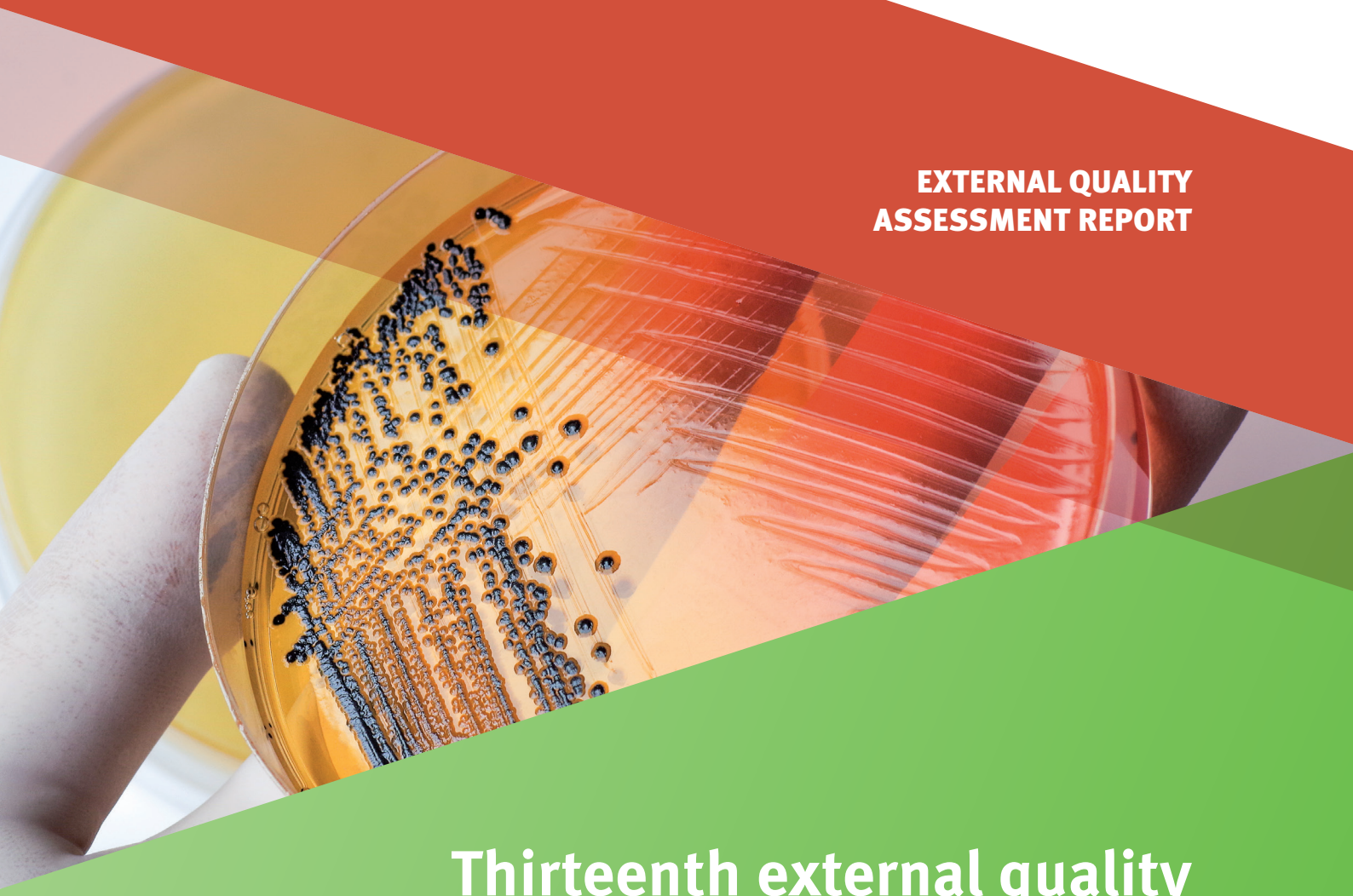
EXTERNAL QUALITY ASSESSMENT REPORT



Thirteenth external quality assessment for *Salmonella* typing

ECDC TECHNICAL REPORT

Thirteenth external quality assessment for *Salmonella* typing

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC) under framework contract ECDC/2021/014-lot 1, coordinated by Taina Niskanen, and produced by the National Institute for Public Health and the Environment (RIVM).

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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
EWRS	Early Warning and Response System
FWD-Net	European Food-and Waterborne Diseases and Zoonosis Network
ID	Identification
IQR	Interquartile range
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, with 65 208 reported cases in the European Union (EU) in 2022. *Salmonella* is also the bacteria associated with most foodborne outbreaks [2]. The overall EU trend in salmonellosis incidence for the years 2017 to 2021 has not changed significantly. To prevent foodborne diseases such as salmonellosis from further spread, it is essential to employ human surveillance systems at different levels to monitor the disease and ensure early detection and response to outbreaks.

ECDC has set surveillance objectives to monitor trends and carry out multinational outbreak detection of salmonellosis and other pathogens. In addition, objectives will 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 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. To monitor the typing methods used, data quality and comparability, and the capability of the laboratories performing these methods, ECDC commissions an annual External Quality Assessment (EQA) scheme for the serotyping and molecular-based cluster analysis of *Salmonella*.

This thirteenth external quality assessment scheme for *Salmonella* typing (EQA-13) 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, two different sets of 10 *Salmonella* isolates were selected (for whole genome sequencing [WGS]/Pulsed-field gel electrophoresis [PFGE] and Multiple locus variable-number tandem repeat analysis [MLVA]), containing cluster and non-cluster isolates. These isolates mimicked a real outbreak situation, originating from a dinner on a cruise ship. In addition, raw reads of five isolates were made available to the participants that used 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, 28 laboratories participated and 54% (15/28) used phenotypical serotyping, based on agglutination with antisera only, while 11% (3/28) used a combination of agglutination with antisera and molecular methods other than WGS. In all, 14% (4/28) of the laboratories used a combination of agglutination with antisera and prediction of serotype with WGS, and 21% (6/28) used prediction of serotype with WGS only. Performance was high for most laboratories, with 16 laboratories achieving performance scores of 100% and six of 92%. The six laboratories that had the lowest performance values (<92%) used phenotypic methods. Overall performance in serotyping was better if the serotype was predicted using WGS than if it was assessed using phenotypic methods alone ($p=0.0090$, χ^2).

Twenty-four laboratories took part in the molecular typing-based cluster analysis, which was an increase compared to the previous two EQAs. In both, EQA-12 (2022) and EQA-11 (2021), 20 laboratories participated in the molecular typing-based cluster analysis. In EQA-13, the proportion of participants that used WGS for their cluster analysis increased from 85% to 96% compared to EQA-12, while the number of participants that applied multiple locus variable-number tandem repeat analysis (MLVA)-based cluster analysis decreased from 15% to 13%. The number of participants using pulsed-field gel electrophoresis (PFGE)-based cluster analysis also decreased from 10% (two participants) to 4% (one participant). All participants that used MLVA also used WGS for cluster analysis.

In the WGS-based cluster analysis, most participants 91% (21/23) applied a gene-by-gene approach, while the other 9% (2/23) 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 96% correct cluster assignment for the isolates and sequences provided. Nineteen laboratories had 100% performance in assigning the provided isolates to clusters and three additional laboratories incorrectly assigned only one of the isolates provided.

Three laboratories applied MLVA-based typing and produced identical MLVA profiles, except for allele SENTR4 in strain EQA2329, indicating a high technical performance of 98% (49/50 alleles). However, overall performance for cluster assignment of provided isolates was lower (82%) than with WGS-based cluster analysis (96%, $p=0.0022$), as two isolates were included in the outbreak cluster based on MLVA profiles by two laboratories, and one isolate was excluded based on MLVA profiles by one laboratory. Therefore, participating laboratories had good capability in applying MLVA, but the resolution of the technique itself was too low to correctly assign isolates to clusters.

Since only one laboratory applied PFGE-based cluster analysis, this laboratory's performance could not be compared with the others. The participant correctly identified all isolates belonging to the cluster but could only exclude two isolates. 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.

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 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-13 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-13, 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.

ECDC has outsourced the organisation of EQA schemes for EU/EEA countries in the disease networks. 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 the EQA schemes are:

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

The provision of an annual EQA scheme for the serotyping and molecular-based cluster analysis of *Salmonella* in 2021−2025 is subcontracted to RIVM by ECDC. This report presents the aggregated results of the EQA *Salmonella* serotyping and molecular-based cluster analysis of 2023 (EQA-13).

1.2 Salmonellosis impact 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 can cause severe invasive infections [1]. Infection with *Salmonella spp.* is the second most reported zoonotic disease in humans, with 65 208 cases reported in the EU in 2022, and accounts for most of the foodborne outbreaks [2]. In the years 2020 and 2021, absolute case numbers for salmonellosis decreased compared to 2017−2019, mainly because of the withdrawal of the United Kingdom from the EU and the impact of COVID-19 control measures. Nevertheless, the overall EU trend in salmonellosis for the years 2017 to 2021 did not changed significantly [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 the FWD-Net [7].

ECDC has set surveillance objectives that have been transferred by the FWD-Net to 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, NPHRLs perform surveillance at national or regional level, based on data and/or submitted samples from clinical microbiology laboratories. The resulting surveillance data is disease-based, 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 portals EpiPulse and the Early Warning and Response System (EWRS) [8, 9]. Data analysis for salmonellosis trends and outbreaks is performed by the 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].

1.3 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 overall aims of integrating molecular typing data into EU-level surveillance are:

- to foster the rapid detection of dispersed international clusters/outbreaks;
- to facilitate the detection and investigation of transmission chains and relatedness of strains across EU/EEA;
- to contribute to global outbreak investigations;
- to detect the emergence of new and/or evolving pathogenic strains;
- to support investigations to trace the source of an outbreak and identify new risk factors;
- to 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.

1.4 Objectives of the EQA-13 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 national level.

1.4.1 Serotyping

The objective of the serotyping part of EQA-13 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.

1.4.2 Molecular typing-based cluster analysis

The objective of the molecular typing-based cluster analysis part of EQA-13 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

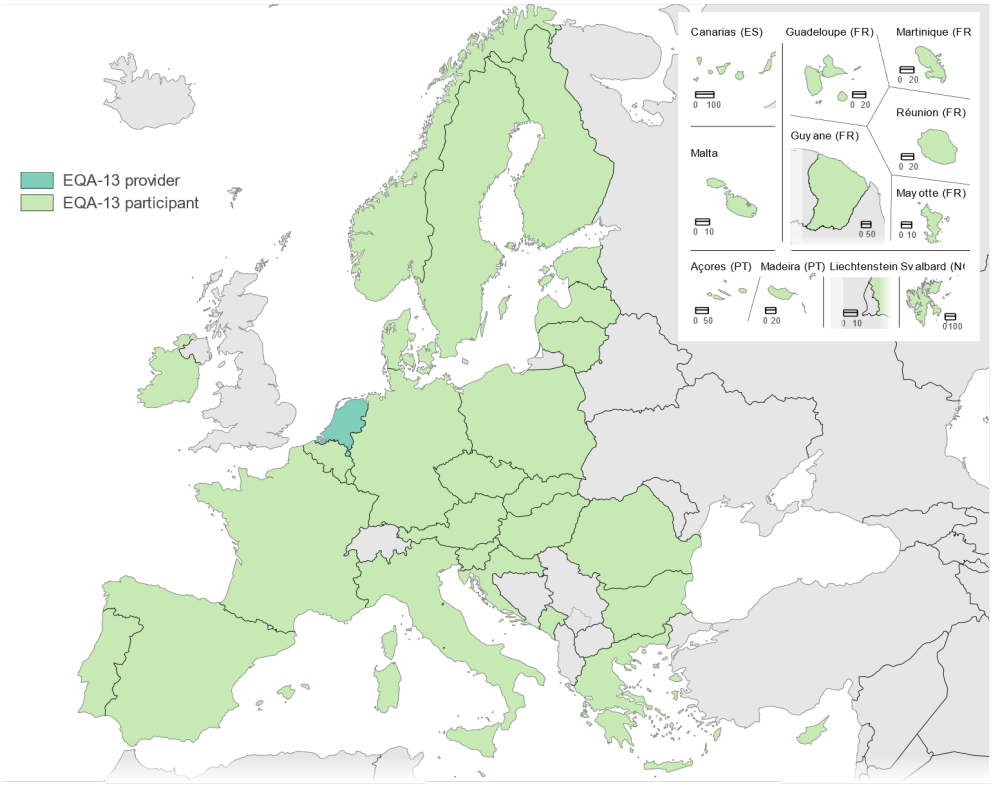
On behalf of ECDC, the EQA-13 was organised by RIVM under framework contract ECDC/2021/014-lot 1 for NPHRLs in the EU/EEA and enlargement countries. Participation of one laboratory per country was funded by ECDC.

Invitations for the EQA-13 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-13 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-13 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 28 (96.6%) of these completed at least one part of the assessment (Figure 1, Annex 2).

Figure 1. Geographical overview of participants in EQA-13



Timeline

The invitation for the EQA-13 was sent on 7 April 2023, and the deadline for registration was 30 April 2023. A reminder was sent on 20 April, and the final participant list, drawn up on 4 May 2023, contained 29 participating laboratories.

The samples were distributed to 29 laboratories on 28 June 2023. A total of 25 laboratories (86.2%) received the parcel two days later and four laboratories (13.8%) received it three days after shipping.

The deadline for result reporting was 15 September 2022. One laboratory did not submit results even after multiple reminders and three laboratories requested an extended deadline. The first results were completed on 21 July and

the last on 13 October 2023, with a median of 78 days (range 23–107 days) from shipping to result completion. Individual evaluation reports were shared with participants on 15 November 2023, 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 as 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 one of the numbers EQA2301–EQA2312. 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-13, including selection rationale

EQA #	Subspecies[a]	Serovar	Formula	Rationale
EQA2301	*enterica*	Infantis	7:r:1,5	[b]
EQA2302	*enterica*	Typhimurium, monophasic	4,5:i:-	[b]
EQA2303	*enterica*	London	3,10:l,v:1,6	To have some diverse O- and H-types
EQA2304	*enterica*	Virchow	7:r:1,2	To have some diverse O- and H-types
EQA2305	*enterica*	Mbandaka	7:z10:e,n,z15	Serotype caused a multi-country outbreak in 2022.
EQA2306	*enterica*	Enteritidis	9:g,m:-	[b]
EQA2307	*enterica*	Goldcoast (or Brikama)	6,8:r:l,w	Challenging serotype when using molecular methods only.
EQA2308	*enterica*	Havana	13,23:f,g:-	To have some diverse O- and H-types
EQA2309	*enterica*	Leeuwarden	11:b:1,5	Serotype shown to be difficult to type in the previous round of this EQA.
EQA2310	*enterica*	Typhimurium	4,5:i:1,2	[b]
EQA2311	*diarizonae*	IIIb 47:k:z35	47:k:z35	Different subspecies as bonus isolate.
EQA2312	*enterica*	Derby	4:f,g:-	[b]

[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 to the participants:

'During a tropical holiday on a cruise ship, an exclusive buffet-style barbecue dinner was served to 50 guests. Two days later, 42 of the dinner guests attending and three restaurant employees on the cruise ship fell ill with diarrhoea. One of the employees was admitted to a hospital at the nearest port. A *Salmonella* isolate was cultured from his faeces, isolate EQA2313 (EQA2328 for participants using MLVA). 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 (EQA2319 and EQA2322), resulting in 10 isolates for the cluster analysis (EQA2313-EQA2322). Participants were requested to report the isolates that clustered with the index case (EQA2313) according to their own cluster cut-off. When using WGS techniques, three of the 10 *Salmonella* isolates clustered closely with the index. Therefore, a participant could report up to three isolates in the cluster, depending on the resulting allele distance and the cluster cut-off used.

Table 2. Selected panel for molecular-based cluster analysis part of EQA-13, specifically for WGS and PFGE techniques

# EQA	Serovar	Part of cluster[a]	Distance to index cgMLST(AD)	Distance to index cSNPs
EQA2313	Dublin	Index	0	0
EQA2314	Dublin	No	96	201
EQA2315	Dublin	Yes	4	4
EQA2316	Dublin	No	258	568
EQA2317	Dublin	No	76	168
EQA2318	Dublin	No	258	615
EQA2319[b]	Dublin	Yes	3	3
EQA2320	Dublin	No	45	88
EQA2321	Enteritidis	No	1744	19905
EQA2322[b]	Dublin	Yes	3	3

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

For participants using MLVA, the outbreak situation was mimicked by selecting 10 *S. Enteritidis* isolates with cluster and non-cluster isolates for the cluster analysis (EQA2328–EQA2337). Participants were requested to report the isolates that clustered with the index case (EQA2328) according to their own cluster cut-off. All 10 isolates were analysed with MLVA (Table 3). Using MLVA, five of the nine isolates cluster to the index EQA2313 because they exhibit exactly the same MLVA profile, one isolate being closely related, with one repeat difference. When analysing the same set of isolates, there is a discrepancy in isolate EQA2333, which differs one allele in MLVA but belongs to the cluster when using WGS analysis (Table 3). This indicates the sub-optimal performance of MLVA as opposed to WGS. 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 3. Selected panel for molecular-based cluster analysis part of EQA-13, specifically MLVA

# EQA	Serovar	Part of cluster based on MLVA	MLVA profile	Part of cluster based on WGS
EQA2328	Enteritidis	Index	02-10-07-04-02	Index
EQA2329	Enteritidis	No	02-08-05-15-NA	No
EQA2330	Enteritidis	No	03-10-05-04-01	No
EQA2331	Enteritidis	Yes	02-10-07-04-02	Yes
EQA2332	Enteritidis	Yes	02-10-07-04-02	Yes
EQA2333	Enteritidis	Yes	02-10-07-05-02	Yes
EQA2334	Enteritidis	Yes	02-10-07-03-02	No
EQA2335	Enteritidis	No	03-11-05-03-01	No
EQA2336	Enteritidis	Yes	02-09-07-04-02	No
EQA2337	Enteritidis	Yes	02-10-07-04-02	Yes

The clustering of isolates using PFGE was not known beforehand. Since RIVM no longer performs PFGE, results from the participant that used PFGE for the cluster analysis could only be compared to cluster assignment with WGS-based cluster analysis.

For the participants that used WGS-based cluster analysis, there was an additional exercise in the mock outbreak situation. 'The menu at the barbecue dinner consisted of different food products from which left-overs were saved in the refrigerator by the service personnel. The left-overs were sampled by the food safety authorities and *Salmonella* isolates (coded EQA2323 – EQA2327) cultured from these food products were sequenced using Illumina WGS techniques. Food products attributed to EQA2323 to EQA2327 were egg salad, sliced cucumber, beef hamburger patty, ice cream and chocolate mousse, respectively. 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 the 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 4. All reads were analysed for quality and clustering with the index case EQA2313 by another team member to reach consensus.

Table 4. Additional raw reads provided for WGS analysis

# EQA	Serovar	Manipulation	Quality	Distance to index cgMLST(AD)
EQA2323	Enteritidis	Reads of a regular non-cluster *S.* Dublin isolate were mixed with *S.* Typhimurium LT2 reads.	Bad quality, multiple serotypes	NA
EQA2324	Enteritidis	None, regular non-cluster sequence.	Good quality	27
EQA2325	Enteritidis	Duplicate of index isolate EQA2313, but contaminated with 50% reads of *Klebsiella.*	Bad quality, contaminated	NA
EQA2326	Enteritidis	Manipulated index isolate EQA2313 to one allele difference.	Good quality	0
EQA2327	Monophasic Typhimurium	Reads of regular non-cluster *S.* Dublin isolate, down-sampled to 30% of the original read count.	Bad quality, too low coverage	NA

cSNPs = core single nucleotide polymorphisms.

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. 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 for WGS and PFGE typing were sequenced at all time points and analysed using cgMLST. All samples fell within the same sample clusters at all timepoints, indicating a stable cluster analysis panel. Specimens for MLVA typing were also sequenced at all time points and analysed using MLVA. 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 a 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' v3.0.2 [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' v0.5.10 [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' (accessed on 02-10-2023) [32] was used to establish core SNP variants against reference EQA2313, 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 SENTR7, SENTR5, SENTR6, SENTR4 and SE3 were analysed using BioNumerics v7.6.3.

Since only one participant performed PFGE typing, which is not supported by the provider, the results from the participant using PFGE were compared to the cluster assessment of the provider using cgMLST based on the Enterobase *Salmonella* enterica v2 scheme.

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). Serovar Goldcoast differs only by variably-expressed O6 from Brikama, which is genetically indistinguishable from O8 [36]. If participants used phenotypic serotyping only 'Goldcoast' was considered correct for EQA2307, and for participants that used WGS prediction, both 'Goldcoast' and 'Goldcoast or Brikama' were considered to be correct results. As a challenging isolate, a subspecies other than *enterica* was added (EQA2311). 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. As long as detected O- and H-antigens were correct, these were considered as correct results, although 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 EQA2313 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=3), correct results were defined as congruence with the EQA provider for MLVA profiles. For the participant that used PFGE for cluster analysis, results were compared to the cluster assignment using WGS.

All descriptive analyses and comparison of groups, including visualisation, were performed using Microsoft Excel, R v4.2.1 and ggplot2 v3.3.6 [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 and PFGE 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 2 January 2023, after distribution of the individual evaluation reports, a feedback survey was sent to participating laboratories that had completed EQA-13 (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 28 laboratories that completed at least one part of the assessment registered for serotyping and submitted results. Their results are described in this section.

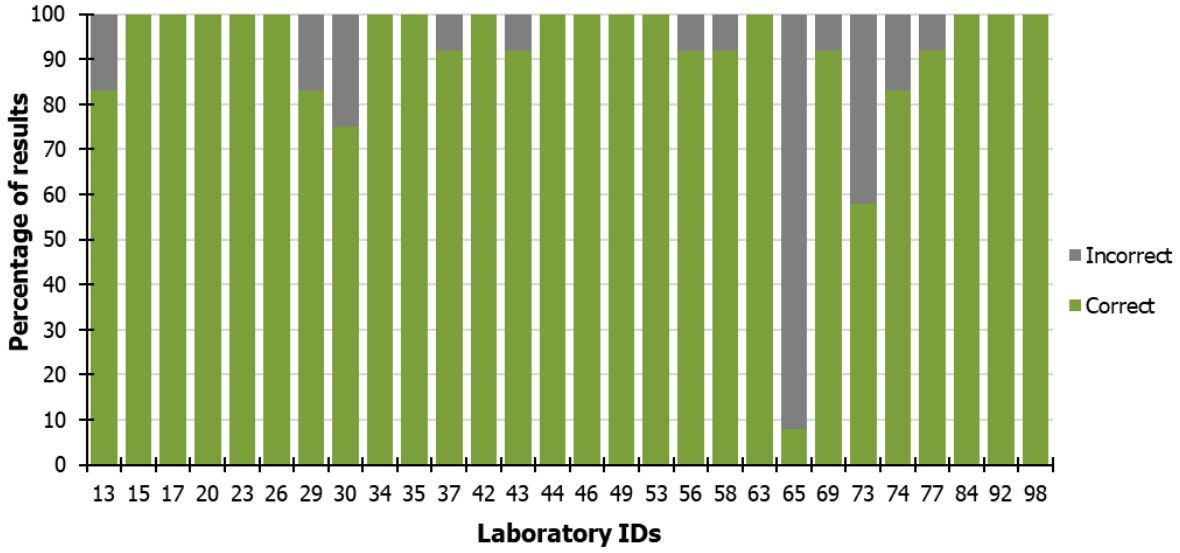
3.1.1 Methods used by participants

Of the 28 laboratories that had completed serotyping results, 15 (54%) used phenotypical serotyping based on agglutination with antisera only, six laboratories (21%) used prediction of serotype with WGS only, four laboratories (14%) used a combination of agglutination with antisera and prediction of serotype with WGS, two laboratories used a combination of agglutination with antisera and molecular genetic serotyping with polymerase chain reaction (PCR) and one laboratory 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

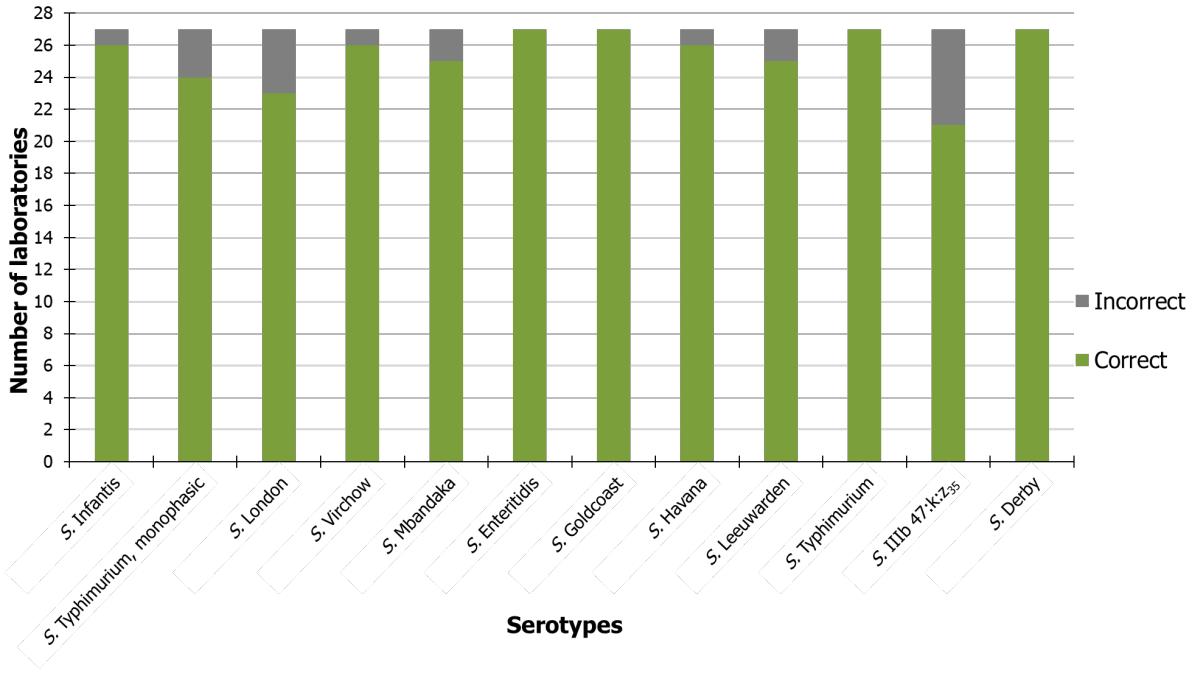
In total, 16 of 28 laboratories (57%) serotyped all isolates correctly, resulting in a performance score of 100%, and six laboratories (21%) had a performance score of 11 out of 12 (92%) (Figure 2). Five laboratories serotyped between two and five isolates incorrectly, resulting in 83%, 75%, 67% and 58% as performance scores (Figure 2). Laboratory 65 indicated that not all antisera were available to them, which hampered their serotyping results, as they were not able to assign conclusive serovars for 11 out of 12 isolates. 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



For calculation of performance values per sample, laboratory 65 was excluded, because of their limited set of antisera. Four of 12 samples were correctly serotyped by all of the remaining 27 laboratories (Figure 3), *S.* Enteritidis (EQA2306), *S.* Goldcoast (EQA2307), *S.* Typhimurium (EQA2310) and *S.* Derby (EQA2312). *S.* Infantis (EQA2301), *S.* Virchow (EQA2304) and *S.* Havana (EQA2308) were correctly serotyped by 26 of 27 laboratories. *S.* Mbandaka (EQA2305) and *S.* Leeuwarden (EQA2309) were correctly serotyped by 25 of 27 laboratories (Figure 3). *S.* Typhimurium monophasic variant (EQA2302), *S.* London (EQA2303) and *S.* IIIb 47:k,z35 (EQA2311) were correctly serotyped by 24, 23 and 21 of 27 laboratories, respectively (Figure 3).

Figure 3. Results of serotyping by serotype



When all results were combined from all 28 laboratories that performed serotyping, 315 of 336 isolates (94%) were correctly assigned to the serotype (Annex 7). For the 21 incorrectly assigned serotypes, 43 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 (86%, 37/43) were made only once, and 14% (6/43) 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 laboratory 65 because of their limited set of antisera, and the seven laboratories that used phenotypic methods in combination with other techniques (WGS, n=4; PCR, n=2; Luminex, n=1) the methods used by 20 laboratories were compared to assess whether the use of phenotypic (n=14) or WGS predictive (n=6) methods has an effect on overall performance. Although in all samples WGS predictive methods have a slightly better performance score, there was no difference observed in the performance score of most samples based on the method used (p-values ranging from 0.1162 to 0.5164, χ^2), except for sample EQA2311 (*S.* IIIb 47:k:z35) for which laboratories using WGS performed better (100%) than laboratories that used phenotypic methods only (53%, p=0.0444 χ^2). When all samples serotyped by laboratories using only phenotypic methods were combined, 149 of 168 serovars were correctly assigned (89%). For the samples serotyped by laboratories using WGS predictive typing, 71 of 72 serovars were correctly assigned (99%), resulting in a better overall performance in assigning the correct serotype when using WGS predictive techniques (p=0.0090).

Laboratories that performed serotyping were also given the option to report the MLST sequence types of the 12 isolates provided. Nine of 28 participants (32%) provided MLST sequence types which were all in concordance with those found by the EQA provider (Annex 9).

3.2 Molecular-based cluster analysis

Of the 28 laboratories that completed at least one part of the assessment, 26 had registered for the molecular-based cluster analysis, 24 of which submitted results. Three 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=1) and only bi-annual participation in cluster analysis proficiency testing 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

Of the 24 laboratories that completed molecular-based cluster analysis results, one used PFGE only (4%), 23 used WGS techniques (96%), with three of these laboratories also submitting MLVA results. Details of methods for each participating laboratory can be found in Annex 11.

Of the 23 laboratories that used WGS for their cluster analysis, 22 (96%) used Illumina as a platform and one used Ion Torrent. A total of 21 laboratories (91%) used a gene-by-gene-approach and two (9%) used SNP-typing (Annex 11). Of the laboratories that used Illumina sequencing (n=22), 10 (45%) used the Nextera XT DNA Library kit, eight used the Illumina DNA Prep kit (36%), one used the NEBNext Ultra™ II FS DNA Library Prep Kit, one used KAPA HyperPlus, one used the MiSeq Reagent Kit v3 and one laboratory reported their sequencing reagent kit (Miseq Reagent kit V3) as a library preparation kit (Annex 11).

Of the 21 laboratories that used a gene-by-gene approach, 11 (52%) used SeqSphere as MLST tool. Enterobase or ChewBBACA were used by three laboratories each (14% each). Bionumerics was used by two laboratories (9.5%) and cgMLSTFinder and an in-house pipeline, based on an unknown tool, were used by one laboratory each (Annex 10). The Enterobase MLST scheme was the most frequently used (15/21, 71%), while other schemes used were cgMLST.org (n=2), INNUENDO (n=1), and an in-house developed scheme (n=1). Two laboratories used the unknown schemes 'Core Genome' (n=1) and 'seqsphere' (n=1). A median of seven allelic distances was used as cluster cut-off (range: 3–20) (Annex 10).

Both laboratories that used SNP-typing used the index EQA2313 as reference genome in their analysis. One laboratory used an in-house SNP tool and the other used CSI phylogeny. Both laboratories used a cluster cut-off of five SNPs (Annex 11).

Species confirmation of the resulting WGS data was performed by 21 (91%) laboratories, mainly (38%) using kraken/kraken2, either alone or in combination with another tool (Annex 11). Other tools used were Mash/Mash Screen (n=5), KmerFinder (n=5), SeqSero (n=2), ConFindr (n=1), SpeciesFinder (n=1), rMLST (n=1), Enterobase (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 23 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. Coverage was the most frequently assessed parameter (20/23, 87%) and thresholds varied from 15 to 80 (median 31x). Coverage of contigs and unassembled reads were both assessed but not always defined as such. Genome size was assessed by 19 laboratories (83%), 18 laboratories (78%) determined the number of contigs, 15 laboratories determined the N50 value (65%) and 12 laboratories (52%) assessed the percentage of good targets in the MLST scheme used. Nine laboratories (39%) assessed contamination of the sample, either directly, by the percentage of species assignment, guanine-cytosine content (GC content) or a combination of these methods. 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 23 participants (100%) provided MLST sequence types which were all in concordance with those found by the EQA provider (Annex 13).

Three 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 two laboratories, while 0 locus difference was used by the third laboratory.

Only one laboratory used PFGE, for which methods and cluster cut-off could not be reported and are therefore unknown (Annex 10).

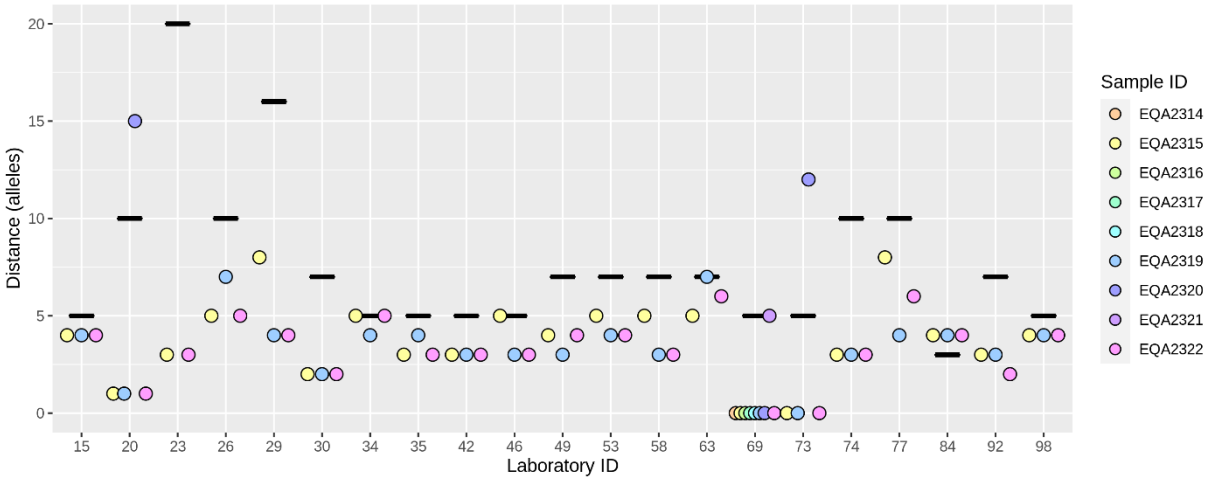
3.2.2 Results WGS-based cluster analysis

Using WGS-based cluster analysis, almost all isolates (244/253, 96%) were assigned correctly to the cluster of index EQA2313 or as singleton, despite the variety of methods used (Annex 12). This performance calculation was based on cluster or singleton assignment of provided isolates EQA2314-EQA2322 and provided good quality raw reads EQA2324 and EQA2326 (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 EQA2315, EQA2319 and EQA2322 have no or very few distances to reference EQA2313. The exceptions were laboratory 23, where an allelic distance of 258 was reported for EQA2319 which resulted in an incorrect exclusion of the isolate, and laboratory 69, where eight of nine isolates clustered at 0 allelic distance (AD) to the reference EQA2313 due to the use of the Achtman 7-gene MLST scheme for cluster analysis. 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 SNP difference to the index EQA2313 for EQA2315, EQA2319 and EQA2322 (Figure 5, Annex 15).

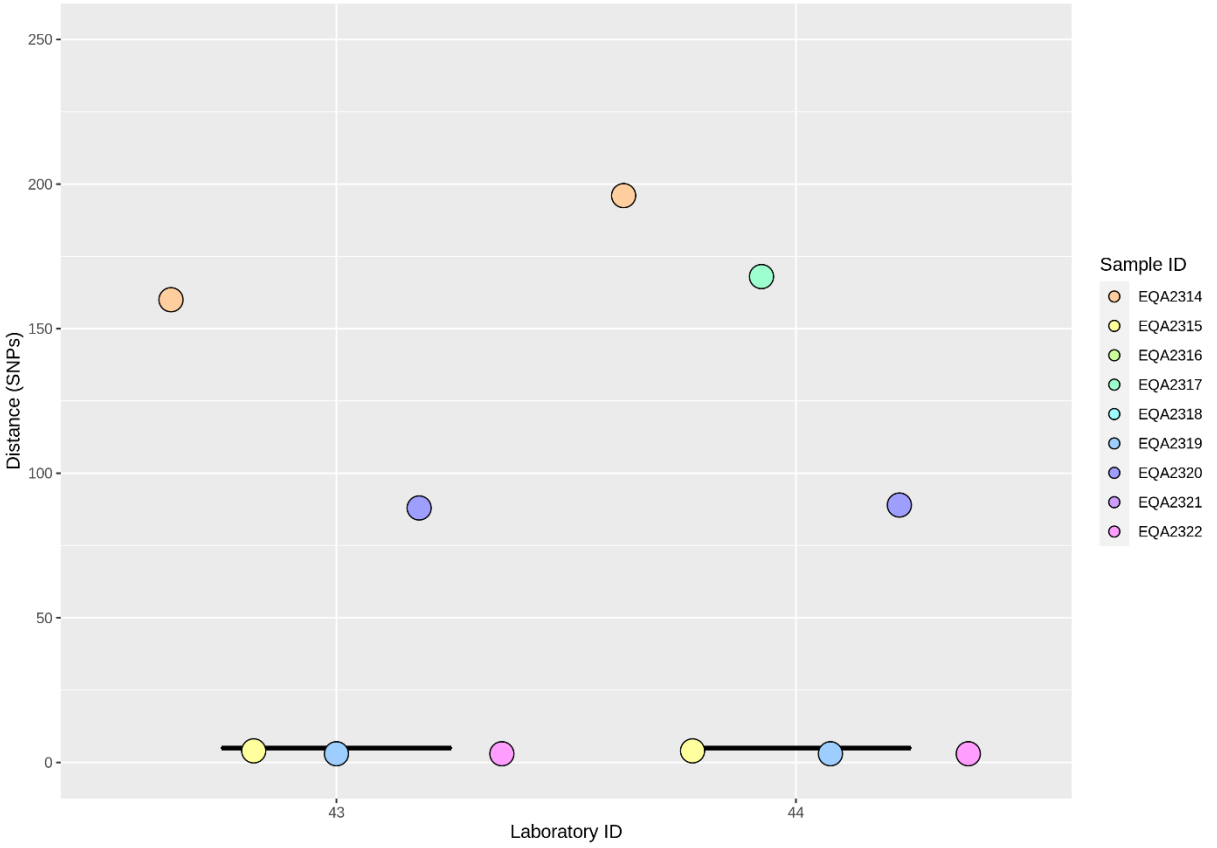
Figure 4. Distance from index EQA2313 in alleles for distances <20 AD, per laboratory



Black lines = cluster cut-offs set by participating laboratories themselves.

To assess the differences of the sequences that participants produced from the isolates EQA2313-EQA2322, 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 EQA2313 in SNPs for distances <250 SNPs, per laboratory

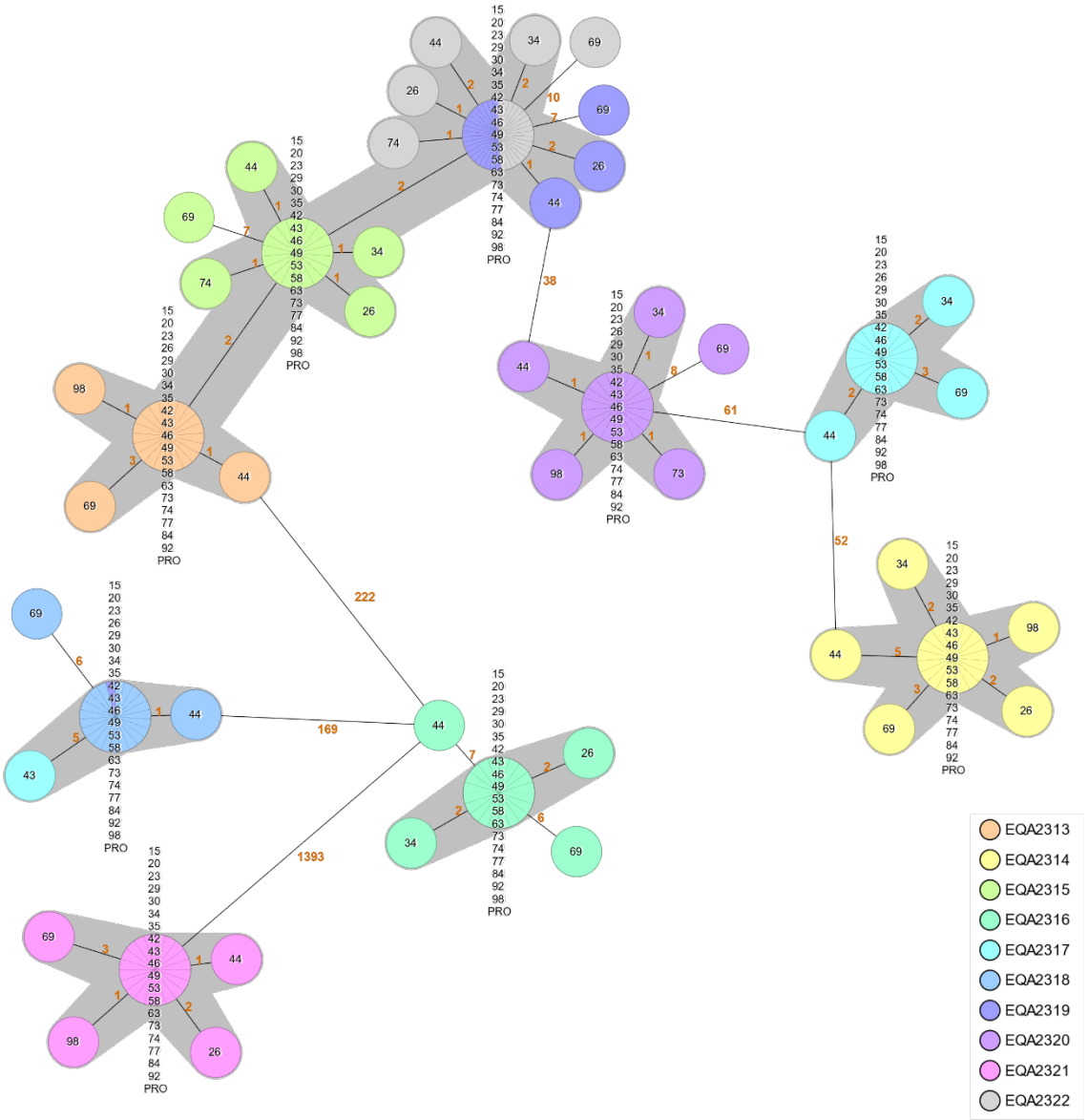


All sequences of the same samples clustered together using the cluster cut-off from provider (≤5 AD), except EQA2316 from laboratory 44 and EQA2315, EQA2316, EQA2318, EQA2319, EQA2320 and EQA2322 from laboratory 69. However, all of these isolates were still within close range (≤10 AD) of the cluster and would be considered as probably related, prompting further investigation in an outbreak situation (Figure 5). Analysis of the uploaded WGS-data showed low contamination with *Escherichia coli* which might have been introduced in the laboratory. In addition, a relatively low coverage was observed for most isolates by laboratory 69, which might contribute to the slightly higher AD observed. Laboratory 43 seemed to have sequenced isolate EQA2318 twice while labelling one

as EQA2317, as they both belong to the cluster of EQA2318. This is supported by the fact that laboratory 43 reported 501 SNP distance to the index for both EQA2317 and EQA2318. This reported distance is comparable to the SNP distance of EQA2318 to the index, as assessed by the EQA provider (Table 2, Figure 5).

All 23 laboratories detected the inferior quality of EQA2323 that had a higher percentage of contamination, indicating the presence of multiple serotypes. One of the 23 laboratories (4%) did not detect the inferior quality of EQA2325 that was artificially contaminated with *Klebsiella* by the provider (Annexes 12 and 13). The laboratory performed no quality control on parameters that assess contamination, either directly through percentage of contamination or indirectly via percentage of species assignment, but did assess the GC content with upper thresholds comparable to that of the provider (Annex 11). With this indicator, a high GC content should have been detected. In all, 10 of 23 laboratories (43%) did not detect the inferior quality of EQA2327 that was sub-sampled by the provider to mimic samples with low coverage (Annexes 12 and 13). Six of those 10 laboratories did employ quality control parameters that assess coverage at similar or higher thresholds to that of the provider, which should have enabled the low coverage to be detected. Laboratory 46 recognised the low coverage of the samples but still included it for cluster analysis in this outbreak investigation only, since all other quality control parameters were acceptable.

Figure 6. Minimum spanning tree of cgMLST by provider for EQA2313-EQA2322



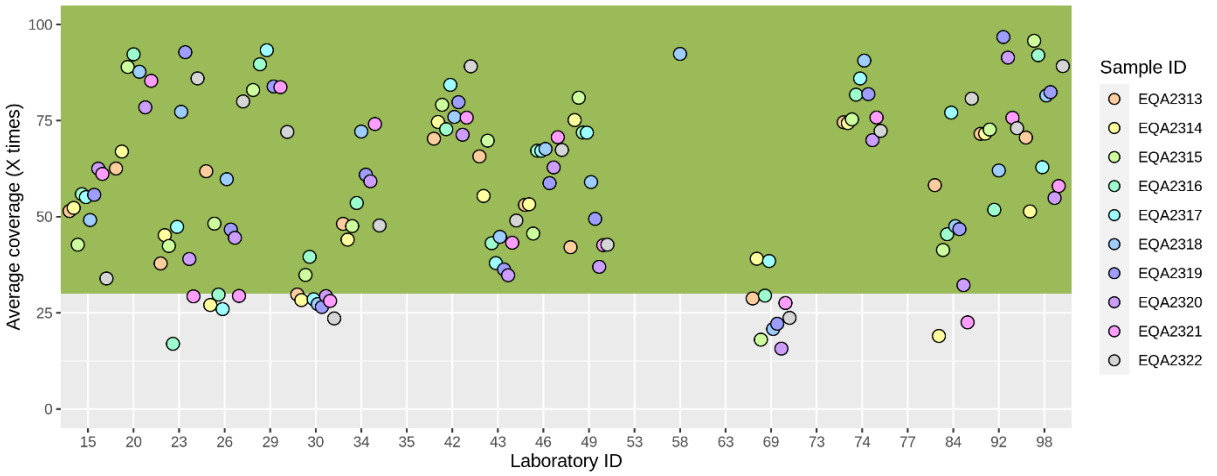
MST for 240 samples, distances based on Enterobase S. enterica V2 cgMLST scheme, pair-wise ignoring missing alleles. Nodes coloured by sample IDs, numbers are laboratories IDs. PRO = provider. Orange numbers = allelic distances. Grey halo = clusters based on ≤5 AD.

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 24 samples from five different laboratories below the threshold set by the provider (≥30x, Figure 7). Six laboratories had a very high coverage for most samples; laboratories 35, 53 and 58 had an average coverage between 100−200x and laboratories 63, 73 and 77 had an average coverage with a median (IQR) of 282x (141−411), 311x (175−533)and 522x (440−641), respectively.

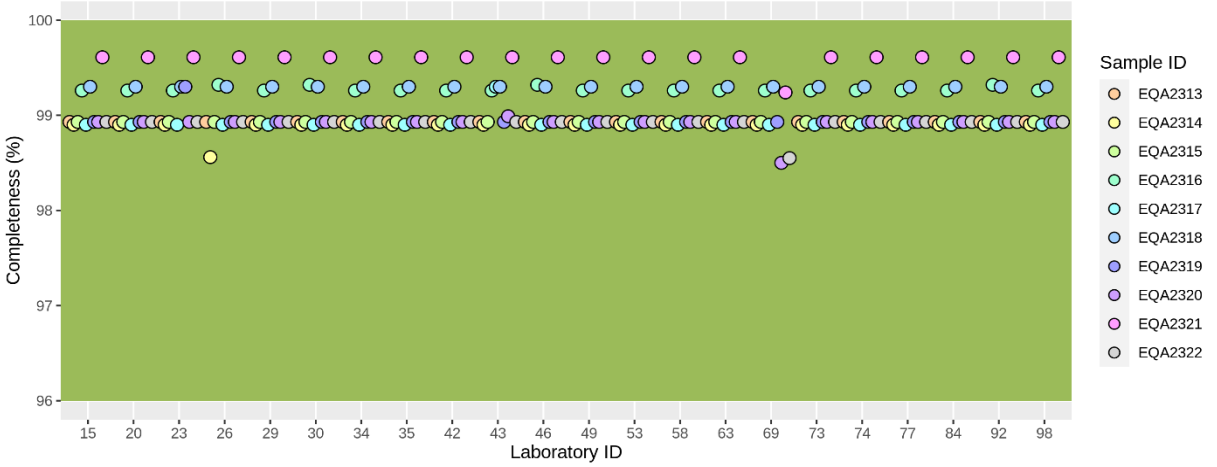
Figure 7. Average coverage of sequenced samples by participating laboratories, <100x



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

The completeness of all sequences was within the provider's thresholds for all samples and all laboratories (Figure 8). The percentage of contamination of most sequences was within threshold of the provider for all samples, except for sample EQA2321, sequenced by laboratory 23 (Figure 9). The genome size of this isolate was 5.2 Mbp for laboratory 23, while the genome size of EQA2321 for the provider and other laboratories was approx. 4.7 Mbp, which indicates a contamination with a second strain. However, this did not affect laboratory 23's allele calling and performance in cluster assignment, probably because enough coverage for most MLST loci was reached for EQA2321 (Figure 4 and Annex 12).

Figure 8. Completeness of sequenced genomes by participating laboratories

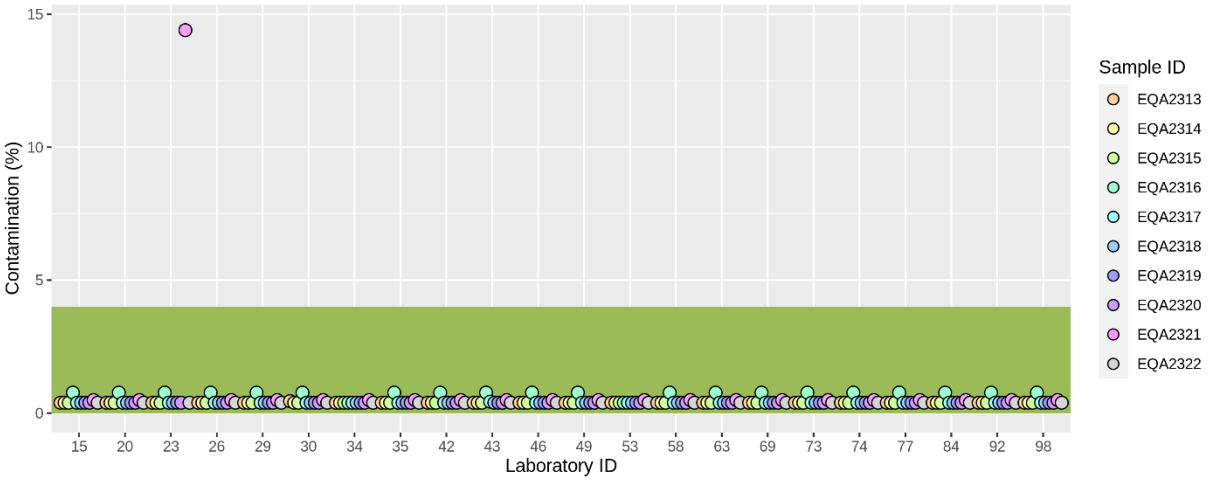


Green range = within quality threshold of provider (>96%).

The GC content of all laboratories' sequences were within the quality threshold of 51.6−52.3%, with a mean content of 52.10% (95% CI; ±0.0053), except for sample EQA2320 from laboratory 69, which was slightly above the upper threshold with a GC content of 52.36.

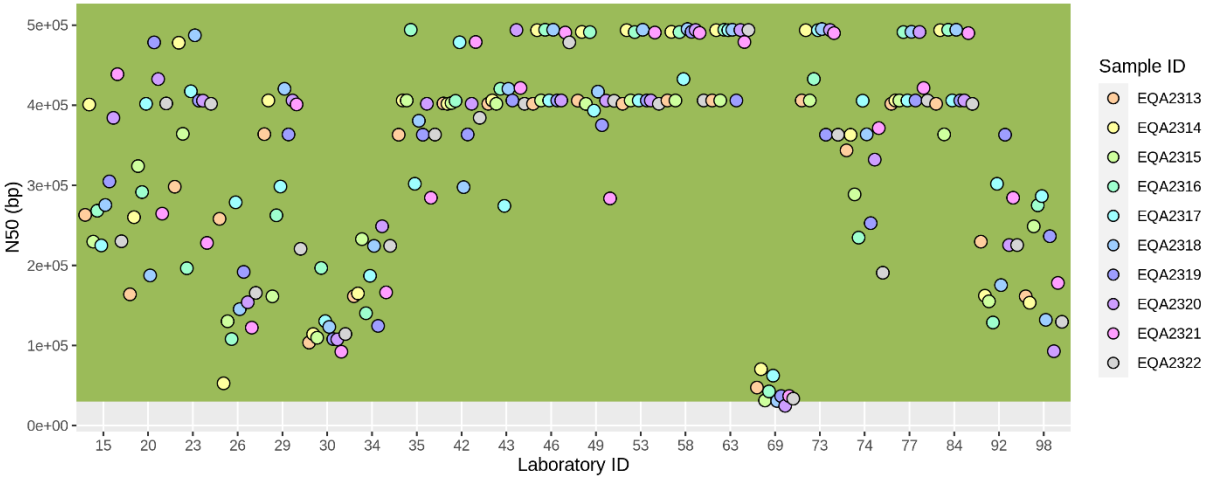
N50 values varied from 30 kbp to 495 kbp, but all but one were above the threshold set (>30 kbp) by the provider (Figure 10). Sample EQA 2320 from laboratory 69 had a N50 of 24.6 kbp, which was below the provider's threshold. Total genome lengths (assembled) were all within the quality threshold and varied from 4.67 Mbp to 4.99 Mbp with a mean of 4 864 310 bp (95% CI; ± 9277). 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 three laboratories, which all performed MLVA in combination with WGS (laboratories 26, 63 and 74). In nine out of 10 isolates (90%) all detected MLVA profiles were in concordance with that of the provider (Table 2). None of the three laboratories were able to correctly determine the SENTR4 allele in EQA2329.

The isolates EQA2331, EQA2332, EQA2333, EQA2334, EQA2336 and EQA2337 were considered to be part of the same cluster as index EQA2328 by laboratories 26 and 74, who used a cluster cut-off of one allele difference (Annex 17). Using a cluster cut-off of 0 alleles, laboratory 63 did not consider EQA2333, EQA2334 and EQA2336 as part of the cluster (Annex 17). Based on WGS, EQA2334 and EQA2336 could be excluded from the cluster, whereas using MLVA with a cut-off of one allele difference, these isolates would be included.

Although all laboratories displayed high technical skill, resulting in 98% (44/45) of MLVA alleles being correctly identified, compared to WGS cluster assignment of isolates using MLVA resulted in erroneous inclusion of EQA2334 and EQA3436 when using a cut-off of one allele (78% concordance), or the erroneous exclusion of EQA2333 when using a cut-off of 0 alleles (89% concordance). Overall, laboratories using MLVA-based cluster analysis assigned 22 of 27 samples (82%) correctly to a cluster, while with WGS-based cluster analysis, 244 of 253 samples were assigned correctly (96%). This indicates a statistical difference in favour of WGS-based cluster analysis ($p=0.0022$, χ^2).

3.2.5 Results PFGE-based cluster analysis

PFGE was performed by one laboratory only. Since the provider do not perform PFGE anymore, the results of the laboratory could not be compared. Unfortunately, this participant did not submit a gel image in TIFF format, so the provider could not assess the quality of the fingerprint or the correctness of the cluster analysis. The participant correctly identified all the isolates belonging to the cluster, but could only exclude two isolates. For four isolates, it was concluded that they "possibly" belonged to the cluster (Annex 18).

3.3 Results feedback survey

In total, 16 of 28 laboratories (57%) responded to the feedback survey. 11 of 16 (69%) used the results of this EQA as documentation for accreditation and/or licensing purposes for the methods used in their laboratory. All the laboratories apart from one were satisfied with their individual EQA reports. The laboratory in question was unable to fully participate in the EQA due to only having a limited number of antisera available, and this resulted in unsatisfactory results for a number of the samples tested in their individual EQA report. Eight of 16 (50%) laboratories indicated that all their analytical test results conformed to the expected results, and four (25%) took corrective action based on the results of this EQA. One specified the corrective action, which involved repeating the tests for the incorrectly assigned EQA samples. Another laboratory revised their SOP on serotyping and intends to procure a wider range of antisera. One laboratory intends to verify reagents and ensure that SOPs and agglutination procedures are followed in the correct order by qualified technicians. One laboratory reviewed their SOP on cluster analysis and will be using the recommended cgMLST in the future. Laboratories were asked to evaluate whether the use of PFGE and/or MLVA for cluster analysis were of added value WGS was also performed. In all, 11 of 16 (69%) did not see an added value for PFGE or MLVA. Three (19%) found that both PFGE and MLVA were of added value and two (12%) only recognised an added value for MLVA.

Two laboratories made comments or suggestions for improving the organisation of the EQA. One suggested that the same strain set should be provided for serotyping, and noted that MLVA and WGS could allow interesting comparisons of results obtained using different methods (n=1). The other laboratory suggested that the timing of the EQA scheme could be improved by not organising it during the summer holiday period. ECDC and the provider will take these suggestions into consideration in the design of EQA-14 for 2024.

4 Discussion

In all, 30 countries were invited to participate in the EQA, and 29 laboratories (97%) registered for at least one part and received the specimen panel(s). One laboratory that registered cancelled its participation before the result submission deadline. In the end, 28 participants (97%) completed at least one part of EQA-13. A total of 28 laboratories completed the serotyping part and 24 completed the molecular typing-based cluster analysis part, which is a higher percentage of participation than for the last two EQAs on *Salmonella* (EQA-11 and EQA-12) (39,40). A total of 24 of the laboratories that participated in the cluster analysis part of EQA-13 also participated in EQA-12. Since the setup of EQA-13 was similar to EQA-12, results can be compared to the previous EQA.

4.1 Serotyping

In EQA-13, 28 laboratories participated in the serotyping of 12 isolates. This resulted in 15 laboratories (54%) using phenotypic typing with antisera only, six laboratories using WGS-predictive serotyping (21%) only, four laboratories using a combination of agglutination with antisera and prediction of serotype with WGS (14%), two laboratories using a combination of agglutination with antisera and molecular genetic serotyping with PCR (7%) and one laboratory using genetic serotyping with Luminex combined with phenotypic typing with antisera (4%).

A total of 16 laboratories (57%) had a performance score of 100%, six laboratories (21%) had a performance sore of 92%. Three laboratories had a performance score of 83% and two laboratories had performance scores of 75% and 58%, respectively. A performance score could not be calculated for the remaining laboratory due to a lack of appropriate antisera.

When corrected for sample size, there was no difference observed in total performance scores per sample, except for EQA2311. Differences in overall performance scores were observed between the group of laboratories that only used phenotypic methods (89%, n=15) and the group that used WGS-predictive methods (99%, n=6), with the laboratories using WGS predictive techniques having a better performance score overall ($p=0.0090$, χ^2) In EQA-12, overall performance was not influenced by only using WGS predictive techniques or phenotypic methods. 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, 43 different error types were made in the serotyping part, the majority (86%) being made only once. Most error types were false-positive detection, false-negative detection or misclassifications of H-antigens in both phases (n=28, 65%). At least eight error types point towards the use of less specific antisera and one to incorrect prediction using WGS data, where a second H-phase 1,2 was erroneously detected. Additional error types were false-positive detection or misclassification of O-antigens in nine cases (21%), at least six of which indicate the use of less specific antisera. In total, indication of the use of less specific antisera was observed for six laboratories (30, 37, 58, 65, 73 and 74). Information regarding the antisera manufacturer was provided for five of the six laboratories. While one laboratory reported that antisera were prepared by the laboratory itself, others used antisera manufactured by SSI Diagnostics (n=3) and Pro-Lab Diagnostics (n=1). 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=5) and type misclassifications of subspecies (n=1).

Overall, the number of laboratories that participated in serotyping for EQA-13 was 17% higher than for EQA-12. Performance of laboratories which completed the serotyping part in this EQA-13 and the EQA-12 were compared (n=23). One laboratory was excluded from the comparison due to incomplete results, stemming from its limited access to antisera. In EQA-12, 12 of the laboratories (55%) had a performance score of 100%, whereas in EQA-13, the same performance score was achieved by 13 laboratories (59%). Assessment of the serovars tested showed that in EQA-12 a rare serotype *S. Leeuwarden* appeared to be challenging, since it was not serotyped correctly by so many laboratories (18 of 22 laboratories (82%)). It was therefore included again in the serotyping panel of EQA-13 and results showed that 20 of 22 laboratories (91%) were now able to correctly type this serovar. Only one laboratory mistyped the serovar again. In EQA-13, the most challenging isolate for serotyping was *S. IIIb 47:k:z35* (EQA2311), which was incorrectly assigned by 21 of 27 laboratories (Annex 19 and 20).

In 2022, for the first time in 10 years, FWD-Net and ECDC commissioned an EQA for *Salmonella* via RIVM which included assessment of serotyping. This was done again for EQA-13 in 2023. 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 EQA-13 than EQA-12. In addition, by comparing results for the two years, laboratories were able to take successful corrective measures for errors in serotyping and achieve better performance scores. Results produced in the coming years will show whether this trend continues.

4.2 Molecular-typing based cluster analysis

In EQA-13, 24 laboratories participated in the molecular-typing based cluster. This resulted in 23 laboratories (96%) using WGS-based cluster analysis, three of which also applied MLVA-based cluster analysis. One laboratory (4%) performed only PFGE-based cluster analysis.

A higher proportion of participants (96%, 23/24) applied WGS-based cluster analysis in this EQA than in 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) of laboratories, and only 4% (1/24) performed solely PFGE in EQA-13. 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 [39, 40].

4.2.1 WGS-based cluster analysis

A total of 23 laboratories used an WGS-based cluster analysis, 22 of them (94%) using Illumina sequencing and one using Ion Torrent sequencing. A gene-by-gene approach was performed by 91% (21/23) of laboratories, while 9% (2/23) performed SNP-typing. In EQA-12, a gene-by-gene approach was applied by 82% (14/17) of laboratories and 18% 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 23 laboratories.

Overall performance of cluster assignment using WGS-based methods was high, 83% of laboratories (19/23) 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. Another three laboratories assigned 10 of the 11 isolates correctly. Only one laboratory had a significantly lower percentage of correctly assigned cluster isolates, which was due to the use of the Achtman 7-gene MLST scheme. The resolution of 7-gene MSLT typing in *Salmonella* is comparable to the resolution of serotyping, as it was introduced as a replacement for serotyping in 2012 [43] and is used as such in in silico serotyping tools such as SISTR [44]. Therefore, 7-locus MSLT typing does not have enough resolution for outbreak investigations and should not be used for this purpose.

For all but two laboratories, the reported distance in alleles or SNPs for the cluster isolates EQA2315, EQA2319 and EQA2322 to index EQA2313 was well below their reported cluster cut-off. Laboratory 23 reported an allelic distance of 258 for isolate EQA2319, which seems to be a reporting mistake as they also reported a 258 AD for isolate EQA2318 and their results cannot be reproduced by the provider using the fastq files submitted for EQA2319. Laboratory 69 reported clustering of nine isolates at 0 AD to the reference EQA2313 which can be explained by the use of the Achtman 7-gene MLST scheme, which is not suitable for cluster analysis of *Salmonella*. When assessing distances inferred by the provider from all submitted raw reads, all sequences of the same isolate clustered within cluster cut-off of ≤5 AD, except for five isolates (EQA2315, EQA2316, EQA2318, EQA2320 and EQA2322) from laboratory 69, which were close to the cluster cut-off (≤10 AD). Analysis of the submitted WGS-data showed low contamination with *Escherichia coli*. This might be due to contamination in the participant's laboratory. Another explanation could be use of a lower quality sequencing kit or one that was past its expiration date.

This confirmed the results of the provider's stability tests – that the selected *S.* Dublin 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 *S.* Dublin 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 17 of 22 (77%) laboratories all sequences passed all the provider's quality criteria. One laboratory submitted data that produced a low average coverage in provider's assembly pipeline and as a result, all of the isolates were below the provider's threshold. Four other laboratories each had two or more samples with a lower coverage just below threshold, observed in different samples. Three 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.

An additional five sequences were made available to participants. Two of these isolates were cluster isolates. One of them was assigned correctly as a cluster isolate by all laboratories. The other was correctly assigned by all but three laboratories. The remaining three isolates were non-cluster sequences that were manipulated by the provider. Reads of EQA2323 were artificially contaminated with *S.* Durham, mimicking contamination with another serotype. Reads of EQA2325 were down-sampled to 50% (25X coverage) and supplemented with 50% (25X coverage) of reads from a *Klebsiella* isolate, mimicking contamination. Reads of EQA2327 were down-sampled to 30% (15X coverage) to mimic a low read count. Of the 23 laboratories that performed WGS-based cluster analysis, 83% (19/23) identified the poor quality of all three isolates. Four laboratories (17%) failed to identify poor quality in one of the samples.

Overall, the number of laboratories that participated in WGS-based cluster analysis was 35% higher than for EQA-12. Performance of laboratories which completed the cluster analysis part of this EQA-13 and EQA-12 were compared (n=17). In EQA-12, 15 of those laboratories (88%) had a performance score of 100% and 99% (201/204) of isolates were correctly assigned to the cluster in EQA-12. When considering only the results for the laboratories that also participated in EQA-12, 16 of 17 laboratories (94%) had a performance score of 100% and 99% (186/187) of isolates were correctly assigned to the cluster in EQA-13. If the results of all participating laboratories are taken into consideration, 96% (244/253) of isolates were correctly assigned to the cluster in EQA-13, while six more laboratories participated in the WGS-based cluster analysis. When excluding the clustering results of laboratory 69, where the Achtman 7-gene MLST scheme was applied, 99% (239/242) of isolates were correctly assigned to the cluster in EQA-13.

4.2.2 MLVA-based cluster analysis

Three laboratories participated in MLVA-based cluster analysis. All three laboratories achieved a 90% performance score in determination of MLVA profiles. For the isolate EQA2329, none of the three laboratories were able to correctly determine the SENTR4 allele. The provider retested this isolate and Sanger sequencing confirmed that the SENTR4 allele in EQA2329 had 15 repeats, thereby confirming the provider's initial result. Two laboratories used an MLVA cluster cut-off of one allele difference, the third laboratory used a cluster cut-off of 0 alleles. However, the cluster assignment of isolates was set using WGS-typing, which is a higher resolution typing technique. This resulted in an assignment of two extra cluster isolates for two laboratories. EQA2334 (n=2) and EQA2336 (n=2) were erroneously considered to be part of the outbreak while using MLVA techniques for cluster identification.

The number of laboratories that participated in MLVA-based cluster analysis was the same as in EQA-12. All three laboratories achieved a performance score of 100% in determination of MLVA profiles. In EQA-12, 23 of 27 isolates (85%) were assigned correctly to the index isolate using MLVA-based cluster analysis, while this score was slightly lower (82%, 22/27) for EQA-13.

For participants applying WGS (or PFGE) for cluster analysis, the serovar *S.* Dublin was chosen for EQA-13. Since cluster analysis on this serovar could not be carried out using MLVA, an additional strain set was selected by the provider 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 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. with regard to case definitions. The use of typing techniques such as MLVA hampers the fulfilment of the surveillance objectives of the FWD-Net, 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

Only one laboratory participated in PFGE-based cluster analysis. Since the provider does not perform PFGE anymore, the results of the laboratory could not be compared. Unfortunately, the participant did not submit a gel image in TIFF format, which meant that the quality of the fingerprint or correctness of the cluster analysis could not be assessed by the provider. The participant correctly identified all isolates belonging to the cluster and excluded two. However, for four isolates, no definitive cluster assignment was provided, and the participant concluded that those isolates 'possibly' belonged to the cluster (Annex 18).

In the previous EQA-12, two laboratories participated in PFGE-based cluster analysis, which allowed comparison of results. It could be concluded that 89% and 56% of isolates respectively were correctly assigned to the cluster by the two laboratories. One laboratory could not reach a definitive conclusion regarding cluster assignment for four of the nine isolates provided. When inferring a dendrogram of the submitted banding patterns, it was observed that isolates cluster more frequently at laboratory level than at sample level, indicating the limited portability and interlaboratory comparability of PFGE.

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 57% (16/28) of participants that had completed at least one of part of EQA-13 filled in the feedback survey. A total of 11 (69%) used the results as documentation for accreditation and/or licensing purposes, showing the added value of this EQA to laboratory quality systems.

Eight of 16 (50%) laboratories indicated that all their analytical results conformed to the expected results, and four laboratories (25%) reported that they had taken a range of corrective actions based on their individual results, with four specifying these actions. One of the actions was to repeat the typing of the incorrectly typed samples. Two laboratories reported that they intend to improve their collection of antisera by procuring either a wider range of antisera or verifying reagents. Two laboratories reported that SOPs will be reviewed/revised and one laboratory intends to use the recommended cgMLST scheme in the future. 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 their opinion on the possible added value of PFGE and/or MLVA for cluster analysis and 11 of 16 laboratories (69%) reported that neither technique was of added value. Two laboratories (12%) recognised an added value for MLVA only, and three laboratories (19%) found that both PFGE and MLVA were of added value for cluster analysis.

5 Conclusions

5.1 Methods and capability of serotyping

For serotyping, 54% 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 21% only use WGS-based methods in which the serotype is inferred from genetic characteristics. Four laboratories (14%) routinely apply WGS-based methods alongside to phenotypic serotyping, two laboratories (7%) apply PCR-based methods alongside phenotypic serotyping and one laboratory (4%) routinely applies a combination of genetic serotyping using Luminex technique, supplemented with phenotypic methods.

The main methods (phenotypic serotyping or WGS-predictive serotyping) influenced the overall capability of the laboratories that apply them, but not the ability to type particular serovars (apart from the non-enterica subspecies). For all samples serotyped by laboratories using WGS predictive typing, 99% (71/72 serovars) were correctly assigned, resulting in a significantly better overall performance for assigning the correct serotype if using WGS predictive techniques. The non-enterica subspecies were challenging for laboratories that used phenotypic serotyping, yet all the labs that used WGS-predictive serotyping typed this isolate correctly.

Performance was high for most laboratories, with 16 (57%) achieving performance scores of 100%, six 92% and three 83%. Another three laboratories with the lowest performance values (<83%) all used phenotypic methods. The results and the type of errors indicated that less specific antisera were used. However, this cannot be definitively concluded on the basis of the information requested in the results form.

5.2 Methods and capability of molecular typing-based cluster analysis

Most participating laboratories (96%) used WGS-based cluster analysis, sometimes combined with MLVA (n=3). It is not known from the information requested whether WGS-based cluster analysis is applied routinely or only in outbreak situations. One laboratory (4%) only used PFGE-based cluster analysis.

Performance was highest among laboratories that used WGS-based cluster analysis, with an overall performance score of 78%. In all, 18 of 23 laboratories had 100% performance in assigning the isolates provided to clusters, and another four laboratories had a performance score of 91% (10/11 isolates assigned correctly to the cluster). One laboratory had a performance score of 45%, which can be attributed to the use of the Achtman 7-gene MLST scheme, which is not suitable for cluster analysis of *Salmonella*. 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 was 90%, as all three participating laboratories were unable to correctly determine the SENTR4 allele for isolate EQA2329. Overall performance for cluster assignment of provided isolates using MLVA was lower (82%) than for WGS-based cluster analysis (96%, $p < 0.0022$, χ^2). Therefore, it can be concluded that participating laboratories had good capabilities in the application of MLVA, even though the resolution of the technique itself was too low to correctly assign isolates to clusters.

Technical performance in PFGE-based cluster analysis cannot be assessed, because the technique is no longer applied by the provider. Only one laboratory participated in PFGE-based cluster analysis, therefore it was also impossible to compare the results with other laboratories. The participating laboratory had difficulty assigning isolates to a cluster, as four of the nine isolates (44%) were not definitively assigned to cluster or singleton.

In the feedback survey, participants of EQA-13 were asked to give their opinion on whether the use of MLVA and/or PFGE for cluster analysis was of added value and the majority (69%) reported that neither technique offered added value. In all, 12% recognised an added value for MLVA only, and 19% found that both PFGE and MLVA were of added value for cluster analysis. Although MLVA and PFGE-based cluster analysis might still be useful for national purposes, for international cluster detection and outbreak investigation they become less important. ECDC will no longer collect this data. The MLVA data reporting has been replaced by WGS real-time reporting.

5.3 Evaluation of EQA-13

The participation rate in the cluster analysis part was relatively high, 80% of the 30 laboratories invited provided results, which is a higher participation rate than that for EQA-12 and EQA-11 (56%). Participation in WGS-based cluster analysis has increased, while participation in MLVA- and PFGE-based cluster analysis has decreased. For the second time in a decade, serotyping capability was assessed and participation rate was higher (93% of the

laboratories invited) compared to EQA-12 (67% 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 one comment was received from a laboratory about there being too many samples.

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-13, 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 national public health reference laboratories

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 92%. These laboratories all performed phenotypic serotyping and were recommended to assess the specificity of their antisera or to transition to WGS predictive methods. If desired, NPHRLs can contact the EQA provider for assistance and the provision of recommendations tailored to their specific needs and resources.

Although technical performance was very strong for all methods used for the molecular typing-based cluster analysis, performance in cluster assignment was much better for WGS (96%) than for MLVA (82%). It is recommended that laboratories use WGS-based cluster analysis for outbreak situations as a minimum. If enough resources are available for the employment of other typing techniques, 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.

6.3 Recommendations for EQA organisation and provider

The second assessment of *Salmonella* serotyping in a decade was successful and prompted laboratories to take action for improvement. The inclusion of rare serovars should continue as this challenges the laboratories. In the EQA, cluster analysis could be performed using three different methods (WGS, MLVA and PFGE). As most laboratories use WGS-based cluster assignment, the EQA provider used *S.* Dublin in the panel, as this serovar can be used for cluster analysis when using WGS or PFGE. For MLVA-based cluster analysis, the range of serovars that can be used is quite limited and therefore a panel containing *S.* Enteritidis was used. However, the use of two panels with different serovars does not allow comparison of clustering outcomes between techniques. In the next EQA, the provider should determine whether to use *S.* Enteritidis or *S.* Typhimurium for cluster analysis to be able to compare clustering results using different clustering techniques, or whether to provide an additional strain panel with a different serovar for WGS- and PFGE-based cluster analysis. To assess the application of different techniques in different EU/EEA countries, information should be gathered on the routine methodology for cluster analysis.

To improve the identification and analysis of error types even further, it is recommended that the EQA provider includes 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* 2023 should be taken into consideration by the provider during the design of EQA *Salmonella* 2024.

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Annex 1. Online registration form

EQA Salmonella 2023

You are hereby invited to participate in the EQA *Salmonella* 2023

Please answer the questions below to register

*Fields marked with a * are mandatory*

* Would you like to participate in the *Salmonella* EQA 2023?

☐ 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?

☐ Phenotypic (using antisera)

☐ WGS

☐ Other, please elaborate:

If using WGS for serotyping part

* 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* 2023 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 / Institute for Medical Microbiology and Hygiene Graz	AGES - Austrian Agency for Health and Food Safety
Belgium	NRC Salmonella & Shigella	Sciensano
Bulgaria	NRL for Enteric Pathogens	National Center for Infectious and Parasitic Diseases
Croatia	Division for microbiology; Department for Diagnostics of Intestinal Infections	Croatian Institute of Public Health
Cyprus	Nicosia General Hospital	General hospital of Nicosia
Czechia	NRL for salmonella	National Institute of Public Health
Denmark	Laboratory of Gastrointestinal Bacteria	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board
Finland	Expert microbiology	Finnish institute for health and welfare (THL)
France	Centre National de Reference des E. coli, Shigella et Salmonella	Institut Pasteur
Germany	NRC for Salmonella and other bacterial enteric pathogens	Robert Koch Institute
Greece	NRL FOR SALMONELLA	UNIVERSITY OF WEST ATTICA
Hungary	FWD-Laboratory	National Center for Public Health and Pharmacy
Ireland	NSSLRL	Galway University Hospital
Italy	Department of Infectious Diseases	Istituto Superiore di Sanità
Latvia	National Microbiology Reference Laboratory	Riga East University Hospital
Lithuania	NVSPL	NVSPL
Luxembourg	Epidemiology and Microbial Genomics	Laboratoire National de Santé
Malta	Microbiology laboratory	Mater Dei Hospital
Montenegro	Centre for Medical microbiology, Department of sanitary microbiology	Institute of Public Health of Montenegro
Norway	National Reference Laboratory	Norwegian Institute of Public Health
Poland	Bacteriology and Biocontamination Control	National Institute of Public Health NIH - NRI
Portugal	URGI	INSA
Romania	Molecular Epidemiology for Communicable Diseases	Cantacuzino National Military Medical Institute for Research and Development
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	Unidad de Enterobacterias	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 molecular: what kind of molecular method did you use?

* If WGS: which sequencing platform did you use?

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
EQA2301						
EQA2302						
EQA2303						
EQA2304						
EQA2305						
EQA2306						
EQA2307						
EQA2308						
EQA2309						
EQA2310						
EQA2311						
EQA2312						

* Did you use a second method for serotyping?

* If yes, what other method did you use for serotyping?

* If phenotypic (using antisera): From which manufacturer did you obtain the antisera you used?

* Do you want to report the serotyping results obtained using the second method separately?

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
EQA2301						
EQA2302						
EQA2303						
EQA2304						
EQA2305						
EQA2306						
EQA2307						
EQA2308						
EQA2309						
EQA2310						
EQA2311						
EQA2312						

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

* If yes:

Isolate	Sequence Type (number)
EQA2301	
EQA2302	
EQA2303	
EQA2304	
EQA2305	
EQA2306	
EQA2307	
EQA2308	
EQA2309	
EQA2310	
EQA2311	
EQA2312	

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 (EQA2313) and whether you would consider the isolate part of the cruise ship outbreak:

Isolate	Distance to index case	Part of the outbreak?
EQA2314		
EQA2315		
EQA2316		
EQA2317		
EQA2318		
EQA2319		
EQA2320		
EQA2321		
EQA2322		

* 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)
EQA2313	
EQA2314	
EQA2315	
EQA2316	
EQA2317	
EQA2318	
EQA2319	
EQA2320	
EQA2321	
EQA2322	

* 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 cruise ship outbreak (part of a cluster with the index case EQA2313)

* What is your assessment of provided genome EQA2323 from an isolate obtained from tropical egg 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 EQA2324 from an isolate obtained from sliced cucumber?

* 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 EQA2325 from an isolate obtained from beef hamburger patty?

* 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 EQA2326 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?

* What is your assessment of provided genome EQA2327 from an isolate obtained from chocolate mousse?

* 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.

* What sizemarker did you use for MLVA?

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

Please report the MLVA profile (SENTR7-SENTR5-SENTR6-SENTR4-SE3) of the isolates and whether you would consider the isolate part of the cruise ship outbreak with index case EQA2328:

Isolate	MLVA profile	Part of the outbreak?
EQA2328		
EQA2329		
EQA2330		
EQA2331		
EQA2332		
EQA2333		
EQA2334		
EQA2335		
EQA2336		
EQA2337		

* Did you determine the serovar of the MLVA isolates?

If yes: How did you determine the serovar?

Isolate	Serovar
EQA2328	
EQA2329	
EQA2330	
EQA2331	
EQA2332	
EQA2333	
EQA2334	
EQA2335	
EQA2336	
EQA2337	

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 cruise ship outbreak, based on PFGE:

Isolate	Part of the outbreak?
EQA2314	
EQA2315	
EQA2316	
EQA2317	
EQA2318	
EQA2319	
EQA2320	
EQA2321	
EQA2322	

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* 2023

Fields marked with a * are mandatory

Dear Participant,

Recently you 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

* **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:** Besides WGS, it was also possible to perform cluster analysis using PFGE or MLVA for this EQA. In your opinion, do these techniques present added value for all participants for this EQA and for those in the coming years, for comparing *Salmonella* isolates in an international setting?

☐ Yes, both PFGE and MLVA have added value

☐ Only PFGE has added value

☐ Only MLVA has added value

☐ No, neither PFGE nor MLVA have added value

Please motivate your answer:

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	Phenotypic (using antisera)	
15[a]	Yes	Yes	Phenotypic (using antisera)	
17[b]	Yes	Yes	Phenotypic (using antisera)	
20[a]	Yes	Yes	Phenotypic (using antisera)	
23	Yes	Yes	Phenotypic (using antisera)	
26	Yes	Yes	Phenotypic (using antisera)	
29	Yes	Yes	Phenotypic (using antisera)	
30	Yes	Yes	Phenotypic (using antisera)	
34	Yes	Yes	Prediction serotype with WGS	Illumina
35[a]	Yes	Yes	Phenotypic (using antisera)	
37	Yes	Yes	Phenotypic (using antisera)	
42	Yes	Yes	Prediction serotype with WGS	Illumina
43	Yes	Yes	Phenotypic (using antisera)	
44	Yes	Yes	Prediction serotype with WGS	Ion Torrent
46	Yes	Yes	Phenotypic (using antisera)	
49	Yes	Yes	Prediction serotype with WGS	Illumina
53[b]	Yes	Yes	Phenotypic (using antisera)	
56	Yes	Yes	Phenotypic (using antisera)	
58	Yes	Yes	Phenotypic (using antisera)	
63[c]	Yes	Yes	Phenotypic (using antisera)	
65	Yes	Yes	Phenotypic (using antisera)	
69	Yes	Yes	Phenotypic (using antisera)	
73	Yes	Yes	Phenotypic (using antisera)	
74	Yes	Yes	Phenotypic (using antisera)	
77	Yes	Yes	Prediction serotype with WGS	Illumina
84	Yes	Yes	Prediction serotype with WGS	Illumina
92	Yes	Yes	Phenotypic (using antisera)	
98[a]	Yes	Yes	Phenotypic (using antisera)	

[a]*Laboratory used WGS alongside phenotypic method for serotyping.*
[b]*Laboratory used PCR alongside phenotypic method for serotyping.*
[c]*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
EQA2301	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2302	enterica	enterica	1,4,5,12	i	-	Typhimurium
EQA2303	enterica	enterica	3,10,15	l,v	1,6	London
EQA2304	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	1,4,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	IIIb	k	z35	IIIb:k:z35
EQA2312	enterica	enterica	1,4,5,12	f,g	-	Derby

Grey = incorrect results ; Green = incorrect notation.

Laboratory ID 15

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	4,5:i:- monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Laboratory ID 17

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	4,5.i- monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 20

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	I 4,5,12:i:-
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 23

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	I 4,5:i:-, monophasic variant
EQA2303	enterica	enterica	3,10,15	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g,s	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,5	f,g	-	Derby

Laboratory ID 26

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Typhimurium monophasic
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 29

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Bradford
EQA2302	enterica	enterica	4	i	-	4,5:i:- monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Heidelberg
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	g,f	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	g,f	-	Derby

Green = incorrect notation.

Laboratory ID 30

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2302	enterica	enterica	1,4,[5],12	i	-	Typhimurium monophasic
EQA2303	enterica	enterica	1,3,19	l,v	1,6	Winterthur
EQA2304	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,13,23	f,g,[s]	-	Havana
EQA2309	enterica	enterica	6,7	b	1,5	Brazzaville
EQA2310	enterica	enterica	1,4,[5],12	i	1,2	Typhimurium
EQA2311	enterica	enterica	-	-	-	-
EQA2312	enterica	enterica	1,4,[5],12	f,g	[1,2]	Derby

Grey = incorrect results.

Laboratory ID 34

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Monophasic variant of Typhimurium
EQA2303	enterica	enterica	3,10,15	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 35

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	Typhimurium, monophasic
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Laboratory ID 37

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	7	r	1,5	7:r:1,5
EQA2302	enterica	enterica	4,5,12	i	-	4,5,12:i:-
EQA2303	enterica	enterica	10	l,v	1,6	10:l,v:1,6
EQA2304	enterica	enterica	7	r	1,5	7:r:1,5
EQA2305	enterica	enterica	6,7	e,h	1,2	6,7:e,h:1,2
EQA2306	enterica	enterica	9,12	g,m	-	9,12:g,m:-
EQA2307	enterica	enterica	6,8	r	l,w	6,8:r:l,w
EQA2308	enterica	enterica	13,23	f,g	-	13,23:f,g:-
EQA2309	enterica	enterica	11	b	1,5	11:b:1,5
EQA2310	enterica	enterica	4,5,12	i	1,2	4,5,12:i:1,2
EQA2311	enterica	diarizonae	47	k	Hz35	47:k:Hz35
EQA2312	enterica	enterica	4,5,12	g,f	-	4,5,12:g,f:-

Grey = incorrect results.

Laboratory ID 42

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2302	enterica	enterica	1,4,[5],12	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,10,15	l,v	1,6	London
EQA2304	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast/Brikama
EQA2308	enterica	enterica	1,13,23	f,g	-	Havana
EQA2309	enterica	enterica	6,7	b	1,5	Brazzaville/Leeuwarden
EQA2310	enterica	enterica	1,4,[5],12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	-	z35	z35	IIIb 47:k:z35/ IIIb 51:k:z35
EQA2312	enterica	enterica	1,9,12[Vi]	g,p	-	Derby

Grey = incorrect results.

Laboratory ID 43

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	I 1,4,[5],12:i:-
EQA2303	enterica	enterica	3	l,v	6	London
EQA2304	enterica	enterica	6,7	r	2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13	f,g	-	Havana
EQA2309	enterica	enterica	11	b	5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	2	Typhimurium
EQA2311	enterica	diarizonae	-	k	-	Salmonella enterica subsp. diarizonae
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Grey = incorrect results.

Laboratory ID 44

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	7	r	1,5	Infantis
EQA2302	enterica	enterica	4	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,1	l,v	1,6	London
EQA2304	enterica	enterica	7	r	1,2	Virchow
EQA2305	enterica	enterica	7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Laboratory ID 46

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	I 4,5,12:i:-
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 49

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2302	enterica	enterica	1,4,[5],12	i	-	Typhimurium monophasic
EQA2303	enterica	enterica	3,{10}{15}	l,v	1,6	London
EQA2304	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	1,4,[5],12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	1,4,[5],12	f,g	-	Derby

Laboratory ID 53

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7,14	r	1,5	Infantis
EQA2302	enterica	enterica	1,4,5,12	i	-	Typhimurium monophasic
EQA2303	enterica	enterica	3,10,15	v	1,6	London
EQA2304	enterica	enterica	6,7,14	r	1,2	Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	1,9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,13,23	f,g,[s]	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	1,4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	1,4,5,12	f,g	[1,2]	Derby

Laboratory ID 56

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,1	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	-	IIIb 47:k:
EQA2312	enterica	enterica	4,5	f,g	-	Derby

Grey = incorrect results.

Laboratory ID 58

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,7	Give
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Grey = incorrect results ; Green = incorrect notation.

Laboratory ID 63

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	7	r	1,2	Virchow
EQA2305	enterica	enterica	7	z10	z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Laboratory ID 65[a]

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301			6,7	e,h		6,7:e,h
EQA2302			4,5	i		4,5:i
EQA2303			10			10
EQA2304			6,7			6,7
EQA2305			6,7			6,7
EQA2306	enterica	enterica	9	g,m		Enteritidis
EQA2307			6,8			6,8
EQA2308			1			1
EQA2309			5			5
EQA2310			4,5,12			4,5,12:i
EQA2311			47			47
EQA2312			4,5,12			4,5,12

Grey = incorrect results [a]Laboratory 65 indicated that not all antisera were available to them, therefore, it was only possible to assess which O- and H-antigens were incorrectly detected.

Laboratory ID 69

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5	i	-	Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,5	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Green = incorrect notation.

Laboratory ID 73

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Monophasic var. Typhimurium
EQA2303	enterica	enterica	3,10	e,h	1,6	Anatum
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	i	1,2	Austenborg
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	1,3,19	f,g	-	Rideau
EQA2309	enterica	enterica	11	b	e,n,z15	Pharr
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Grey = incorrect results ; Green = incorrect notation.

Laboratory ID 74

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Salmonella Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Salmonella enterica ssp. enterica ser. 4,5,12:i:-
EQA2303	enterica	enterica	3,10	Sp	-	Salmonella enterica ssp. enterica ser. 3,10:Sp
EQA2304	enterica	enterica	6,7	r	1,2	Salmonella Virchow
EQA2305	enterica	enterica	6,7,14	z10	e,n,z15	Salmonella Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Salmonella Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Salmonella Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Salmonella Havana
EQA2309	enterica	enterica	11	b	1,5	Salmonella Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Salmonella Typhimurium
EQA2311	enterica	diarizonae	6,7	k	-	Salmonella ssp. diarizonae ser. 6,7:k:-
EQA2312	enterica	enterica	4,12	f,g	-	Salmonella Derby

Grey = incorrect results.

Laboratory ID 77

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	7	r	1,5	Infantis
EQA2302	enterica	enterica	4	i	1,2	Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	7	r	1,2	Virchow
EQA2305	enterica	enterica	7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	8	r	l,w	Goldcoast or Brikama
EQA2308	enterica	enterica	13	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Grey = incorrect results.

Laboratory ID 84

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	7	r	1,5	Infantis
EQA2302	enterica	enterica	4	i	-	4,[5],12:i:-
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	7	r	1,2	Virchow
EQA2305	enterica	enterica	7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9	g,m	-	Enteritidis
EQA2307	enterica	enterica	8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4	f,g	-	Derby

Laboratory ID 92

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	4,[5],12:i:-
EQA2303	enterica	enterica	3,10,15	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Laboratory ID 98

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2301	enterica	enterica	6,7	r	1,5	Infantis
EQA2302	enterica	enterica	4,5,12	i	-	Monophasic Typhimurium
EQA2303	enterica	enterica	3,10	l,v	1,6	London
EQA2304	enterica	enterica	6,7	r	1,2	Virchow
EQA2305	enterica	enterica	6,7	z10	e,n,z15	Mbandaka
EQA2306	enterica	enterica	9,12	g,m	-	Enteritidis
EQA2307	enterica	enterica	6,8	r	l,w	Goldcoast
EQA2308	enterica	enterica	13,23	f,g	-	Havana
EQA2309	enterica	enterica	11	b	1,5	Leeuwarden
EQA2310	enterica	enterica	4,5,12	i	1,2	Typhimurium
EQA2311	enterica	diarizonae	47	k	z35	IIIb 47:k:z35
EQA2312	enterica	enterica	4,12	f,g	-	Derby

Annex 7. Assigned serovar per sample

Lab ID	EQA2301	EQA2302[a]	EQA2303	EQA2304	EQA2305	EQA2306
Provider	**Infantis**	**Typhimurium, monophasic**	**London**	**Virchow**	**Mbandaka**	**Enteritidis**
13	Infantis	Typhimurium	London	Virchow	Mbandaka	Enteritidis
15	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
17	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
20	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
23	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
26	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
29	Bradford	Typhimurium, monophasic	London	Heidelberg	Mbandaka	Enteritidis
30	Infantis	Typhimurium, monophasic	Winterthur	Virchow	Mbandaka	Enteritidis
34	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
35	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
37	7:r:1,5	Typhimurium, monophasic	10:l,v:1,6	7:r:1,5	6,7:e,h:1,2	9,12:g,m:-
42	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
43	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
44	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
46	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
49	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
53	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
56	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
58	Infantis	Typhimurium, monophasic	Give	Virchow	Mbandaka	Enteritidis
63	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
65[b]	O:6, O:7, H:e, H:h	O:4, O:5, H:i	O:10	O:6, O:7	O:6, O:7	Enteritidis
69	Infantis	Typhimurium	London	Virchow	Mbandaka	Enteritidis
73	Infantis	Typhimurium, monophasic	Anatum	Virchow	Austenborg	Enteritidis
74	Infantis	Typhimurium, monophasic	Salmonella enterica ssp. enterica ser. 3,10:Sp	Virchow	Mbandaka	Enteritidis
77	Infantis	Typhimurium	London	Virchow	Mbandaka	Enteritidis
84	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
92	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis
98	Infantis	Typhimurium, monophasic	London	Virchow	Mbandaka	Enteritidis

[a]Notation of serovars equalised.
[b]Laboratory 65 indicated that not all antisera were available to them, therefore it was only possible to assess which O- and H-antigens were incorrectly detected.
Grey=incorrect serovar.

Lab ID	EQA2307	EQA2308	EQA2309	EQA2310	EQA2311[a]	EQA2312
Provider	**Goldcoast**	**Havana**	**Leuwarden**	**Typhimurium**	**IIIb 47:k:z35**	**Derby**
13	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb:k:z35	Derby
15	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
17	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
20	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
23	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
26	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
29	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
30	Goldcoast	Havana	Brazzaville	Typhimurium	-	Derby
34	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
35	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
37	6,8:r:l,w	13,23:f,g:-	11:b:1,5	4,5,12:i:1,2	IIIb 47:k:z35	4,5,12:g,f:-
42	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
43	Goldcoast	Havana	Leeuwarden	Typhimurium	Salmonella enterica subsp. diarizonae	Derby
44	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
46	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
49	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
53	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
56	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:	Derby
58	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
63	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
65[b]	O:6, O:8	O:1	O:5	O:4, O:5, O:12	O:47	O:4, O:5, O:12
69	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
73	Goldcoast	Rideau	Pharr	Typhimurium	IIIb	Derby
74	Goldcoast	Havana	Leeuwarden	Typhimurium	Salmonella ssp. diarizonae ser. 6,7:k:-	Derby
77	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
84	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
92	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby
98	Goldcoast	Havana	Leeuwarden	Typhimurium	IIIb 47:k:z35	Derby

[a]Notation of serovars equalised.
[b]Laboratory 65 indicated that not all antisera were available to them, therefore it was only possible to assess which O- and H-antigens were incorrectly detected.
Grey=incorrect serovar.

Annex 8. Concordance and errors per sample

EQA #	Intended serovar name or seroformula	Concordance (%)	Type of errors
EQA2301	Infantis	93	Non-standard nomenclature, serovar name incorrectly assigned as Bradford (n=1, lab 29) Type misclassification of He,h instead of Hr in 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2302	Typhimurium, monophasic	86	Non-standard nomenclature, serovar name incorrectly assigned as Typhimurium (n=2, lab 13 and 69) False-positive H1,2 detection in 2nd phase (n=1, lab 77)
EQA2303	London	82	Type misclassification of O1,3,19 instead of O3,10 (n=1, lab 30) Type misclassification of He,h instead of Hl,v in 1st phase (n=1, lab 73) Type misclassification of HSp instead of Hl,v in 1st phase (n=1, lab 74) Type misclassification of H1,7 instead of H1,6 in 2nd phase (n=1, lab 58) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2304	Virchow	93	Non-standard nomenclature, serovar name incorrectly assigned as Heidelberg (n=1, lab 29) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2305	Mbandaka	89	Type misclassification of He,h instead of Hz10 in 1st phase (n=1, lab 37) Type misclassification of Hi instead of Hz10 in 1st phase (n=1, lab 73) Type misclassification of H1,2 instead of He,n,z15 in 2nd phase (n=2, lab 37 and 73) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2306	Enteritidis	100	None
EQA2307	Goldcoast	96	False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2308	Havana	93	Type misclassification of O1,3,19 instead of O13,23 (n=1, lab 73) False-negative O-antigen O13,23 (n=1, lab 65) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2309	Leeuwarden	89	Type misclassification of O6,7 instead of O11 (n=2, lab 30 and 42) Type misclassification of O5 instead of O11 (n=1, lab 65) Type misclassification of He,n,z15 instead of H1,5 in 2nd phase (n=1, lab 73) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2310	Typhimurium	96	Type misclassification of O1 instead of O13,23 (n=1, lab 65) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)
EQA2311	IIIb 47:k:z35	75	Non-standard nomenclature, seroformula name incorrectly assigned as IIIb (n=1, lab 73) Type misclassification of OIIIb instead of O47 (n=1, lab 13) Type misclassification of O6,7 instead of O47 (n=1, lab 74) Type misclassification of subspecies *enterica* instead of *diarizonae* (n=1, lab 30) False-negative O-antigen (n=2, lab 30 and 43) False-negative H-antigen 1st phase (n=2, lab 30 and 65) False-negative H-antigen 2nd phase (n=5, lab 30 43, 56, 65 and 74)
EQA2312	Derby	96	Non-standard nomenclature, seroformula indicates serovar Dublin (n=1, lab 42) False-negative H-antigen 1st phase (n=1, lab 65) False-negative H-antigen 2nd phase (n=1, lab 65)

Annex 9. Reported MLST sequence types of serotyping strain panel

Lab ID	MLST reported	EQA2301	EQA2302	EQA2303	EQA2304	EQA2305	EQA2306	EQA2307	EQA2308	EQA2309	EQA2310	EQA2311	EQA2312
Provider	**Yes**	**32**	**34**	**155**	**197**	**413**	**11**	**358**	**1526**	**4347**	**19**	**430**	**40**
13	No												
15	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
17	No												
20	No												
23	No												
26	No												
29	No												
30	No												
34	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
35	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
37	No												
42	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
43	No												
44	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
46	No												
49	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
53	No												
56	No												
58	No												
63	No												
65	No												
69	No												
73	No												
74	No												
77	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
84	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40
92	No												
98	Yes	32	34	155	197	413	11	358	1526	4347	19	430	40

Green = No reporting of MLST results.

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	Yes	No				
15	Yes	Yes			X	
17	No	No				
20	Yes	Yes			X	
23	Yes	Yes			X	
26	Yes	Yes		X	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	Yes	Yes				X
44	Yes	Yes				X
46	Yes	Yes			X	
49	Yes	Yes			X	
53	Yes	Yes			X	
56	Yes	Yes	X			
58	Yes	Yes			X	
63	Yes	Yes		X	X	
65	No	No				
69	Yes	Yes			X	
73	Yes	Yes			X	
74	Yes	Yes		X	X	
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]
15	Illumina	MLST	Nextera XT	Ridom SeqSphere+	Enterobase	5
20	Illumina	MLST	Nextera Xt	RidomSeqSphere	in-house cgMLST for S.Enteritidis	10
23	Illumina	MLST	DNA Prep	SeqSphere	Enterobase	20
26	Illumina	MLST	Nextera XT	Enterobase	EnteroBase	10
29	Illumina	MLST	Nextera XT	chewBBACA 3.1.2	wgMLST schema for S.enterica from INNUENDO project	16[b]
30	Illumina	MLST	Nextera XT	SeqSphere+	EnteroBase	7
34	Illumina	MLST	Nextera XT	EnteroBase	cgMLST	5
35	Illumina	MLST	Nextera XT	Ridom SeqSphere+	EnteroBase	5[c]
42	Illumina	MLST	KAPA HyperPlus	SeqSphere+ v. 8.3.0	EnteroBase	5
43	Illumina	SNP[a]	DNA Prep	CSI Phylogeny		5
44	Ion Torrent	SNP[a]		In-house		5
46	Illumina	MLST	DNA Prep	SeqSphere, cgMLST. Chewbacca	EnteroBase	5
49	Illumina	MLST	DNA Prep (Nextera Flex)	SeqSphere	Enterobase	7
53	Illumina	MLST	DNA prep	cgMLSTFinder	Enterobase	7
58	Illumina	MLST	Illumina DNA prep (M) Tagmentation	SeqSphere	Enterobase	7
63	Illumina	MLST	MiSeq Reagent Kit v3 - 500 cycles	In house pipeline	Enterobase	7
69	Illumina	MLST	Nextera XT	Enterobase	Achtman 7 gene MLST	5
73	Illumina	MLST	NEBNext Ultra™ II FS DNA Library Prep Kit	chewBBACA Allele Call	Enterobase	5
74	Illumina	MLST	DNA prep	SeqSphere	Enterobase	10
77	Illumina	MLST	DNA prep	Chewbbaca version 3.1.2	S.enetrica cgMLST v2 scheme from cgmlst.org obtained on 2023-05-15	10
84	Illumina	MLST	DNA prep	BioNumerics	Core (EnteroBase)	3[d]
92	Illumina	MLST	Nextera XT	Ridom SeqSphere+ cgMLST	EnteroBase	7
98	Illumina	MLST	Nextera XT	Bionumerics 8.1	Applied Maths/Enterobase	5[e]

[a] *All laboratories using SNP analysis, have used EQA2313 as reference genome.*

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 reads’ 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 16G database. MLST prediction was determined using mlst v2.18.1 software. Cluster analysis: This gene-by-gene analysis was performed with chewBBACA v3.1.2 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 v2.0.3 using both GrapeTree and single-linkage clustering algorithms, defining the core cgMLST loci using a threshold of 90%. Samples with less than 90% 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 3821 cgMLST loci), as suggested by the INNUENDO project (Llarena et al., 2018).*

[c] *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

[d] *We don't normally analyse food isolates, only human isolates*

[e] *The cutoff distance here is set to 5 AD, however this will be an ongoing evaluation dependent on the serotype, clone, time, place and type of outbreak.*

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

Lab ID	Species confirmation	Q score (Phred)	Coverage	% Good targets MLST	#contigs
15[a]	Included in Ridom SeqSphere+		~50x	~98%	
20	SeqSero2 and Kraken2		>29 (avg)	>90%	No threshold provided
23	SeqSero2 and MLST		40x		
26[b]	Kraken				<600
29	Confindr and Kraken with the standard 16G database		30x (as implemented in INNUca v4.2.2)	≥90%	According to default criteria with standard 16G database
30	MashDistance		≥30x	≥90%	<300
34	Kmer				<600
35	Mash (implemented in SeqSphere) and KRAKEN		30-fold	95% (aiming for 98% with own data)	
42	Kraken and Mash Distance		>30	>90%	
43	Enterobase, CGE-SpeciesFinder and CGE-KmerFinder		>50x		<250
44	BLAST towards an in-house database with reference sequences		≥20x		
46	Kmer finder	30	30x	95%	<150
49	Mash Screen		>50	>95%	500
53[c]	KmerFinder		40x	>95%	<300
58[d]	K-mer		30x		<500
63	Kraken2		30x		
69	rMLST				300
73	Kmer finder		80x		<300 bp
74	Mash Screen (included in SeqSphere)	>30	>50x	>95%	<200
77	Kraken2 and rMLST			>90%	<200
84[e]		≥30	≥30	>97%	
92[f]	rMLST and Mash Distance		>40x	>95%	<300
98[g]	Kraken and Bracken analysis		>50	≥95%	No threshold provided
Median (range)[h]	**NA**	**30 (30)**	**31 (20-80)**	**95 (90-98)**	**<300 (199-599)**

Green = Not reported.
[a]Laboratory 15 also assessed contig size with threshold >200 bp.
[b]Laboratory 26 also assessed the proportion of scaffolding placeholders with a cut-off of <3%.
[c]Laboratory 53 also assessed the MLST and samples were accepted when all seven loci were present and no multiple variants were found.
[d]Laboratory 58 also assessed the average read length, which was set at 150 bp.
[e]Laboratory 84 also assessed the NrBAFperfect with threshold >4000 and the NrBAFMultiple with threshold <20.
[f]Laboratory 92 also assessed number of reads with threshold >1000000.
[g]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.
[h]Calculated by laboratories that reported numerical values.

Lab ID	Genome size	N50	Species assignment	Contamination	% GC
15[a]	4.6-5.3 MB				
20					
23		<500			
26[b]	4-5.8 Mbp	>20 kb	>70% contigs assigned		
29	Approx. 5.0 MB	According to default criteria with standard 16G database		According to default criteria with standard 16G database	
30	Within 10% of *Salmonella* genome size	≥30000	≥95%		
34	4.0-5.8 Mbp	>20 Kb	>70%		
35					
42	4.9+1.2	>50000			
43	4-5,5 Mbp (rationally)	>30000			
44	3.6-5.4 Mbp		No set threshold		
46	5 Mb	80000			50 (approx.)
49	Length of contigs assembled <ref genome +10%				
53[c]	Similar to expected				
58[d]	4.4-5.8 Mb	>30000 bp			
63				No obvious contamination (other than plasmid, etc.)	
69	4.4-5.8 Mb	>30000 bp			
73	4.4-5.8 Mb	>30000			
74	4.6-5.6 Mbp	>100 kb			
77	4627000 < x ≤ 5006000	>50000			51.8 <x ≤52.3
84[e]	4.5-5.5 Mb	>100000			
92[f]	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[g]	4510000-5300000 bp	No threshold provided		<5%	
Median (range)[h]	**4.4-5.6 Mb (3.6-4.9 – 5.0-6.1)**	**30 kb (20-100)**	**>70% (70-95)**	**5% (5)**	**51.8-52.2 (50-51.8 – 50-52.3)**

Green = Not reported.
[a]*Laboratory 15 also assessed contig size with threshold >200 bp.*
[b]*Laboratory 26 also assessed the proportion of scaffolding placeholders with a cut-off of <3%.*
[c]*Laboratory 53 also assessed the MLST and samples were accepted when all seven loci were present and no multiple variants were found.*
[d]*Laboratory 58 also assessed the average read length, which was set at 150 bp.*
[e]*Laboratory 84 also assessed the NrBAFperfect with threshold >4000 and the NrBAFMultiple with threshold <20.*
[f]*Laboratory 92 also assessed number of reads with threshold >1000000.*
[g]*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.*
[h]*Calculated by laboratories that reported numerical values.*

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

Lab ID	MLST reported	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Provider	**Yes**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**3233**	**10**
15	Yes	10	10	10	10	10	10	10	10	3233	10
20	Yes	10	10	10	10	10	10	10	10	3233	10
23	Yes	10	10	10	10	10	10	10	10	3233	10
26	Yes	10	10	10	10	10	10	10	10	3233	10
29	Yes	10	10	10	10	10	10	10	10	3233	10
30	Yes	10	10	10	10	10	10	10	10	3233	10
34	Yes	10	10	10	10	10	10	10	10	3233	10
35	Yes	10	10	10	10	10	10	10	10	3233	10
42	Yes	10	10	10	10	10	10	10	10	3233	10
43	Yes	10	10	10	10	10	10	10	10	3233	10
44	Yes	10	10	10	10	10	10	10	10	3233	10
46	Yes	10	10	10	10	10	10	10	10	3233	10
49	Yes	10	10	10	10	10	10	10	10	3233	10
53	Yes	10	10	10	10	10	10	10	10	3233	10
58	Yes	10	10	10	10	10	10	10	10	3233	10
63	Yes	10	10	10	10	10	10	10	10	3233	10
69	Yes	10	10	10	10	10	10	10	10	3233	10
73	Yes	10	10	10	10	10	10	10	10	3233	10
74	Yes	10	10	10	10	10	10	10	10	3233	10
77	Yes	10	10	10	10	10	10	10	10	3233	10
84	Yes	10	10	10	10	10	10	10	10	3233	10
92	Yes	10	10	10	10	10	10	10	10	3233	10
98	Yes	10	10	10	10	10	10	10	10	3233	10

Annex 14. Results reported WGS-based cluster assignments based on index EQA2313

Belonging to cluster yes/no

Lab ID	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322	EQA2323	EQA2324	EQA2325	EQA2326	EQA2327	% correctly assigned per lab[a]
Provider	**No**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**No**	**Yes**	**IQ**	**No**	**IQ**	**Yes**	**IQ**	**NA**
15	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
20	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
23	No	Yes	No	No	No	No	No	No	Yes	IQ	No	IQ	Yes	IQ	91
26	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
29	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
30	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
34	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
35	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
42	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
43	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
44	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
46	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
49	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
53	No	Yes	No	No	No	Yes	No	No	Yes	IQ	Yes	IQ	Yes	Yes	91
58	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
63	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
69[b]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	IQ	Yes	IQ	Yes	Yes	45
73	No	Yes	No	No	No	Yes	No	No	Yes	IQ	Yes	IQ	Yes	No	91
74	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
77	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	No	Yes	IQ	100
84	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
92	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
98	No	Yes	No	No	No	Yes	No	No	Yes	IQ	No	IQ	Yes	IQ	100
% Correctly assigned per sample	100	100	100	100	100	100	100	94	94	100	100	NA	NA	94	

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

[a]Calculation based on cluster or singleton assignment of provided isolates EQA2314 -EQA2322 and provided good quality raw reads EQA2324 and EQA2326.

[b]Laboratory used Achtman 7 gene MLST scheme for cluster analysis.

Annex 15. Distances reported based on index EQA2313

Gene-by-gene approach, allelic distances

Lab ID	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322	EQA2323	EQA2324	EQA2325	EQA2326	EQA2327
Provider	**96**	**4**	**258**	**76**	**258**	**3**	**45**	**1744**	**3**	**NA**	**27**	**NA**	**0**	**NA**
15	97	4	259	76	258	4	46	1740	4	IQ	28	IQ	1	IQ
20[b]	41	1	106	27	112	1	15	772	1	IQ	10	IQ	0	IQ
23	96	3	253	75	258	258	45	1729	3	IQ	27	IQ	0	IQ
26	196	5	328	124	544	7	47	2138	5	IQ	31	IQ	0	125
29	139	8	373	114	384	4	63	2220	4	IQ	37	IQ	4	IQ
30	174	2	469	110	288	2	43	1733	2	IQ	27	IQ	0	98
34	106	5	286	87	293	4	47	1803	5	IQ	31	IQ	0	125
35	96	3	257	75	258	4	45	1742	3	IQ	27	IQ	0	IQ
42	96	3	257	75	258	3	45	2690	3	IQ	27	IQ	0	95
46	179	5	479	115	297	3	47	2021	3	IQ	27	IQ	0	110
49	96	4	257	75	258	3	45	1738	4	IQ	27	IQ	0	IQ
53	101	5	278	85	283	4	45	1793	4	IQ	3	IQ	1	5
58	179	5	479	115	297	3	47	2026	3	IQ	27	IQ	0	104
63	111	5	296	91	307	7	52	1806	6	IQ	32	IQ	2	IQ
69[a]	0	0	0	0	0	0	0	5	0	IQ	0	IQ	0	0
73	40	0	268	48	266	0	12	1932	0	IQ	4	IQ	0	15
74[c]	96	3	257	75	258	3	45	1733	3	IQ	27	IQ	0	IQ
77	102	8	269	41	270	4	48	1741	6	IQ	33	547	3	IQ
84	103	4	279	83	274	4	45	1785	4	IQ	27	IQ	0	89
92	96	3	257	75	257	3	45	1737	2	IQ	27	IQ	0	IQ
98[d]	104	4	278	84	281	4	46	1785	4	IQ	27	IQ	0	IQ
Median	**101**	**4**	**269**	**76**	**270**	**4**	**45**	**1785**	**3**	**NA**	**27**	**NA**	**0**	**NA**

IQ = insufficient quality. Orange = insufficient quality not detected.
[a]Laboratory used Achtman 7 gene MLST scheme for cluster analysis.

Comments made by participants about allele calling and cluster cut-off
[b]EQA2324: allelic distance to index case by using cgMLST was 10 which is our cut-off value for cluster analysis. However, by using cgMLST and accessory genes the allellic distance was 29.
[c]The allele distance of EQA2327 sample is >10 (95) if we try to analyse against the low quality data
[d]Strain EQA2324 has a read coverage < 50 (45), which is the threshold we use, but the cgMLST core% is > 95 and the strain can be used for cluster analysis. Strain 2327 has low read coverage, but a borderline core% and could, in case of an outbreak, be included as suspected case. We included the stain in both a cgMLST and a supporting SNP analysis and found it not related to the outbreak strains. However, the strain should be resequenced and rerun in new cluster analysis for confirmation of results.

SNP-typing, SNP distances

Lab ID	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322	EQA2323	EQA2324	EQA2325	EQA2326	EQA2327
Provider	**201**	**4**	**568**	**168**	**615**	**3**	**88**	**19905**	**3**	**NA**	**27**	**NA**	**0**	**NA**
43	160	4	561	166	562	3	89	789	3	IQ	63	IQ	5	IQ
44	196	4	555	168	598	3	89	20886	3	IQ	66	IQ	1	IQ
Median	**178**	**4**	**558**	**167**	**580**	**3**	**89**	**10838**	**3**	**NA**	**65**	**NA**	**3**	**NA**

IQ = insufficient quality.

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

Laboratory ID 15

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	51.481	52.286	42.719	55.867	55.096	49.132	55.701	62.500	61.120	33.930
N50	263066	400907	229658	268268	224933	275453	304843	384275	438797	230133
GC%	52.09	52.09	52.09	52.05	52.1	52.09	52.09	52.09	52.14	52.09
Total length	4867301	4871735	4868195	4993825	4870230	4870548	4873016	4872497	4696939	4872637
# reads	1155894	1186420	940040	1301930	1282788	1163674	1324402	1441932	1325342	774012
Mean read length	216.5	214.5	221	214	209	206	205	211	217	214

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 20

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	62.532	66.943	88.941	92.230	154.376	87.688	112.655	78.439	85.335	104.267
N50	163863	260213	324010	291583	401809	187471	478524	432723	264617	402251
GC%	52.09	52.09	52.09	52.05	52.09	52.09	52.08	52.09	52.14	52.09
Total length	4866247	4871749	4867005	4992966	4871790	4870530	4872365	4871902	4697050	4873394
# reads	2200612	2327864	3354650	3412136	5434852	3112762	4079734	2676488	2834834	3561012
Mean read length	138	140	129	135	138	137.5	134.5	142	141	142

Laboratory ID 23

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	99.3	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	14.4	0.39
Avg coverage	37.845	45.196	42.390	16.945	47.361	77.280	92.781	38.997	29.282	85.965
N50	298265	478057	364291	196454	417434	487369	405767	405740	228083	401825
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.09	52.09	52.01	52.08
Total length	4866365	4871424	4868529	4988731	4871681	4871434	4870410	4872053	5231432	4871501
# reads	1431378	1697628	1604692	669006	1795352	2899794	3494052	1496406	1201468	3223566
Mean read length	128	129.5	128	125.5	128	129.5	129	126.5	127	129.5

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 26

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.56	98.93	99.32	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	61.824	27.043	48.200	29.743	25.971	59.753	46.671	44.513	29.398	79.964
N50	258170	52733	130210	108031	278771	145355	191862	153991	122259	165648
GC%	52.1	52.25	52.11	52.09	52.09	52.12	52.09	52.11	52.15	52.09
Total length	4865079	4831792	4863719	4980415	4868954	4867119	4870149	4868789	4693411	4871247
# reads	2504392	1091832	1967982	1243226	1068526	2388416	1881170	1804948	1161788	3198746
Mean read length	120	119.5	119	119	118	121.5	120.5	119.5	118.5	121.5

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 29

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	103.360	100.440	82.946	89.640	93.302	111.821	83.872	112.516	83.690	72.052
N50	363899	405971	161336	262546	298500	420525	363520	405971	401029	220699
GC%	52.09	52.09	52.1	52.06	52.09	52.09	52.09	52.09	52.14	52.09
Total length	4867668	4872746	4866206	4990916	4871455	4873044	4873735	4873088	4696780	4872582
# reads	3679852	3590180	2972668	3276540	3342482	3993698	2992668	4084446	2920290	2599482
Mean read length	136	136	135.5	136	136	136	136.5	134	134	134.5

Laboratory ID 30

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.32	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.45	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	29.750	28.311	34.850	39.566	28.535	27.290	26.511	29.356	28.088	23.546
N50	103618	114228	109674	196794	130351	123174	107998	107559	92200	114312
GC%	52.11	52.1	52.11	52.05	52.12	52.12	52.11	52.11	52.18	52.1
Total length	4862432	4870164	4863775	4992722	4865824	4866174	4868049	4866886	4687493	4867929
# reads	714288	678864	822886	964146	688986	660892	646114	707544	659926	579564
Mean read length	203	204.5	206	205.5	202	202	201.5	202.5	200	198.5

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 34

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	48.142	44.028	47.559	53.564	100.557	72.142	60.926	59.208	74.099	47.717
N50	161494	165003	232802	140294	187005	224333	124428	248818	166311	224423
GC%	52.09	52.09	52.09	52.15	52.2	52.13	52.17	52.08	52.2	52.08
Total length	4863291	4868346	4864836	4887499	4839899	4859402	4849614	4860589	4681243	4870863
# reads	1936002	1774854	1914298	2166848	4006516	2883698	2132830	2378722	2867140	1936318
Mean read length	120.5	120.5	120.5	120.5	121	121	138.5	120.5	120.5	120

Laboratory ID 35

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	177.554	165.298	168.193	160.961	149.812	137.421	195.019	137.342	171.924	154.074
N50	363207	405971	405959	494242	301919	380616	363207	401853	284462	363359
GC%	52.09	52.09	52.09	52.05	52.09	52.09	52.08	52.09	52.14	52.09
Total length	4867271	4871999	4868166	4993467	4872994	4873119	4872816	4872693	4697619	4872894
# reads	6358802	5903366	5992036	5883246	5346540	4893248	7009576	4894466	5928334	5494172
Mean read length	135.5	136.5	136	136	136	136.5	135.5	136.5	135.5	136.5

Laboratory ID 42

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	70.286	74.636	79.101	72.758	84.283	75.947	79.779	71.306	75.767	89.102
N50	402251	401786	402846	405492	478645	297743	363520	401835	478985	384338
GC%	52.09	52.09	52.09	52.05	52.09	52.09	52.08	52.09	52.14	52.08
Total length	4864514	4870335	4864830	4989750	4869590	4869932	4871495	4870891	4696244	4871976
# reads	3218072	3433262	3603500	3427010	3884550	3450392	3639268	3261166	3336676	4029518
Mean read length	106	105.5	106.5	106	105.5	107	106.5	106	106	107.5

Laboratory ID 43

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.3	99.3	99.26	98.93	98.99	99.61	98.93
Contamination	0.39	0.39	0.39	0.43	0.39	0.77	0.39	0.39	0.49	0.39
Avg coverage	65.682	55.398	69.773	37.938	44.762	43.103	36.281	34.773	43.235	49.003
N50	401824	405971	401825	274325	420525	420546	405959	493971	421606	401825
GC%	52.09	52.09	52.09	52.09	52.09	52.05	52.09	52.09	52.14	52.08
Total length	4866989	4872512	4867474	4877941	4871581	4993642	4867940	4872028	4695981	4872957
# reads	2294446	1924938	2421090	1319202	1580984	1534834	1260370	1221330	1449106	1694980
Mean read length	139	140	140.5	140.5	138	140	140	138.5	139.5	140.5

Laboratory ID 46

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.32	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	53.085	53.236	45.616	67.167	67.146	67.628	58.735	62.819	70.668	67.356
N50	401825	493790	405959	494242	405971	494251	405959	405971	490801	478524
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.09	52.09	52.14	52.08
Total length	4867247	4872121	4867452	4988239	4871444	4871823	4872710	4872576	4696804	4873028
# reads	1487052	1397328	1206356	1836730	1856342	1815830	1539960	1667738	1827424	1830674
Mean read length	173.5	185.5	183.5	182	176.5	181.5	185.5	183.5	181	179.5

Laboratory ID 49

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	42.064	75.136	80.941	71.895	71.864	59.011	49.441	36.968	42.600	42.722
N50	405785	491573	401748	491340	393292	417006	375172	405773	283627	405559
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.08	52.09	52.14	52.08
Total length	4866722	4871135	4867590	4992492	4870202	4870183	4871881	4870764	4696213	4870848
# reads	1464384	2639984	2851236	2589434	2515818	2087158	1723570	1316198	1458004	1506544
Mean read length	139.5	138.5	138	138	138.5	137.5	139.5	136.5	137	138

Laboratory ID 53

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	191.628	146.949	191.813	155.003	184.258	175.297	178.328	168.499	191.102	187.748
N50	401825	493790	405959	491384	405971	494251	405959	405971	490801	401825
GC%	52.09	52.09	52.09	52.12	52.1	52.09	52.08	52.09	52.14	52.09
Total length	4867253	4872433	4867554	4895031	4871179	4872978	4873004	4872837	4697629	4872948
# reads	7116736	5395362	6971170	5681048	6723176	6423324	6520256	6182586	6744742	6865430
Mean read length	131	132.5	133.5	133	133	132.5	133	132.5	133	133

Laboratory ID 58

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	170.337	166.023	297.179	174.587	174.194	92.328	127.652	146.022	246.170	327.118
N50	405950	491679	405959	491388	432712	495151	491693	493971	490374	405959
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.08	52.09	52.14	52.09
Total length	4867519	4872957	4867453	4993794	4872200	4873597	4873968	4872961	4696868	4872578
# reads	5797390	5698536	10068970	6300220	6048872	3159952	4404184	5059734	8212100	11207594
Mean read length	142.5	142	143.5	138	140.5	142	141	140.5	141	142

Laboratory ID 63

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	234.797	322.791	433.515	175.472	312.756	255.054	532.663	438.784	272.466	308.639
N50	405950	493790	405959	494242	493790	494251	405959	493971	478985	493972
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.08	52.09	52.14	52.08
Total length	4867153	4872383	4867426	4994244	4872458	4873709	4873418	4872430	4696952	4874228
# reads	8094962	11112600	14851640	6254038	10743110	8757900	18310676	15094316	9045332	10629542
Mean read length	141	141	141.5	140	141.5	141.5	141	141	141	141

Laboratory ID 69

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.5	99.24	98.55
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	28.739	39.069	18.021	29.477	38.475	20.797	22.165	15.697	27.560	23.690
N50	47452	70402	31344	42595	62350	30659	36794	24634	36826	33543
GC%	52.17	52.12	52.25	52.16	52.15	52.25	52.2	52.36	52.25	52.27
Total length	4848980	4864873	4829887	4964331	4859767	4834237	4847458	4803295	4673800	4828371
# reads	976648	1332956	610832	1023630	1300212	710694	762478	543420	910604	809514
Mean read length	142	142.5	142	143	143.5	141.5	141	138.5	141.5	141.5

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 73

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	445.175	574.835	519.977	470.482	439.798	471.657	612.038	641.080	524.912	570.369
N50	405950	493777	405959	432775	493790	495151	363207	493971	490086	363207
GC%	52.09	52.09	52.09	52.04	52.09	52.09	52.09	52.09	52.14	52.09
Total length	4867110	4872579	4870668	4994185	4872428	4872873	4871403	4872757	4696824	4876133
# reads	15320108	19899676	18135582	16843014	15304990	16386892	21122074	22052364	17628638	20042718
Mean read length	141	140.5	139.5	139.5	140	140	141	141.5	139.5	138.5

Laboratory ID 74

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	74.567	74.342	75.310	81.733	85.950	90.592	81.877	69.898	75.795	72.317
N50	343641	363069	288668	234689	405718	363650	252766	332076	371449	190789
GC%	52.08	52.09	52.09	52.04	52.09	52.09	52.08	52.09	52.12	52.08
Total length	4860185	4864024	4858862	4984578	4863610	4864076	4865507	4863415	4686592	4859370
# reads	2926114	2946938	2946462	3262444	3321562	3491566	3208210	2753728	2880806	2790090
Mean read length	123	122	124	125	126	126	124	123	123	126

Laboratory ID 77

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	141.169	315.694	342.403	244.820	271.146	292.437	234.607	243.905	379.926	410.817
N50	401825	405971	405959	491391	405971	491711	405959	491477	421574	405959
GC%	52.09	52.09	52.09	52.05	52.09	52.09	52.09	52.09	52.14	52.08
Total length	4867059	4872289	4868003	4993254	4872503	4873178	4872761	4872828	4697835	4872828
# reads	4855686	10802366	11719100	8647292	9361260	10078978	8058970	8466408	12745822	14048186
Mean read length	141	142	142	141	140.5	141	141.5	140	139.5	142

Laboratory ID 84

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	58.206	18.987	41.337	45.444	77.072	47.552	46.771	32.208	22.524	80.710
N50	401825	493790	363586	494242	405971	494251	405959	405971	490086	401748
GC%	52.09	52.09	52.09	52.05	52.09	52.09	52.08	52.09	52.14	52.08
Total length	4867288	4871691	4868407	4992665	4869897	4871983	4874027	4873188	4696664	4872146
# reads	1343798	453122	950294	1071140	1910174	1094022	1089062	750992	508970	1987762
Mean read length	210.5	203.5	211.5	211	196	211.5	209	209	207.5	198

Grey = does not pass quality criteria of EQA provider.

Laboratory ID 92

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.32	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	71.554	71.614	72.669	51.810	111.521	62.020	96.727	91.381	75.728	73.022
N50	229619	162145	155088	128676	301875	175362	363207	225415	284462	225415
GC%	52.09	52.1	52.1	52.06	52.09	52.1	52.09	52.1	52.14	52.09
Total length	4866358	4871615	4865378	4990142	4871241	4871075	4873074	4872282	4696845	4873173
# reads	1810742	1799444	1810324	1411690	2882250	1550880	2501426	2345712	1913160	1828942
Mean read length	192	193.5	195	183	188	195	188.5	189.5	186	194

Laboratory ID 98

QC parameter	EQA2313	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Completeness	98.93	98.9	98.93	99.26	98.9	99.3	98.93	98.93	99.61	98.93
Contamination	0.39	0.39	0.39	0.77	0.39	0.39	0.39	0.39	0.49	0.39
Avg coverage	70.582	51.377	95.737	91.995	62.867	81.521	82.388	54.864	57.979	89.156
N50	161417	153367	248737	275013	286533	131997	236462	92816	178102	129530
GC%	52.09	52.09	52.09	52.05	52.09	52.1	52.08	52.1	52.14	52.09
Total length	4866314	4869510	4866810	4990912	4869494	4869994	4870769	4868268	4695716	4870931
# reads	2885128	2115612	3933056	3885980	2595668	3350700	3402588	2274450	2299138	3664338
Mean read length	118.5	118	118	118	117.5	118	117.5	117	118	118

Annex 17. Reported results MLVA-based cluster assignments to index EQA2328

Lab ID	EQA2329	EQA2330	EQA2331	EQA2332	EQA2333	EQA2334	EQA2335	EQA2336	EQA2337
Provider WGS	**No**	**No**	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**	**Yes**
Provider MLVA	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**
26	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes
63	No	No	Yes	Yes	No	No	No	No	Yes
74	No	No	Yes	Yes	Yes	Yes	No	Yes	Yes

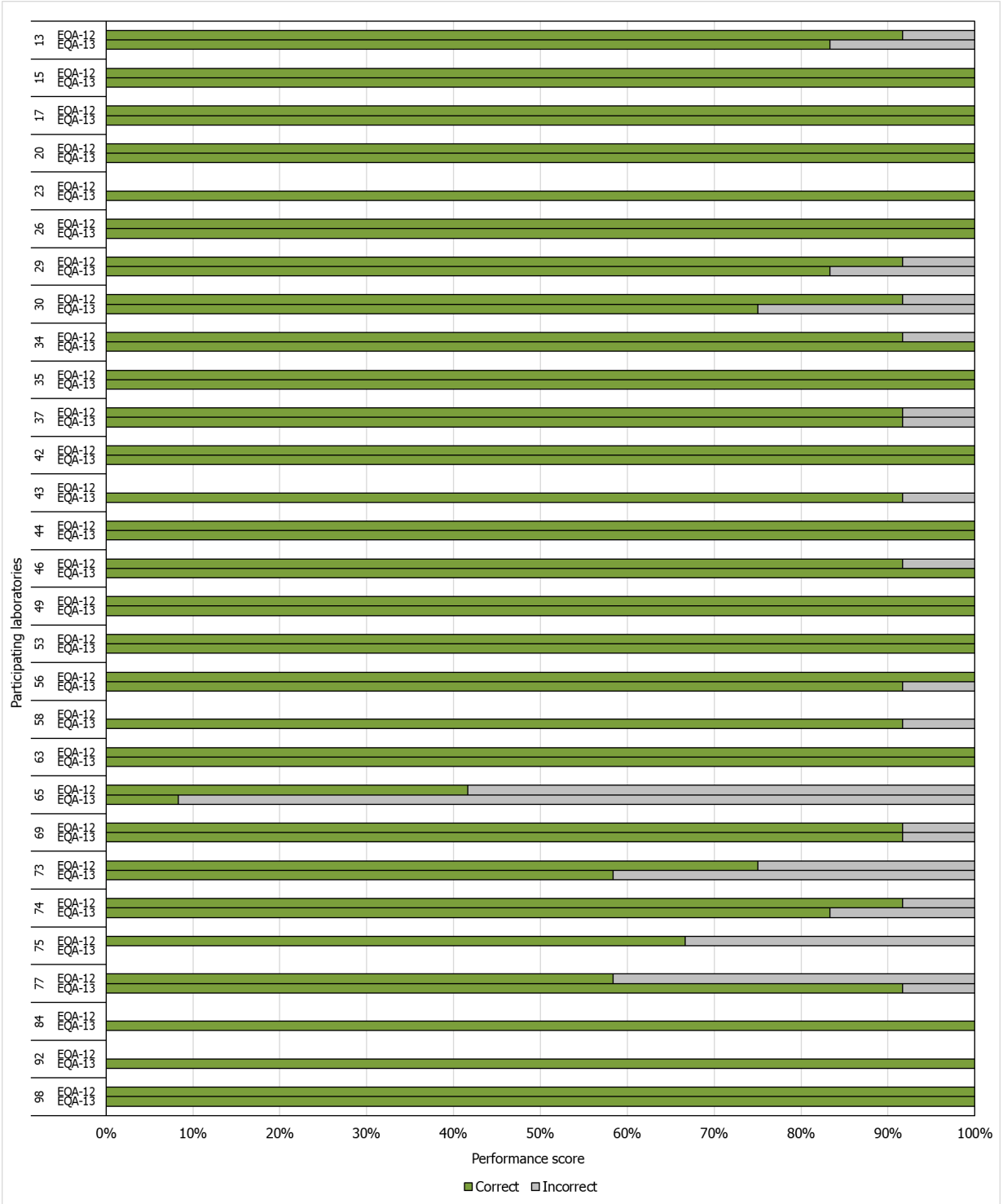
Green = provider. Grey = incorrectly assigned, based on cluster identification with WGS data.

Annex 18. Reported results PFGE-based cluster assignments to index EQA2313

Lab ID	EQA2314	EQA2315	EQA2316	EQA2317	EQA2318	EQA2319	EQA2320	EQA2321	EQA2322
Provider WGS	**No**	**Yes**	**No**	**No**	**No**	**Yes**	**No**	**No**	**Yes**
92	Possibly	Yes	No	Possibly	Possibly	Yes	Possibly	No	Yes

Green = provider, WGS-based clustering. Grey = incorrectly assigned, based on cluster identification with WGS data.

Annex 19. Comparison serotyping results between EQA-12 and EQA-13 per laboratory



Annex 20. Comparison serotyping results between EQA-12 and EQA-13 per serovar

Lab ID	Infantis		Typhimurium, monophasic		Enteritidis		Goldcoast		Leeuwarden		Typhimurium		Derby	
	EQA-12	EQA-13	EQA-12	EQA-13	EQA-12	EQA-13	EQA-12	EQA-13	EQA-12	EQA-13	EQA-12	EQA-13	EQA-12	EQA-13
13				[a]										
15														
17														
20														
26														
29		[a]												
30														
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42														
44														
46														
49														
53														
56														
63														
65[b]														
69				[a]										
73														
74														
77			[a]											
98														

[a]Correct seroformula determined, but wrong serovar reported.

[b]Laboratory 65 indicated that not all antisera were available to them which affected serotyping performance.

Grey=incorrect serovar; Green=correct serovar.

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