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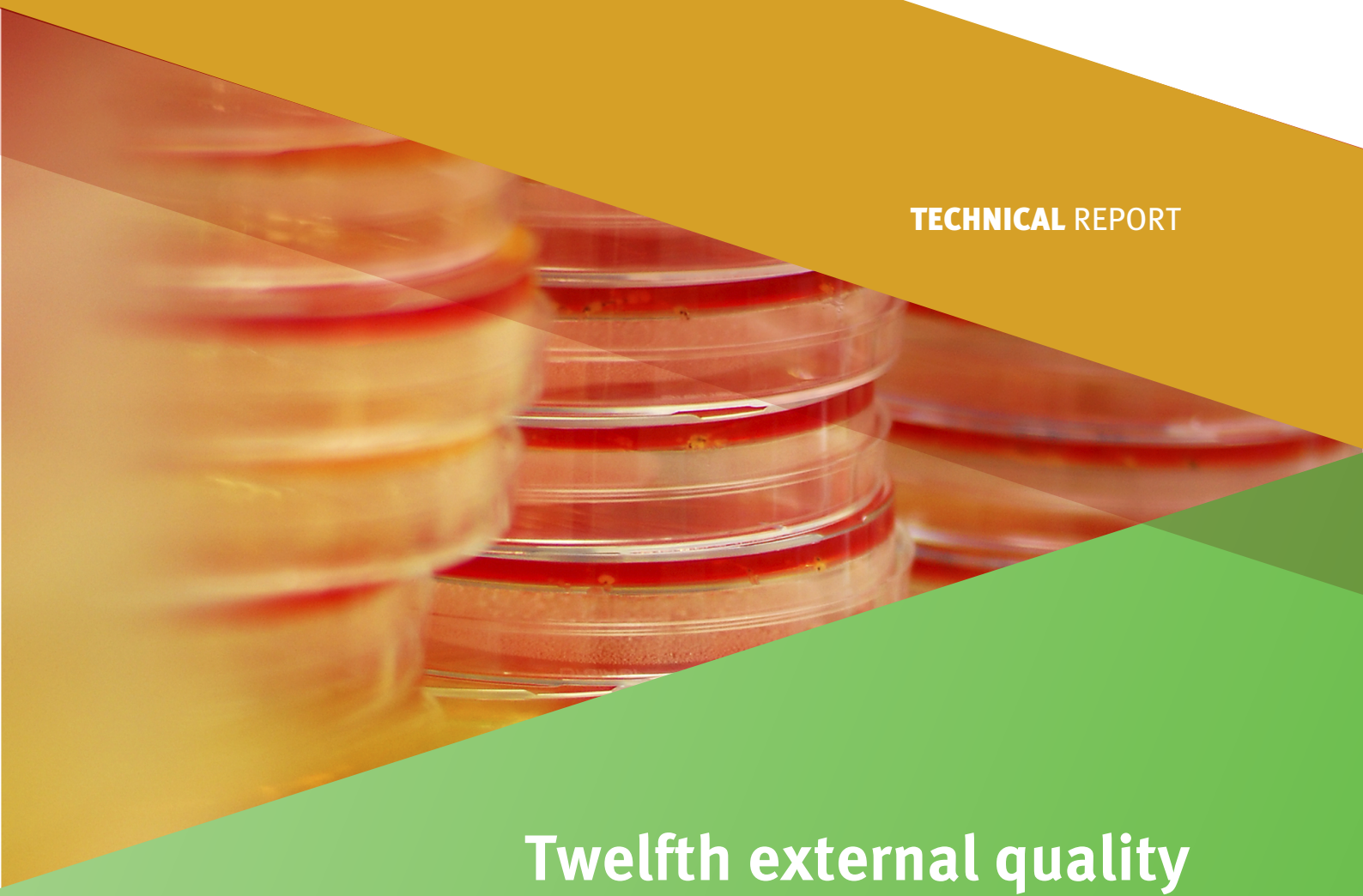

TECHNICAL REPORT

Twelfth external quality assessment scheme for *Salmonella* typing

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Twelfth external quality assessment scheme 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 Dutch National Institute for Public Health and the Environment (RIVM).

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Acknowledgements

Alejandra Hernandez Segura, Wilma Jacobs-Reitsma and Eelco Franz are acknowledged for their contribution to this EQA.

Suggested citation: European Centre for Disease Prevention and Control. Twelfth external quality assessment scheme for *Salmonella* typing. Stockholm: ECDC; 2023.

Stockholm, April 2023

ISBN 978-92-9498-631-3
doi: 10.2900/696916
Catalogue number TQ-03-23-120-EN-N

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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
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 60 050 reported cases in 2021 in the European Union (EU) and *Salmonella* is associated with the highest number of foodborne outbreaks. The overall EU trend of salmonellosis incidence for the years 2017 to 2021 have not changed significantly. To prevent foodborne diseases such as salmonellosis, human surveillance systems at different levels are essential to monitor the disease and to have an early detection and response to outbreaks.

ECDC has set surveillance objectives to monitor trends and perform multinational outbreak detection of salmonellosis and other foodborne pathogens. In addition, objectives are to contribute to 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 the burden of disease, to generate hypotheses on sources and transmission modes, and to 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 their comparability as well as 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 12th external quality assessment scheme for *Salmonella* typing (EQA-12) was subcontracted to the National Institute for Public Health and the Environment (RIVM) in the Netherlands and comprised a serotyping part and a molecular typing-based cluster analysis part. Participants were expected to use their routinely applied methods for both parts of the EQA and were assessed for their performance. Serotyping consisted of 12 isolates with different carefully selected serovars. For cluster analysis 10 *S.* Enteritidis isolates were selected, containing cluster and non-cluster isolates, mimicking a real outbreak situation originated at a wedding dinner. In addition, raw reads of five isolates were made available to the participants that use whole genome sequencing (WGS) for cluster-analysis. These isolates acted as food isolates and participants were asked which food product was likely causing the outbreak.

For serotyping, 24 laboratories participated and 79% (19/24) of these laboratories used phenotypic serotyping based on antisera agglutination, 17% (4/24) used serotype prediction from WGS data and 4% (1/24) applied a combination of genetic serotyping using Luminex, supplemented with phenotypic methods. Performance was high for most laboratories, with 12 laboratories achieving performance scores of 100% and eight of 92%. The four laboratories that have the lowest performance values (<92%) were all using phenotypic methods, and for three out of four, their type of errors indicate that less specific antisera were used. However, the choice of phenotypic serotyping or WGS-predictive serotyping did not influence overall performance ($p=0.1134$) or the ability to type particular serovars ($p=0.418$-0.818).

Twenty laboratories took part in the molecular typing-based cluster analysis, which was the same number of participants than for the last EQA (EQA-11, 2021). The proportion of participants that used WGS for their cluster analysis increased from 70% to 85% compared to EQA-11, while the number of participants that applied multiple locus variable-number tandem repeat analysis (MLVA)-based cluster analysis decreased from 40% to 15% and pulsed-field gel electrophoresis (PFGE)-based cluster analysis participants decreased from 30% to 10%.

In the WGS-based cluster analysis, most participants 14/17 (82%) applied a gene-by-gene approach, while the other 18% (3/17) applied single nucleotide polymorphism (SNP) typing. In total, 15 different combinations of platforms, approaches, kits, cluster analysis tools, typing schemes, and cluster cut-offs were used. However, methods used did not affect the high performance, with an overall performance score of 98% correct cluster assignment for provided isolates and provided sequences. All but one laboratory had 100% performance in assigning provided isolates to clusters, and one additional laboratory did not assess a sequence because it was considered to be of insufficient quality.

Three laboratories applied MLVA-based typing and all produced identical MLVA profiles, indicating a high technical performance of 100%. However, overall performance for cluster assignment of provided isolates was lower (74%) than with WGS-based cluster analysis (98%, $p=0.0021$), as two genetically unrelated isolates would be included in the outbreak cluster based on MLVA profiles. Therefore, participating laboratories had good capability in applying MLVA, but the resolution of the technique itself is too low to correctly assign isolates to clusters.

Two laboratories applied PFGE-based cluster analysis. The performance of these laboratories was compared to each other, which confirmed that PFGE is not portable and interlaboratory comparability was low. Overall performance for cluster assignment of provided isolates was lower (72%) than with WGS-based cluster analysis (98%, $p<0.0001$). In addition, difficulty with definitive cluster assignment was observed.

Laboratories are recommended to use WGS-based cluster analysis at least in outbreak situations. The use of PFGE-based cluster analysis is not recommended because of the inferior resolution, the poor portability, and the limited

use which hampers the use in (inter)national outbreak assessments in which multiple institutes are involved and therefore hampers the fulfilment of the surveillance objectives of ECDC.

With the provision of the EQA-12, the typing methods used, including their quality and comparability as well as the capability of the performing laboratories were monitored and assessed. After distribution of the individual evaluation reports, a feedback survey was sent to participating laboratories. Results from a feedback survey showed that multiple laboratories took corrective actions based on the results of EQA-12, proving the added value of this EQA to typing capability of the NPHRLs in the EU/EEA and enlargement countries. Maximum capability and capacity of the NPHRLs contributes to surveillance and outbreak detection on a regional and national level as well as to the fulfilment of 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 with a mission to identify, assess, and communicate current and emerging threats to human health from infectious diseases. ECDC's founding regulation outlines its mandate as fostering the development of sufficient capacity within EU/EEA-dedicated surveillance networks for the diagnosis, detection, identification, and characterisation of infectious agents that may threaten public health. ECDC maintains and extends such cooperation and supports the implementation of quality assurance schemes [1].

EQAs are an essential part of laboratory quality management and use an external contractor to assess the performance of laboratories on test samples supplied specifically for the purpose of quality assessment.

ECDC has outsourced the organisation of EQA schemes for EU/EEA countries in disease networks. EQAs aim to identify areas for improvement in laboratory diagnostic capacities relevant for epidemiological surveillance of communicable diseases as in Decision No 1082/2013/EU [2] and ensure the reliability and comparability of results generated by laboratories across all EU/EEA countries. The main objectives of EQA schemes are to:

- assess the general standard of performance ('state-of–the-art');
- assess the effects of analytical procedures (method principle, instruments, reagents, calibration);
- support method development;
- evaluate individual laboratory performance;
- identify problem areas;
- provide continuing education; and
- 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 the RIVM by ECDC. In this report, the aggregated results of the EQA *Salmonella* serotyping and molecular-based cluster analysis of 2022 (EQA-12) are presented.

1.2 Salmonellosis impact and surveillance objectives

Salmonellosis is caused by non-typhoidal *Salmonella* serovars and presents usually 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 60 050 reported cases in the EU in 2021, and it accounts for the highest number of 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 of salmonellosis for the years 2017 to 2021 have 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, which is regulated by the EU in 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 further, employment of surveillance systems at different levels is essential to monitor disease and for early outbreak detection and response [4]. International networks for human surveillance were set up following EU Decision 1082/2013/EU3 'on serious cross-border threats to health' [5].

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

ECDC has set surveillance objectives that were translated by the FWD-Net to specifically food-and waterborne diseases and zoonoses such as salmonellosis [6, 7]. First, trends in disease and antimicrobial resistance for Salmonella are monitored over time and across EU/EEA countries. In each country, NPHRLs perform surveillance on a national or regional level based on data and/or submitted samples from clinical microbiology laboratories. The resulting disease-based surveillance data are reported to ECDC by mandate of the EU using The European Surveillance System (TESSy) [7]. Second, multinational outbreaks of salmonellosis are detected and monitored with respect to source, time, population and place to provide a rationale for public health action [7]. 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]. Salmonellosis data reported by the FWD-Net is analysed for trends and outbreaks by ECDC and performed and summarised in annual epidemiological reports and EU One Health Zoonoses Reports [2, 10]. Using and analysing all these data specifically collected for salmonellosis, ECDC and FWD-Net can pursue their remaining surveillance objectives. These objectives are: contributing to evaluation and monitoring of prevention and control programmes, identifying population groups at risk and in need of targeted prevention, contributing to the assessment of the burden of disease, and generating hypotheses on sources, transmission modes and identifying needs for research projects [6, 7].

1.3 Microbiological methods and quality assessment

Microbiological surveillance for salmonellosis takes place in the EU/EEA countries, mostly in 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 in which 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.

Next to establishing serovars, relatedness of encountered isolates is often assessed by the NPHRLs using molecular-based clustering techniques. Traditionally, PFGE and MLVA were frequently used molecular subtyping methods. However, over the past 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 the surveillance objectives of ECDC and FWD-Net relies heavily on the data provided by the NPHRLs of the EU/EEA countries. Therefore, it is important to monitor the typing methods used, data quality and comparability as well as the capability of the laboratories performing these methods. ECDC organises EQAs for NPHRLs to facilitate harmonisation and increase 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 the 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]. Specific priority for the implementation of WGS for continuous surveillance and multi-country outbreak investigations was conferred to food and waterborne diseases and zoonoses, such as *Salmonella* enterica [18].

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

- foster the rapid detection of dispersed international clusters/outbreaks;
- facilitate the detection and investigation of transmission chains and relatedness of strains across EU/EEA
- and contribute to global outbreak investigations;
- detect the emergence of new and/or evolving pathogenic strains;
- support investigations to trace the source of an outbreak and identify new risk factors; and
- aid the study of particular pathogen's characteristics and behaviour in 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-12 on *Salmonella*

EQA schemes offer quality support to those NPRLs 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-12 was to assess the capabilities regarding identification of the *Salmonella* serovars within 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-12 was to assess the ability regarding cluster designations of *Salmonella* isolates within NPHRLs of the EU/EEA and enlargement countries. Laboratories were able to use WGS, MLVA or PFGE techniques to perform the cluster analysis on provided isolates. This made it possible to monitor the methods used and their performance regarding 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-12 was organised by RIVM under the 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-12 were distributed by ECDC to the FWD-Net contact points for EU/EEA countries. In addition, the enlargement countries Albania, Bosnia and Herzegovina, Kosovo[i], Montenegro, North Macedonia, Serbia, and Türkiye were requested to nominate a laboratory for the EQAs in 2022-2025.

Participating laboratories were able to register for the EQA-12 via an online form using a link in the invitation. The online form contained questions including contact person, shipping address, whether the participant will participate in both parts (serotyping and cluster analysis) and the main methods used (Annex 1).

The EQA-12 comprised of two parts, serotyping and molecular typing-based cluster analysis. Laboratories were encouraged to participate in both parts, but participation in one of the parts was possible. In total, 36 countries were invited, 30 (83%) of them registered for participation in at least one part, of which 27 (75%) completed at least one part of the assessment (Annex 2).

2.1.1 Timeline

The invitation for the EQA-12 was sent on 8 April 2022, and deadline for registration was set at 30 April 2022. A reminder was sent on 28 April, and the final participant list was composed on 4 May 2022 which contained 30 participating laboratories.

The samples were distributed to 30 laboratories on 4 July 2022. A total of 22 laboratories (73%) received the parcel the day after, seven laboratories (23%) received it two days after shipping, and one laboratory received the parcel after 21 days because of a delay in customs clearance.

The deadline for result reporting was 22 September 2022. One laboratory cancelled their participation before deadline and two did not submit results even after multiple reminders. Three laboratories requested an extended deadline. The first results were completed on 1 August and the last on 30 September 2022, with a median of 79 days (range 28–88 days) from shipping to result completion. Individual evaluation reports were shared with participants on 22 November 2022 as scheduled.

2.2 Sample preparation

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

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 EQA2201-EQA2212. The selected isolates were tested blindly using traditional agglutination by another team member to reach an expert consensus about 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.

For the cluster analysis, a mock outbreak situation was provided to the participants: 'A wedding dinner was served to 50 people. Two days after, 43 of the attending party guests and three restaurant employees fell ill with diarrhea. A *Salmonella* isolate was cultured from the feces of the head chef, isolate EQA2213. After this, a microbiological investigation in the remaining cases rendered nine isolates of nine more cases.' To mimic this outbreak situation, nine *S.* Enteritidis 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 (EQA2216 and EQA2221), resulting in ten isolates for the cluster analysis (EQA2213-EQA2222). Participants were requested to report the isolates that clustered with the index case (EQA2213) according to their own cluster cut-off. When using WGS techniques, three out of 10 *S.* Enteritidis isolates clustered closely. Two other

[i] This designation is without prejudice to positions on status, and in line with UNSCR 1244/99 and the ICJ Opinion on the Kosovo Declaration of Independence.

isolates were related around the cluster cut-off in the hands of the provider. Therefore, a participant could report three to five isolates in the cluster, depending on the resulting allele distance and their used cluster cut-off.

Table 1. Selected panel for serotyping part of EQA-12, including selection rationale

EQA #	Subspecies[a]	Serovar	Formula	Rationale
EQA2201	*enterica*	Enteritidis	1,9,12:g,m:-	Five most reported serovars of human salmonellosis in Europe [2], of which some also caused multi-country outbreaks in 2020-2022
EQA2202	*enterica*	Typhimurium	1,4,[5],12:i:1,2	
EQA2203	*enterica*	Typhimurium, monophasic	1,4,[5],12:i:-	
EQA2204	*enterica*	Infantis	6,7,14:r:1,5	
EQA2205	*enterica*	Derby	1,4,[5],12:f,g:[1,2]	
EQA2206	*enterica*	Braenderup	6,7,14:e,h:e,n,z_{15}	Serovars that caused multi-country outbreaks in 2020-2022
EQA2207	*enterica*	Montevideo	6,7,14, [54]:g,m,[p],s:[1,2,7]	
EQA2208	*enterica*	Goldcoast (or Brikama)	6,8:r:l,w	Challenging serovar when using molecular methods only
EQA2209	*enterica*	Leeuwarden	11:b:1,5	Challenging serovar present in EURL-*Salmonella* PT for veterinary and food NRLs
EQA2210	*enterica*	Amsterdam	10:g,m,s:-	To include some diverse O- and H-types
EQA2211	*enterica*	Senftenberg	1,3,19: g,s,t: -	To include some diverse O- and H-types
EQA2212	*houtenae* (or *arizonae*/ *diarizonae*)	IV 50:z_4,z_{23}:-	IV 50:z_4,z_{23}:-	Different subspecies as challenging isolate

[a]*All isolates were Salmonella enterica.*

All 10 isolates were analysed with MLVA (Table 2). Using MLVA, five out of nine isolates cluster to the index EQA2213 because they exhibit exactly the same MLVA profile, with one isolate closely related with one repeat difference. This indicates the lower resolution of the typing technique as opposed to WGS. Another team member analysed the same data to reach consensus about the assigned clustering using cgMLST and MLVA. All isolates were stored in agar slants at room temperature until bulk culturing.

Table 2. Selected panel for molecular-based cluster analysis part of EQA-12

# EQA	Serovar	Part of cluster[a]	Distance to index cgMLST(AD)	Distance to index cSNPs	MLVA profile	AMR markers
EQA2213	Enteritidis	Index	0	0	03-10-04-04-01	aac(6')-Iaa
EQA2214	Enteritidis	No	70	132	03-10-04-04-01	aac(6')-Iaa, tet(A)
EQA2215	Enteritidis	No	219	470	02-09-09-04-02	aac(6')-Iaa
EQA2216[b]	Enteritidis	Yes	0	1	03-10-04-04-01	aac(6')-Iaa
EQA2217	Enteritidis	Borderline[c]	8	10	03-10-04-04-01	aac(6')-Iaa
EQA2218	Enteritidis	No	252	554	02-10-08-05-01	aac(6')-Iaa
EQA2219	Enteritidis	Borderline[c]	6	9	03-10-04-04-01	aac(6')-Iaa
EQA2220	Enteritidis	No	85	152	03-10-05-04-01	aac(6')-Iaa
EQA2221[b]	Enteritidis	Yes	0	1	03-10-04-04-01	aac(6')-Iaa
EQA2222	Enteritidis	No	670	1824	02-11-09-03-01	aac(6')-Iaa

cSNPs = core single nucleotide polymorphisms. [a]Cluster assignment of EQA provider based on cgMLST [b]Technical duplicates. [c]Related to index around cluster cut-off, belonging to cluster or not depending on the allele distance of participants and their used cluster cut-off.

The clustering of isolates using PFGE was not known beforehand. Because the RIVM does not perform PFGE anymore, results of participants that used PFGE for the cluster analysis were compared to each other and 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 wedding consisted of different food products from which left-overs were saved in the refrigerator by the caterer. The left-overs were sampled by the food authorities and *Salmonella* isolates (coded EQA2223-EQA2227) cultured from these food products were sequenced using Illumina WGS techniques.' To mimic this additional outbreak investigation, raw reads of five additional isolates were selected or manipulated and made available to the participants that use WGS for cluster-analysis. These isolates acted as the food isolates and participants were asked which food product was likely causing the outbreak. The characteristics of these reads are depicted in Table 3. All reads were analysed for quality and clustering with the index case EQA2213 by another team member to reach consensus.

Table 3. Additional raw reads provided for WGS analysis

# EQA	Serovar	Manipulation	Quality	Distance to index cgMLST(AD)	Distance to index cSNPs	AMR markers
EQA2223	Enteritidis	None, technical duplicate of index	Good quality	0	0	aac(6')-Iaa
EQA2224	Enteritidis	None, regular non-cluster sequence	Good quality	853	3334	aac(6')-Iaa
EQA2225	Enteritidis	80% of reads of a regular non-cluster *S.* Enteritidis were combined with 20% *Escherichia coli* reads	Bad quality, contaminated	NA	NA	NA
EQA2226	Enteritidis	Reads of regular non-cluster *S.* Enteritidis down sampled to 5% of the original read number	Bad quality, too few reads	NA	NA	NA
EQA2227	Monophasic Typhimurium	None, regular non-cluster sequence of a different serotype	Good quality	2577	31407	aac(6')-Iaa, aph(6)-Id, aph(3")-Ib, blaTEM-1B, sul2, tet(B)

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 criterion 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 passing criterion 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. For assessment of 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 the last day 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 time points, indicating a stable serotyping panel. The specimens of the molecular typing-based panel were sequenced at all time points and analysed using cgMLST. All samples fell within the same sample clusters at all time points, indicating a stable 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 3373 regulation.

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 White-Kauffman-Le Minor [11].

For production of WGS data, DNA from pure isolates was extracted using the Sigma Genelute Bacterial Genomic 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 2x 150 bp paired-end reads. Reads were processed using the in-house developed quality control and assembly pipeline 'Juno-assembly' v2.0.6 [19] based on SPAdes 3.15.3 [20], consisting of FastQC v0.11.9 [21] to assess the quality of the raw reads, FastP v0.20.1 [22] to remove poor quality data and adapters, Picard v2.26.0 [23] for library fragment determination, QUAST v5.0.2 [24], Bbtools v38.86 [25] and MultiQC v1.11 [26] for assessing and visualising 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. Using the filtered and trimmed reads output the *Salmonella* serotype was predicted using the in-house developed pipeline 'Juno-typing' v0.5.0 [30] based on SeqSero2 v1.1.1 in micro-assembly mode. Antimicrobial resistance (AMR) markers were detected from the filtered and quality trimmed reads using 'Juno-AMR' v0.4 [31], based on ResFinder and PointFinder v4.1.3.

For cluster analysis, *de novo* assemblies were used for cgMLST and imported into Ridom SeqSphere v8.3.1, in which the Enterobase *S. enterica* cgMLST V2 scheme (3,002 loci) was used. Hamming distances were calculated, ignoring pairwise missing alleles and distances were visualised with a Minimum Spanning Tree (MST). For SNP-analysis, the in-house developed pipeline 'Juno-SNP' (accessed on 24-10-2022) [32] was used to establish core SNP variants against reference EQA2213, 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, SENTRE6, SENTRE4, and SE3 were analysed using BioNumerics v7.6.3.

PFGE typing is not performed by the provider, so results of participants using PFGE were compared between each other. For this comparison, a dendrogram using an unweighted pair group method with arithmetic mean (UPGMA) was created using BioNumerics v7.6.3 based on the Dice coefficient with a position tolerance of 1.5% and an optimisation setting of 0.5%.

2.4 Results assessment and reporting

Participants were expected to use their routinely applied methods for both parts of the EQA. Information about their analytical methods and their 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 requested for more information via email if 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 EQA2208, and for participants that used WGS prediction both 'Goldcoast' and 'Goldcoast or Brikama' were considered as correct results. As a challenging isolate, another subspecies than *enterica* was added (EQA2212). Confirmatory biochemical testing of EQA2212 by the provider already showed that this isolate produced atypical results, and subspecies *houtenae* and *arizonae/diarizonae* were indistinguishable. Therefore, all three subspecies were considered correct if using phenotypical methods. With serotype prediction using WGS, the result of this isolate was unambiguously subspecies *houtenae*, and this was the only result considered correct if participants used WGS prediction.

Some laboratories reported genus instead of species in the result form or did not use the correct notation for seroformulas. 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.

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 routinely used analysis pipelines to evaluate genetic relatedness including the raw reads provided by the organiser. In addition, participants should be able to assess quality of the provided raw reads, including indicating the specific issues if quality was insufficient. For the isolates that clustered around cluster cut-off (EQA2217 and EQA2219), cluster or singleton assignment were both considered correct, as long as they were in concordance with their own cut-off. In addition, all participants were welcomed to optionally report detected AMR markers if WGS methods were used. As methods for the AMR analysis were not requested in the online results form, an assessment of the correctness of AMR detection was not performed by the EQA provider. Performance can be assessed by the participants themselves based on overall reported AMR markers. For a thorough assessment on AMR detection in *Salmonella*, we would like to refer to the results of ECDCs EQA-4 on antimicrobial susceptibility testing and detection of ESBL-, acquired AmpC-, and carbapenemase-production of *Salmonella* [37] and the EQAs organised within the FWD AMR-RefLabCap project that have a focus on AMR detection in *Salmonella* and *Campylobacter*.

Participants were required to upload their raw reads (.fastq or .fastq.gz) to the Research Drive sharing platform. The quality and distances to index EQA2213 of the sequences generated by participants using Illumina techniques was assessed by the EQA provider, using methods for quality assessment as described in chapter 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. It is already known from the provider's results that for the selected panel a 100% correct cluster assignment cannot be achieved using MLVA, regardless of performance on the technique. For participants that used PFGE for cluster analysis (n=2), results were compared within this group of participants to determine performance limits as well as to the cluster assignment using WGS.

All descriptive analyses and comparison of groups, including visualisation, were performed using Microsoft Excel, IBM SPSS Statistics 28 and R v4.2.1 and ggplot2 v3.3.6 [38].

2.4.2 Reporting of results

For serotyping, all results were analysed per participant and reported in the individual evaluation reports, including a percentage of correctly reported serovars. In case serovars were incorrectly reported, specific comments by the EQA provider were made. In this way, participants were able to easily interpret their own performance. If participants reported an incorrect serovar for two or more samples, assistance from the EQA provider was offered in the individual evaluation report.

For the molecular-based cluster analysis part, results were analysed per participant and reported 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 troubleshooting advice if necessary.

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

2.5 Feedback survey

On 16 December 2022, following distribution of the individual evaluation reports, a feedback survey was sent to participating laboratories that had completed the EQA-12 (Annex 4). In this survey, experiences of the participants 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

Of the 27 laboratories that completed at least one part of the assessment, 25 had registered for serotyping, of which 24 submitted results. Two laboratories registered for the molecular-based cluster analysis but did not participate in the serotyping. Reasons for not participating were that they would like to focus on the WGS-based cluster analysis (n=1) or that the costs for performing WGS in this EQA are very high to sequence all distributed 22 isolates for both parts (n=1).

In this section the results of the 24 laboratories that have completed the serotyping part are described.

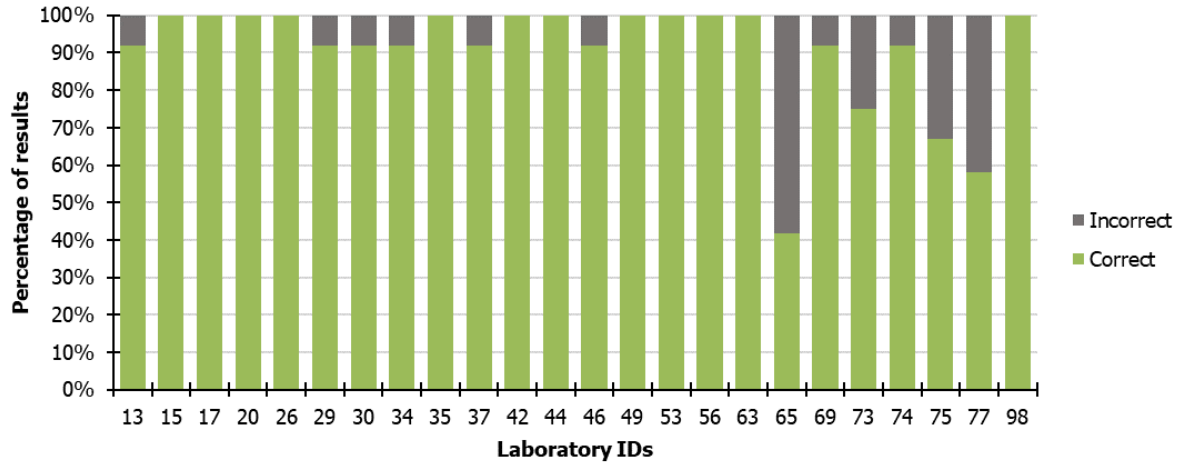
3.1.1 Methods used by participants

Of the 24 laboratories that had completed serotyping results, 19 (79%) used phenotypical serotyping based on agglutination with antisera, four laboratories (17%) used prediction of serotype with WGS and one laboratory used a combination of molecular genetic serotyping with Luminex techniques combined with phenotypical serotyping based on agglutination. Details of methods per participating laboratory can be found in Annex 5.

3.1.2 Results of participants

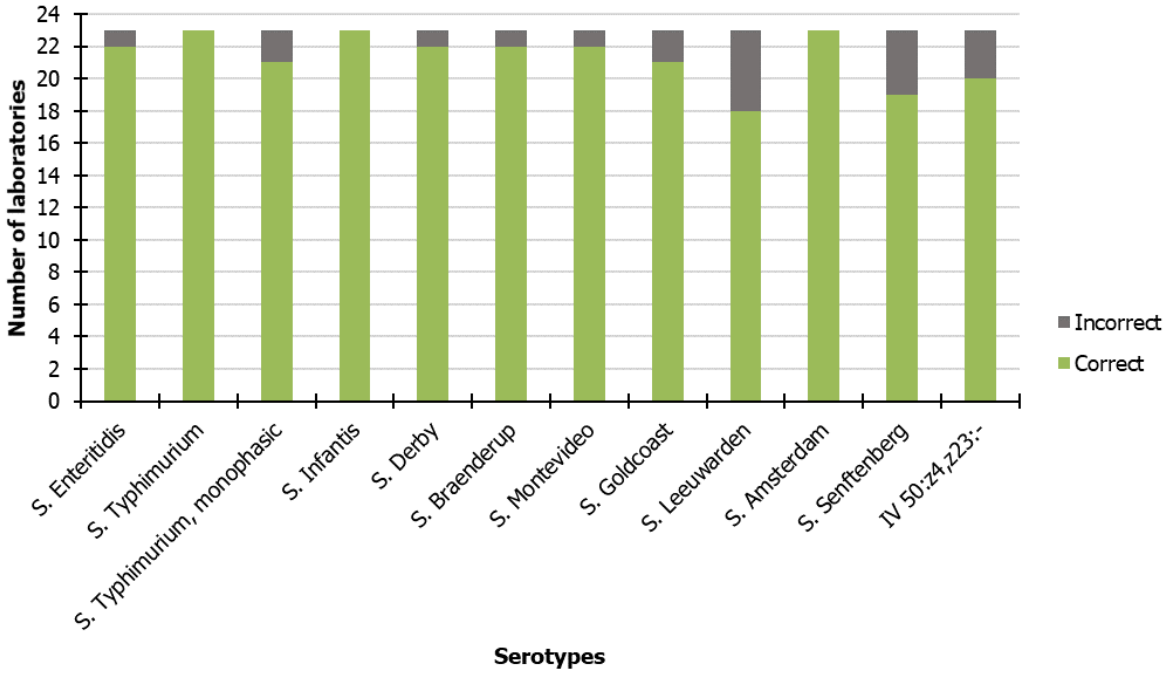
In total, 12 out of 24 laboratories (50%) serotyped all isolates correctly, resulting in a performance score of 100%, and eight laboratories (33%) had a performance score of 11 out of 12 (92%) (Figure 1). Three laboratories serotyped three to five isolates incorrectly, resulting in 75%, 67% and 58% as performance scores (Figure 1). 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. Therefore, it was only possible to assess which O- and H-antigens were false-positively detected by this laboratory. Their results indicated that for seven out of 12 isolates (58%) O- and H-antigens were detected that should not be present; therefore at least 58% of isolates were considered assigned incorrectly (Figure 1). A detailed description of all serotyping results per participating laboratory is shown in Annexes 6 and 7.

Figure 1. Results of serotyping by participating laboratories



For the calculation of performance values per sample, laboratory 65 was excluded due to its limited set of antisera. Three out of 12 samples were correctly serotyped by all the remaining 23 laboratories (Figure 2), *S.* Typhimurium (EQA2202), *S.* Infantis (EQA2204) and *S.* Amsterdam (EQA2210). *S.* Enteritidis (EQA2201), *S.* Derby (EQA2205), *S.* Braenderup (EQA2206) and *S.* Montevideo (EQA2207) were correctly serotyped by 22 of 23 laboratories. *S.* Typhimurium monophasic variant (EQA2203) and *S.* Goldcoast (EQA2208) were correctly serotyped by 21 of 23 laboratories (Figure 2). *S.* IV 50:z4,z23:- (EQA2212), *S.* Senftenberg (EQA2211) and *S.* Leeuwarden (EQA2209) were correctly serotyped by 20, 19 and 18 out of 23 laboratories, respectively (Figure 2).

Figure 2. Results of serotyping by serotype



Combining all results from all 24 laboratories that performed serotyping together, 261 of 288 isolates (91%) were correctly assigned to the serotype (Annex 7). For the 27 incorrectly assigned serotypes, 41 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 (88%, 36/41) were made only once, 10% (4/41) of the errors were made twice by two different laboratories, and one error, the misclassification of O3,10 instead of O3,19 for *S.* Senftenberg (EQA2211) was made by three different laboratories. A detailed description about concordance and error type per sample is shown in Annex 8.

After excluding laboratory 65 because of their limited set of antisera and laboratory 63 because of the uniquely used typing technique (genetic serotyping based on Luminex), the methods used by 22 laboratories were compared to assess if the use of phenotypic (n=18) or WGS predictive (n=4) methods has an effect on overall performance. There was no difference observed in performance score of any sample based on the method used (p values ranging from 0.418 to 0.818, Fisher's exact test). Combining all samples serotyped by laboratories using phenotypic methods, 197 out of 216 serovars were correctly assigned (91.2%). For all samples serotyped by laboratories using WGS predictive typing, 47 out of 48 serovars were correctly assigned (97.9%). There is no statistically significant difference between these proportions ($p=0.1134, \chi 2$). These results indicate that the method did not influence the overall performance of serotyping.

3.2 Molecular-based cluster analysis

Of the 27 laboratories that have completed at least one part of the assessment, 22 had registered for the molecular-based cluster analysis, of which 20 submitted results. Five laboratories registered for the serotyping but did not register for the cluster analysis. Reasons for not participating were that laboratories do not perform molecular typing (n=2), a lack of resources or capacity (n=2) and that set-up for such analysis was not in place in time for this EQA (n=1).

In this section the results of the 20 laboratories that have completed the molecular-based cluster analysis part are described per used technique.

3.2.1 Methods used by participants

Of the 20 laboratories that have completed molecular-based cluster analysis results, two used PFGE only (10%), one used MLVA only (5%), and 17 used WGS techniques (85%) of which two laboratories also submitted MLVA results. Details of methods per participating laboratory can be found in Annex 9.

Of the 17 laboratories that used WGS for their cluster analysis, 16 (94%) used Illumina as a platform and one used Ion Torrent. A total of 14 laboratories (82%) used a gene-by-gene-approach and three (18%) used SNP typing (Annex 9). Of the laboratories that used Illumina sequencing (n=16), eight (50%) used the Nextera XT DNA Library kit, five used the Illumina DNA Prep kit (31%), one used the NEBNext Ultra ™ II FS DNA Library Prep Kit,

one used KAPA HyperPlus and one laboratory reported their sequencing reagent kit (Miseq Reagent kit V3) as library preparation kit (Annex 10).

Of the 14 laboratories that used a gene-by-gene approach, 7 (50%) used SeqSphere as MLST tool. Enterobase, Bionumerics or ChewBBACA were used by two laboratories each (14% each) and one laboratory used an in-house pipeline based on an unknown tool (Annex 10). Enterobase MLST schemes were used most frequently (12/14, 86%), one laboratory used the INNUENDO scheme and one an in-house developed scheme. A median of 5 allelic distances was used as cluster cut-off (range: 1-12), although five laboratories commented on their used cluster cut-off that this is not a fixed number and also depends on other circumstances (Annex 10).

All of the three laboratories that used SNP typing, used the index EQA2213 as reference genome in their analysis. Unfortunately, no field in the electronic results form was available reporting about the SNP tools used, therefore this is unknown. Cluster cut-off varied: two, five, or 30 SNPs were used (Annex 10).

Species confirmation of the resulting WGS data is performed by 16 (94%) laboratories, most of them (53%) use kraken/kraken2, either alone or in combination with another tool (Annex 10). Other tools used are Mash/Mash Screen (n=4), SeqSero (n=2), KmerFinder (n=2), Jspecies (n=1), SpeciesFinder (n=1), rMLST (n=1), Enterobase (n=1) and BLAST towards an in-house database (n=1).

In conclusion, for WGS-typing 15 different combinations of platforms, approaches, kits, tools, schemes, and cluster cut-off were used by the 17 participating laboratories, showing very diverse methods and combinations of those used for WGS-based cluster analysis (Annex 10).

Laboratories were asked to report their routinely used parameters and thresholds for quality control of WGS data. Laboratory 29 reported that they performed quality control, however they did not report the parameters assessed and their thresholds. The other 16 laboratories reported their parameters. Coverage was the most frequently assessed parameter (13/16, 81%) and thresholds varied from 20 to 80 (median 40x), however coverage of contigs and unassembled reads were both assessed and not always defined as such. Genome size was assessed by 12 laboratories (75%), 10 laboratories (63%) determined the number of contigs or N50 value, nine laboratories (56%) assessed the percentage of good targets in the MLST scheme used and two laboratories (19%) checked the quality of reads using a Q score (Phred). Seven laboratories (44%) assessed contamination of the sample, either directly, by the percentage of species assignment, GC content or a combination of these methods. More details on parameters used and their threshold assigned by the participants can be found in Annex 11.

Although optional if using WGS-based cluster analysis, 13 laboratories also reported their detected AMR markers (Annex 9). As this part was optional, no enquiries were made about the methods used and are therefore unknown.

Three laboratories have performed MLVA-based cluster analysis, of which two laboratories did so in addition to their WGS-based cluster analysis (Annex 9). As MLVA cluster cut-off, 1 locus difference was used by one laboratory, the second laboratory used not more than 1 technical repeat difference on a maximum of 2 loci as cut-off and the last laboratory has not reported their used cluster cut-off.

Two laboratories used PFGE, for which methods and cluster cut-off could not be reported and are therefore unknown (Annex 9).

3.2.2 Results WGS-based cluster analysis

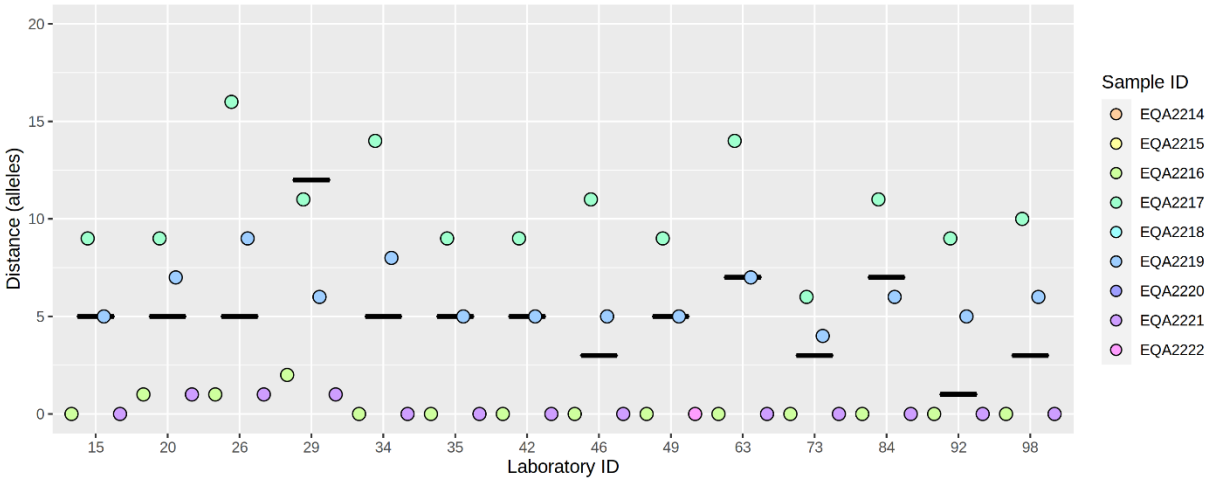
Using WGS-based cluster analysis, almost all isolates (167/170, 98.2%) were assigned correctly to the cluster of index EQA2213 or as singleton, despite all various methods used (Annex 12). This performance calculation was based on cluster or singleton assignment of provided isolates EQA2214-EQA2222 and provided good quality raw reads EQA2223, EQA2224 and EQA2227. For borderline cluster isolates EQA2217 and EQA2219 assignment to cluster or singleton isolate were both considered correct. Two isolates were incorrectly assigned by laboratory 49, which seems to be caused by a sample swap of EQA2221 and EQA2222. Laboratory 63 decided that provided raw reads of EQA2227 were of insufficient quality because of contamination with *Escherichia coli*, while the contamination percentage of *E. coli* was <0.5%. All other laboratories assigned all isolates correctly to a cluster or singleton (Annex 12).

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 EQA2216 and EQA2221 have none or a few distances to reference EQA2213, except for laboratory 49, where EQA2221 seemed to be swapped with EQA2222 (Figure 3). For all laboratories, EQA2217 and EQA2219 differentiate from EQA2213 around cluster cut-off, with EQA2219 being closer to the index (Figure 3). However, assignment to the cluster is dependent on the measured allelic distance (AD) and cut-off used by the participants (Figure 3).

Assessing the reported distance by laboratories that used SNP typing also showed none or one SNP difference of EQA2216 and EQA2221 to the index EQA2213 and distance around cluster cut-off for EQA2217 and EQA2219, except for laboratory 77 (Figure 4). Laboratory 77 found lower SNP differences for all samples than the provider and other laboratories. This, combined with the higher reported cluster cut-off, caused a closeness to the cut-off of

isolates EQA2214 and EQA2220 that was not reported by other laboratories (Figure 4). Although it did not cause incorrect cluster assignment of isolates, this might prompt laboratory 77 into further investigations of these isolates if this were a real outbreak situation. More information about the reported distances to index EQA2213 and their medians per sample can be found in Annex 13.

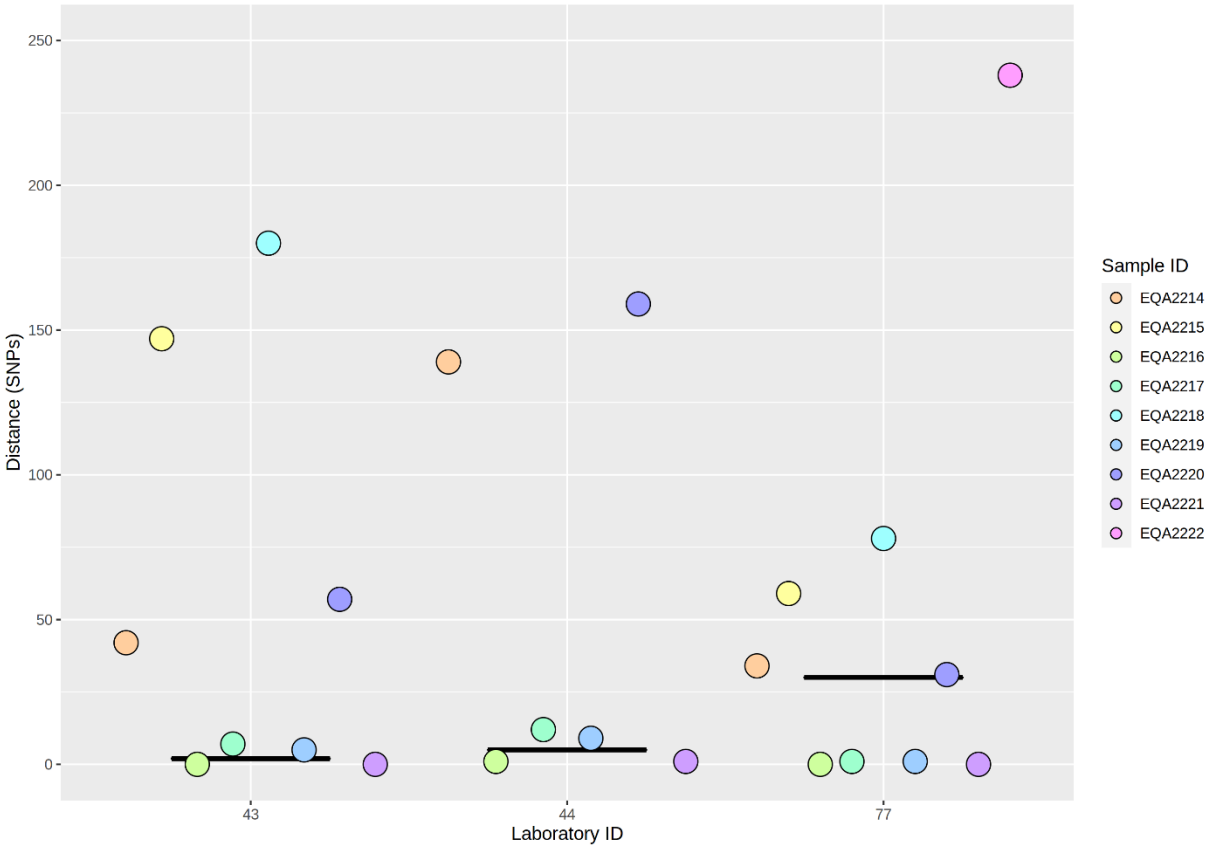
Figure 3. Distance from index EQA2213 in alleles for distances <20 AD, per laboratory



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

To assess the differences of the sequences that participants produced from the isolates EQA2213-EQA2222, without taking all the different analysis methods into account, all submitted raw reads of the participants were analysed using the cgMLST methods of the provider as described in chapter 2.3. A minimum spanning tree (MST) was produced using Ridom SeqSphere (Figure 5).

Figure 4. Distance from index EQA2213 in SNPs for distances <250 SNPs, per laboratory

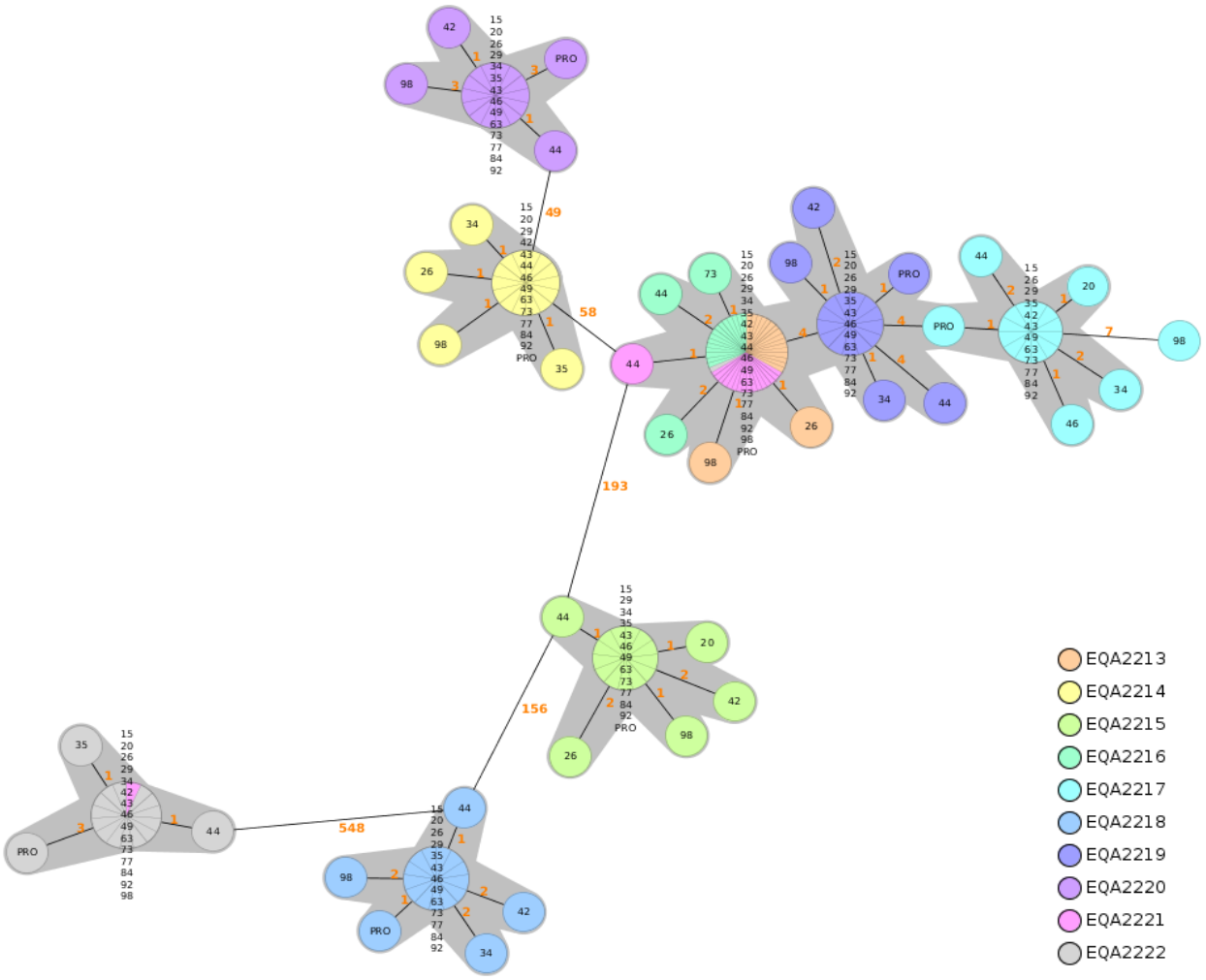


All sequences of the same samples clustered together using the cluster cut-off from provider (≤5 AD), except EQA2217 from laboratory 98. However, this isolate was still in a close range (7 AD) of the cluster and would be considered as probably related and would prompt further investigation in an outbreak situation. The analysis confirmed that samples EQA2221 and EQA2222 from laboratory 49 were swapped as their presence in the sample cluster was also swapped (Figure 5).

Three out of 17 laboratories (18%) did not detect the inferior quality of EQA2225 that was artificially contaminated with E. coli by the provider (Annexes 12 and 13). These laboratories performed no quality control on parameters that assess contamination, either directly or indirectly via percentage of species assignment or GC content (Annex 11). However, one of them reported that they assessed genome size, which could point to inferior quality because the genome is too large for the genus *Salmonella*. In addition, two other laboratories (12%) did not detect the inferior quality of EQA2226 that was subsampled by the provider to mimic samples with low read count (Annexes 12 and 13). Laboratory 34 assessed the N50 value and laboratory 73 the N50 value and the coverage (Annex 11). With these indicators, a low read count should have been detected by both laboratories.

Participants that were using WGS for cluster analysis had the opportunity to report their detected AMR markers. A total of 13 out of 17 laboratories have reported these (Annex 14). It was not possible to assess correctness because information about methods and databases used was unavailable. However, all laboratories assessed AMR markers in the sequence that had insufficient quality because of contamination (EQA2225), but only laboratory 92 commented on the low quality. It would not be correct to report AMR markers from contaminated sequences as the species origin of the detected AMR markers would be unknown (Annex 14).

Figure 5. Minimum spanning tree of cgMLST by provider for EQA2213-EQA2222



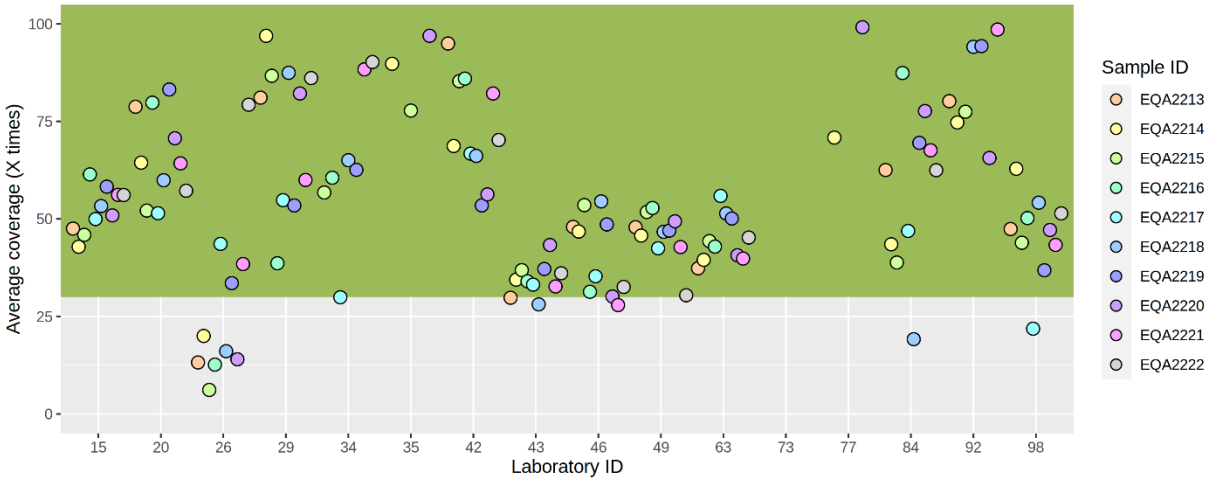
MST for 180 samples, distances based on Enterobase S. enterica V2 cgMLST scheme, pairwise ignoring missing alleles. Nodes coloured by sample IDs, numbers are laboratories IDs. PRO = provider. Orange numbers = allelic distances. Grey halo = clusters based on ≤5 AD

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 chapter 2.3. In this section, the results of these 16 laboratories are described.

Average coverage varied strongly among laboratories, with 11 samples from six different laboratories below threshold set by the provider (≥30x, Figure 6). Laboratories 73 and 77 had a very high coverage for most samples with a median (IQR) of 335x (313-370) and 542x (428-609), respectively.

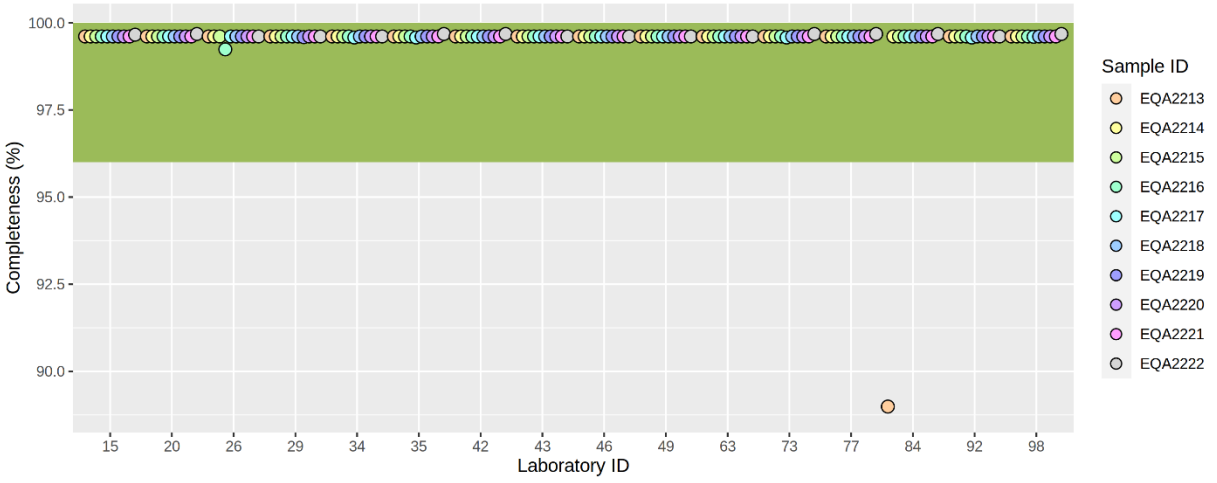
Figure 6. Average coverage of sequenced samples by participating laboratories, <100x



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

The completeness and contamination of most sequences were within the threshold set up by the provider for all samples, except index sample EQA2213 sequenced by laboratory 84 (Figure 7 and 8). Analysis with Kraken2/Bracken showed that 33% of reads were assigned to *Salmonella enterica*, while a higher percentage of reads (46%) was assigned to *Neisseria subflava*, a non-pathogenic commensal [39]. However, this has not affected the allele calling and performance in cluster assignment of laboratory 84, probably because enough coverage for most MLST loci was reached for EQA2213 (Figure 3 and Annex 12).

Figure 7. Completeness of sequenced genomes by participating laboratories



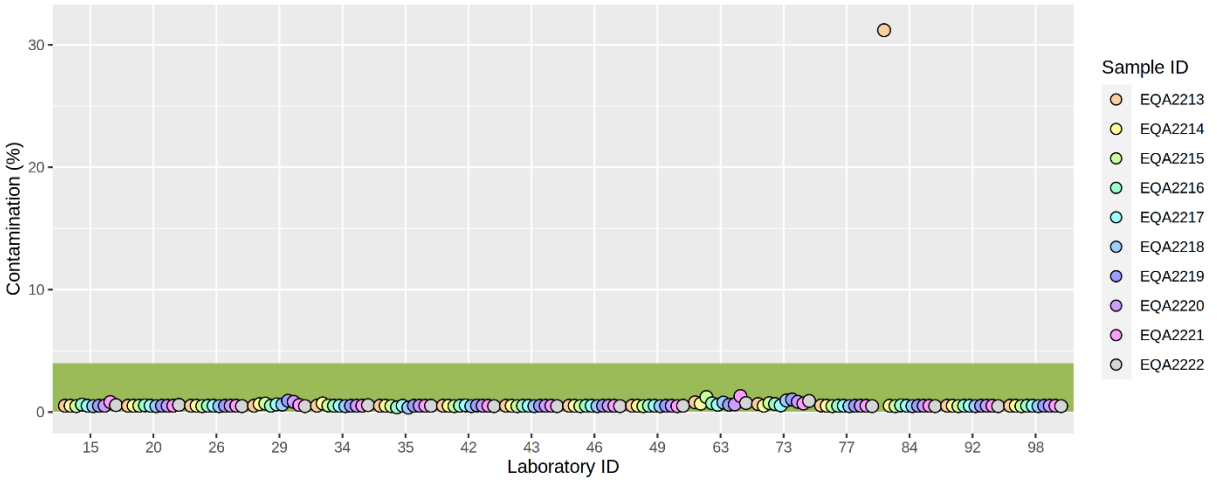
Green range = within quality threshold of provider (>96.0%).

The GC content of the sequences of all laboratories were within the quality threshold of 51.6-52.3% with a mean (95% CI) of 52.14% (± 0.0001). The incomplete and contaminated sample EQA2213 of laboratory 84 was also within the threshold. This can be explained, because the contaminant *N. subflava* was reported to have a GC content in the range of 49.0-52.8% that overlaps the range of GC content of *Salmonella spp*.

N50 values varied from 32 kbp to 694 kbp, but all were above the threshold set (>30 kbp) by provider (Figure 9). Total genome lengths (assembled) were all within the quality threshold and varied from 4.6 Mbp to 4.9 Mbp with a

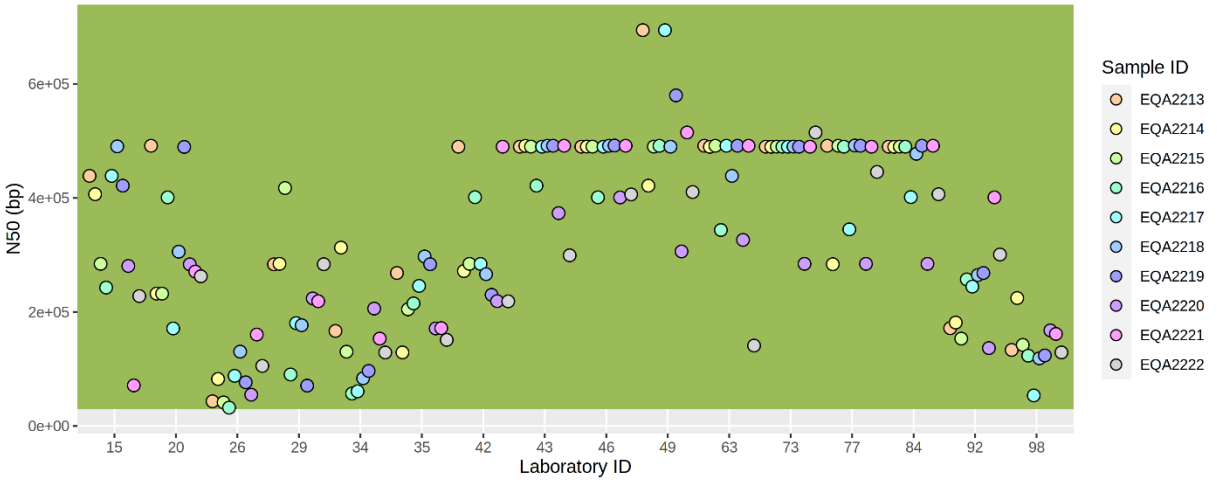
mean (95% CI) of 4,728,779 bp (± 311). For detailed results of quality assessment of provider from raw sequences submitted by participants, see Annex 15.

Figure 8. Contamination in sequenced samples by participating laboratories



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

Figure 9. 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, two of which performed MLVA in combination with WGS (laboratories 26 and 63). Laboratory 74 performed cluster analysis based on MLVA only. All detected MLVA profiles were 100% in concordance with the provider (Table 2) and with each other.

The isolates EQA2214, EQA2216, EQA2217, EQA2219 and EQA2221 were considered as part of the same cluster as index EQA2213 by laboratories 26 and 63 (Annex 16). Laboratory 74 also considered EQA2220 as part of the cluster (Annex 16). With WGS analysis, the isolates EQA2214 and EQA2220 do not belong to the cluster with 70 AD and 85 AD to the index EQA2213, respectively (Table 2). EQA2214 (n=3) and EQA2220 (n=1) were erroneously considered as part of the outbreak while using MLVA techniques for cluster identification.

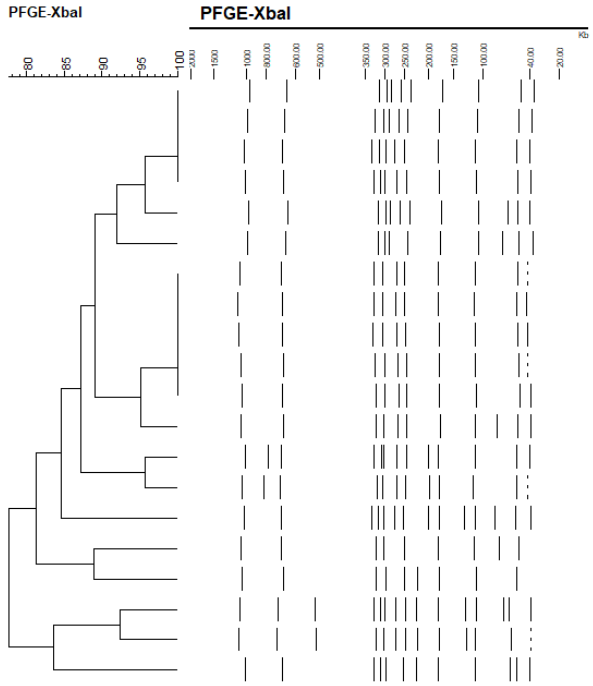
Although the technique was correctly applied by all laboratories, resulting in identical MLVA profiles, cluster assignment of isolates EQA2214 and EQA2217 was different when using MLVA compared to WGS (p=<0.001 and 0.018 respectively). For all samples analysed by laboratories using MLVA-based cluster analysis, 23 out of 27 were assigned correctly (85.2%), while with WGS-based cluster analysis 117 out of 119 samples (98.3%, excluding borderline samples EQA2217 and EQA2219) were assigned correctly. Overall, the proportion of correctly assigned cluster or singleton assignment is higher if using WGS (p=0.0021, $\chi 2$).

3.2.5 Results PFGE-based cluster analysis

PFGE was performed by two laboratories and results were compared with each other, because the EQA provider does not perform PFGE anymore. Laboratory 17 considered EQA2214, EQA2216, EQA2219 and EQA2221 as part of the same cluster as index EQA2213 (Figure 10, Annex 17). With WGS analysis, isolate EQA2214 does not belong to the cluster with 70 AD to the index EQA2213. Laboratory 75 considered EQA2216, EQA2219 and EQA2221 as part of the same cluster as index EQA2213, but was also not able to rule out EQA2214, EQA2215, EQA2217 and EQA2218 (Figure 10, Annex 17). Both laboratories agreed on 56% of isolates for cluster assignment, the assignment of EQA2216, EQA2219, EQA2221 and the singletons of EQA2220 and EQA2222. For four isolates laboratory 75 was not able to draw a definitive conclusion (Figure 10, Annex 17).

A dendrogram of the submitted banding profiles shows that the profiles of isolates EQA2217 and EQA2222 of the two participants cluster together. For the other isolates, clustering was seen on participant level rather than on sample level (Figure 10), illustrating the low comparability of PFGE results between laboratories.

Figure 10. Dendrogram of submitted PFGE banding profiles by participating laboratories, UPGMA, Dice coefficient



Sample	Lab ID	Cluster assignment
EQA2213	75	Index case
EQA2216	75	Cluster
EQA2219	75	Cluster
EQA2221	75	Cluster
EQA2214	75	Possible cluster
EQA2215	75	Possible cluster
EQA2213	17	Index case
EQA2214	17	Cluster
EQA2216	17	Cluster
EQA2219	17	Cluster
EQA2221	17	Cluster
EQA2220	17	Not part of cluster
EQA2217	75	Possible cluster
EQA2217	17	Not part of cluster
EQA2220	75	Not part of cluster
EQA2215	17	Not part of cluster
EQA2218	17	Not part of cluster
EQA2222	75	Not part of cluster
EQA2222	17	Not part of cluster
EQA2218	75	Possible cluster

For all samples analysed by laboratories using PFGE-based cluster analysis, 13 out of 18 were assigned correctly (72.2%), while with WGS-based cluster analysis 117 out of 119 samples (98.3%, excluding borderline samples EQA2217 and EQA2219) were assigned correctly. Overall, the proportion of correctly assigned cluster or singleton assignment is lower for PFGE-based cluster analysis than with using WGS ($P<0.0001$, $\chi 2$).

3.3 Results feedback survey

A total of 13 out of 27 laboratories (48%) responded to the feedback survey. Ten out of 13 (76%) used the results of this EQA as documentation for accreditation and/or licensing purposes for the methods used in their laboratory. All laboratories were satisfied with their individual EQA report and considered the difficulty level just right. Five out of 13 (38%) laboratories indicated that all their analytical test results conformed to the expected results, and eight (62%) have taken corrective actions based on the results of this EQA. Seven specified the corrective actions which comprise repeating the tests for the incorrectly assigned EQA samples (n=3), paying more attention to the preparation of samples for analysis (n=1), performing a contamination check for the contaminated sample EQA2225 (n=1), implementing a double-check control to prevent result interpretation bias (n=1) and improving their flagellins database (n=1).

Three laboratories made comments or suggestions for improvements for the EQA organisation: the sharing platform Research Drive was not easily accessible (n=1), there were too many questions about sequencing parameters (n=1) and one suggestion was made about being able to participate in the serotyping part based on both phenotypic methods and WGS. The suggestions made will be considered in the design of EQA *Salmonella* 2023 by ECDC and the provider.

4 Discussion

Of the 36 countries invited to participate, 30 laboratories (83%) registered for at least one part and received the specimen panel(s). One registered laboratory cancelled its participation before the result submission deadline and two laboratories did not submit results despite multiple reminders. Finally, 27 participants (75%) completed at least one part of the EQA-12. A total of 24 laboratories completed the serotyping part and 20 completed the molecular typing-based cluster analysis part, which is the same number as in the previous EQA about *Salmonella* (EQA-11) [40]. A total of 17 of the laboratories that participated in the cluster analysis part of EQA-12 also participated in EQA-11. Three laboratories participating in EQA-11 were replaced by three others. Because this is the first EQA for *Salmonella* since EQA-3 in 2012 that comprises a serotyping part, participation on this part cannot be compared to previous EQAs.

4.1 Serotyping

In EQA-12, 24 laboratories participated in the serotyping of 12 provided isolates. This resulted in 19 laboratories (79%) using phenotypic typing with antisera, 4 laboratories using WGS-predictive serotyping (17%) and one laboratory using genetic serotyping using Luminex combined with phenotypic typing with antisera.

Twelve laboratories (50%) had a performance score of 100%, eight laboratories (33%) had a performance score of 92%, three laboratories had performance scores of 75%, 67%, and 58% respectively, and for the remaining laboratory a performance score could not be calculated due to a lack of necessary antisera.

Corrected for sample size, there was no difference observed in total performance score per sample or per laboratory between de group of laboratories that used phenotypic methods (n=19) and the group that used WGS-predictive methods (n=4).

In total, 41 different error types were made in the serotyping part, of which the majority (88%) was made only once. Most of error types were false-positive detection, false-negative detection or misclassifications of H-antigens in both phases (n=27, 66%), of which at least four error types point towards the use of less specific antisera and one to incorrect prediction using WGS data where a 2nd H-phase 1,5 was not detected. Additional error types were false-positive detection or misclassification of O-antigens in 11 cases (27%), of which at least three point towards the use of less specific antisera. In total, indication of the use of less specific antisera was observed among six laboratories (13, 29, 65, 73, 74, and 77). However, as data about types of antisera used and the manufacturers are not available, no definitive conclusions about causes for error could be made. The remaining three error types were type misclassifications of subspecies (n=2) and use of non-standard nomenclature (n=1).

As serotyping was not performed in the last decade in former EQAs, no trend analyses for performance can be made for the group of NPHRLs. However, the provider contributes to the EURL-*Salmonella* Proficiency Tests (PTs) serotyping which are organised for quality assessment of European National Reference Laboratories (NRLs) in the animal and food sector. As salmonellosis is a zoonotic disease, a lot of serovars occur in both animal reservoirs and its derived foods and environments as well as in human reservoirs. To assess the capability of laboratories in a One Health setting, EQA2209 (*S.* Leeuwarden) was selected because it is the same isolate as sample S-7 in EURL-*Salmonella* PT serotyping 2018 [41]. In EQA-12, this sample had the lowest performance score of all samples: 78%. In EURL-*Salmonella* PT serotyping 2018, *S.* Leeuwarden was assigned correctly by 34 of 36 laboratories (94%), which is better but not significantly (p=0.0631, χ^2).

For the first time in 10 years, in 2022 ECDC commissioned, via the RIVM, an EQA for *Salmonella* including assessment of serotyping. With the provision of this EQA, a starting point for assessing capability of NPHRLs regarding serotyping of *Salmonella* was established, to monitor trends in methods used and performance for the coming years.

4.2 Molecular-typing based cluster analysis

In EQA-12 20 laboratories participated in the molecular-typing based cluster. This resulted in 17 laboratories (85%) using WGS-based cluster analysis, three laboratories (15%) applied MLVA-based cluster analysis, of which two also applied WGS-based cluster analysis. Two laboratories (10%) performed only PFGE-based cluster analysis.

A higher proportion of participants (85%, 17/20) applied WGS-based cluster analysis in this EQA compared to the previous EQA-11 (70%, 14/20). The proportion of participants that applied MLVA-based cluster analysis decreased from 40% (8/20) to 15% (3/20) laboratories compared to EQA-11 and only 5% (1/20) performed MLVA only. The proportion of participants that used PFGE-based cluster analysis decreased from 30% (6/20) to 10% (2/20) in EQA *Salmonella* 2022 compared to EQA-11 [40].

4.2.1 WGS-based cluster analysis

A total of 17 laboratories used a WGS-based cluster analysis, of which 16 (94%) with Illumina sequencing and one with Ion Torrent sequencing. A gene-by-gene approach was performed by 82% (14/17) of laboratories, while 18% (3/17) performed SNP typing. In EQA-11, a gene-by gene approach was applied by 93% (13/14) of laboratories and 14% performed SNP typing, of which one in addition to a gene-by-gene approach.

A very diverse set of platforms, approaches, kits, cluster analysis tools, typing schemes, and cluster cut-offs was used, in which 15 different combinations of methods were employed by the 17 laboratories.

The overall performance of cluster assignment while using WGS-based methods was very high: 94% of laboratories (16/17) assigned all provided cluster isolates correctly, regardless of using a gene-by-gene or a SNP-approach and despite the variety of methods and cluster cut-offs used within these approaches. This is comparable with EQA-11, where 93% (13/14) of laboratories assigned all cluster isolates correctly. The laboratory that had a performance score of 89% has produced good quality sequences but seemed to have swapped two isolates. This probably had occurred during pre-analysis because reported distances and uploaded FASTQ files were both swapped.

For all laboratories, the reported distance in alleles or SNPs for the cluster isolates EQA2216 and EQA2221 to index EQA2213 was well below their reported cluster cut-off, except for laboratory 49, which had swapped sample EQA2221. Isolates EQA2217 and EQA2219 had distances around cluster cut-off for all laboratories, their conclusions of cluster assignment depended on their measured distance combined with the cut-off. This proves that in a real outbreak situation epidemiological data are necessary to support conclusions about inclusion or exclusion of a case to a cluster. Other isolates were well above cluster cut-off for most laboratories, except one. Laboratory 77 reported overall lower distances to the index EQA2213, while using a high cluster cut-off, causing isolates EQA2214 and EQA2220 to come closer to cluster cut-off. 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 EQA2217 from laboratory 98, which had a small distance of 7 AD to the cluster. This confirmed the results of the stability tests by the provider that the selected *S.* Enteritidis genomes were very stable; after storing, transport, culturing procedures and sequencing by different laboratories it was still possible 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*, as at least *S.* Enteritidis sequences can be easily shared and produce meaningful results when used in analyses by another laboratory.

A quality assessment was performed on the submitted Illumina reads using methods and thresholds of the provider. For 10 out of 16 (63%) laboratories, all sequences passed quality criteria of the provider. One laboratory submitted data that produced a low average coverage in provider's assembly pipeline, of which six out of 10 isolates below threshold. Four other laboratories, each had one sample with a lower coverage just below threshold, observed in different samples. One laboratory uploaded contaminated reads for index EQA2213, although this did not affect the results of the cluster analysis of this laboratory. Two laboratories had very high average coverage. While this is not harmful, they can potentially reduce sequencing costs per sample by including more samples in each sequence run.

An additional five sequences were made available to participants. Three of these isolates were non-manipulated sequences, consisting of one cluster isolate (technical duplicate of index EQA2213) and two non-cluster isolates. All but one laboratory assigned these isolates correctly as cluster or non-cluster isolates. One laboratory did not perform cluster analysis for one of the non-cluster isolates because they reported contamination with *E. coli* in the sample, while in fact, the contamination percentage was <0.5%. The remaining two isolates were non-cluster sequences that were manipulated by the provider. Reads of EQA2225 were down-sampled to 80% and supplemented with 20% of reads from an *E. coli* isolate, mimicking contamination. Reads of EQA2226 were down-sampled to 5% to mimic a low read count. Of the 17 laboratories that performed WGS-based cluster analysis, 71% (12/17) identified the poor quality of both EQA2225 and EQA2226, the other 29% identified poor quality in only one of those samples. However, 13 of the 17 laboratories reported AMR markers, and all had reported AMR markers in the contaminated sample, only one of the laboratories commented about the quality. In fact, detection of AMR markers in sequences that are contaminated should not be performed unless contamination is filtered out, as the species of origin of these markers is unknown.

4.2.2 MLVA-based cluster analysis

Three laboratories participated in MLVA-based cluster analysis, of which one used MLVA only. All three laboratories achieved a 100% performance score in determination of MLVA profiles. However, the cluster assignment of isolates was set using WGS-typing which is a higher resolution typing technique. This resulted in an assignment of one extra cluster isolate for two laboratories and two extra cluster isolates for one laboratory when performing MLVA-based cluster analysis. EQA2214 (n=3) and EQA2220 (n=1) were erroneously considered as part of the outbreak while using MLVA techniques for cluster identification. These results are comparable to the results of EQA-11 in which also all MLVA profiles were correctly identified, but the expected cluster could not be identified by any of the laboratories (n=8) while using MLVA.

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 causes inefficient employment of outbreak investigation as more cases need to be interviewed. In addition, data of interviewed cases that do not actually belong to the outbreak dilute the source tracing data, which prompts a need to interview more cases to have a statistically sound foundation for epidemiological analyses [15].

In addition, more and more laboratories are in transition into WGS typing, at least in outbreak situations. Therefore, MLVA will be less used in communication for international outbreaks, for instance in case definitions. The use of typing techniques such as MLVA hampers the fulfilment of the surveillance objectives of the FWD-Net, such as improvement of 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 [42].

4.2.3 PFGE-based cluster analysis

Two laboratories have participated in PFGE-based cluster analysis, technical performance cannot be assessed as the provider does not perform PFGE anymore. The results of the two laboratories were compared with each other and to WGS-based clustering. Both laboratories assigned a different combination of isolates to the cluster of index EQA2213. The use of a lower resolution typing technique as PFGE resulted in correct cluster assignment of 89% of isolates for one laboratory and 56% of isolates for the other laboratory. This is comparable to the results of EQA-11, in which also none of the participants that used PFGE (n=6) was able to reach a correct cluster identification.

In addition, using PFGE, one laboratory was not able to reach definitive conclusions regarding cluster assignment for four out of nine isolates (44%). When inferring a dendrogram of the submitted banding patterns, it was observed that isolates cluster more frequently on laboratory level than on 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 10% of the participating laboratories performed it, make this method unsuitable for multi-country outbreak investigations.

4.3 Feedback from participants

A total of 48% (13/27) of participants that have completed at least one of the parts of EQA-12, completed the feedback survey. Ten of them (76%) used the results as documentation for accreditation and/or licensing purposes, showing the added value of this EQA to laboratory quality systems.

Eight out of 13 laboratories (62%) reported that they have taken a range of corrective actions based on their individual results, of which seven specified these actions. Four of those actions were repeating the tests for the incorrectly assigned EQA samples. Three laboratories reported that they have implemented structural improvements in their workflows. 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 enlargement countries.

5 Conclusions

5.1 Methods and capability of serotyping

For serotyping, 79% of laboratories in the EU/EEA and enlargement countries routinely apply phenotypic serotyping based on slide agglutination with O- and H-antisera. A total of 17% uses WGS-based methods in which the serotype is inferred from genetic characteristics. 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) did not influence overall capability of the laboratories that apply them or the ability of typing particular serovars.

Performance is high for most laboratories, with 12 (50%) laboratories achieving performance scores of 100% and eight of 92%. The four laboratories that have the lowest performance values (<92%) all used phenotypic methods, and for most (75%) there is indication from their type of errors that less specific antisera were used. However, based on the information that was requested in the result form, this cannot be definitively concluded.

5.2 Methods and capability of molecular typing-based cluster analysis

Most participating laboratories (85%) used WGS-based cluster analysis, sometimes combined with MLVA (n=2). It is not known from the information that was requested if WGS-based cluster analysis is applied routinely or only in outbreak situations. One laboratory (5%) used MLVA only, and two laboratories (10%) used PFGE-based cluster analysis only.

Performance was highest among laboratories that used WGS-based cluster analysis, with an overall performance score of 98%. All but one laboratory had 100% performance in assigning provided isolates to clusters. For the laboratory with a performance score of 89%, the error was probably made in the pre-analysis part, because sequence quality was high and it seemed that two samples were swapped. 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 100%, all three laboratories had identical MLVA profiles identified. However, overall performance for cluster assignment of provided isolates using MLVA is lower (85%) compared to WGS-based cluster analysis (98%, $p < 0.0021$, χ^2). Therefore, it can be concluded that participating laboratories had good capability in applying MLVA, however the resolution of the technique itself is too low to correctly assign isolates to clusters.

Technical performance in PFGE-based cluster analysis cannot be assessed, because the technique is not (anymore) applied by the provider. Overall performance for cluster assignment of provided isolates using PFGE is lower (72%) compared to WGS-based cluster analysis (98%, $p<0.0001$). When comparing performance of the two laboratories that used PFGE, it can be concluded that one laboratory had difficulty with assigning isolates to a cluster, as four out of nine isolates (44%) were not definitively assigned to cluster or singleton. In addition, the comparison confirmed that PFGE is not portable and interlaboratory comparability was low.

Although MLVA an PFGE-based cluster analysis might still be useful for national purposes, for cluster detection and outbreak investigation they become less important. ECDC will no longer regularly analyse MLVA data, and collection of this data will eventually be discontinued. The MLVA data reporting will be replaced by the WGS real-time reporting.

5.3 Evaluation of EQA-12

Participation rate in the cluster analysis part was stable, 56% of 36 invited laboratories completed results. The same percentage of laboratories participated as in the previous EQA (EQA-11). Participation in WGS-based cluster analysis has increased, while participation in MLVA- and PFGE-based cluster analysis decreased. For the first time in a decade, serotyping capability was assessed and as a starting point, participation rate was higher (67% of invited laboratories) than participation in the molecular typing-based cluster analysis part.

The EQA design was approved by ECDC, and prepared according to standards ISO 15189, ISO 17043 and chapter 11 from ISO 13528. The difficulty level was evaluated in the feedback survey and was assessed as suitable by all participating laboratories. The number of samples was appropriate to draw conclusions about performance, although one comment was received from a laboratory about too large amounts of 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. Regardless, individual reporting was evaluated as satisfactory by all laboratories that responded to the feedback survey.

Multiple laboratories took corrective actions based on the results of EQA-12, proving the added value of this EQA to typing capability of the NPHRLs in the EU/EEA and enlargement countries. Maximum capability of the NPHRLs contributes to surveillance and outbreak detection on a regional and national level as well as to the fulfilment of the international surveillance objectives of ECDC and FWD-Net.

6 Recommendations

6.1 Recommendations for NPHRLs

Fulfilment of the EU level surveillance objectives starts with good monitoring of trends and cluster and outbreak detection on a national level. For EU/EEA countries to be able to perform this trend and outbreak monitoring, good performance in typing is essential, for *Salmonella* in serotyping as well as in molecular typing for cluster analysis.

Most laboratories had a good performance on serotyping, but a few laboratories scored performances below 92%. These laboratories all perform phenotypic serotyping and are recommended to assess the specificity of their antisera used. If desired, NPHRLs can contact the EQA provider for assistance and the provision of recommendations tailored to the needs and resources of the specific laboratories.

Although technical performance for all methods used for the molecular typing-based cluster analysis is very high, performance in cluster assignment is much higher for WGS (98%) as opposed to MLVA (74%). Laboratories are recommended to use WGS-based cluster analysis at least in outbreak situations. If enough resources are available for the employment of other typing techniques, PFGE-based cluster analysis should ideally not be used because the inferior resolution and the non-portability hampers the use in (inter)national outbreak assessments in which multiple institutes are involved. In addition, because many laboratories transitioned to WGS-based typing, the use of PFGE- and MLVA-based cluster analysis becomes less suitable in multi-country outbreak investigations because of the limited backwards compatibility. ECDC will no longer regularly analyse MLVA data, and collection of these data will eventually be discontinued. The MLVA data reporting will be replaced by the WGS real-time reporting.

Because EQAs can help identify opportunities for improving quality of typing methods and their harmonisation, it is encouraged to NPHRLs to participate in the EQAs organised on behalf of and funded by ECDC.

6.2 Recommendations for FWD-Net and ECDC

ECDC will keep stimulating NPHRLs to participate in EQAs to maximise typing capabilities and harmonisation to fulfil the surveillance objectives. In addition, ECDC is actively 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 over other typing techniques and by promoting submission of high-quality data to TESSy.

6.3 Recommendations for EQA organisation and provider

The first assessment of *Salmonella* serotyping in a decade was successful and prompted laboratories to actions for improvement. The inclusion of rare serovars should be continued as it challenges laboratories.

As most laboratories use WGS-based cluster assignment, the EQA provider should consider implementing a different serovar in the panel instead or in addition to *S.* Enteritidis or *S.* Typhimurium in the next EQA.

To improve the identification and analysis of error types even further, it is recommended to the EQA provider to include more questions about the methods used for phenotypical serotyping, WGS-predictive serotyping and SNP typing-based cluster analysis and its applications.

Comments from participants about design and organisation of EQA *Salmonella* 2022 should be considered by the provider during the design of EQA *Salmonella* 2023.

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Annex 1. Online registration form

EQA Salmonella 2022-2023

You are hereby invited to participate in the EQA Salmonella 2022-2023

Please answer the questions below to register

*Fields marked with a * are mandatory*

*Would you like to participate in the Salmonella EQA 2022-2023?

- Yes
- No

If participation is not desired

*Please mention the reason if not to participate:

If participation is desired

*Name of contact person:

*Email of contact person:

*Name of institute or organisation:

*Country:

*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.

*What method will you use for serotyping?

- Phenotypic serotyping (using antisera)
- Molecular
- Other, please elaborate:

If using molecular serotyping method

*What kind of molecular method do you use in your regular protocols?

- WGS
- Other, please elaborate:

If using WGS for serotyping part

*What sequencing platform will you use?

- Illumina
- Other, namely:

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
- Other, namely:

*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):

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

Email address contact person:

Email address second contact person *(optional)* :

Name of laboratory – in full:

Name of laboratory - acronym:

Name of institution or organisation – in full:

Name of institution or organisation – acronym:

Shipping address *(please include street name and number, postal code, city and country)* :

*Do you have a different postal address for correspondence (the certificate)?

- Yes
- No

If different postal address

*Postal address *(please include street name and number, postal code, city and country)* :

If registered

*Do you have any additional comments?

Yes

No

If yes, please enter your comments here:

Your submitted personal data are only used for the purpose of the execution of the EQA Salmonella 2022-2023 and is handled with care. Original data are 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	Austrian Agency for Health and Food Safety
Belgium	Lab of Human Bacterial Diseases	Sciensano
Bulgaria	National Reference Laboratory for Enteric infections, Pathogenic Cocci and Diphteria	National Centre of Infectious and Parasitic Diseases
Cyprus	National Reference Laboratory for Salmonella and other Enteric Pathogens, Microbiology Department	Nicosia General Hospital
Czechia	National Reference Laboratory for Salmonella	The National Institute of Public Health
Denmark	Tarmbakteriologisk Laboratorium	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board, Estonia
Finland	Expert Microbiology Unit	Finnish Institute for Health and Welfare
France	Centre National de Référence des E. coli, Shigella et Salmonella	Institut Pasteur
Germany	National Reference Centre for Salmonella and other bacterial enteric pathogens	Robert Koch Institute
Greece	National Reference Centre for Salmonella	University of West Attica
Hungary	FWD-Reference Laboratory Hungary	National Public Health Centre
Ireland	Galway Reference Laboratory Services	University Hospital Galway
Italy	Dep. of Infectious Diseases	Istituto Superiore di Sanità
Latvia	National Reference laboratory	Riga East University hospital, Infectology Centre of Latvia
Lithuania	National public health surveillance laboratory	National public health surveillance laboratory
Luxembourg	Epidemiology and Microbial Genomics	Laboratoire National de Santé
Malta	Bacteriology Laboratory	Mater Dei Hospital
Poland	Laboratory of Bacteriology and Biocontamination Control	National Institute of Public Health NIH - National Research Institute
Portugal	National Reference Laboratory of Gastrointestinal Infections	Instituto Nacional de Saúde Doutor Ricardo Jorge
Romania	Molecular Epidemiology for Communicable Diseases	'Cantacuzino' National Military Medical Institute for Research and Development
Slovakia	National Refference Centre for Salmonelloses	Public Health Authority of the Slovak Republic
Slovenia	Department for Public Health Microbiology	National Laboratory of Health, Environment and Food
Sweden	Enheten för laborativ bakterieövervakning	Folkhälsomyndigheten
Norway	National Reference Laboratory Enteropathogenic Bacteria	Norwegian Institute of Public Health
Montenegro	Centre for Medical Microbiology	Institute of Public Health of Montenegro
Serbia	Department for Molecular Microbiology	Institute of Public Health of Serbia 'Dr Milan Jovanovic Batut'

Annex 3. Online results form

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
EQA2201						
EQA2202						
EQA2203						
EQA2204						
EQA2205						
EQA2206						
EQA2207						
EQA2208						
EQA2209						
EQA2210						
EQA2211						
EQA2212						

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?

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?

How many loci are included in this scheme?

If you use a SNP typing approach for cluster 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 from the head chef (EQA2213) and whether you would consider the isolate part of the wedding dinner outbreak:

Isolate	Distance to index case	Part of the outbreak?
EQA2214		
EQA2215		
EQA2216		
EQA2217		
EQA2218		
EQA2219		
EQA2220		
EQA2221		
EQA2222		

Does your WGS analysis include a confirmation of species?

Which method do you use to confirm the species?

Which criteria and thresholds do you use to assess the quality of your WGS reads or assemblies? (possibility to fill in up to ten 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 wedding dinner outbreak (part of a cluster with the index case)

What is your assessment of provided genome EQA2223 from an isolate obtained from bavarois dessert?

What is the distance of this genome to the index case?

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

What is your assessment of provided genome EQA2224 from an isolate obtained from smoked salmon?

What is the distance of this genome to the index case?

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

What is your assessment of provided genome EQA2225 from an isolate obtained from raw milk cheese?

What is the distance of this genome to the index case?

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

What is your assessment of provided genome EQA2226 from an isolate obtained from a garnish of parsley?

What is the distance of this genome to the index case?

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

What is your assessment of provided genome EQA2227 from an isolate obtained from steak tartare?

What is the distance of this genome to the index case?

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

Does your WGS analysis include identification of AMR markers?

Optional part if you detected AMR markers

Please report which AMR markers you identified for isolate 2213:

Please report which AMR markers you identified for isolate 2214:

Please report which AMR markers you identified for isolate 2215:

Please report which AMR markers you identified for isolate 2216:

Please report which AMR markers you identified for isolate 2217:

Please report which AMR markers you identified for isolate 2218:

Please report which AMR markers you identified for isolate 2219:

Please report which AMR markers you identified for isolate 2220:

Please report which AMR markers you identified for isolate 2221:

Please report which AMR markers you identified for isolate 2222:

Please report which AMR markers you identified in genome 2223:

Please report which AMR markers you identified in genome 2224:

Please report which AMR markers you identified in genome 2225:

Please report which AMR markers you identified in genome 2226:

Please report which AMR markers you identified in genome 2227:

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 cut-off do you use for cluster analysis with MLVA?

Please report the MLVA profile (SENTR7-SENTR5-SENTR6-SENTR4-SE3) of the isolates and whether you would consider the isolate part of the wedding dinner outbreak:

Isolate	MLVA profile	Part of the outbreak?
EQA2213		
EQA2214		
EQA2215		
EQA2216		
EQA2217		
EQA2218		
EQA2219		
EQA2220		
EQA2221		
EQA2222		

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.

Please report which of the isolates you would consider to be part of the wedding dinner outbreak, based on PFGE:

Isolate	Part of the outbreak?
EQA2214	
EQA2215	
EQA2216	
EQA2217	
EQA2218	
EQA2219	
EQA2220	
EQA2221	
EQA2222	

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 2022

Fields marked with a * are mandatory

Dear Participant,

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

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

- Yes
- No

Please explain:

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

- Yes
- No
- Not applicable

Please explain:

* **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.

Please specify what corrective actions were taken:

* **Question 4:** What is your opinion on the difficulty level of this EQA exercise?

- Too difficult
- Too easy
- Just right
- Other (please specify)

Please specify:

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

Annex 5. Methods used serotyping

Lab ID	Registered serotyping	Participated serotyping	Method used	Sequencing platform
13	Yes	Yes	Phenotypic (using antisera)	
15	Yes	Yes	Phenotypic (using antisera)	
17	Yes	Yes	Phenotypic (using antisera)	
20	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	Yes	Yes	Phenotypic (using antisera)	
37	Yes	Yes	Phenotypic (using antisera)	
42	Yes	Yes	Prediction serotype with WGS	Illumina
43	No	No		
44	Yes	Yes	Prediction serotype with WGS	Ion Torrent
46	Yes	Yes	Phenotypic (using antisera)	
49	Yes	Yes	Prediction serotype with WGS	Illumina
53	Yes	Yes	Phenotypic (using antisera)	
56	Yes	Yes	Phenotypic (using antisera)	
63	Yes	Yes	Molecular genoserotyping (Luminex) and agglutination serotyping	
65	Yes	Yes	Phenotypic (using antisera)	
69	Yes	Yes	Phenotypic (using antisera)	
73	Yes	Yes	Phenotypic (using antisera)	
74	Yes	Yes	Phenotypic (using antisera)	
75	Yes	Yes	Phenotypic (using antisera)	
77	Yes	Yes	Phenotypic (using antisera)	
84	No	No		
92	Yes	No		
98	Yes	Yes	Phenotypic (using antisera)	

Annex 6. Serotyping results reported per laboratory

Laboratory ID 13

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	1,9,12	g,m	-0	Enteritidis
EQA2202	*S. enterica*	*enterica*	1,4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	1,4,5,12	i	-0	1,4,5,12:i:-
EQA2204	*S. enterica*	*enterica*	6,7,14	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	1,4,5,12	f,g	-0	Derby
EQA2206	*S. enterica*	*enterica*	6,7,14	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7,14	g,m,s	-0	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10	g,m,s	-0	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,10	g,s,t	-0	Westhampton
EQA2212	*S. enterica*	*arizonae*	50	z4,z23	-0	50:z4,z23:-

Grey = incorrect results.

Laboratory ID 15

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	4,5,12:i:-
EQA2204	*S. enterica*	*enterica*	7	r	5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	7	h	z15	Braenderup
EQA2207	*S. enterica*	*enterica*	7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	10	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	S. IV (S. enterica subsp.houtenae) 50:z4,z23:-

Laboratory ID 17

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	1,9,12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	1,4,[5],12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	1,4,[5],12	i	-	Monofasic S.1,4,[5],12:i:- variant
EQA2204	*S. enterica*	*enterica*	6,7,14	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	1,4,[5],12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7,14	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7,14	g,m,[p],s	[1,2,7]	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,{10}{15}{15,34}	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4z23	-	IV 50 :z4z23:-

Laboratory ID 20

EQA#	Species	Subspecies	O	H (phase 1)	H (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5,12	i	-	Group B (monophasic S. Typhimurium)
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8:r:l,w	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	Salmonella ssp.IV

Green = incorrect notation.

Laboratory ID 26

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	4,5,12 : i : -
EQA2204	*S. enterica*	*enterica*	7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV 50:z4,z23:-

Laboratory ID 29

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4	i	2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	4,5:i:- monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*salamae*	3,10	g,m,s,t	-	10:g,m,s,t:-
EQA2212	*houtenae*	*arizonae or houtenae*	50	z4,z15	-	IV 50:z4,z23:-

Grey = incorrect results. Green = incorrect notation.

Laboratory ID 30

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	1,9,12	g,m	-0	Enteritidis (1,9,12:g,m:-)
EQA2202	*S. enterica*	*enterica*	1,4,5,12	i	H2	Typhimurium (1,4,[5],12:i:1,2)
EQA2203	*S. enterica*	*enterica*	1,4,12,5	i	-0	Typhimurium monophasic (1,4,[5],12:i:1,2)
EQA2204	*S. enterica*	*enterica*	6,7,14	r	H5	Infantis (6,7,14:r:1,5)
EQA2205	*S. enterica*	*enterica*	1,4,12	g,f	-0	Derby (1,4,[5],12:f,g:[1,2])
EQA2206	*S. enterica*	*enterica*	6,7	e,h	Hz15	Braenderup (6,7,14:e,h:e,n,z15)
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-0	Montivideo (6,7,14:g,m,[p],s:[1,2,7])
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast (6,8:r:l,w)
EQA2209	*S. enterica*	*enterica*	11	b	H5	Leeuwarden (11:b:1,5)
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-0	Amsterdam (3{10}{15}{15,34}:g,m,s:-)
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-0	Senftenberg (1,3,19:g,[s],t:-)
EQA2212	*S. enterica*	*enterica*	6,7	Hz4, z23	H6	Planckendael (6,7:z4,z23:1,6)

Grey = incorrect results

Laboratory ID 34

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	4,5,12:i.- (monophasic)
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	-	I. 11:b:-
EQA2210	*S. enterica*	*enterica*	3,10,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV. 50:z4,z23:-

Grey = incorrect results.

Laboratory ID 35

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	Typhimurium, monophasic
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,19	g,s,t	0	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	Ssp. IV (50:z4,z23:-)

Laboratory ID 37

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	4,5:i:- monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,19	g,t	-	Senftenberg
EQA2212	*S. enterica*	*diarizonae*	50	r	e,n,z15	IIIb 50:r:e,n,z15

Grey = incorrect results.

Laboratory ID 42

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4	i	-	Monophasic Salmonella Typhimurium
EQA2204	*S. enterica*	*enterica*	7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	8	r	l,w	Goldcoast or Brikama
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV 50:z4,z23:-

Laboratory ID 44

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4	i	-	4,5:i.- monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*Arizonae* or *houtenae*	50	z4,z23	-	IV. 50:z4,z23:-

Laboratory ID 46

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	/	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5,12	i	/	I 4,5,12:i:-
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	/	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e.n.z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	/	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	6,7	b	1,5	Edinburg
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	/	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,t	/	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	Z4,z23	/	V 50:z4,z23:-

Grey = incorrect results. Green = incorrect notation.

Laboratory ID 49

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9.12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,12	i	-	Monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	(IV) O50:z4,z23:-

Laboratory ID 53

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9; 12	g, m	-0	Enteritidis
EQA2202	*S. enterica*	*enterica*	4; 5; 12	i	2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4; 5; 12	i	-0	Typhimurium monophasic
EQA2204	*S. enterica*	*enterica*	6; 7	r	5	Infantis
EQA2205	*S. enterica*	*enterica*	1; 4; 12	f, g	-0	Derby
EQA2206	*S. enterica*	*enterica*	6; 7	e, h	e, n, z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6; 7	g, m, s	-0	Montevideo
EQA2208	*S. enterica*	*enterica*	6; 8	r	l, w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3; 15	g, m, s	-0	Amsterdam
EQA2211	*S. enterica*	*enterica*	1; 3; 19	g, t	-0	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4, z23	-0	Group O:50 (Z)

Green = incorrect notation.

Laboratory ID 56

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	-0	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-0	Monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-0	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-0	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3	g,m,s	-0	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,19	g,s,t	-0	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-0	IV 50:z4,z23:-

Laboratory ID 63

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	-	Monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV: 50: z4,z23: -

Laboratory ID 65[a]

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	Group O:9	No	O:9	H:G; H:m	No	Salmonella Enteritidis
EQA2202	Group O:4	No	O:4, O:5, O:12	H:G	No	O:4, O:5, O:12, H:g
EQA2203	Group O:4	No	O:4	No	No	O:4
EQA2204	No	No	O:6	No	No	O:6
EQA2205	Group O:4	No	O:1, O:4, O:12	H:G, H:f	No	O:1, O:4, O:12, H:G, H:f
EQA2206	Group O:4	No	O:4, O:5	H:c	H:e,n,x	O:4, O:5, H:c, H:e,n,x
EQA2207	Group O:4	No	O:4, O:5, O:12, O:27	H:a	H:5	O:4, O:5, O:12, O:27, H:a, H:5
EQA2208	Group O:8	No	O:6, O:8	z:4	H:7	O:6, O:8, z:4, H:7
EQA2209	Group O:4	No	O:4	No	No	O:4
EQA2210	Group O:3,10	No	O:3,10,15; O:15	H:b	No	O:3,10,15; O:15, H:b
EQA2211	Group O:4	No	O:1, O:4	H:g, H.f	No	O:1, O:4, H:g, H.f
EQA2212	Group O:50	No	O:50	H:k	No	O:50, H:k

Grey = incorrect results. Green = incorrect notation. [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 antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9	g,m	-0	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5	i	2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5	i	2	Typhimurium
EQA2204	*S. enterica*	*enterica*	7	r	5	Infantis
EQA2205	*S. enterica*	*enterica*	4	f,g	-0	Derby
EQA2206	*S. enterica*	*enterica*	7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	m,s	-0	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,10,15	g,m,s	-0	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-0	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-0	50: z4,z23: -

Grey = incorrect results.

Laboratory ID 73

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5,12	i	-	O:4,5,12 H:i:-
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	l,v	1,7	Manchester
EQA2209	*S. enterica*	*enterica*	1,6,14,25	b	1,5	Kuntair
EQA2210	*S. enterica*	*enterica*	3,15,34	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,15,19	g,s,t	-	Dessau
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	O:50 H:z4,z23:-

Grey = incorrect results.

Laboratory ID 74

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5,12	i	-	4,5,12 : i : -
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	i	1,2	Aberdeen*
EQA2210	*S. enterica*	*enterica*	3,10	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	Z4,z23	-	Salmonella enterica ssp. houtenae ser. 50 : z4,z23 : - **

**weak 2nd phase. **OR Salmonella enterica ssp. Aarizonae ser. 50:z4,z23:- because of atypical biochemical results. Grey = incorrect results*

Laboratory ID 75

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,46	g,m	-	Hillingdon
EQA2202	*S. enterica*	*enterica*	1,4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	1,4,5,12	i	-	I 1,4,5,12:i:-
EQA2204	*S. enterica*	*enterica*	6,7,14	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	1,4,12	i	-	I 1,4,12:i:-
EQA2206	*S. enterica*	*enterica*	6,7	r	e,n,z15	Papuana
EQA2207	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2208	*S. enterica*	*enterica*	6,8	r	l,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV. 50:z4,z23:-

Grey = incorrect results.

Laboratory ID 77

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	1;9;12	m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	1;4;5;12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	1;4;5;12	i	-	Typhimurium
EQA2204	*S. enterica*	*enterica*	6;7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	1;4;5;12	f	1,2	Derby
EQA2206	*S. enterica*	*enterica*	6;7;14	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6;7;14	m,s	1,2	Montevideo
EQA2208	*S. enterica*	*enterica*	6;8	e,h	e,n,x	Fillmore
EQA2209	*S. enterica*	*enterica*	6;8	d	1,2	Muenchen
EQA2210	*S. enterica*	*enterica*	3;10;15	m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	3;10	t	1,5	Bloomsbury
EQA2212	*S. enterica*	*enterica*	50	l,v	1,2	Fass

Grey = incorrect results. Green = incorrect notation.

Laboratory ID 98

EQA#	Species	Subspecies	O antigens	H antigens (phase 1)	H antigens (phase 2)	Serovar name or seroformula
EQA2201	*S. enterica*	*enterica*	9,12	g,m	-	Enteritidis
EQA2202	*S. enterica*	*enterica*	4,5,12	i	1,2	Typhimurium
EQA2203	*S. enterica*	*enterica*	4,5,12	i	-	Monophasic Typhimurium
EQA2204	*S. enterica*	*enterica*	6,7	r	1,5	Infantis
EQA2205	*S. enterica*	*enterica*	4,12	f,g	-	Derby
EQA2206	*S. enterica*	*enterica*	6,7	e,h	e,n,z15	Braenderup
EQA2207	*S. enterica*	*enterica*	6,7	g,m,s	-	Montevideo
EQA2208	*S. enterica*	*enterica*	6,8	r	1,w	Goldcoast
EQA2209	*S. enterica*	*enterica*	11	b	1,5	Leeuwarden
EQA2210	*S. enterica*	*enterica*	3,15	g,m,s	-	Amsterdam
EQA2211	*S. enterica*	*enterica*	1,3,19	g,s,t	-	Senftenberg
EQA2212	*S. enterica*	*houtenae*	50	z4,z23	-	IV 50:z4,z23:-

Annex 7. Assigned serovar per sample

Lab ID	EQA2201	EQA2202	EQA2203[a]	EQA2204	EQA2205	EQA2206
Provider	**Enteritidis**	**Typhimurium**	**Typhimurium, monophasic**	**Infantis**	**Derby**	**Braenderup**
13	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
15	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
17	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
20	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
26	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
29	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
30	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
34	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
35	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
37	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
42	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
44	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
46	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
49	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
53	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
56	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
63	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
65[b]	Enteritidis	O:4, O:5, O:12, H:g	O:4	O:6	O:1, O:4, O:12, H:G, H:f	O:4, O:5, H:c, H:e,n,x
69	Enteritidis	Typhimurium	Typhimurium	Infantis	Derby	Braenderup
73	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
74	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup
75	Hillingdon	Typhimurium	Typhimurium, monophasic	Infantis	I 1,4,12:i:-	Papuana
77	Enteritidis	Typhimurium	Typhimurium	Infantis	Derby	Braenderup
98	Enteritidis	Typhimurium	Typhimurium, monophasic	Infantis	Derby	Braenderup

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

Lab ID	EQA2207	EQA2208	EQA2209	EQA2210	EQA2211	EQA2212[a]
Provider	**Montevideo**	**Goldcoast**	**Leeuwarden**	**Amsterdam**	**Senftenberg**	**IV 50:z4,z23:-**
13	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Westhampton	IV 50:z4,z23:-
15	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
17	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
20	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
26	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
29	Montevideo	Goldcoast	Leeuwarden	Amsterdam	10:g,m,s,t:-	IV 50:z4,z23:-
30	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	Planckendael
34	Montevideo	Goldcoast	I. 11:b:-	Amsterdam	Senftenberg	IV 50:z4,z23:-
35	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
37	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IIIb 50:r:e,n,z15
42	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
44	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
46	Montevideo	Goldcoast	Edinburg	Amsterdam	Senftenberg	IV 50:z4,z23:-
49	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
53	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
56	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
63	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
65	O:4, O:5, O:12, O:27, H:a, H:5	O:6, O:8, z:4, H:7	O:4	O:3,10,15; O:15, H:b	O:1, O:4, H:g, H.f	O:50, H:k
69	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
73	Montevideo	Manchester	Kuntair	Amsterdam	Dessau	IV 50:z4,z23:-
74	Montevideo	Goldcoast	Aberdeen	Amsterdam	Senftenberg	IV 50:z4,z23:-
75	Infantis	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-
77	Montevideo	Fillmore	Muenchen	Amsterdam	Bloomsbury	Fass
98	Montevideo	Goldcoast	Leeuwarden	Amsterdam	Senftenberg	IV 50:z4,z23:-

[a]Notation of serovars equalised. [b]Laboratory 65 indicated that not all antisera were available to them, so 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
EQA2201	Enteritidis	96	False-positive O46 detection (n=1, lab75)
EQA2202	Typhimurium, diphasic	96	Type misclassification of Hg instead of Hi in phase 1 (n=1, lab 65)
EQA2203	Typhimurium, monophasic	92	False-positive H1,2 detection in phase 2 (n=1, lab 69) Non-standard nomenclature, serovar name incorrectly assigned as Typhimurium (n=1, lab 77)
EQA2204	Infantis	100	None
EQA2205	Derby	96	Type misclassification of Hi instead of Hf,g in phase 1 (n=1, lab 75)
EQA2206	Braenderup	92	Type misclassification of O4,5 instead of O6,7 (n=1, lab 65) Type misclassification of Hc instead of He,h in phase 1 (n=1, lab 65) Type misclassification of Hr instead of He,h in phase 1 (n=1, lab 75) False-positive detection of He,n,x in phase 2 (n=1, lab 65)
EQA2207	Montevideo	92	Type misclassification of O 4,5,12,27 instead of O6,7 (n=1, lab 65) Type misclassification of Ha instead of Hg,m,s in phase 1 (n=1, lab 65) False-positive H1,5 detection in phase 2 (n=2, lab 65 and 75) Type misclassification of Hr instead of Hg,m,s in phase 1 (n=1, lab 75)
EQA2208	Goldcoast (or Brikama)	88	Type misclassification of Hz_4 instead of Hr in phase 1 (n=1, lab 65) Type misclassification of H1,7 instead of Hl,w in phase 2 (n=2, lab 65 and 73) Type misclassification of Hl,v instead of Hl,w in phase 1 (n=1, lab 73) Type misclassification of He,h instead of Hl,w in phase 1 (n=1, lab 77) Type misclassification of He,n,x instead of Hl,w in phase 2 (n=1, lab 77)
EQA2209	Leeuwarden	75	False-negative H-antigen 2nd phase (n=1, lab 34) Type misclassification of O6,7 instead of O11 (n=1, lab 46) Type misclassification of O4 instead of O11 (n=1, lab 65) Type misclassification of O6,14 instead of O11 (n=1, lab 73) Type misclassification of Hi instead of Hb in phase 1 (n=1, lab 74) Type misclassification of H1,2 instead of H1,5 in phase 2 (n=2, lab 74 and 77) Type misclassification of O6,8 instead of O11 (n=1, lab 77) Type misclassification of Hd instead of Hb in phase 1 (n=1, lab 74)
EQA2210	Amsterdam	100	None
EQA2211	Senftenberg	79	Type misclassification of O3,10 instead of O3,19 (n=3, lab 13 and 29 and 77) Type misclassification of subspecies *salamae* instead of *enterica* (n=1, lab 29) False-positive detection of Hm in phase 1 (n=1, lab 29) Type misclassification of O1,4 instead of O3,19 (n=1, lab 65) Type misclassification of Hg,f instead of Hg,s,t in phase 1 (n=1, lab 65) False-positive O15 detection (n=1, lab 73) False-negative Hg,s in phase 1 (n=1, lab 77) False-positive H1,5 detection in phase 2 (n=1, lab 77)
EQA2212	IV 50:z_4,z_{23}:- (or IIIa 50:z_4,z_{23}:-)	83	Type misclassification of subspecies *enterica* instead of *houtenae*[a] (n=2, lab 30 and 77) Type misclassification of O6,7 instead of O50 (n=1, lab 30) False-positive H6 detection in phase 2 (n=1, lab 30) Type misclassification of Hr instead of Hz_4,z_{23} in phase 1 (n=1, lab 37) False-positive He,n,z_{15} detection in phase 2 (n=1, lab 37) Type misclassification of Hk instead of Hz_4,z_{23} in phase 1 (n=1, lab 65) Type misclassification of Hl,v instead of Hz_4,z_{23} in phase 1 (n=1, lab 77) False-positive H1,2 detection in phase 2 (n=1, lab 77)

[a] *or arizonae/diarizonae.*

Annex 9. Techniques used molecular typing-based cluster analysis

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

Green = No participation in molecular typing-based cluster analysis

Annex 10. Reported methods used for WGS-based cluster analysis

Lab ID	Platform	Approach	Library prep	MLST Tool	MLST scheme	Cluster cut-off
15	Illumina	MLST	Nextera XT DNA Library Kit	Ridom SeqSphere+	Enterobase	5
20	Illumina	MLST	Nextera Xt	RidomSeqSphere	in-house cgMLST for S.Enteritidis	5
26	Illumina	MLST	Nextera XT DNA library preparation kit	Enterobase	Enterobase	5
29	Illumina	MLST	Nextera XT Illumina library	chewBBACA 3.0.0	wgMLST schema for S.enterica from INNUENDO project	12 alleles
34	Illumina	MLST	Nextera (Illumina)	EnteroBase	cgMLST V2 + HierCC V1	5 AD
35	Illumina	MLST	Nextera XT	Ridom SeqSphere	EnteroBase cgMLST	5 AD (see comment below)[b]
42	Illumina	MLST	KAPA HyperPlus	SeqSphere+ v. 8.3.0	EnteroBase Salmonella cgMLST v2	5 AD
43	Illumina	SNP[a]	Illumina DNA Prep			2
44	Ion Torrent	SNP[a]				approximately 5 SNPs but it also depends on the characteristics of the cluster and the epidemiological data available
46	Illumina	MLST	Illumina DNA Prep	SeqSphere, cgMLST	EnteroBase	3[c]
49	Illumina	MLST	DNA Prep (Nextera Flex)	SeqSphere	Enterobase	5[d]
63	Illumina	MLST	MiSeq Reagent Kit v3 - 500 cycles	In house pipeline	cgMLST Enterobase scheme	7[e]
73	Illumina	MLST	NEBNext Ultra™ II FS DNA Library Prep Kit for Illumina.	chewBBACA Allele Call	Enterobase	3
77	Illumina	SNP[a]	Illumina DNA prep			30
84	Illumina	MLST	Illumina DNA Prep	BioNumerics 8.1	Core (EnteroBase)	approx 7 cgMLST AD[f]
92	Illumina	MLST	Nextera XT DNA Library Prep	Ridom SeqSphere	EnteroBase *S.* enterica cgMLST v2 scheme	1
98	Illumina	MLST	Nextera XT Kit	Bionumerics	Applied Maths/Enterobase cgMLST scheme	3 AD

[a] *All laboratories using SNP analysis, have used EQA2213 as reference genome.*

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

[b] *The 5 AD cut-off for cluster definition does only mean, that we have a closer look into it but not that we regard it as an outbreak cluster. Depending on serotype, cluster characteristics (number of isolates, spatio-temporal distribution etc.) and epi data outbreaks are defined on an individual basis. For point-source outbreaks a 5 AD match would not be considered as part of the outbreak (even less for S. Enteritidis), unless there is persuasive epidemiological evidence. In such a case we would perform additional SNP analysis.*

[c] *Guiding suggestions in the 'Proposed protocol for whole genome sequencing based analysis for detection and tracing of epidemic clones of antimicrobial resistant Salmonella and Campylobacter - to be used for national surveillance and integrated outbreak investigations by NRLs for public health', 8 July 2022, FWD AMR-RefLabCap were used for cluster identification. We have considered the following suggestion: 'For Campylobacter isolates, 5 or less ADs/SNPs can be considered as genetic clusters (Brehony et al. 2021, Joensen et al. 2021), while for Salmonella this depends on the serovar, with 2-3 ADs/SNPs in clonal serovars and up to 5 AD/SNP in other serovars.' (Payne et al., 2021, Gymoese et al. 2017.)*

[d] *in real life, the cut-off for cluster analysis should be adjusted to the context of the outbreak. Here, the time frame is very short and S. Enteritidis is a very clonal serotype.*

[e] *Allelic differences are defined on 100% exact matches with the current nomenclature. Uncalled loci are excluded from the comparison.*

[f] *In our routine surveillance we would sometimes use a ? cluster to indicate loose clusters.*

Annex 11. Reported quality criteria used for assessment of WGS data

Lab ID	Species confirmation	Q score (Phred)	Coverage	% Good targets MLST	#contigs
15[a]	JSpecies, SeqSero		50x	>98%	
20	Kraken2		>29 (avg)	>90%	none
26	Kraken				< 600
29[b]	Kraken v.2.0.7 (with 8Gb database available at https://ccb.jhu.edu/software/kraken/) for both raw reads and final polished assemblies	NA	NA	NA	NA
34	Kraken				<600
35	Mash (implemented in SeqSphere) and KRAKEN		20x (avg assembled)	98% (max. 1% with warnings)	
42	Kraken and Mash Distance		>30 (avg assembled)	>90%	
43	SpeciesFinder; KmerFinder; Enterobase		>50x		<250
44	Species is detected by BLAST towards an in-house database with reference sequences		20x		
46	KmerFinder	30	40x	95%	less than 150 contigs
49	Mash Screen		>30	>95%	500
63	Kraken2		30x		
73	seq sero		80x		<500
77	kraken2		> 40 (depth)		< 216
84[c]		>= 30		>=97%	
92[d]	rMLST (pubmlst.org), Mash Screen (Ridom SeqSphere)		>40x (avg unassembled)	>95%	<300 (contigs >=200)
98[e]	Kraken		Minimum x 50 (avg read)	Minimum 95% core percent and maximum 15% loci with multiple consensus	Available from QC analysis but no threshold
Median (range)[f]	**NA**	**30 (30)**	**40 (20-80)**	**95 (90-98)**	**<400 (216-600)**

Green = Not reported
[a]Laboratory 15 also assessed contig size with threshold >200 bp.
[b]Laboratory 29 performed quality control and reported methods of assessing quality criteria, but not which parameters were assessed and their tresholds.
[c]Laboratory 84 also assessed multiple alleles with threshold 20.
[d]Laboratory 92 also assessed number of reads with threshold >1000000, and average read length with threshold >140 bases (for 2x150 chemistry).
[e]Laboratory 98 also assessed number of unidentified bases or ambiguous sites, but uses no threshold.
[f]Calculated from laboratories that reported numerical values.

Lab ID	Genome size	N50	Species assignment	Contamination	% GC
15[a]	4.6-5.3 MB				
20					
26	4.0 Mb- 5.8 Mb	>20 kb	>70% contigs		
29[b]	NA	NA	NA	NA	NA
34	4-5,8 Mbp	>20 kb	>70% contigs assigned		
35					
42	4,9+1,2	>50000			
43	Rationally, around 4 - 5,5 Mbp	>30000 bp			
44	3.6-5,4 Mbp				
46	app. 5 MB	100000			app. 50
49	length of contigs assembled <ref genome + 10%				
63				no obvious contamination (other than plasmid, etc)	
73		>30000			
77	4627000 < x ≤ 5006000	53027	> 95%		51.8 < x ≤ 52.3 (in contigs)
84[c]	4.5 to 5.5 MB	>100000			
92[d]	4.3 - 5.8 Mb	>30000		<5% other species, genome size out of range, no. of contigs out of range	
98[e]	Between 4510000-5300000 bp	Available from QC analysis but no threshold		Below 5% contamination with other genus	
Median (range)[f]	**4.4-5.5 Mb (3.6-4.9 – 5.0-6.1)**	**30 kb (20-100)**	**>70% (70-95)**	**5% (5)**	**NA**

Green = Not reported
[a]Laboratory 15 also assessed contig size with threshold >200 bp.
[b]Laboratory 29 performed quality control and reported methods of assessing quality criteria, but not which parameters were assessed and their tresholds.
[c]Laboratory 84 also assessed multiple alleles with threshold 20.
[d]Laboratory 92 also assessed number of reads with threshold >1000000, and average read length with threshold >140 bases (for 2x150 chemistry).
[e]Laboratory 98 also assessed number of unidentified bases or ambiguous sites, but uses no threshold.
[f]Calculated from laboratories that reported numerical values.

Annex 12. Results reported WGS-based cluster assignments based on index EQA2213

Belonging to cluster yes/no

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222	EQA2223	EQA2224	EQA2225	EQA2226	EQA2227	% correctly assigned/ lab[a]
Provider	**No**	**No**	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**No**	**Yes**	**No**	**IQ**	**IQ**	**No**	**NA**
15	No	No	Yes	No	No	Yes	No	Yes	No	Yes	No	IQ	IQ	No	100
20	No	No	Yes	No	No	No	No	Yes	No	Yes	No	No	IQ	No	100
26	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
29	No	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No	No	IQ	No	100
34	No	No	Yes	No	No	Yes[b]	No	Yes	No	Yes	No	IQ	No	No	100
35	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
42	No	No	Yes	Yes[b]	No	Yes	No	Yes	No	Yes	No	No	IQ	No	100
43	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
44	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
46	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
49	No	No	Yes	No	No	Yes	No	No	Yes	Yes	No	IQ	IQ	No	83
63	No	No	Yes	No	No	Yes	No	Yes	No	Yes	No	IQ	IQ	IQ	92
73	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	No	No	100
77	No	No	Yes	Yes	No	Yes	No	Yes	No	Yes	No	IQ	IQ	No	100
84	No	No	Yes	No	No	Yes	No	Yes	No	Yes	No	IQ	IQ	No	100
92	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
98	No	No	Yes	No	No	No	No	Yes	No	Yes	No	IQ	IQ	No	100
% Correctly assigned/ 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 EQA2214 -EQA2222 and provided good quality raw reads EQA2223, EQA2224 and EQA2227. [b]EQA2217 and EQA2219 were around cluster cut-off, therefore yes and no were both considered correct, however, these laboratories did not adhere to their own reported cluster cut-off.

Annex 13. Distances reported based on index EQA2213

Gene-by-gene approach, allelic distances

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222	EQA2223	EQA2224	EQA2225	EQA2226	EQA2227
Provider	**70**	**219**	**0**	**8**	**252**	**6**	**85**	**0**	**670**	**0**	**897**	**NA**	**NA**	**2643**
15	70	233	0	9	264	5	86	0	684	0	872	IQ	IQ	2660
20	104	326	1	9	364	7	118	1	929	0	1100	63	IQ	3269
26	75	243	1	16	441	9	139	1	714	1	921	IQ	IQ	2676
29	97	290	2	11	326	6	119	1	873	1	1098	62	IQ	3140
34	75	240	0	14	275	8	93	0	713	0	920	IQ	284	>2000[a]
35	70	233	0	9	264	5	86	0	682	0	873	IQ	IQ	2659
42	70	231	0	9	243	5	85	0	648	0	871	43	IQ	2652
46	71	236	0	11	424	5	125	0	872	0	1077	IQ	IQ	3711
49	70	233	0	9	264	5	85	682	0	0	873	IQ	IQ	2652
63	74	241	0	14	276	7	93	0	717	0	926	IQ	IQ	IQ
73	76	257	0	6	278	4	85	0	755	0	922	IQ	221	2933
84	71	238	0	11	273	6	90	0	702	0	902	IQ	IQ	2669[a]
92	70	233	0	9	264	5	85	0	682	0	873	IQ	IQ	2653
98	70	232	0	10	268	6	90	0	694	0	897	IQ	IQ	2643
Median	**71**	**236**	**0**	**10**	**273**	**6**	**90**	**0**	**708**	**0**	**900**	**NA**	**NA**	**2660**

IQ = insufficient quality. Orange = insufficient quality not detected. [a]Laboratories commented correctly that raw reads EQA2227 were of a different serovar (monophasic Typhimurium)

SNP typing, SNP distances

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222	EQA2223	EQA2224	EQA2225	EQA2226	EQA2227
Provider	**132**	**470**	**1**	**10**	**554**	**9**	**152**	**1**	**1824**	**0**	**3334**	**NA**	**NA**	**31407**
43	42	147	0	7	180	5	57	0	529	0	1056	IQ	IQ	9962[a]
44	139	501	1	12	670	9	159	1	2605	0	4490	IQ	IQ	40674
77	34	59	0	1	78	1	31	0	238	0	279	IQ	IQ	177
Median	**42**	**147**	**0**	**7**	**180**	**5**	**57**	**0**	**529**	**0**	**1056**	**NA**	**NA**	**9962**

IQ = insufficient quality. [a]Laboratory commented correctly that raw reads EQA2227 were of a different serovar (monophasic Typhimurium)

Annex 14. AMR markers reported

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220
Provider	aac(6′)-Iaa	aac(6′)-Iaa, tet(A)	aac(6′)-Iaa	aac(6′)-Iaa	aac(6′)-Iaa	aac(6′)-Iaa	aac(6′)-Iaa
15	tet(A); aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa
26	amikacin, tobramycin	amikacin, tobramycin, tetracycline	amikacin, tobramycin	tobramycin, amikacin	tobramycin, amikacin	tobramycin, amikacin	tobramycin, amikacin
29	aac(6')-Iaa	aac(6')-Iaa tet(A)	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa
34	aac(6')-Iaa[a]	- aac(6')-Iaa[a] - tet(A)	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]
42	None	Tetracycline resistance: tet(A)	None	None	None	None	None
43	aac(6')-Iaa	aac(6')-Iaa; tet(A)	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa
46	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa
49	mdsA - mdsB	mdsA - mdsB - tet(A)	mdsA - mdsB	mdsA - mdsB	mdsA - mdsB	mdsA - mdsB	mdsA - mdsB
73	aac(6')-Iaa_1	aac(6')-Iaa_1, tet(A)_6	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1
77	aac(6')-Iaa	aac(6')-Iaa tet(A)	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa
84	none	tet(A)	none	none	none	none	none
92	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy
98	aac(6')-Iaa[a]	aac(6')-Iaa[a], tet(A)	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]

[a]*Participant commented that aac(6′)-Iaa is a cryptic gene that does not confer resistance.*

Lab ID	EQA2221	EQA2222	EQA2223	EQA2224	EQA2225	EQA2226	EQA2227
Provider	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	NA, IQ	NA, IQ	aac(6')-Iaa, aph(6)-Id, aph(3")-Ib, blaTEM-1B, sul2, tet(B)
15	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa; qnrB19	blaTEM-1B; tet(B); sul2; aac(6')-Iaa; aph(3")-Ib; aph(6)-Id
26	tobramycin, amikacin	tobramycin, amikacin	tobramycin, amikacin	tobramycin, amikacin	amikacin, tobramycin	low coverage	amikacin, tobramycin, ampicilin, sulfamethoxazole, tetracycline
29	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa qnrB19	aph(3")-Ib aph(6)-Id aac(6')-Iaa tet(B) sul2 blaTEM-1B
34	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	- aac(6')-Iaa[a] - qnrB19	- aac(6')-Iaa[a] - aph(6)-Id - aph(3")-Ib - tet(B) - blaTEM-1B - sul2
42	None	None	None	None	None	-(qnrB19)[b]	Aminoglycoside resistance: strA, srtB Beta-lactam: blaTEM-1 Sulfonamide: sul2 Tetracycline: tetB
43	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa; qnrB19	aac(6')-Iaa; aph(6)-Id; aph(3")-Ib; tet(B); sul2; blaTEM-1B
46	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa, qnrB19	aac(6')-Iaa, blaTEM-1B, sul2, tet(B), aph(3")-Ib, aph(6)-Id
49	mdsA - mdsB	mdsA - mdsB	mdsA - mdsB	mdsA - mdsB	blaEC - emrD - acrF- mdsA - mdsB	qnrB19 - mdsA	aph(6)-Id - aph(3"-Ib - blaTEM-1 - sul2 - tet(B) - mdsA - mdsB
73	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1	aac(6')-Iaa_1qnrB19	aac(6')-Iaa_1, aph(3")-Ib, aph(6)-Id, blaTEM-1B, sul2, tet(B)
77	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	aac(6')-Iaa	qnrB19	aac(6')-Iaa aph(3")-Ib aph(6)-Id blaTEM-1B sul2 tet(B)
84	none	none	none	none	none	qnrB19	blaTEM-1B, sul2, tet(B)
92	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy	aac(6')-Iaa/Iy, parC_T57S (based on the low-quality sequence data)	aac(6')-Iaa/Iy, qnrB19 (based on the low-quality sequence data)	tet(B), sul2, blaTEM-1B, aac(6')-Iaa/Iy, aph(3")-Ib, aph(6)-Id
98	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a]	aac(6')-Iaa[a], mdf(A)	aac(6')-Iaa[a], qnrB19	aac(6')-Iaa[a], blaTEM-1B, sul2, aph(6)-Id, tet(B), aph(3")-Ib

IQ = insufficient quality. Orange = insufficient quality not detected. [a]Participant commented that aac(6')-Iaa is a cryptic gene that does not confer resistance.

Annex 15. Quality assessment of submitted Illumina WGS data per laboratory

Laboratory ID 15

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.66
Contamination	0.52	0.52	0.49	0.61	0.52	0.49	0.52	0.52	0.82	0.57
Avg coverage	47.511	42.859	45.914	61.403	49.962	53.291	58.260	50.899	56.162	56.122
N50	438811	406609	284469	242855	438811	490516	421618	280737	71221	227920
GC%	52.13	52.07	52.14	52.13	52.13	52.12	52.13	52.14	52.13	52.2
Total length	4701890	4750572	4697711	4702639	4702919	4715351	4701456	4717904	4716431	4911351
# reads	1376936	1291760	1313816	1767318	1443584	1533226	1698936	1576164	1613562	1706188
Mean read length	162	157.5	164	163	162.5	163.5	161.5	154	164	161.5

Laboratory ID 20

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.68
Contamination	0.52	0.52	0.52	0.55	0.52	0.49	0.52	0.52	0.52	0.59
Avg coverage	78.741	64.416	52.129	79.795	51.447	59.910	83.170	70.665	64.238	57.219
N50	491607	232196	232189	401033	171153	305775	489575	283660	270873	262588
GC%	52.13	52.08	52.14	52.13	52.13	52.12	52.13	52.14	52.13	52.2
Total length	4701391	4749571	4696691	4704450	4700362	4714584	4700772	4714342	4702305	4910214
# reads	2724774	2185988	1772550	2745252	1758102	2095480	2801406	2422452	2176668	2038854
Mean read length	135.5	139.5	137.5	136.5	137	134.5	139	137.5	138.5	137

Laboratory ID 26

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.24	99.61	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	13.200	19.995	6.155	12.643	43.591	16.084	33.521	14.006	38.426	79.261
N50	43285	82413	41409	32209	87994	130429	76676	54883	160465	105348
GC%	52.22	52.1	52.21	52.21	52.16	52.13	52.16	52.18	52.14	52.22
Total length	4678355	4744176	4678339	4684257	4696875	4712661	4694585	4703735	4701053	4908025
# reads	497464	798238	243414	512820	1578782	597924	1269490	579674	1311334	2882222
Mean read length	124.5	118.5	118	115	129.5	126.5	124	113.5	137	135

Grey = does not pass quality criteria of EQA provider

Laboratory ID 29

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.59	99.61	99.61	99.61
Contamination	0.52	0.65	0.7	0.52	0.63	0.62	0.93	0.84	0.58	0.49
Avg coverage	81.079	96.912	86.686	38.623	54.804	87.443	53.443	82.148	59.979	86.099
N50	283659	284467	417427	90350	180449	176772	70816	223796	218815	283825
GC%	52.09	52.07	52.13	52.15	52.08	52.08	52.13	52.11	52.1	52.2
Total length	4719708	4751071	4703577	4699017	4723652	4736470	4709567	4740402	4713550	4912234
# reads	2203978	2648910	2388930	1150842	1535610	2385578	1526858	2337812	1651210	2440144
Mean read length	173.5	174	170.5	157.5	168.5	173.5	164.5	167.5	171	173

Laboratory ID 34

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.58	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.71	0.54	0.52	0.52	0.49	0.52	0.52	0.52	0.57
Avg coverage	115.610	125.673	56.766	60.553	29.909	65.040	62.568	100.016	88.324	90.205
N50	166692	312904	130299	56876	60804	83666	96568	206004	153305	128908
GC%	52.16	52.1	52.19	52.29	52.24	52.2	52.2	52.17	52.19	52.26
Total length	4693570	4745865	4683206	4661940	4665309	4698717	4682565	4706131	4685981	4895291
# reads	4290786	4727708	2106050	2232644	1107296	2416078	2327790	3740210	3265328	3517170
Mean read length	126	126	126	126	126	126	125.5	125.5	126.5	125

Grey = does not pass quality criteria of EQA provider

Laboratory ID 35

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.58	99.61	99.61	99.61	99.61	99.68
Contamination	0.52	0.52	0.49	0.39	0.52	0.37	0.52	0.52	0.52	0.52
Avg coverage	132.587	89.757	77.768	109.879	107.693	118.403	103.074	96.931	120.720	111.170
N50	268434	129035	204736	215051	245765	297409	283824	171160	171710	151066
GC%	52.13	52.12	52.16	52.14	52.13	52.13	52.13	52.15	52.15	52.22
Total length	4702307	4741201	4692698	4700756	4698134	4715651	4702356	4714717	4698432	4906060
# reads	4746810	3190792	2739452	3886792	3807048	4203254	3634508	3452668	4244978	4063132
Mean read length	131	133.5	133	132.5	132.5	132.5	133.5	132	133	134

Laboratory ID 42

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.68
Contamination	0.52	0.52	0.49	0.52	0.55	0.49	0.54	0.52	0.52	0.49
Avg coverage	94.957	68.680	85.297	85.962	66.733	66.151	53.455	56.283	82.149	70.221
N50	489947	271832	284469	401355	284277	266344	230144	219035	489948	218678
GC%	52.13	52.07	52.14	52.13	52.13	52.12	52.13	52.13	52.13	52.2
Total length	4702180	4748916	4695575	4702862	4702454	4714983	4701546	4713689	4702568	4909461
# reads	2312510	2946446	3569780	3580732	2783002	2854616	2255120	2406856	3457012	3051560
Mean read length	193	110	112	113	112.5	109	111	110	111.5	112.5

Laboratory ID 43

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	29.787	34.415	36.865	33.993	33.147	28.089	37.168	43.314	32.683	36.060
N50	489948	491393	490086	421585	489948	491607	491608	373429	491607	299390
GC%	52.13	52.08	52.14	52.13	52.13	52.12	52.13	52.14	52.13	52.2
Total length	4701272	4750918	4697306	4702809	4702342	4715399	4701303	4716022	4701734	4911428
# reads	990454	1134220	1202062	1115706	1085102	929710	1213146	1419364	1076900	1230262
Mean read length	141	144	143.5	142.5	143.5	142	144	143.5	142.5	143.5

Grey = does not pass quality criteria of EQA provider

Laboratory ID 46

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	47.946	46.761	53.531	31.322	35.290	54.483	48.579	30.108	27.912	32.563
N50	489947	490160	490086	401110	489948	491597	492034	400956	491606	406258
GC%	52.13	52.13	52.14	52.13	52.13	52.12	52.13	52.14	52.13	52.2
Total length	4702140	4705275	4696354	4701126	4701387	4716251	4701865	4715700	4701480	4909604
# reads	1358898	1356082	1527984	980236	1117560	1611814	1475006	944912	871440	1017108
Mean read length	166	162	164.5	150	148	159	154.5	149.5	150	157

Grey = does not pass quality criteria of EQA provider

Laboratory ID 49

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.49	0.52
Avg coverage	47.870	45.725	51.777	52.798	42.454	46.691	46.997	49.382	42.779	30.419
N50	694425	421588	490086	491496	694372	489980	580033	306216	514862	410468
GC%	52.13	52.08	52.14	52.13	52.13	52.13	52.13	52.14	52.2	52.13
Total length	4701086	4749518	4697023	4701697	4699512	4716069	4701342	4715012	4911227	4700151
# reads	1546430	1496626	1670764	1703496	1373976	1515866	1518492	1605102	1454654	987292
Mean read length	145.5	144.5	145.5	145.5	145	145	145	144.5	144	144.5

Laboratory ID 63

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61
Contamination	0.8	0.69	1.23	0.72	0.6	0.78	0.6	0.63	1.31	0.74
Avg coverage	37.335	39.496	44.308	42.893	55.899	51.385	50.096	40.668	39.835	45.221
N50	491606	489733	491595	343800	491607	438786	491607	326440	491606	141064
GC%	52.15	52.09	52.14	52.13	52.14	52.14	52.14	52.15	52.16	52.21
Total length	4709133	4753731	4702678	4710693	4703632	4723277	4707804	4721529	4712434	4924438
# reads	869682	930356	1052428	995734	1285296	1187772	1151848	941194	921288	1103178
Mean read length	202	201.5	197.5	203	205	204	204.5	203.5	203.5	202

Laboratory ID 73

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.58	99.61	99.61	99.61	99.61	99.68
Contamination	0.66	0.52	0.73	0.66	0.55	0.95	1.03	0.84	0.7	0.91
Avg coverage	354.738	334.886	299.187	375.378	335.415	404.893	331.568	306.174	380.633	272.987
N50	489947	489733	490086	489947	489948	490088	489948	284467	489948	514862
GC%	52.13	52.08	52.14	52.13	52.12	52.12	52.13	52.14	52.13	52.2
Total length	4702224	4749848	4698126	4702626	4699112	4718836	4702214	4715940	4702581	4913405
# reads	11807622	11278054	9958468	12492596	11189064	13516998	11061602	10299574	12651646	9622534
Mean read length	141	141	141	141	140.5	141	141	139.5	141	139

Laboratory ID 77

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.68
Contamination	0.54	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	542.708	70.843	540.518	480.007	626.739	693.373	557.524	99.123	751.199	410.333
N50	491606	283824	491595	489948	344944	492025	491607	284467	489947	445656
GC%	52.13	52.08	52.14	52.13	52.13	52.12	52.13	52.14	52.13	52.2
Total length	4704441	4749958	4700569	4702670	4703344	4719708	4703549	4719426	4704555	4912722
# reads	18155624	2390626	17902080	16104346	20777156	23075374	18499592	23635126	25092708	14351442
Mean read length	140	140.5	141.5	140	142	141.5	141.5	141	140.5	140

Laboratory ID 84

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	88.99	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.61	99.68
Contamination	31.2	0.52	0.49	0.55	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	62.529	43.489	38.819	87.396	46.915	19.158	69.493	77.645	67.584	62.486
N50	489957	489733	490086	489947	401544	477534	491607	284467	491607	406594
GC%	52.11	52.08	52.14	52.13	52.13	52.13	52.13	52.14	52.13	52.2
Total length	4739942	4750402	4697405	4702615	4703262	4716934	4702376	4715716	4702274	4911984
# reads	1689614	1086342	953100	2129276	1198932	488662	1709862	1939618	1669770	1606918
Mean read length	175.5	190	191	192.5	183.5	184.5	191	188.5	190	190.5

Grey = does not pass quality criteria of EQA provider

Laboratory ID 92

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.58	99.61	99.61	99.61	99.61	99.61
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	80.180	74.719	77.475	119.521	118.011	94.111	94.287	65.616	98.538	111.564
N50	171610	181476	153296	257159	244526	264786	268385	136638	401033	300802
GC%	52.14	52.14	52.15	52.13	52.13	52.13	52.14	52.16	52.13	52.2
Total length	4701394	4704237	4694993	4701405	4696960	4715891	4701784	4710462	4701277	4911251
# reads	2671282	2493884	2579002	4158244	4005070	3171688	3157920	2219156	3322162	3932238
Mean read length	141	141	140.5	135	138	139.5	140	139	139.5	139

Laboratory ID 98

QC parameter	EQA2213	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Completeness	99.61	99.61	99.61	99.61	99.6	99.61	99.61	99.61	99.61	99.68
Contamination	0.52	0.52	0.49	0.52	0.52	0.49	0.52	0.52	0.52	0.49
Avg coverage	47.399	62.850	43.884	50.211	21.856	54.152	36.835	47.155	43.331	51.413
N50	133432	224445	142259	123377	53602	118687	123790	167723	161475	129071
GC%	52.13	52.08	52.14	52.13	52.15	52.12	52.13	52.13	52.13	52.2
Total length	4700089	4749516	4695009	4699440	4694460	4714057	4700207	4712982	4699809	4907655
# reads	2049400	2758814	1893686	2193708	949624	2353150	1597244	2056486	1881884	2320756
Mean read length	108.5	108	108.5	107.5	107.5	108	108	107.5	107.5	108

Grey = does not pass quality criteria of EQA provider

Annex 16. Reported results MLVA-based cluster assignments to index EQA2213

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Provider WGS	**No**	**No**	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**No**
Provider MLVA	**Yes**	**No**	**Yes**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
26	Yes	No	Yes	Yes	No	Yes	No[a]	Yes	No
63	Yes	No	Yes	Yes	No	Yes	No	Yes	No
74	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No

Green = provider. Grey = incorrectly assigned, based on cluster identification with WGS data. [a]Despite laboratory 26 reported a cluster cut-off of 1 allele, EQA2220 is not considered as cluster isolate, while the MLVA profile differed 1 allele.

Annex 17. Reported results PFGE-based cluster assignments to index EQA2213

Lab ID	EQA2214	EQA2215	EQA2216	EQA2217	EQA2218	EQA2219	EQA2220	EQA2221	EQA2222
Provider WGS	**No**	**No**	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**No**
17	Yes	No	Yes	No	No	Yes	No	Yes	No
75	Possibly	Possibly	Yes	Possibly	Possibly	Yes	No	Yes	No

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

TQ-03-23-120-EN-N

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Publications Office of the European Union

ISBN 978-92-9498-631-3