	52.17	51.92	52.17	52.17	52.17
Total length	4906890	4907196	4923802	4907426	4908389	4912619	5074385	4906955	4914335	4906853
# reads	12792220	10190142	11798000	11819784	7985604	9847564	8733534	6667384	8624520	6852284
Mean read length	143	144.5	144	144.5	141	144	143.5	144	144	144.5

Laboratory ID 63

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.68	99.61	99.61	99.68	99.61	99.61	99.68	99.61	99.68	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	45.696	52.694	40.627	46.213	45.225	52.060	36.783	31.683	43.837	50.440
N50	271352	273245	278517	224333	182720	150380	320519	280410	279976	277124
GC%	52.17	52.17	52.14	52.17	52.15	52.18	51.93	52.17	52.17	52.17
Total length	4905617	4906743	4922572	4906002	4906396	4908886	5072005	4904312	4910672	4905802
# reads	1315470	1321532	1096390	1254220	1038778	1170086	856708	727366	1005806	1121112
Mean read length	170.5	195	182	180.5	213.5	218.5	218	213.5	214	220.5

Laboratory ID 69

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.07	99.17	99.61	99.61	99.61	99.61	99.68	99.57	99.61	99.61
Contamination	0.67	0.55	0.53	0.53	0.53	0.73	1.2	1.16	0.53	0.53
Avg coverage	8.937	11.779	33.578	26.550	34.179	28.009	28.154	10.563	30.737	19.322
N50	16666	26916	104772	50818	102859	53514	51720	20165	121985	37936
GC%	52.37	52.25	52.13	52.2	52.16	52.15	52.04	52.21	52.18	52.24
Total length	4839479	4873848	4913825	4896238	4897591	4955678	5043539	4878080	4904198	4882521
# reads	327208	443858	1295488	968356	1306704	1058168	1098554	419078	1109002	702554
Mean read length	132.5	129	127.5	134	128.5	131	131	123	135.5	134

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 73

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.68	99.61	99.61	99.61	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.53	0.53
Avg coverage	93.653	89.048	89.387	92.228	84.564	84.651	81.417	81.041	81.660	83.154
N50	280410	277410	282725	282725	184718	282725	390312	315508	282725	280410
GC%	52.17	52.17	52.13	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4906892	4907251	4926762	4906921	4908219	4914528	5073852	4906824	4914203	4906883
# reads	3535384	3398444	3391350	3475476	3193304	3234550	3183340	3061130	3078736	3138226
Mean read length	129.5	128.5	129.5	129.5	129.5	128	129.5	129.5	130	129.5

Laboratory ID 74

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.68	99.68	99.68	99.68	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.61	0.53	0.89	0.53	0.55	0.53
Avg coverage	90.827	91.939	87.071	91.736	97.152	99.694	89.517	92.544	111.816	101.274
N50	282725	282607	282493	285042	182716	277410	320401	315878	282493	315988
GC%	52.16	52.17	52.13	52.16	52.15	52.16	51.92	52.17	52.17	52.16
Total length	4901366	4901660	4917598	4901600	4900031	4906467	5071743	4901595	4903900	4901736
# reads	3547714	3549860	3398480	3541480	3742966	3838598	3593940	3564248	4298166	3908454
Mean read length	125	126.5	126	126.5	127	127	126	126.5	127.5	126.5

Laboratory ID 77

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.68	99.61	99.61	99.68
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.53	0.53
Avg coverage	106.416	123.737	130.826	129.552	125.067	166.539	146.142	190.491	117.155	138.648
N50	280410	282725	282493	280410	210471	282725	320287	280410	279976	282724
GC%	52.17	52.17	52.13	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4905715	4905992	4922283	4906543	4907233	4910545	5072864	4905658	4913814	4906390
# reads	4161742	4858808	5070306	5013434	4850266	6471440	5872394	7372290	4548386	5361492
Mean read length	125	124.5	127	126.5	126	126	126	126.5	126	126

Laboratory ID 84

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.68	99.61	99.61	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.53
Avg coverage	149.110	127.060	154.613	179.000	121.132	133.560	186.928	170.258	137.397	120.600
N50	280410	239192	316028	271352	184718	278517	265150	277410	272811	280410
GC%	52.17	52.17	52.14	52.17	52.16	52.16	51.92	52.17	52.17	52.17
Total length	4906634	4905845	4920992	4905339	4906372	4912690	5074470	4904215	4910064	4906612
# reads	3055438	2624470	3491246	3707192	2418808	2683004	4208952	3843730	2832254	2458964
Mean read length	239	237.5	218	236.5	246	244.5	225	217	238	240.5

Laboratory ID 92

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.61	99.68	99.68	99.61	99.61	99.68	99.58	99.68	99.61
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.89	0.53	0.55	0.6
Avg coverage	69.691	106.282	62.852	99.643	103.335	116.355	111.834	76.193	89.846	98.148
N50	277410	214586	282725	224333	150015	226352	234491	201216	279976	277410
GC%	52.17	52.17	52.14	52.17	52.16	52.17	51.94	52.17	52.17	52.17
Total length	4906177	4907333	4923817	4907290	4906419	4910684	5069994	4905802	4914097	4906219
# reads	1734614	2757694	1574010	2519788	2578566	2941010	2942052	1980738	2292510	2467252
Mean read length	197	189	196.5	193.5	196	194	192	188	192	194.5

Laboratory ID 98

QC parameter	EQA2413	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Completeness	99.61	99.68	99.61	99.61	99.65	99.68	99.68	99.61	99.56	99.31
Contamination	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.55	0.55	0.91
Avg coverage	58.448	93.113	71.350	64.735	53.510	129.981	122.553	67.972	69.195	57.574
N50	59630	129776	45719	44607	32849	96307	129785	62047	53455	35164
GC%	52.24	52.17	52.26	52.26	52.32	52.17	52.19	52.26	52.24	52.1
Total length	4882521	4899985	4883931	4878829	4858298	4903802	4896008	4878683	4881131	5022520
# reads	2403724	3852252	2948130	2680936	2204066	5455436	5026924	2802858	2843470	2456428
Mean read length	118.5	118	118	117.5	118	116.5	119	118	118	117.5

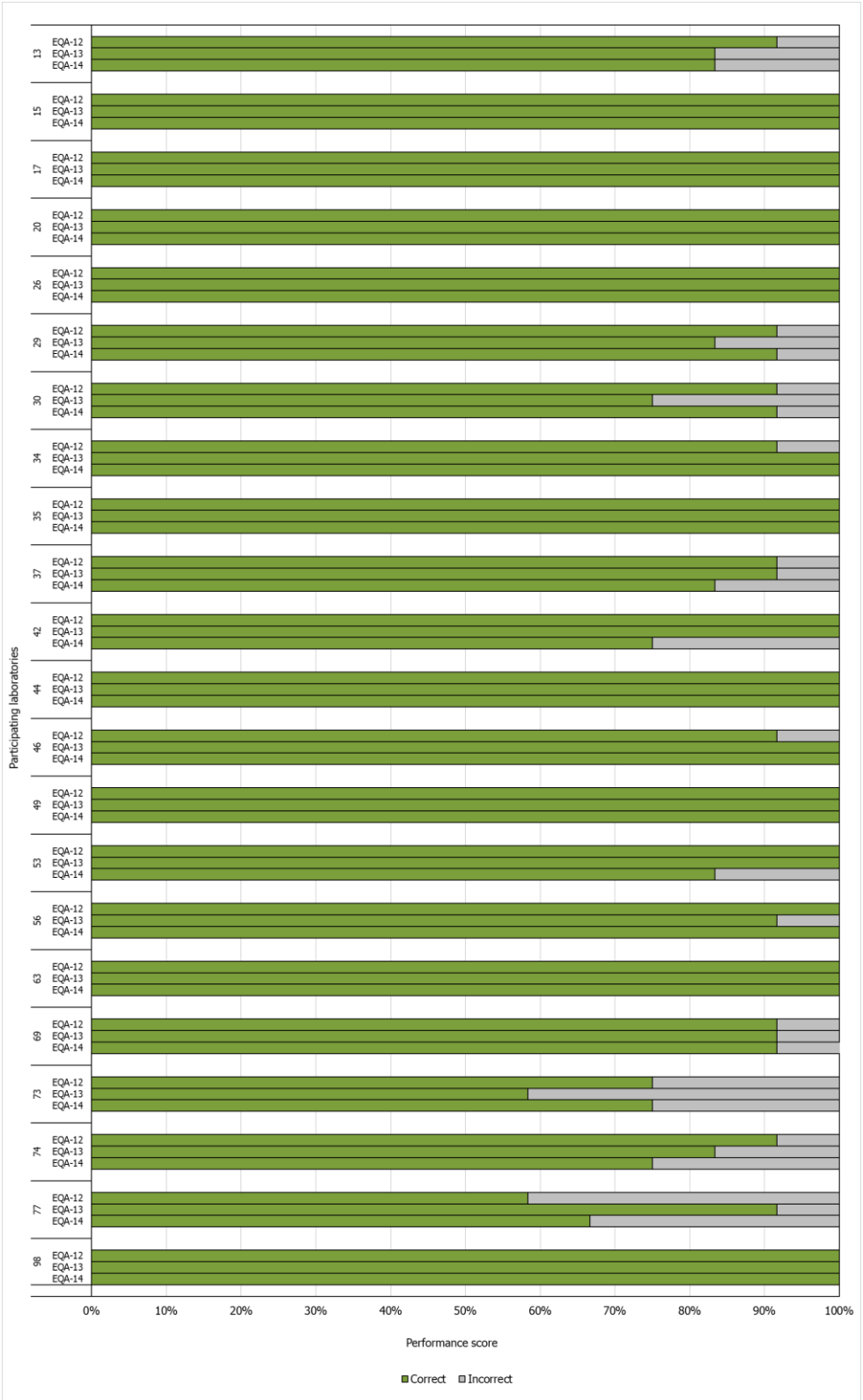
Grey = does not pass quality criteria of EQA provider.

Annex 17. Reported results MLVA-based cluster assignments to index EQA2413

Lab ID	EQA2414	EQA2415	EQA2416	EQA2417	EQA2418	EQA2419	EQA2420	EQA2421	EQA2422
Provider WGS	**Yes**	**No**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**Yes**
Provider MLVA	**Yes**	**No**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**Yes**
63	Yes	No	Yes	No	No	No	Yes	No	Yes
74	Yes	No	Yes	No	No	No	Yes	No	Yes

Green = provider.

Annex 18. Comparison serotyping results between the last three rounds of EQA's per laboratory



Annex 19. Comparison serotyping results between previous EQA's per serovar

Lab ID[a]	Infantis			Typhimurium, monophasic			Enteritidis			Typhimurium			Derby			IIIb 47:k:z35		
	EQA-12	EQA-13	EQA-14	EQA-12	EQA-13	EQA-14	EQA-12	EQA-13	EQA-14	EQA-12	EQA-13	EQA-14	EQA-12	EQA-13	EQA-14	EQA-12	EQA-13	EQA-14
13					b											nd	b	
15																nd		
17																nd		
20																nd		
26																nd		
29		b														nd		
30																nd		b
34																nd		
35																nd		
37																nd		b
42																nd		
44																nd		
46																nd		
49																nd		
53																nd		
56																nd		
63																nd		
69					b											nd		b
73																nd	b	
74																nd		
77				b												nd		b
98																nd		

[a] *Laboratories that participated in all three rounds of EQA's.*

[b] *Correct seroformula determined but wrong serovar reported.*

Grey=incorrect serovar; Green=correct serovar; nd=not included in EQA-round.

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