TECHNICAL REPORT

Eighth external quality assessment scheme for *Salmonella* typing

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Abbreviations

BN	BioNumerics
cgMLST	Core genome multilocus sequence type
wgMLST	Whole genome multilocus sequence type
EFSA	European Food Safety Authority
EQA	External Quality Assessment
EURL	European Union Reference Laboratory
FWD	Food- and waterborne diseases and zoonoses
FWD-Net	Food- and Waterborne Diseases and Zoonoses Network
PFGE	Pulsed field gel electrophoresis
PH NRL	Public health national reference laboratories
QC	Qualitative control
SNP	Single nucleotide polymorphism
SSI	Statens Serum Institut
ST	Sequence type
TESSy	The European Surveillance System
WGS	Whole genome sequence

Executive summary

This report presents the results of the eighth round of the external quality assessment (EQA-8) scheme for typing of *Salmonella enterica* subsp. *enterica* organised for public health national reference laboratories (PH NRLs) in ECDC's Food- and Waterborne Diseases and Zoonoses network (FWD-Net). Since 2012, the EQA scheme has covered molecular typing methods used for EU-wide surveillance. The EQA-8 scheme was arranged by the Section for Foodborne Infections at the Statens Serum Institut (SSI) in Denmark and the current EQA represents the first round of a new ECDC-funded contract.

Salmonellosis was the second-most commonly reported zoonotic disease in EU, with a notification rate of 20.4 cases per 100 000 population in 2016. From 2012 to 2016, the annual number of reported salmonellosis cases has been in the range of 92 012–94 597 except for 2013, when 87 453 cases were reported [3]. Since 2007, ECDC's Food- and waterborne diseases and zoonoses (FWD) programme has been responsible for EU-wide surveillance of salmonellosis, including facilitating the detection and investigation of food-borne outbreaks. Surveillance data, including certain basic typing parameters, are reported by Member States to the European Surveillance System (TESSy). In 2012, more advanced and discriminatory molecular typing data were incorporated into TESSy to improve surveillance of food-borne infections.

The reporting of molecular surveillance data relies on the capacity of PH NRLs in FWD-Net to produce comparable typing results. Currently, data from pulsed-field gel electrophoresis (PFGE) and multiple-locus variable number of tandem repeat analysis (MLVA) are collected in TESSy. The previous EQA schemes from EQA-4 to EQA-7 included assessment of the PFGE typing methods for all *Salmonella* serovars and MLVA for *Salmonella* Typhimurium (STm). In order to ensure the EQA is contemporary with the development of surveillance methods used by PH NRLs in Europe, EQA-8 contains two new features: assessment of performance of MLVA for *S.* Enteritidis (SE) and ability to identify a cluster based on molecular typing by PFGE, MLVA and/or whole genome sequencing- (WGS) derived data.

The objectives of the EQA scheme are to assess the quality and comparability of molecular typing data produced by PH NRLs in FWD-Net. Test isolates for the EQA were selected to cover isolates currently relevant for public health in Europe. Four sets of 10 to 12 isolates were selected, including different *Salmonella* serovars for PFGE, *S.* Typhimurium and *S.* Enteritidis isolates for the two MLVA methods and a mixture of different sequence types (ST) in the cluster analysis.

Twenty-five laboratories signed up and 24 completed the exercise despite some only completing part of the methods for which the laboratory had signed up. This is a minor decrease of 8% in overall participation compared with the previous EQA-7. Implementation of the already scheduled removal of the PFGE part (gel quality and analysis) in the coming EQAs will possibly result in a further decrease. A minority (21%) of participants completed the full EQA scheme (PFGE, MLVA and molecular typing-based cluster analysis). In total, 17 (71%) participated in the PFGE part, 13 (54%) in at least one of the MLVA methods (12 for STm and 12 for SE) and 13 (54%) in the molecular typing-based cluster analysis. Eleven (46%) laboratories submitted WGS-based typing results for cluster analysis.

A PFGE gel of sufficiently high quality was produced by the majority of laboratories (14/17; 82%) and almost all (10/11; 91%) made the gel analysis in accordance with guidelines for producing inter-laboratory comparable gels. For both MLVA schemes (STm and SE), the overall performance was high, as nine laboratories of 12 (75%) reported correct allelic profiles for all test isolates in each set. In the new MLVA part for *S.* Enteritidis, two participants had low scores, as only 40% and 70% of the test isolates were reported with correct allelic profiles.

Out of the twenty-four laboratories participating in EQA-8, 13 (54%) performed molecular typing-based cluster analysis. The idea of the cluster analysis part of the EQA was to assess the PH NRL's ability to identify a cluster of genetically closely related isolates given the fact that a multitude of different laboratory and analytical methods are used as the primary cluster detection approach in Member States. This part of the EQA was atypical in the sense that the aim was to assess the participants' ability to reach the correct conclusion, i.e. correctly categorise cluster test isolates, not the ability to follow a specific procedure.

The cluster of closely related *S.* Enteritidis ST11 isolates could be identified by PFGE, MLVA and WGS-derived data. The expected cluster was based on a predefined categorisation by the organiser and contained seven isolates based on PFGE, five isolates by MLVA and four if the identification was based on WGS-derived data. All four cluster isolates had been part of the multi-country outbreak linked to eggs [7].

Four laboratories used PFGE for cluster analysis and two of them reported a cluster analysis based on WGS data. Only one laboratory did not identify the correct cluster using PFGE. Four laboratories used MLVA for cluster analysis and all also reported a cluster analysis based on WGS data. All laboratories identified the correct cluster using MLVA.

Performance was high using WGS-derived data for cluster analysis, with 10/11 of the participants (91% correctly identifying the cluster of four closely related isolates. However, one of these laboratories did not report a complete cluster analysis data set due to low sequencing quality for two test isolates. Another laboratory identified a cluster of only three isolates, but indicated that possible inclusion of the fourth isolate should depend on epidemiological information. The participants were free to choose their preferred analytical method for WGS-based cluster

identification. The majority of participants preferred a whole core genome multilocus sequence typing (allele-based) method, with 7/11 (64%) using cgMLST and only 4/11 (36%) using single nucleotide polymorphism (SNP-based) analysis as the main method for cluster analysis. Allele-based and SNP methods seemed equally suitable for cluster identification.

For inter-laboratory comparability and communication about cluster definitions, cgMLST using a standard scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results, whereas the use of non-standardised SNP analysis may be more challenging for comparison and communication between laboratories. This issue is further complicated because many laboratories still use other methods (PFGE or MLVA) and will probably not switch to WGS in the near future.

1 Introduction

1.1 Background

The European Centre for Disease Prevention and Control (ECDC) is an independent European Union (EU) agency with a mandate to operate dedicated surveillance networks. The mission of ECDC is to identify, assess and communicate current and emerging threats to human health from communicable diseases. ECDC fosters the development of sufficient capacity within the community network 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].

External quality assessments (EQA) are an essential part of laboratory quality management and uses an external evaluator to assess the performance of laboratories on test samples supplied specifically for the purpose.

ECDC's disease-specific networks organise a series of EQAs for EU/European Economic Area (EEA) countries. EQAs aim to identify areas for improvement in laboratory diagnostic capacities relevant to epidemiological surveillance of communicable diseases as set forth 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 the 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)
- evaluate individual laboratory performance
- identify and justify problem areas
- provide continuing education; and
- identify needs for training activities.

Since 2012, the Section for Foodborne Infections at the Statens Serum Institut (SSI) in Denmark has been the EQA provider for the typing of *S. enterica* subsp. *enterica*. Shiga toxin/verocytotoxin-producing *Escherichia coli* (STEC/VTEC) and *Listeria monocytogenes*. In 2016, SSI was also granted the new round of tenders (2017 to 2020) for all three lots. The contracted EQA-8 scheme for *Salmonella* covers PFGE, MLVA typing of both *S.* Typhimurium and *S*. Enteritidis and molecular typing-based cluster analysis. This report presents the results of the *Salmonella* EQA-8.

1.2 Surveillance of non-typhoidal salmonellosis

In 2016, non-typhoidal salmonellosis (later 'salmonellosis') was the second-most commonly reported zoonotic disease in the EU, with a total of 94 530 cases reported by the 28 EU Member States (EU notification rate of 20.4 cases per 100 000 population) similar to 2015. As in previous years, the most commonly reported *Salmonella* serovars were *S*. Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium, *S*. Infantis and *S*. Derby. In the period 2012 to 2016, the annual number of reported salmonellosis cases was in the range of 92 012 to 94 597, except for 2013, when 87 453 cases were reported [3].

Since 2007, ECDC's Food- and waterborne diseases and zoonoses (FWD) programme has been responsible for EU-wide surveillance of salmonellosis and facilitating the detection and investigation of foodborne outbreaks. One of the key objectives of the FWD programme is to improve and harmonise the surveillance system in the EU and increase scientific knowledge of aetiology, risk factors and the burden of FWD. The surveillance data, including some basic typing parameters for the isolated pathogen, are reported by Member States to the European Surveillance System (TESSy). In addition to the basic characterisation of the pathogens isolated from human infections, there is a public health value in using more discriminatory typing techniques in the surveillance of foodborne infections. In 2012, ECDC initiated enhanced EU-level surveillance by incorporating molecular typing data into reporting ('molecular surveillance'). Three priority FWD pathogens were selected for the pilot: *Salmonella enterica* subsp. *enterica*, *L. monocytogenes* and STEC/VTEC. The overall aims of integrating molecular typing data into EU-level surveillance are to:

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

Molecular typing-enhanced surveillance gives Member State users access to EU-wide molecular typing data for the pathogens included. It also provides users with the opportunity to perform cluster searches and cross-sector

comparability of EU-level data to determine whether isolates characterised by molecular typing at the national level(s) are part of a multinational cluster that may require cross-border response collaboration.

EQA schemes are targeted to public health national reference laboratories (PH NRL) already expected to be performing molecular typing-enhanced surveillance at the national level.

1.3 Objectives

1.3.1 Pulsed field gel electrophoresis typing

The objectives of the *Salmonella* EQA-8 were to assess the quality of standard PFGE typing and comparability of collected test results among participating laboratories. The exercise focused on the production of high-quality raw PFGE gels, normalisation of PFGE images and band assignment.

1.3.2 Multiple locus variable number of tandem repeats analysis typing of *S.* Typhimurium and *S.* Enteritidis

The *Salmonella* EQA-8 aimed to determine and ensure the quality and integrity of *S. enterica* subsp. *enterica* serovar Typhimurium and serovar Enteritidis MLVA results in the participating laboratory. The MLVA part covered both the laboratory procedure and subsequent data analysis (calibration of raw data into correct MLVA alleles according to the nomenclature [4–5]).

1.3.3 Molecular typing-based cluster analysis

The objective of the molecular typing-based cluster analysis of *Salmonella* EQA-8 was to assess the ability to detect clusters of closely related isolates. Laboratories could perform the analyses using PFGE, MLVA and/or derived data from WGS.

2 Study design

2.1 Organisation

The *Salmonella* EQA-8 was funded by ECDC and arranged by SSI following the requirements in ISO/IEC 17043:2010 [6]. EQA-8 included PFGE of different serovars, MLVA of *S.* Typhimurium and *S.* Enteritidis and molecular typing-based cluster analysis. EQA-8 was conducted between September 2017 and March 2018.

Invitations were emailed to ECDC contact points in FWD-Net (30 countries) by 14 June 2017 with a deadline to respond 28 June 2017. In addition, invitations were sent to EU candidate and potential candidate countries Albania, Montenegro, the former Yugoslav Republic of Macedonia, Serbia, Turkey, Bosnia and Herzegovina and Kosovo[i].

Twenty-five PH NRLs in the EU/EEA and EU candidate countries accepted the invitation to participate, but only 24 submitted results (Annex 1). Among the 24 participants, one new participant was included. In Annex 2, details of participation in EQA-7 and EQA-8 are listed to give an overview of the trend in the number of participants.

EQA test isolates were sent to the laboratories on 30 August 2017. The participants were asked to submit their results to an SSH File Transfer Protocol (SFTP) site and complete the online form by 20 November 2017 (Annex 21).

EQA submission protocol, preconfigured BioNumerics (BN) databases, XML export, Excel sheets for the MLVA reference isolates and MLVA allele calling were available at the online site.

2.2 Selection of test isolates

One hundred *Salmonella* test isolates were selected to fulfil the following criteria:

- represent commonly reported isolates in Europe
- remain stable during the preliminary test period at the organising laboratory
- include repeat isolates from EQA-4 through 8; and
- include closely related isolates.

The 100 selected isolates were analysed using the methods in the EQA before and after having been re-cultured 10 times. All candidate isolates remained stable using these methods and the final test isolates were selected. The 11 test isolates for the PFGE part were selected to include both 'easy' and more 'difficult' profiles with double bands. A variety of different serovars relevant for the epidemiological situation in Europe, including recent outbreak isolates of *S.* Enteritidis [7], were selected (Table 1). For the MLVA part, 10 *S.* Typhimurium and 10 *S.* Enteritidis were selected to cover common MLVA profiles (Annexes 7–8). The 12 isolates for cluster analysis were selected to include isolates with different (or varying) relatedness and comprised different multilocus sequence types (ST 10, 11, 183, and 1925). The cluster contained seven isolates if based on PFGE derived data, five if based on MLVA derived data and four isolates (one technical duplicate) if based on WGS-derived data. The characteristics of the test isolates used are listed as Original/REF in Annexes 5 and 7–11.

Table 1. Serovars of test isolates

Method	Number of test isolates	Serovars	Annex
PFGE	11	Enteritidis, Infantis*, Kentucky, Newport, O:4,5,12;H:i:-, Oranienburg, Poona*, Szentes, Typhimurium and Virchow	3–6
MLVA *S.* Typhimurium	10	Typhimurium *STm6 (3-12-9-NA-211), *STm9 (3-13-NA-NA-211)	7
MLVA *S.* Enteritidis	10	Enteritidis	8
Cluster	12	Enteritidis (ST11, ST183 and ST1925), Dublin (ST10)	9–20

**: repeat isolates included in EQA-4 to 8.*
NA: designates a locus not present (-2 by submission, Annex 7).

[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.

2.3 Carriage of isolates

All test isolates were blinded and shipped on 30 August 2017. The protocol for the EQA exercise and a letter stating the unique isolate IDs were included in the packages and distributed individually to participants by e-mail on 29 August as an extra precaution. Twenty participants received their dispatched isolates within one day, three within three days and only two participants received the isolates late after shipment, six and 13 days respectively. The parcels were shipped from SSI labelled as UN 3373 Biological Substance. No participants reported damage to the shipment or errors in the unique specific isolate IDs.

On 20 September 2017, instructions to the submission of results procedure were e-mailed to the participants. This included the links to the online uploading/downloading site and submission form.

At the site, participants should have downloaded the preconfigured BioNumerics (BN) databases with the correct experiment settings (PFGE part), an XML export file and four Excel sheets; a compensatory table for MLVA reference isolates and a sheet for the subsequent calculation of MLVA alleles for both *S.* Typhimurium and *S.* Enteritidis (MLVA part).

2.4 Testing

In the PFGE part, participants could choose to perform the laboratory part only (submit TIFF image of the PFGE gel) or to further complete an analysis of a PFGE profile made by the EQA provider. This change was introduced in order to truly evaluate the band assignment without the influence of the participants' own gel quality. For the laboratory procedures, the participants were instructed to use the laboratory protocol 'Standard PulseNet *Salmonella* PFGE-One-Day (24–28 h) Standardised Laboratory Protocol for Molecular Subtyping of *Escherichia coli* O157:H7, *Salmonella* serotypes*, Shigella sonnei* and *Shigella flexneri* by Pulsed Field Gel Electrophoresis (PFGE)' [8]. For the gel analysis, participants were instructed to use the distributed preconfigured BN database and analyse the PFGE gel made by the EQA provider, including normalisation and band assignment. Submission of results included online uploading of PFGE images as either TIFF or XML export files including the BN analysis. Guidelines for setting up the BN database and exporting XML files from BN were included in the EQA submission instruction.

In the MLVA part, the 10 *S.* Typhimurium and 10 *S.* Enteritidis test isolates were tested to assess the participants' ability to obtain the true number of repeats in each of the five MLVA loci for each scheme. The participants were instructed to use ECDC's laboratory standard operating procedure for MLVA of *Salmonella enterica* serotype Typhimurium [4] and MLVA of *Salmonella enterica* serotype Enteritidis [5]. The distributed Excel sheets could be used to convert the measured fragment sizes to true allele numbers based on the results obtained for the 33 *S.* Typhimurium and 16 *S.* Enteritidis reference isolates. The allelic profiles should be submitted using the online submission form, -2 was used instead of NA when a locus was missing [4–5].

In the cluster analysis part, the participants could choose to perform the laboratory part using PFGE, MLVA and/or WGS derived data. The participants were instructed to report the IDs of the isolates included in the cluster of closely related isolates by method. A pdf version of the online form was also available for the participants. (Annex 21). If PFGE analysis was conducted, the participant reported the number of shared bands with an isolate that was found as a representative of the cluster. If MLVA was performed, the participants were instructed to report the MLVA scheme used and the number of repeats in each of the loci per isolate.

Laboratories performing WGS could use their own analysis pipeline for the cluster analysis, e.g. SNP-based or allele based and were asked to submit the isolates, identified as cluster of closely related isolates, based on the analysis used. The laboratories could report results from up to three analyses (1 main and 0 to 2 additional), but the detected cluster had to be based on results from the main analysis. The laboratories reported SNP distance or allelic differences between a selected cluster isolate and each test isolate, and uploaded the raw reads (FASTQ files).

2.5 Data analysis

As the participating laboratories submitted their results, the PFGE, MLVA and cluster analysis results, as well as the participants' uploaded raw reads, were imported to a dedicated *Salmonella* EQA-8 BN database.

No errors were identified in the submission process, but several participants needed reminders to upload the raw reads.

PFGE gel quality was evaluated according to a modified version of ECDC's FWD MolSurv Pilot – 'SOPs 1.0, PulseNet US protocol PFGE Image Quality Assessment (TIFF Quality Grading Guidelines EQA-7, Annex 3)' – by scoring the gel according to seven parameters (scores in the range of 1 to 4). The BN analysis was evaluated according to the 'BioNumerics Gel Analysis Quality Guidelines EQA-8' developed at SSI (Annex 4) to grade the BN analysis according to five parameters (scores in the range of 1–3). A score of 1 [Poor] in any of the parameters in the two guidelines corresponded to a gel analysis that could be used for inter-laboratory comparison. The BioNumerics Gel Analysis

Quality Guidelines were slightly modified from EQA-7, giving a more accurate evaluation of the band assignment (Annex 4).

The MLVA results were evaluated according to the percentage of correctly assigned allelic profiles generating a score from 0 to 100% correct profiles.

The molecular typing-based cluster analysis part was evaluated according to correct or incorrect identification of the cluster of closely related isolates based on a predefined categorisation by the EQA provider.

The EQA provider's PFGE results were based on *Xba*I profiles and included seven of the 12 test isolates (REF1, REF2, REF3, REF4, REF6, REF9 and REF11; REF2 and REF11 were technical duplicates). The EQA provider's MLVA results were based on the *S.* Enteritidis scheme [5] and included five ST11 isolates: REF1, REF2, REF4, REF9 and REF11 with the MLVA profile 2-9-7-3-2. The EQA provider's cluster analysis based on WGS-derived data was based on an allele-based (cgMLST, [9]) and SNP analysis (NASP, [10]). The correct number of closely related *S*. Enteritidis isolates were four out of the 12 isolates (REF2, REF4, REF9 and REF11) and all four were part of an European *S*. Enteritidis outbreak [7]. The EQA provider found at most a 0–3-allele difference or 0–6 SNP distances between any two isolates in the cluster. The rest of the cluster test isolates were an additional five ST11 isolates, one ST10, one ST183 and one ST1925.

Individual evaluation reports were distributed to the participants in the beginning of March 2018 and certificates of attendance in April 2018. If WGS data were used, the evaluation report included a quality assessment made by the EQA provider's in-house quality control pipeline (e.g. coverage, N50, sequence length and number of contigs). The evaluation report did not include an evaluation based on quality thresholds.

3 Results

3.1 Participation

The laboratories could participate in either the full EQA scheme or one part only (PFGE quality, PFGE analysis, MLVA *S.* Typhimurium, MLVA *S.* Enteritidis and/or molecular typing-based cluster analysis based on PFGE, MLVA and/or WGS-derived data). Out of the 25 participants who signed up for the exercise, 24 managed to complete and submit their results. Only five laboratories completed PFGE, MLVA (STm and/or SE) and molecular typing-based cluster analysis (PFGE, MLVA and/or WGS). Most laboratories participated in the PFGE part, where 17 (71%) laboratories produced a PFGE gel image, and 11 of them also completed the analysis of the provided PFGE gel. Thirteen (54%) laboratories participated in the MLVA part, 12 out of 13 (92%) submitted results of the *S.* Typimurium test isolates and 12 (92%) laboratories submitted results of the *S.* Enteritidis test isolates. Thirteen laboratories (54%) participated in the cluster analysis part and most of them (11, 85%) reported cluster analysis based on WGS-derived data. Two participants (15%) reported cluster identification using only PFGE data. Furthermore, two participants submitted cluster data based on both MLVA and WGS and two participants reported the cluster using all three methods (Table 2).

Table 2. Number and percentage of laboratories submitting results for each method

	PFGE			MLVA				Cluster					All
	Gel+ analysis	Gel only	Total	STm only	SE only	Both	Total	PFGE only	WGS only	MLVA + WGS	All	Total	Total
Number of participants	11	6	17	1	1	11	13	2	7	2	2	13	24
Percentage of participants	65%	35%	71%*	8%	8%	85%	54%*	15%	54%	15%	15%	54%*	-

Five participants (21%) completed both PFGE, MLVA and cluster analysis of the EQA scheme. Eight participants (33%) completed both PFGE and MLVA. Nine participants (34%) completed both MLVA and cluster analysis of the EQA scheme. None of the laboratories participating in the cluster analysis used only MLVA.
**: Percentage of the total number of participating laboratories (24)*
STm: S. Typhimurium
SE: S. Enteritidis.

3.2 Pulsed field gel electrophoresis

Seventeen laboratories (71%) produced a PFGE gel image and 11 of them also analysed the PFGE profile made by the EQA provider and thus submitted the analysed data in XML export format.

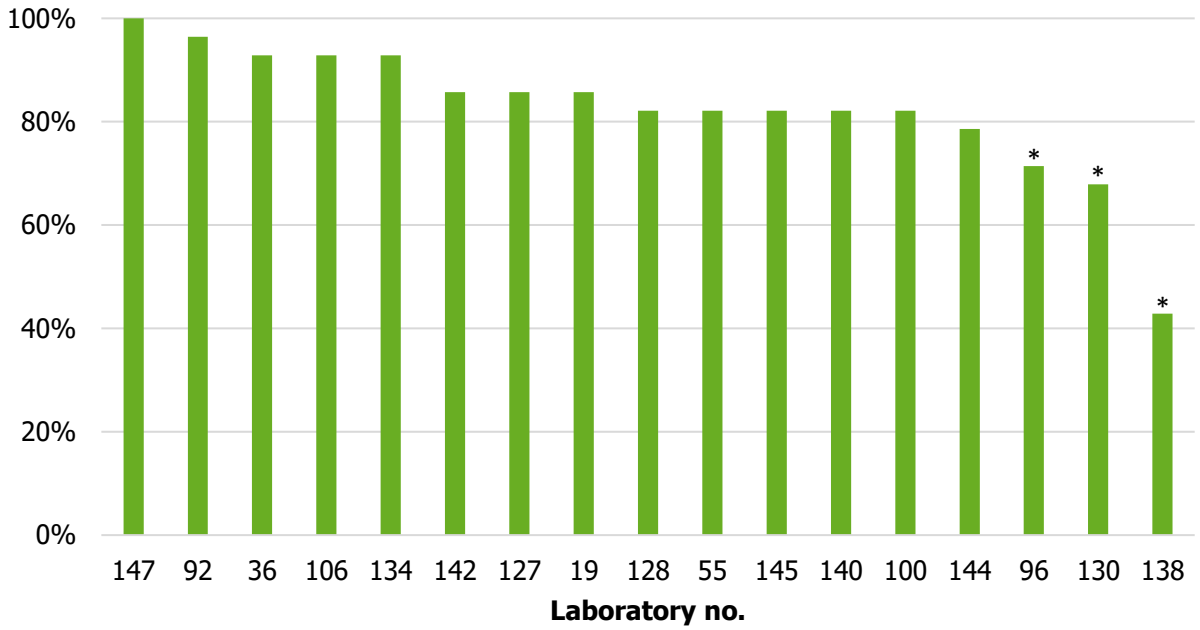
Annex 5 shows the profiles generated by the laboratories for two selected test isolates, PFGE C and H, including the profile produced by the EQA provider.

3.2.1 Gel quality

Gel quality varied considerably among the laboratories (Figure 1) based on a highly variable quality of the profiles for the individual test isolates (Annex 5). Gels were graded according to the TIFF Quality Grading Guidelines EQA-8, evaluating seven gel parameters using four scores from 1 to 4 (Annex 3). An acceptable gel quality (score of 2 [Fair] or better) should be achieved in each parameter since a low quality score of 1 [Poor] in just one parameter would have an impact on the ability to further analyse the image and compare profiles across laboratories. It is important to note that since a score of 1 in any parameter reflects an unacceptable gel that is incomparable on an inter-laboratory basis, the total gel quality score alone cannot be used as a measure for quality.

Fourteen (82%) of the participating laboratories were able to produce a gel of sufficient quality to enable profile detection and inter-laboratory comparison (Figure 1, Annex 6). Laboratories 96 and 130 produced gels with bands that were too fuzzy and laboratory 138 had incorrect running conditions, restriction and DNA degradation issues that made them not acceptable for inter-laboratory comparison. Only laboratory 147 produced a gel of excellent quality with respect to all parameters.

Figure 1. Participant scores for PFGE gel quality



Participating laboratories represented by arbitrary numbers.
Bars represent the total as a percentage of the maximum score of 28 points, according to evaluation of the gels using seven parameters graded 1–4.
**: gels unacceptable for inter-laboratory comparison, score of 1 [Poor] in at least one parameter.*

Table 3 shows the seven gel parameters, evaluated by the TIFF Quality Grading Guidelines EQA-8 (Annex 3), the percentage of laboratories scoring 1–4 and the average score for all laboratories. In general, the average score was above 3 (i.e. between good and excellent). However, one parameter ('DNA Degradation'), obtained an average score just below 3 (i.e. between fair and good) and only a minor percentage (29%) of the laboratories were able to obtain an Excellent [4] score. Furthermore, one gel (laboratory 138) unsuitable for inter-laboratory comparison obtained a score of 1 [Poor] in the parameters 'Image acquisition and running conditions', 'Restriction' and 'DNA degradation'. Two additional laboratories (96 and 130) also obtained a score of 1 [Poor] in the parameter 'Bands'. On average, the majority of the laboratories (11/17; 65%) obtained 3 [Good] or 4 [Excellent] in all parameters, reflecting a generally good gel performance (Table 3). None of the parameters evaluates the number of included reference lanes in the produced gel. Four laboratories disregarded the very important factor of including a reference lane for every 3 to 4 (maximum 5) test isolates.

Table 3. Results of PFGE gel quality

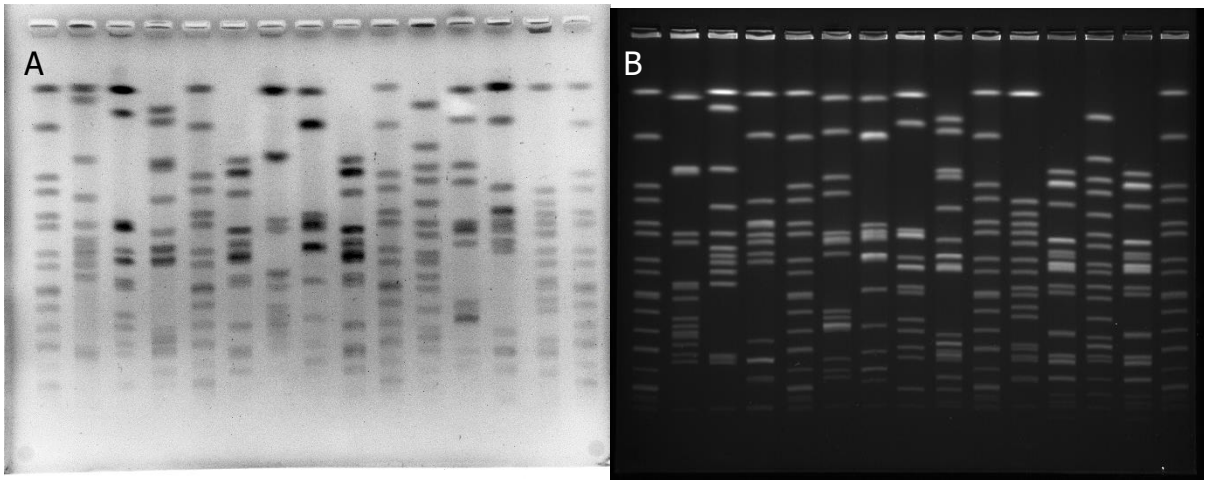
	Grade [score in points] *XbaI*				
Parameter	**1 [Poor]**	**2 [Fair]**	**3 [Good]**	**4 [Excellent]**	**Average**
Image acquisition and running conditions	6%	0%	53%	41%	3.3
Cell suspension	0%	12%	29%	59%	3.5
Bands	12%	12%	35%	41%	3.1
Lanes	0%	0%	59%	41%	3.4
Restriction	6%	6%	6%	82%	3.6
Gel background	0%	18%	35%	47%	3.3
DNA degradation	6%	24%	41%	29%	2.9

The average score and the percentage of laboratories obtaining scores 1–4 in the seven TIFF Quality Grading Guidelines parameters.

Figure 2 shows gels of varying quality with low and high scores in the parameter 'Bands' in this EQA.

Figure 2 (left) shows a gel with a score of 1 [Poor] in 'Bands' due to the fuzziness of the bands. The comparison with a gel with a high score in the same parameter shows how clear bands affect the possibility of correct band assignment.

Figure 2. TIFF file example of gel with running condition problems and gel that scored excellent

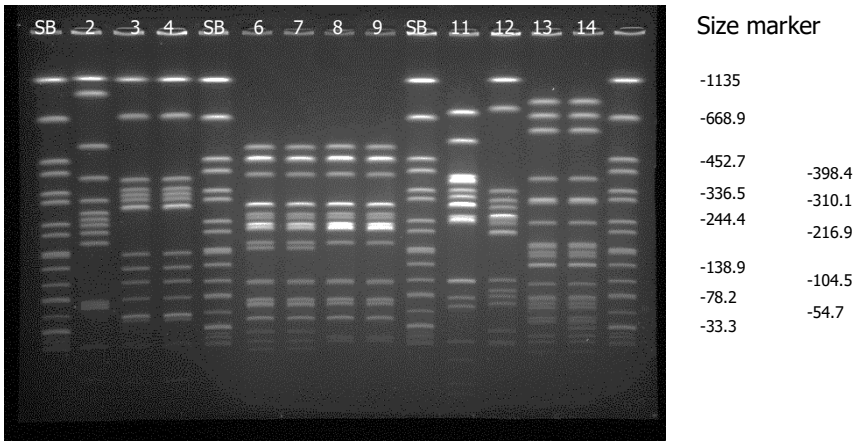


A: gels with fuzzy bands scoring 1 [Poor] in the parameter bands.
B: gel scoring 4 [Excellent] in all parameters.

3.2.2 Gel analysis

BioNumerics (BN) is a software initially developed for PFGE gel analysis. One of the critical steps in analysis is the normalisation of the gel, but all steps in the analysis impact the final profiles and thereby possibility of performing an inter-laboratory comparison. To ensure identical experimental settings in BN, the EQA provider distributed preconfigured BN databases to the participants. Despite the analysis part being separate from the gel quality part, the parameter 'Band assignment´ has been dependent on the participants' gel quality in previous EQAs. In order to standardise and systematically compare the band assignment, a new approach was introduced in EQA-8 and the participants were tasked with analysing a PFGE TIFF file with excellent quality provided by the EQA provider (Figure 3).

Figure 3. EQA-provided profile



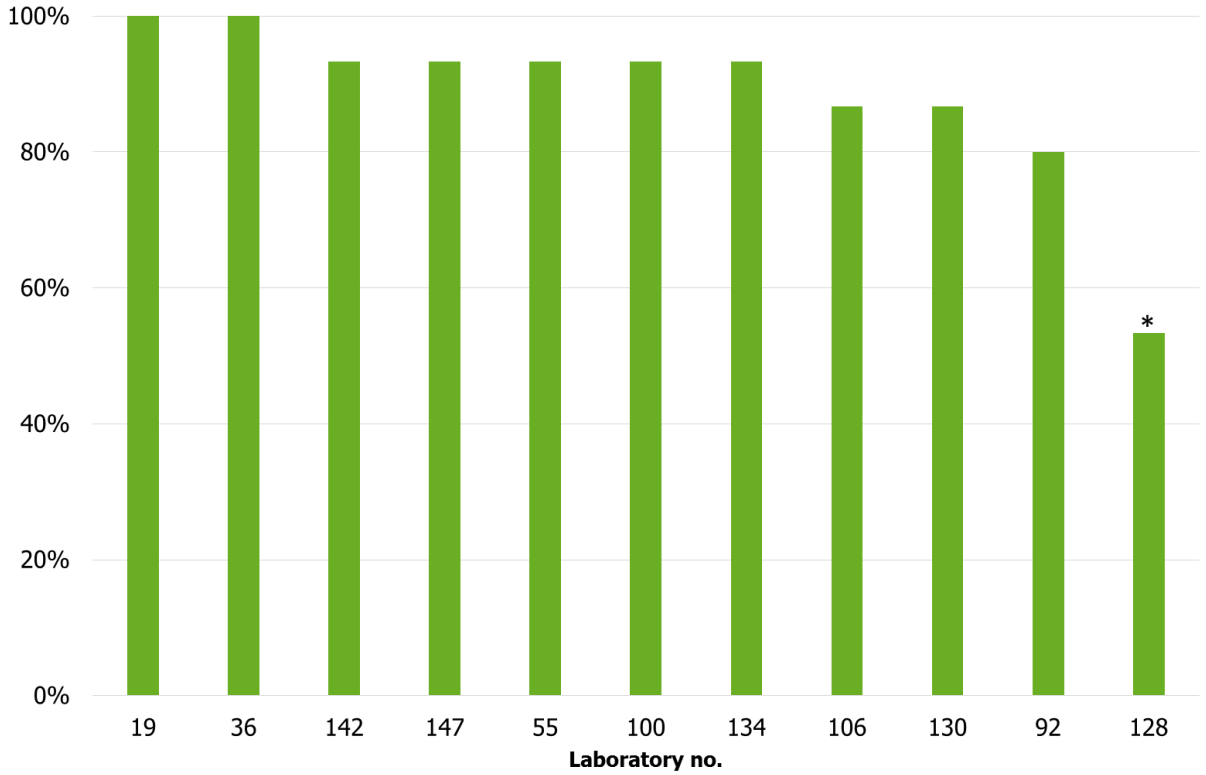
SB: S. *Braenderup position in lanes 1, 5, 10 and 15.*

Eleven laboratories (65%) analysed the provided PFGE TIFF file in BN and were able to produce XML export files according to the protocol. No resubmission of results was necessary. The participants' ability to perform gel analysis was graded according to the updated BioNumerics Gel Quality Grading Guidelines EQA-8. The grading was made for five parameters with scores ranging from 1–3 (Annex 4).

Compared to the varying gel quality observed among the participants, the quality of the gel analysis was more even and demonstrated a very high quality performance (Figure 4). Laboratories 19 and 36 produced a gel analysis

with 'excellent' [3] quality in all parameters including the new more challenging band assignment. Laboratories 142, 147, 55, 100 and 134 achieved a total score of 14 of the maximum 15 points (93%) (Figure 4, Annex 6).

Figure 4. Participant scores for PFGE gel analysis



Participating laboratories are represented by arbitrary numbers. Bars represent the total as a percentage of the maximum score of 15 points according to gel analysis evaluation using five parameters graded 1–3.
**: gels unacceptable for inter-laboratory comparison, score of 1 [Poor] in at least one parameter.*

Table 4 shows the five gel analysis parameters evaluated using the BioNumerics Gel Quality Grading Guidelines EQA-8, the percentage of laboratories scoring 1–3 and the average score for all laboratories.

Ten out of the 11 laboratories performed a gel analysis of 2 (fair) to 3 (excellent) quality (Table 4). Laboratory 128 made an unusual error: mixing inverted and not inverted TIFF in the submission, which also resulted in incorrect normalisation. This average level is on a par with the level of the previous EQA (Table 4, Annex 6). In the previous EQAs, the quality of the band assignment was graded according to the quality of the gel, i.e. a laboratory producing a gel that could not be used for inter-laboratory comparison in terms of gel quality could still achieve an "excellent" score in the BN analysis. In this EQA, the band assignment criteria were slightly changed in order to fully assess the quality of the band assignment (Annex 4). The parameter band assignment obtained the lowest average score of 2.2, but no laboratory scored 1 [Poor] even with these changes.

Table 4. Results of PFGE gel analysis

	Grade [score in points]			
Parameter	**Poor [1]**	**Fair [2]**	**Excellent [3]**	**Average**
Position of gel frame	9%	18%	73%	2.6
Strips	0%	18%	82%	2.8
Curves	0%	18%	82%	2.8
Normalisation	9%	0%	91%	2.8
Band assignment	0%	82%	18%	2.2

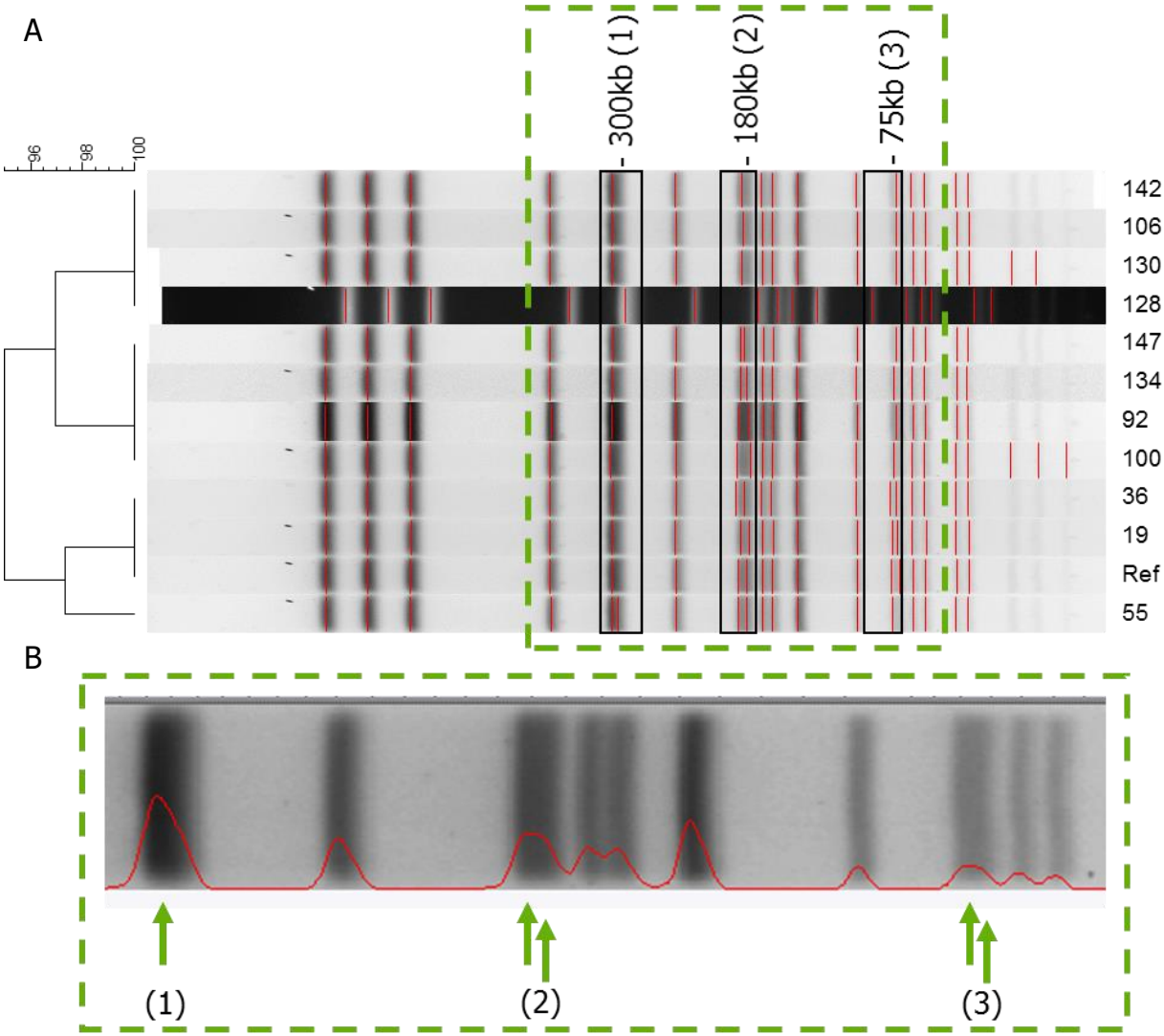
Average scores and percentage of laboratories obtaining scores 1–3 for the five BioNumerics Gel Analysis Quality Guidelines parameters.

With the new design of gel analysis, the 'band assignment' parameter is no longer dependent on the participants' own gel quality. All the laboratories were able to assign correct bands in six of the 11 profiles when observing bands above 33kb, but laboratories 100 and 130 continually assigned bands below 33kb in almost all profiles.

In profile 2 (Figure 3, lane 2), laboratories 55 and 106 missed the middle band in the bottom of the gel (approximately 70kb) where three close bands should have been. In profile 11, laboratories 55 and 100 assigned a double band on single band at position approximately 370kb and laboratory 55 also assigned a double band on a single band in position 229kb. In profile 12, laboratory 55 also assigned a double band on a single band position approximately 250kb.

The profiles 13 and 14 are almost identical: one small band at approximately 33kb is the only difference, however the profiles have two difficult double bands positioned at approximately 75kb and 180kb respectively. Apart from the small band at the bottom, each participant assigned identical profiles for profiles 13 and 14. In Figure 5a, a view of the laboratories' band assignment of profile 13 is shown. Only laboratory 55 assigned a double band on a single band (Figure 5a, position 1). Laboratories 106, 128, 130 and 142 assigned one single band on a double band at position 2 (Figure 5a) and the same four laboratories and laboratories 92, 100, 134 and 147 assigned a single band on the double band at position 3 (Figure 5). Figure 5b shows a zoomed view of the double bands positioned at 75kb and 180kb. Each of the double bands show a small stripe of white between the two bands and indentation in each side. Indentation is seen more easily if the width of the lane is wider than the bands.

Figure 5. Band assignment of profile 13



Participating laboratories represented by arbitrary numbers.
A: comparison of results from participants
B: zoom view of 'difficult bands'
Green arrows: bands at positions 1, 2 and 3.

3.3 Multiple locus variable number of tandem repeats analysis

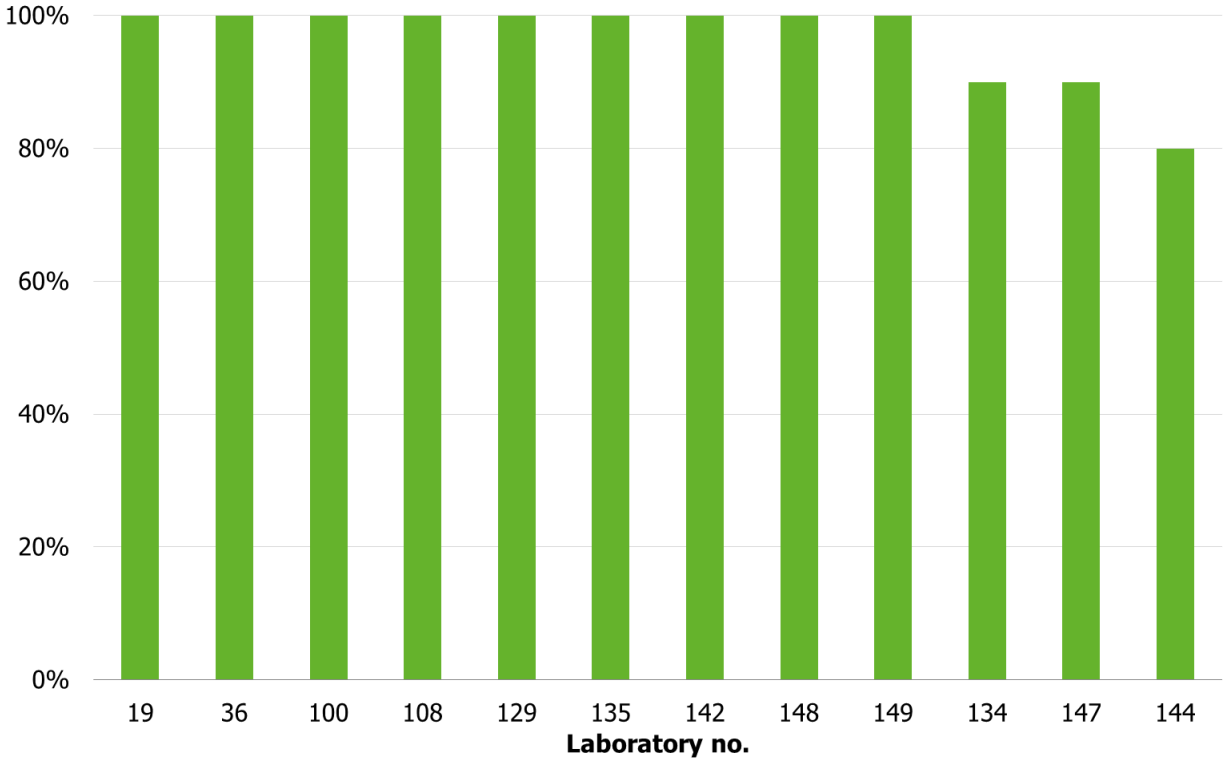
For the first time in ECDC EQA history, MLVA for *S.* Enteritidis was included in the EQA. In total, 13 laboratories (54%) participated in the MLVA part of the EQA, 12 (92%) in MLVA for *S.* Typhimurium and 12 (92%) in MLVA for *S.* Enteritidis. Laboratory 36 participated only in MLVA *S.* Typhimurium and laboratory 55 only in MLVA *S.* Enteritidis (Annex 2).

3.3.1 MLVA for *S.* Typhimurium

Twelve out of the 24 participants in EQA-8 (50%) performed the MLVA typing of *S.* Typhimurium, and nine (75%) of these reported the correct allelic profiles for all ten test isolates (Figure 6).

Laboratory 144 had two errors, both missing the presence of a fragment in locus STTR3. In EQA-5, laboratory 144 had the same mistake and also missed a locus fragment for STTR3 for two different test isolates. Laboratories 134 and 147 had one error each, both reporting a fragment in absent loci (STTR6 and STTR10 respectively).

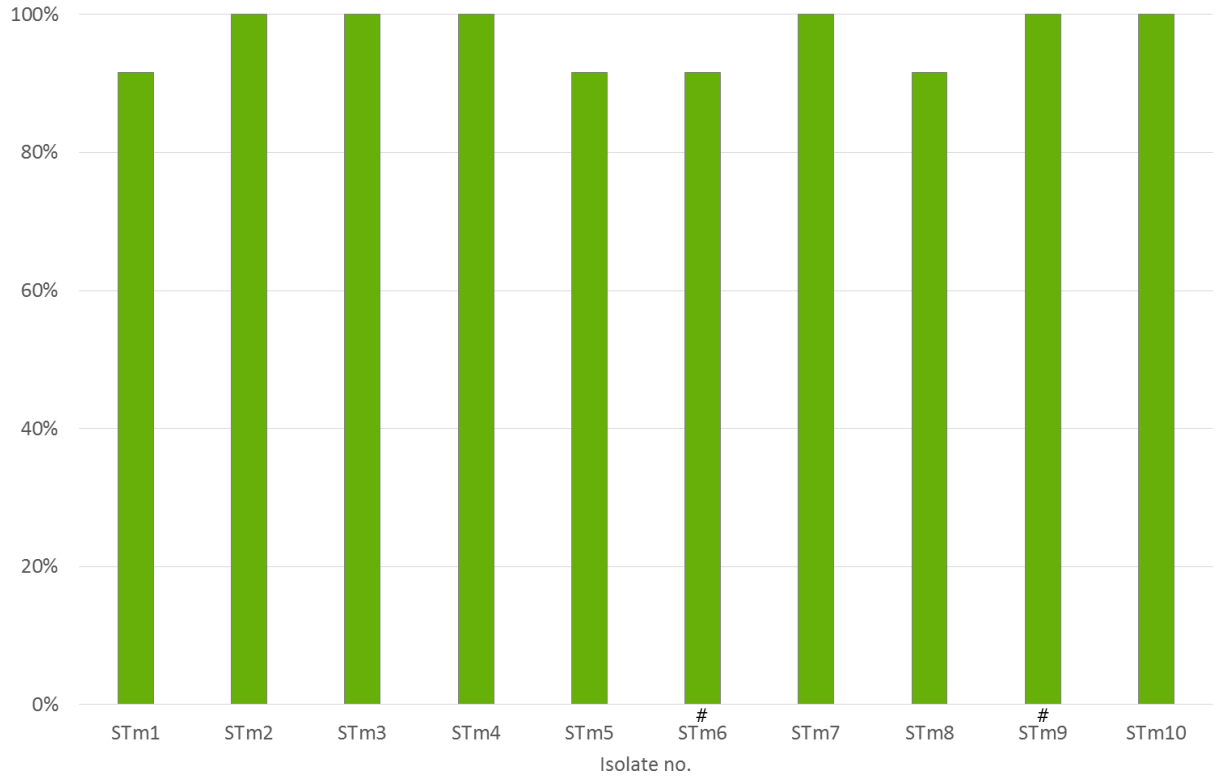
Figure 6. Participant scores for MLVA typing of the 10 *S.* Typhimurium test isolates



Arbitrary numbers represent the participating laboratories. Bars represent the number of correctly assigned MLVA profiles.

The results for each test isolate are summarised in Figure 7. The correct MLVA profile was reported for six of the 10 *S.* Typhimurium test isolates by all participants. No common isolate characteristics caused the problems (Annex 7) as the four incorrect MLVA profiles concerned four different isolates (STm1, 5, 6 and 8).

Figure 7. Average percentage scores of the 10 *S.* Typhimurium test isolates



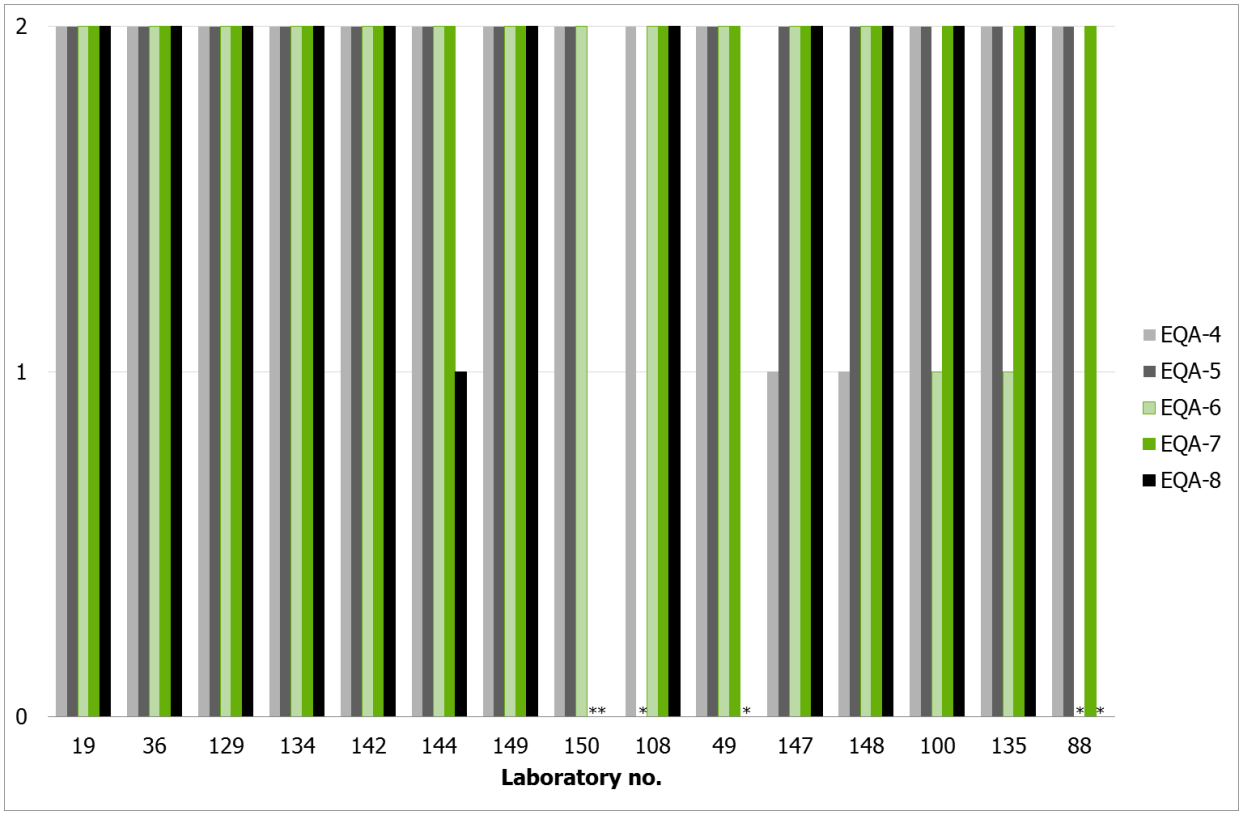
Bars represent the percentage of MLVA profiles correctly assigned by the participants.
#: repeat isolates (STm6 and STm9) in EQA-4 to 8.

To follow the development of individual laboratory performance, two isolates with different allelic profiles were included in EQA-4 through 8: isolate STm6 (3-12-9-NA-211) and STm9 (3-13-NA-NA-211). Figure 8 shows the individual performance by the laboratories of these two repeated isolates during the five EQAs. The MLVA results on the repeated isolates show stability and high performance among the participants.

The majority of participants (11/12; 92%) performed at the same or a better level than the last time they participated. Laboratory 144, which obtained incorrect results in EQA-8, had not previously generated errors on the repeated isolates.

In the previous EQA, three repeat isolates were included. One isolate (18, EQA-7) had changed in the highly variable STTR10 locus, from 23 in EQA-6 to 24 repeats in EQA-7. This isolate was therefore excluded.

Figure 8. Correct MLVA typing of three repeated *S.* Typhimurium isolates from EQA-4 to 8



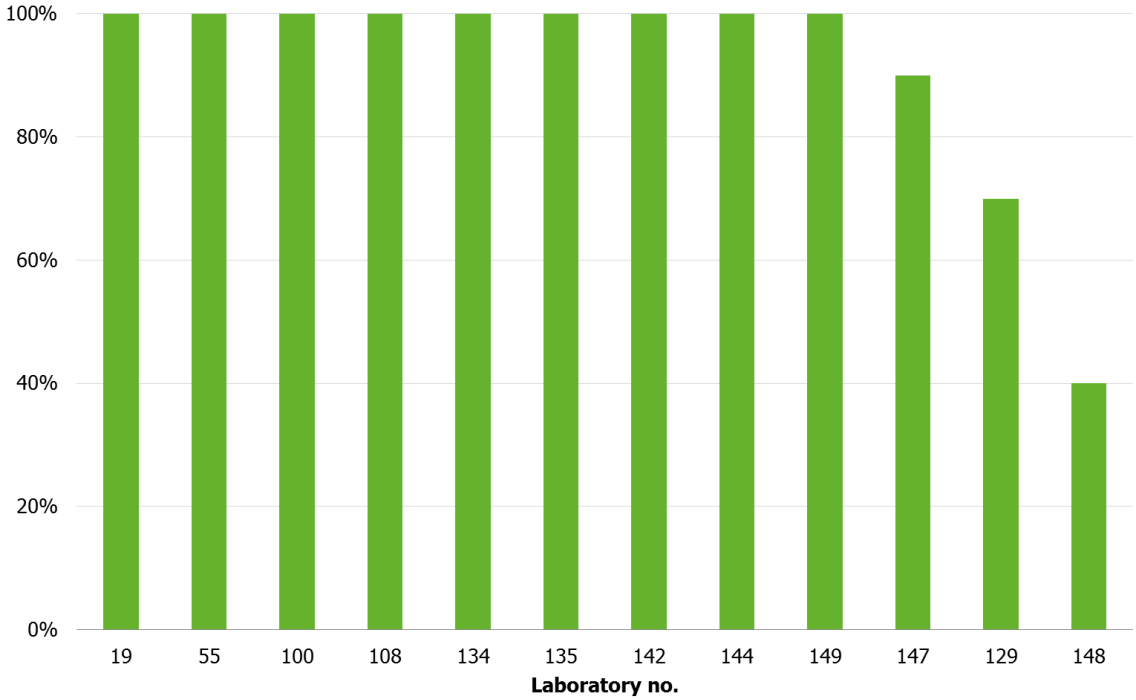
Arbitrary numbers represent the participating laboratories. Bars represent the number of correctly assigned allelic profiles for the two repeated isolates (STm6 and STm9).
**: laboratory not participating in this round of EQA.*

3.3.2 MLVA for *S.* Enteritidis

Twelve laboratories (50%; 11 of which participated in MLVA on *S.* Typhimurium) performed MLVA typing of *S.* Enteritidis and nine (75%) of these reported the correct allelic profiles for all 10 test isolates (Figure 9).

Laboratory 148 had six errors all linked to locus SENTR6 (Annex 8). For test isolate SE8, this laboratory missed a fragment present in locus SENTR6 and wrongly reported 11 as the allele number five times. Laboratory 129 reported three errors by missing the presence of a locus fragment for SENTR6 and laboratory 147 missed a fragment present in locus SENTR7 for test isolate SE8.

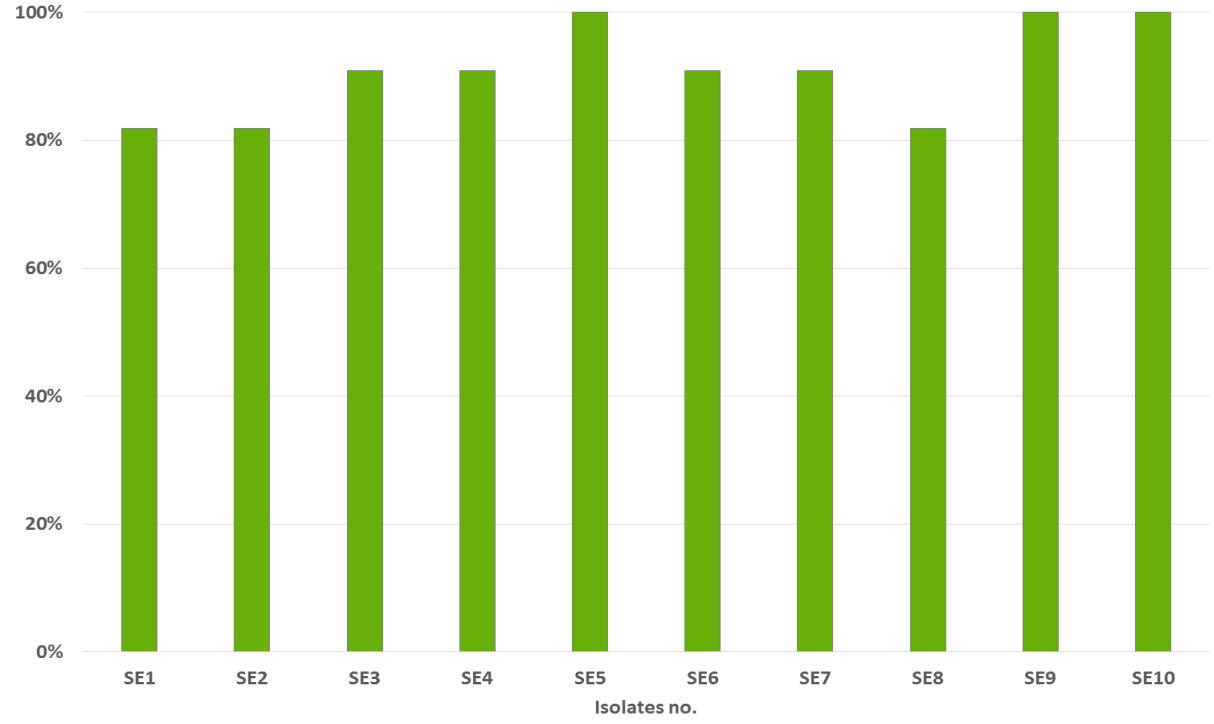
Figure 9. Participant scores for MLVA typing of the 10 *S.* Enteritidis test isolates



Arbitrary numbers represent participating laboratories. Bars represent number of correctly assigned MLVA profiles.

The results for the test isolates are summarised in Figure 10. The correct MLVA profile was reported for only three of the 10 *S.* Enteritidis test isolates by all participants. No common isolate characteristics caused the same problems (Annex 8), but nine of the 10 reported errors were connected to SENTR6 and the two laboratories with 90% of the errors made the same mistake several times. Since the majority of incorrect MLVA profiles (60%) were caused by one laboratory, the overall impression of the quality of *S.* Enteritidis MLVA was good (Annex 8).

Figure 10. Average percentage score of the 10 MLVA *S.* Enteritidis test isolates



Bars represent percentage of MLVA profiles correctly assigned by the participants.

3.4 Molecular typing-based cluster analysis

In this part of the EQA, the participants should correctly identify a cluster of closely related isolates among 12 test isolates by using either PFGE, MLVA and/or WGS-derived data. The cluster test isolates were pre-categorised by the EQA provider.

Based on WGS-derived data, the cluster consisted of four ST11 isolates: REF2, REF4, REF9 and REF11 (Annex 11). The analysis was based on an allele-based (cgMLST [9]) and SNP analysis (NASP [10]). The EQA provider found 0–3 allele differences and a distance of 0–6 SNPs between any two isolates in the cluster. The rest of the cluster test isolates were additional ST11 (5), ST10 (1), ST183 (1) and ST1925 (1).

Based on PFGE (*Xba*I profiles), the EQA provider defined a cluster of seven *S.* Enteritidis ST11 isolates: REF1, REF2, REF3, REF4, REF6, REF9 and REF11 (REF2 and REF11 were technical duplicates). The seven isolates were indistinguishable from each other by PFGE and two other ST11 isolates, REF12 and REF7, differed only by one and two bands respectively from the cluster PFGE-pattern (Annex 9). The last three test isolates, ST1925, ST183 and ST10 (Dublin), had several band differences and REF5 and REF8 in particular had a clearly unrelated PFGE profile (Annex 9).

Based on MLVA-derived data, the cluster consisted of five ST11 isolates, REF1, REF2, REF4, REF9 and REF11 with the MLVA profile 2-9-7-3-2 (Annex 10). Two other isolates, REF6 (2-10-7-3-2) and REF8 (2-9-7-3-1), differed in only one locus from the cluster MLVA-profile. The last five test isolates had variation in two to five loci (Annex 10).

3.4.1 PFGE-derived data

Four (31%) participants performed the cluster analysis using PFGE-derived data. Performance was high, 3 (75%) of the participants correctly identified the cluster of closely related isolates, defined by a pre-categorisation by the EQA provider, among the 12 cluster test isolates. Table 5 provides an overview of the isolates each participant included (yes) or excluded (no) in their cluster identification. Laboratory 92 reported 10 out of 12 cluster isolates as being a part of the cluster of closely related isolates.

Table 5. Results of cluster identification based on PFGE-derived data

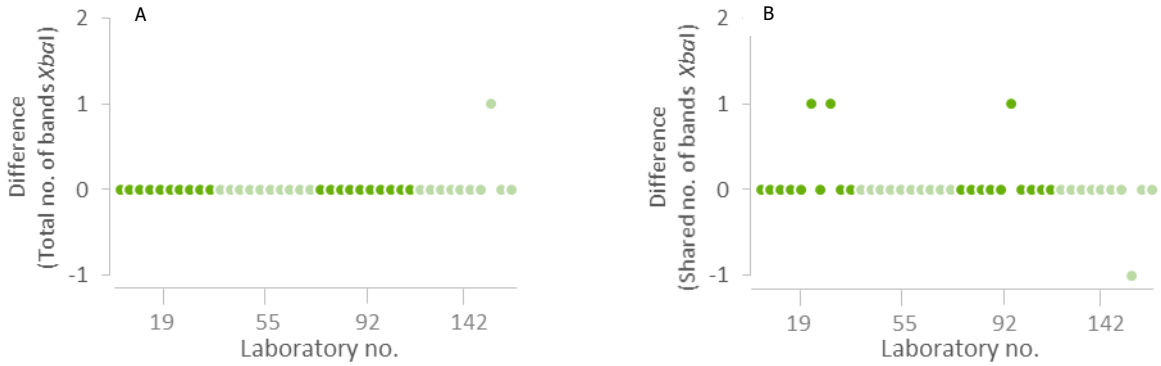
		Laboratory			
Isolate number	**ST**	**19**	**55**	**92**	**142**
REF1‡	11	Yes	Yes	Yes	Yes
REF2‡#	11	Yes	Yes	Yes	Yes
REF3‡	11	Yes	Yes	Yes	Yes
REF4‡	11	Yes	Yes	Yes	Yes
REF5	10	No	No	No	No
REF6‡	11	Yes	Yes	Yes	Yes
REF7	11	No	No	Yes	No
REF8	183	No	No	No	No
REF9‡	11	Yes	Yes	Yes	Yes
REF10	1925	No	No	Yes	No
REF11‡#	11	Yes	Yes	Yes	Yes
REF12	11	No	No	Yes	No
Cluster-identified		Yes	Yes	No	Yes

‡: closely related isolates
#: technical duplicate isolates.

The participants were also instructed to report the total number of bands in each isolate and then report the number of bands shared between each test isolate and the selected cluster representative (Figure 11). All data is available in Annexes 12 and 13.

Figure 11A shows the total number of band differences between the reported total number of bands by the participants and the total number of bands observed by the EQA provider for *Xba*I. Only laboratory 142 reported a higher number of bands in isolate REF10 (ST1925) than expected by the EQA provider. Figure 11B shows the number of band differences between the reported number of shared bands with a selected cluster representative by the participants and the number of shared bands observed by the EQA provider for *Xba*I. Only four differences were reported, as laboratory 19 reported one band more than expected for two isolates, laboratory 92 reported one band more than expected for one isolate and laboratory 142 reported one band less than expected for one isolate. Band differences above 1 were not observed.

Figure 11. Difference between reported total number of bands (A) and shared bands (B) for each isolate to selected isolates



Data from all nine ST11 isolates (REF1, REF2, REF3, REF4, REF6, REF7, REF9, REF11 and REF12) and one ST1925 (REF10). Data from REF5 (ST10, S. *Dublin) and REF8 (ST183) with clearly unrelated PFGE profiles (Annex 9) were not included.*

3.4.2 MLVA-derived data

All participants selected the *S.* Enteritidis scheme and reported the loci in the correct order: SENTR7, SENTR5, SENTR6, SENTR4 and SE-3.

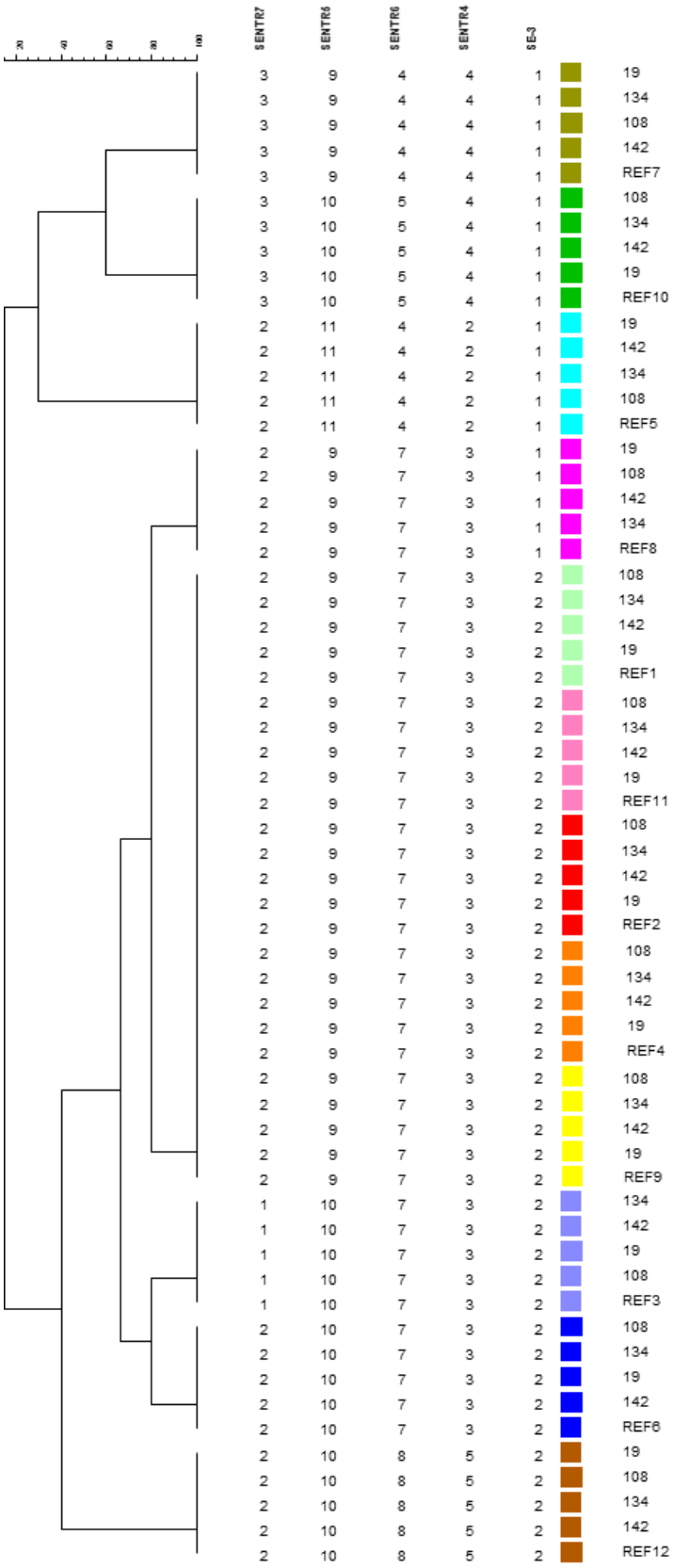
Four participants (31%) performed cluster analysis using MLVA-derived data. Performance was high, with all participants (100%) correctly identifying the cluster of closely related isolates (MLVA profile: 2-9-7-3-2) defined by a pre-categorisation by the EQA provider among the 12 cluster test isolates. Table 6 shows the overview of the isolate each participant included (Yes) and excluded (No) in their cluster analysis. Figure 12 shows a dendrogram of the reported MLVA results. All data are available in Annexes 15 and 16.

Table 6. Results of cluster identification based on MLVA-derived data

			Laboratory ID			
Isolate number	**ST**	**MLVA-profile**	**19**	**108**	**134**	**142**
REF1[ǂ]	11	2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes
REF2[ǂ#]	11	2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes
REF3	11	1 - 10 - 7 - 3 - 2	No	No	No	No
REF4[ǂ]	11	2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes
REF5	10	2 - 11 - 4 - 2 - 1	No	No	No	No
REF6	11	2 - 10 - 7 - 3 - 2	No	No	No	No
REF7	11	3 - 9 - 4 - 4 - 1	No	No	No	No
REF8	183	2 - 9 - 7 - 3 - 1	No	No	No	No
REF9[ǂ]	11	2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes
REF10	1925	3 - 10 - 5 - 4 - 1	No	No	No	No
REF11[#]	11	2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes
REF12	11	2 - 10 - 8 - 5 - 2	No	No	No	No
Cluster-identified		2 - 9 - 7 - 3 - 2	Yes	Yes	Yes	Yes

ǂ: closely related isolates
#: technical duplicate isolates.

Figure 12. Reported MLVA results of each test isolate



Dendrogram from BioNumerics of MLVA profiles reported by laboratories 19, 108, 134 and 142. Each of the 12 test isolates has a different colour.
REF1 to REF12: results from EQA provider.

3.4.3 WGS-derived data

Reported results from participants

Eleven participants (46%) performed cluster analysis using WGS-derived data. Three laboratories reported using external assistance for sequencing. Different sequencing platforms were listed among the participants: one MiniSeq, five MiSeq, one HiSeq, three NextSeq and one Ion Torrent. All reported using commercial kits for library preparation. Of the 11 participants, nine (82%) used Illumina's Nextera kit. Two reported volume changes from the manufacturer's protocol and one laboratory listed increased input DNA (5ng), altered PCR protocol to favour longer fragment sizes, adjustment of extension temperature from 72°C to 65°C and 'manual' normalisation using library concentration and fragment size (as opposed to bead-based normalisation, Annex 14).

Performance was high in cluster analysis with WGS-derived data, with 10 (91%) participants correctly identifying the cluster of closely related isolates defined by a pre-categorisation by the EQA provider among the 12 test isolates. However, laboratory 106 was unable to obtain WGS data for two of the test isolates and therefore missed one of the cluster isolates (marked as ND in Table 7). Another of the 10 laboratories (108) reported REF4 as not being part of the cluster, with a note that there is a need for further epidemiological information to make a conclusion (marked as '(No)[a]' in Table 7). Despite these deviations, cluster identification was considered correct for both of these laboratories. Laboratory 148 included three additional ST11 isolates as part of the cluster of closely related isolates and did not identify the correct cluster.

Table 7. Results of cluster identification based on WGS-derived data

		Laboratory ID										
Isolate number	ST	19	36	49	106	108	129	134	142	147	148	150
REF1	11	No	No	No	No	No	No	No	No	No	Yes	No
REF2‡#	11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF3	11	No	No	No	No	No	No	No	No	No	Yes	No
REF4‡	11	Yes	Yes	Yes	Yes	(No)[a]	Yes	Yes	Yes	Yes	Yes	Yes
REF5	10	No	No	No	No	No	No	No	No	No	No	No
REF6	11	No	No	No	No	No	No	No	No	No	Yes	No
REF7	11	No	No	No	No	No	No	No	No	No	No	No
REF8	183	No	No	No	No	No	No	No	No	No	No	No
REF9‡	11	Yes	Yes	Yes	ND	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF10	1925	No	No	No	No	No	No	No	No	No	No	No
REF11‡#	11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF12	11	No	No	No	ND	No	No	No	No	No	No	No
Main analysis		Allele (cgMLST)	Allele ("wgMLST")	Allele (cgMLST)	SNP	SNP	Allele ("wgMLST")	Allele (cgMLST)	Allele (cgMLST)	Allele (cgMLST)	SNP	SNP
Additional analysis		SNP		Allele (wgMLST)	SNP						Allele (cgMLST)	Allele (cgMLST)
3. analysis				SNP	Allele (cgMLST)							
Identified cluster		Yes	Yes	Yes	(Yes)	(Yes)	Yes	Yes	Yes	Yes	No	Yes

‡: closely related isolates
#: technical duplicate isolates
ST: sequence type
ND: not evaluated due to data quality not meeting laboratory's own QC limits
Allele: allele-based analysis
SNP: single-nucleotide polymorphism analysis
a: inclusion depending on epidemiology data.

Laboratories were instructed to report the data analysis used for cluster identification and select a representative isolate in the cluster for reporting SNP distance or allelic differences between the selected isolate and each test isolate included in analysis. Laboratories could report results from up to three analyses (one main and 1 to 2 additional), but the detected cluster had to be based on results from the main analysis. Only laboratories 49 and 106 reported three analyses.

Of the six participants using SNP analysis, four (66%) used SNP as the main analysis for cluster detection, two reported SNP as an additional analysis and laboratory 106 reported two SNP-based analyses (one as main, the other as additional). All used a reference-based approach with different ST11 isolates as reference. As read mapper, four used Burrows-Wheeler Aligner (BWA), two used Bowtie, one used CLC and one also used a combination of BWA and Bowtie. Four laboratories reported the use of GATK as variant caller, but VarScan2, SAMtools, CLC and BioNumerics own tools were also used.

Tables 8 and 9 show the overview of the submitted data. For laboratory reported SNP distance/allelic differences by isolate, see Annex 18.

Table 8. Reported results of SNP-based cluster analysis

	SNP-based					
Laboratory	**Approach**	**Reference**	**Read mapper**	**Variant caller**	**Distance within cluster**	**Distance outside cluster**
Provider	Reference-based	ST11 REF2	BWA	GATK	0–6	61–462
19*	Reference-based	ST11 in-house strain 1606T13806	BWA	GATK	0–7	78–525
49*	Reference-based	ST11 6302	BWA, Bowtie2	BioNumerics own tools	0–6	65–488
106	Reference-based	CPOO7332 strain EC20120916 ST11	BWA-MEM 0.7.12	GATK 3.8.0	0–3[#]	15–117
106*	Reference-based	CP007332 strain EC20120916 ST11	BOWTIE2 vs 2.2.5	VarScan2	0–9[#]	43–300
108	Reference-based	In-house strain resp ST	CLC assembly cell v4.4.2	CLC assembly cell v4.4.2	0–7	65–510
148	Reference-based	ST11_(NC_011294.1)	Bowtie2	SAMtools	0–97	None reported
150	Reference-based	AM933172 (EBI), ST11, EBG4	BWA	GATK	0–7	68–493

**: additional analysis*
[#]: only three isolates included due to data quality not meeting laboratory's own QC limits.
Distance outside cluster is for reported ST11 isolates. For detailed data, see Annex 18.

Of the 10 participants using an allele-based analysis, seven selected the method as the main analysis for cluster detection. Five of 10 (50%) used only an assembly-based allele calling method and three (30%) used both assembly- and mapping-based allele calling methods. Six used SPAdes as the assembler and two used Velvet. The remaining two laboratories (20%) used only a mapping-based allele calling method.

Eight of 10 laboratories (80%) reported using Enterobase (cgMLST) as the scheme for analysis. The remaining two laboratories (20%) reported the use of wgMLST (an ad hoc scheme for *Salmonella* enterica based on 2.143/1.423 core and 2.201/2.055 accessory loci respectively). Furthermore, laboratory 49, which had reported the use of Enterobase (cgMLST) as the main analysis, also reported results using wgMLST/Applied Maths.

Table 9. Reported results of allele-based cluster analysis

	Allele-based analysis					
Laboratory	**Approach**	**Allelic calling method**	**Assembler**	**Scheme**	**Difference within cluster**	**Difference outside cluster¤**
Provider	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Maths (cgMLST/Enterobase)	0–3	33–240 (1 779)
19	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Enterobase)	0–3	33–239 (1 774)
36	SeqPhere	Mapping-based only	-	Ad hoc scheme for *Salmonella* enterica based on 2.143 core and 2.201 accessory loci ('wgMLST')	0–6	53–336 (2 310)
49	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Enterobase)	0–2	33–227 (1 727)
49*	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (wgMLST)	0–5	55–348 (2 364)
106*	SeqPhere	Assembly-based only	SPAdes vs 3.5.0	Enterobase (cgMLST)	0–3#	31–233 (1 741)
129	SeqPhere	Assembly-based only	Velvet	§Ad hoc scheme for *Salmonella* enterica based on 1.423 core and 2.055 accessory loci ("wgMLST")	0–4	36–252 (1 804)
134	SeqPhere	Assembly-based only	Velvet	Implementation of Enterobase scheme (3002 genes) in SeqSphere	0–3	32–233 (1 701)
142	Enterobase	Assembly-based only	SPAdes	Enterobase (cgMLST)	0–3	33–240 (1 793)
147	SeqPhere	Assembly- and mapping-based	SPAdes 3.9.0	Enterobase (cgMLST)	0–3	32–234 (1 745)
148*	Enterobase	Assembly-based only	SPAdes	Enterobase (cgMLST)	0–36	96–206 (639)
150*	Enterobase	Mapping-based only	-	Enterobase (cgMLST)	0–9	33–36 (256)

**: additional analysis*
#: only three isolates included due to data quality not meeting laboratory's own QC limits
§: modified from submitted information
¤: reported differences to ST11 (non-ST11). For detailed data, see Annex 18.

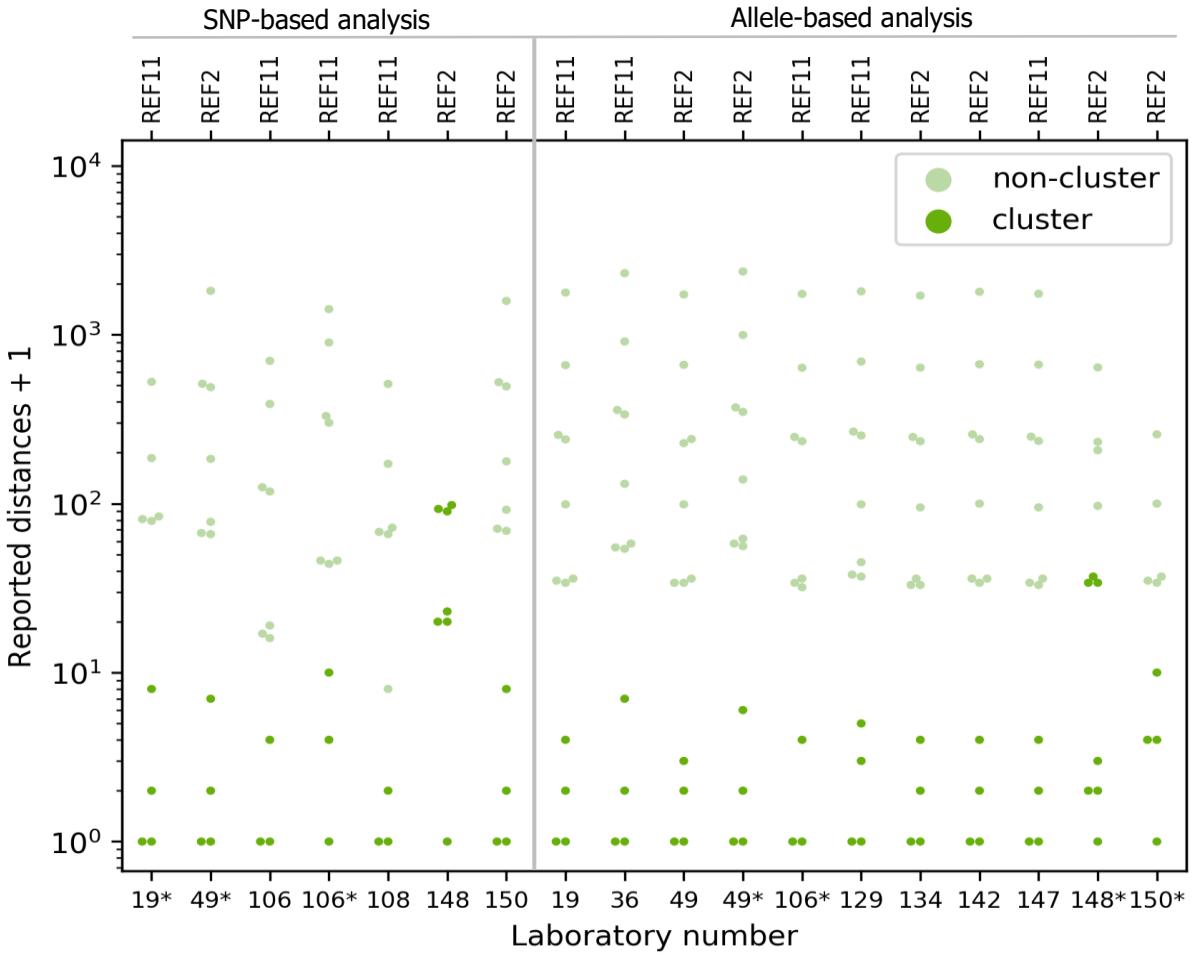
All seven laboratories using an allele-based analysis as the main method could identify the correct cluster of closely related isolates (Figure 13). The overall reported allele difference within the cluster was at 0–9 for nine of 10 of the laboratories using an allele-based analysis. The last laboratory (148) used cgMLST as an additional analysis and reported an allele difference within the cluster at 0–36.

All eight laboratories performing cgMLST used the same scheme as the EQA provider (cgMLST/Enterobase, [9]) and seven identified 0–3 allele differences within the cluster. Laboratory 150 reported a higher number of allele differences based on cgMLST/Enterobase and counted nine allele differences between REF2 and REF11 (technical duplicates). This difference was not seen when the EQA provider analysed the uploaded raw reads.

The three laboratories performing wgMLST identified 0–4, 0–5 and 0–6 allele differences within the cluster.

The allele differences or SNP distances reported could depend on the isolate selected as cluster representative, but all laboratories had selected one of the technical duplicates (REF2 or REF11).

Figure 13. Reported SNP distances or allele differences for each test isolate to selected cluster representative isolate



**: additional analysis*
SNP: single-nucleotide polymorphism analysis
Selected cluster representative marked as REF in dark green: reported cluster of closely related isolates
Light green: reported not part of cluster.

Five other test isolates (REF1, REF3, REF6, REF7 and REF12) were also ST11, but not predefined by the EQA provider as part of the cluster. Seven of eight laboratories performing cgMLST reported allele differences to the selected cluster isolate at 31–240 for this group of isolates (difference outside cluster), but laboratory 148 reported allele differences to the selected cluster isolate at 96-206 for this group of isolates based on cgMLST. Laboratories 36, 49 and 129 reported the 'outside' allele differences to the selected cluster representative isolate at 36-348 based on different wgMLST schemes.

Three test isolates (REF5, REF8 and REF10) were not ST11 and allele differences to the selected cluster isolate at 231–1793 were reported using cgMLST and 266–2364 using wgMLST (Table 9, Annex 18).

Five laboratories (19, 49, 106, 108 and 150) of the six performing SNP analysis identified the correct cluster of closely related isolates (Figure 13), but only three of them (106, 108 and 150) used SNP as main analysis. Furthermore, laboratory 106 did not report the fully correct cluster, as one cluster isolate and one non-cluster isolate was excluded from the results due to low sequencing quality.

Laboratory 108 performed only SNP analysis and reported three of the four cluster isolates as part of the expected cluster. The last cluster isolate (REF4) was reported with 7 pure SNPs difference to the selected cluster representative isolate and the laboratory added that 'depending on the investigation and epidemiological context, this isolate could also be linked to the cluster and thereby a part of the outbreak investigation'.

Laboratory 148 used SNP as main analysis and did not identify the correct cluster, reporting three additional ST11 isolates as belonging to the cluster in addition to the four correct isolates.

The reported SNP distances within the cluster varied from 0–9 for laboratories 19, 49, 106, 108 and 150. Laboratory 148 reported the SNP distances within the cluster as 0–97. Based on reported results from this

laboratory, the SNP distance for the correct cluster of closely related isolate was 0–22 SNPs. Overall, laboratory 148 reported higher SNP distances than expected and an SNP distance of 19 was reported for the technically duplicated isolate (REF2 and REF11).

Laboratory 148 was the only one that did not report an SNP distance outside the cluster. The overall reported SNP distances outside the cluster were 15–525. Laboratory 106 reported two different SNP results, both with significantly shorter distances for non-cluster isolates. A likely cause could be the inclusion of the distant *S.* Dublin isolate (REF5) in the analysis, resulting in a reduced core genome. The results reported for the Bowtie/Varscan analysis included three SNPs between the technical replicates. The reported SNP distances outside the cluster for each laboratory were more uniform (65–525) when disregarding the SNP result from laboratory 106 (15–117 and 43–300).

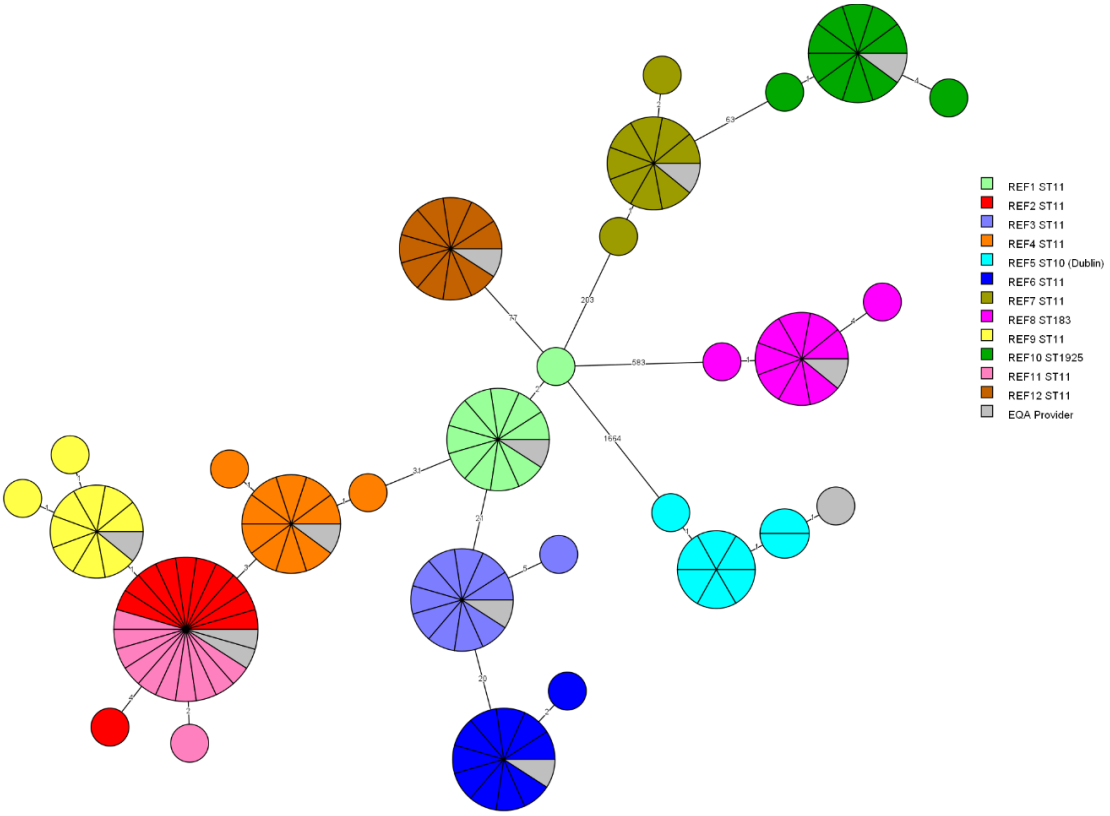
Analysis of raw reads uploaded by participants

In addition to the reported cluster identification, participants submitted their FASTQ files to be evaluated by the EQA provider. The data was initially evaluated using the EQA provider's QC pipeline [11] and FASTQ files were uploaded to an Applied Maths calculation engine for allele calling (cgMLST/Enterobase, [9]). Isolates from laboratories 134, 147 and 150 failed the QC and were excluded from figures derived from the submitted data.

In addition, laboratory 106 only submitted 10 FASTQ files due to data quality not meeting the laboratory's own QC limits and laboratory 108 did not submit FASTQ files for REF5 (ST10, Dublin).

The overall cgMLST analysis shown in the minimum spanning tree (MST, Figure 14) based on submitted raw reads from 11 laboratories shows clear clustering of the results for each test isolate.

Figure 14. Minimum spanning tree of core genome multilocus sequence typing, participant FASTQ files

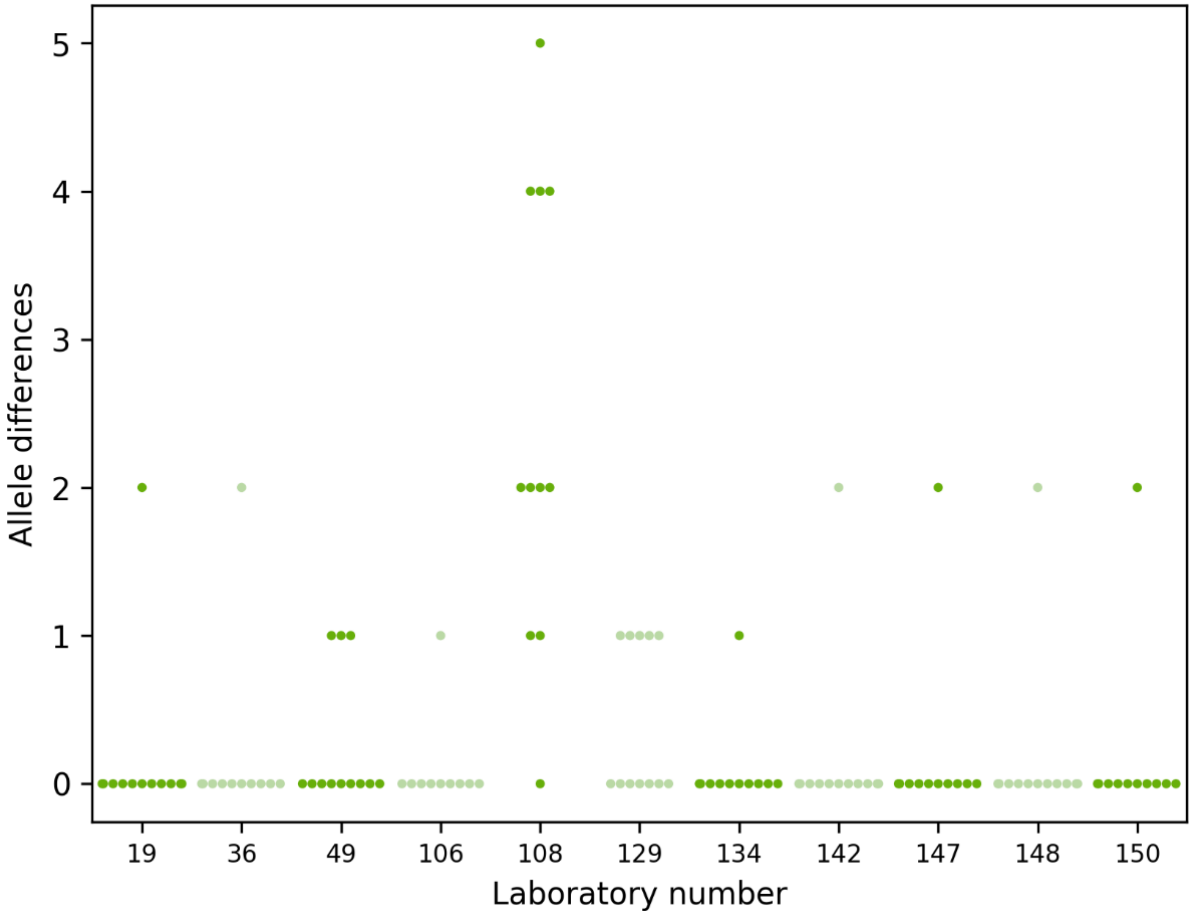


Minimum spanning tree (MST) in log scale of core genome multilocus sequence typing (cgMLST, [9]) based on submitted raw reads (FASTQ files).
Each REF1–REF12 test isolate has a different colour.
REF results from the EQA provider are in grey.
Of 11 laboratories, laboratory 106 only submitted 10 FASTQ files due to data quality not meeting the laboratory's own QC limits.
For laboratories 134, 147 and 150, only 11 of the 12 submitted FASTQ files were used due to data quality not meeting the provider's QC limits.
Laboratory 108 did not submit FASTQ files for REF5 (ST10, Dublin).

The allele differences in Figure 14 do not exactly match those illustrated in the individual reports and consequently in Figures 15 and 16, where the same data are used. This discrepancy is caused by loci being left out if they do not pass QC in all isolates in the analysis, thus the joint analysis contains fewer loci.

For each laboratory, cgMLST was performed on the submitted raw reads (FASTQ files) applying Applied Maths allele calling with the Enterobase scheme [9]. For each laboratory, a hierarchical single linkage clustering was performed on the submitted data along with the EQA provider's reference isolates. Figure 15 shows the allele differences between each submitted sequence and the corresponding reference. As seen in Figure 14, the provider isolate REF5 is 1–3 alleles removed from all participant isolates.

Figure 15. Participant allele difference from reference result (EQA provider) for each test isolate



Allele difference of participant isolates from the corresponding REF isolates (EQA-provider) based on the submitted raw reads (FASTQ files).
Only 10 FASTQ files were used for laboratory 106 due to data quality not meeting the laboratory's own QC limits.
Only 11 FASTQ files were used for laboratories 134, 147 and 150 due to data quality not meeting the provider's QC limits.
Laboratory 108 did not submit FASTQ files for REF5 (ST10, Dublin).

For 100 of 126 results (79%), no differences were identified. For 22 results (18%), a difference of 1–2 alleles from the REF isolate was calculated. For four results (3%), a difference of 4–5 alleles was seen, all by laboratory 108. Data from three of the 11 laboratories (49, 108 and 129) covered 18/26 (69%) of all allele differences. Laboratory 108 had 10 isolates with one or more allele differences. Excluding the REF5 isolate, all but three laboratories had no differences from the reference isolates and only 11 (10%) results had a difference of 1–2 alleles from the remaining REF isolates (data not shown).

Additionally, the laboratories listed the quantitative and qualitative QC parameters used to evaluate their data. As seen in Table 10, coverage is the most widely used QC parameter, with acceptance thresholds ranging from 20–50X coverage. For full QC data, see Annex 19.

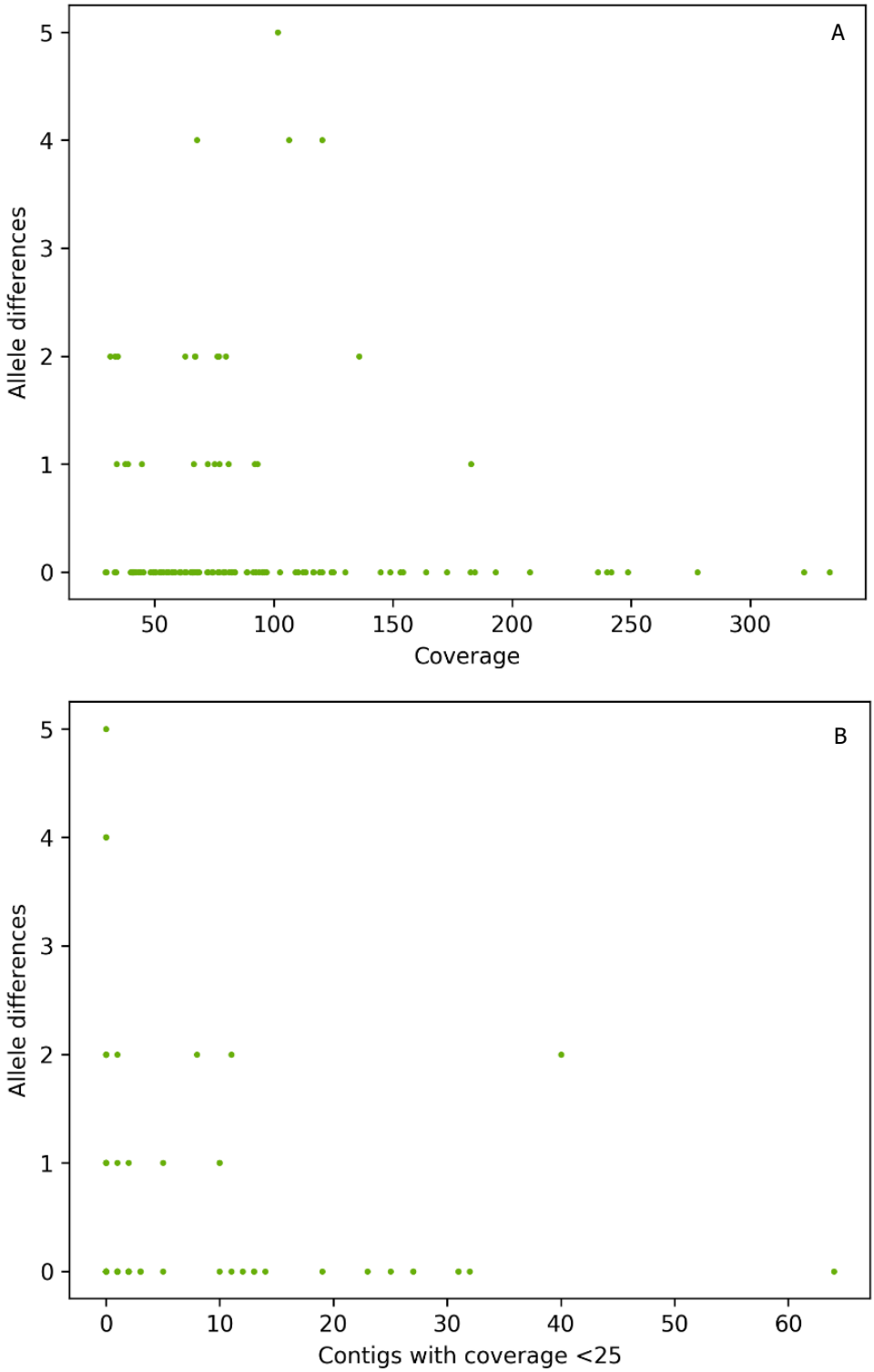
Table 10. Participants' reported quantitative and qualitative parameters

Parameters	Number of laboratories
Coverage	10
Assembly quality	6
Assembly length	6
Allele calling	6
Confirmation of organism	6
Read length	5
Read quality	4
Allele calling	2
Contamination	2
Read trimming	2
GC content	1
Run performance	1
Sequence present	1
SNP pruning	1

Figure 16A shows the allele differences from Figure 15 plotted against the coverage of the individual sample.

Laboratory 108 accounts for most of the differences from the reference isolates. Figures 16A and B show that low coverage cannot account for low accuracy.

Figure 16. Calculated allele difference between participant and REF isolates compared with selected QC parameters



Allele difference of participant isolates from corresponding REF isolates from Figure 15 plotted.
A: against average coverage of the submitted raw reads (FASTQ files) calculated by the EQA provider QC pipeline
B: against number of contigs with minimum coverage <25 when reads are mapped back against a SPAdes de novo assembly.

For each laboratory, the submitted raw reads (FASTQ files) were evaluated by the EQA provider's in-house quality control pipeline [11]. Table 11 shows the QC parameters and range of QC values per laboratory. For the full QC evaluation of all isolates, see Annex 20. Three isolates were discarded due to low quality. One laboratory withheld the results from two isolates due to data quality not meeting the laboratory's own QC limits. Coverage was high overall. Certain laboratories had high variation between isolates of up to 8X.

Table 11. Results of participants' raw sequence data evaluated by EQA provider's QC pipeline

		Laboratory ID										
Parameters	**Ranges**	**19**	**36**	**49**	**106**	**108**	**129**	**134**	**142**	**147**	**148**	**150**
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1
Detected species	{*Se*}	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.79–2.85	0.36–5.32	0.81–3.92	0.95–11.55	0.74–1.76	0.42–1.2	1.39–3.86	0.27–2.09	0.62–2.56	0.66–1.48	1.28–6.44
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.7–4.9	4.7–4.9	4.6–4.9	4.1–4.9	4.7–4.9	4.1–4.9	1.3–4.9	4.7–4.9	0–4.9	4.5–4.8	4.4–4.8
Length [0–25] x minimum. coverage (Mbp)	{<0.25}	0	0	0–0.1	0–1.3	0	0–0.5	0–3.5	0	0–4.5	0–0.1	0–0.4
Number of contigs at 25 x minimum coverage	{>0}	42–81	23–49	33–118	31–50	155–461	31–123	19–61	21–29	1–184	62–162	44–103
Number of contigs [0–25] x minimum coverage[#]	{<1 000}	0–1	0	0–14	0–3	0–8	0–64	0–44	0–2	0–1019	11–32	0–40
Average coverage	{>50}	124–207.4	48.1–125	33.2–75.1	41.4–333.2	31.4–120.2	29.3–93.7	24.1–78.9	33.6–68.1	13.2–95.4	52–116.7	33.4–74.4
Number of reads (x1 000)		2161–3656	479–1544	838–1908	883–6117	506–1696	494–1544	416–1326	366–851	150–1071	903–2085	24–1908
No. of trimmed reads (x1 000)		2134–3610	469–1519	838–1908	874–6039	465–1554	483–1534	411–1313	353–841	144–1041	890–2055	24–1908
Maximum read length		151–151	301–301	101–101	151–151	193–306	151–151	151–151	251–251	300–301	151–151	101–101
Mean read length		138–141	184–258	93–99	134–146	168–228	141–145	135–146	196–231	218–235	143–146	87–95
Read insert size		290–314	301–405	177–343	257–428	NA	380–572	310–418	327–457	300–381	328–392	175–232
Insert size StdDev		116–133	154–185	82–195	142–177	NA	147–235	111–173	166–178	121–146	88–101	70–83
N50 (kbp)		120–229	171–494	63–345	220–297	16–58	47–399	127–322	299–494	6–492	46–128	65–217
N75 (kbp)		75–129	89–234	37–144	95–154	9–31	27–171	93–171	180–406	3–230	26–78	35–128

**: indicative QC range*
Se: Salmonella enterica
#: number of contigs with coverage < 25 (Figure 16B, Annex 20)
NA: not analysed.

4 Discussion

The total number of participants decreased from 26 in EQA-7 to 24 in EQA-8. Among the 24 participants was one new and another participating again after a break of three years. Three laboratories that participated in the PFGE part of EQA-7 did not participate in EQA-8 at all (neither in the independent PFGE part nor in the cluster part by PFGE). One laboratory that only participated in the MLVA part of EQA-7 did not participate in EQA-8 at all.

4.1 Pulsed field gel electrophoresis

Seventeen laboratories participated in the PFGE gel part and their gels were graded according to the TIFF Quality Grading Guidelines EQA-8, where seven parameters are used for grading given scores between 1 and 4 (Poor, Fair, Good and Excellent). The majority of the participating laboratories (14/17; 82%) produced an acceptable quality TIFF gel image for inter-laboratory comparison, representing an increase from 72% in EQA-6 and 76% in EQA-7. Three laboratories (18%) scored 1 [Poor] in one or three parameters ('Bands' or 'Image acquisition and running condition', 'Restriction' and 'DNA degradation'), having generated a gel of insufficient quality for inter-laboratory comparisons.

For the parameter 'Bands' in particular, seven participants (41%) were able to obtain a score of 4 [Excellent], representing an increase from 28% in both EQA-6 and EQA-7. Two of the three laboratories that produced a gel deemed unsuitable for inter-laboratory comparison scored 1 [Poor] in this parameter. The same trend was observed in the previous EQA and laboratories need to improve the quality of bands to ensure onward inter-laboratory comparability of PFGE profiles. The low 'Band' scores were due to thick or fuzzy bands. The easiest and often best way to improve the sharpness of the bands is to use wider wells.

Four laboratories disregarded the very important factor of including a reference lane for every three or four test isolates (no more than five isolate lanes between reference lanes). This strongly affected the subsequent normalisation and band assignment. Furthermore, it is important that equipment is properly maintained, works within specifications and the buffer temperature complies with protocol. The electrophoresis time should also be adjusted in each laboratory, as failure to do so will result in a bottom band that is not 1–1.5 cm from the base of the gel. Following the gel electrophoresis, proper image capture of the gel is critical to obtaining a good quality TIFF image.

Among the six laboratories (96, 129, 130, 132, 138 and 144) scoring 1 [Poor] in the gel-producing part of EQA-7, two (129, 144) improved their performance to 3 [Good] in the current EQA. One did not participate (132) and three (96, 130 and 138) still achieved 1 [Poor] for some of the same parameters as previously in EQA-7 despite many recommendations for improvements. Laboratories were encouraged to resubmit a new PFGE gel, but no laboratories responded that they would.

The performance of the gel analysis was generally very good. Almost all laboratories performed gel analysis in accordance with guidelines. Only one laboratory used an inverted gel in the analysis, which resulted in incorrect normalisation. The performance of PFGE typing has been part of the EQA scheme for *Salmonella* since EQA-4, but for the first time, the gel analysis part was performed with a gel preproduced by the EQA provider. In each previous round, three laboratories (EQA-4 to EQA-6) or none (EQA-7) were unable to produce gel analysis in accordance with the guidelines. Only laboratory 128 scored a Poor performance in gel analysis three times (EQA-8, EQA-6 and EQA-5), two related to normalisation, despite the laboratory receiving recommendations for improvements in the individual evaluation reports. In general, the average score of the parameter 'band assignment' was lower in EQA-8 than in previous EQAs, caused by the new strict evaluation criteria (see Annex 4) and because the parameter is no longer dependent on the quality of the participants' gel. Not surprisingly, the double bands were the most difficult to assign correctly, emphasising the necessity of a curated ECDC/EFSA joint database.

The participation rate in the PFGE part was lower than in the previous schemes (24 in EQA-7 and 17 in EQA-8), and several laboratories (6/17; 35%) did not perform gel analysis (i.e. normalisation and band assignment of the EQA-provided gel). None of the former participants substituted the quality PFGE assessment with the PFGE cluster part, but four previous PFGE participants switched to the cluster part using WGS-derived data. Despite the increased use of WGS-based typing tools for investigating larger outbreaks, some laboratories still use PFGE for their primary surveillance and outbreak investigation. In order to perform good national surveillance as well as submit profiles to EU-wide surveillance, it is important to have the capacity to properly analyse and interpret PFGE profiles at the national level and compare human and food isolates. In this EQA, with improved gel analysis, participants received very detailed band assignment feedback in their individual reports in order for them to improve their performance on assigning double bands correctly.

4.2 Multiple-locus variable number of tandem repeats analysis

Thirteen laboratories participated overall in the MLVA part of the EQA: 12 in MLVA for *S.* Typhimurium and 12 in MLVA for *S.* Enteritidis. MLVA for *S.* Enteritidis was included in the EQA for the first time and the relatively high number of participants shows that it was relevant to include this method. The number of participants in MLVA for *S.* Typhimurium was two laboratories fewer than in EQA-7. By adding MLVA for *S.* Enteritidis, there was one new MLVA participant, but this laboratory only performed MLVA for *S.* Enteritidis.

Nine laboratories (75%) obtained a total score of 100% for *S.* Typhimurium and reported the correct MLVA types for all 10 test isolates. This is a high performance, but a slight decrease compared with EQA-7, where 80% of participants obtained a score of 100%. From EQA-4 to EQA-8, the overall performance in each round was 60%, 71%, 79%, 80% and 75% respectively of the participants reporting correct MLVA *S.* Typhimurium types for all test isolates. There were no obvious reasons for the decrease this round – fewer laboratories participated, but the same number of laboratories reported incorrectly (not the same laboratories).

The MLVA results of the two repeated *S.* Typhimurium isolates from EQA-4 through EQA-8 showed high performance by the participants. The majority of participants (92%; 11/12) performed at the same level as the last time they participated. Laboratory 144 made a mistake for the first time on one of the repeated isolates.

MLVA of *S.* Enteritidis was a new part of EQA-8. Nine laboratories (75%) obtained a total score of 100% and reported correct MLVA types for all 10 test isolates.

No common characteristics of the isolates caused problems among the participants. Mistakes in the MLVA for both *S.* Typhimurium and *S.* Enteritidis were mainly caused by reporting absent alleles where fragments should have been detected or alleles in a locus with no fragment present, and less frequently by assigning a wrong allele in a present fragment. However, laboratory 148 seemed to make a systematic error reporting allele number 11 for *S.* Enteritidis in locus SENTR6 several times.

The reasons for missing the presence of a locus or vice versa (false positive allele number for an absent locus) could be from not using a freshly prepared primer mix. Amplification signals (peaks) decrease as the primer mix gets older and the use of control/reference isolates should indicate whether the primer mix produces readable signals. Another reason could be an unbalanced primer mix, resulting in very different peak heights and a signal being mistakenly recognised as background noise or background noise being identified as a signal.

For a highly discriminatory method such as MLVA that includes fast-changing loci, there is always a risk that the allelic profile may change during passage or transport. Changes in highly variable loci are impossible to avoid or foresee. All test isolates were stability-tested by being passaged 10 times and the one-repeat variant was stable, as also reported by all participants identifying the locus. In general, changes only occur in fast-changing loci (e.g. STTR5, STTR6 and STTR10 for *S.* Typhimurium) and one-repeat changes in these loci would be accepted as correct when evaluating the results of the EQA. However, no one-locus variants were reported by participants this year, so the rule was never applied.

4.3 Molecular typing-based cluster analysis

In the present EQA scheme, a molecular typing-based cluster analysis was included for the first time. Participants were free to choose their preferred method between PFGE, MLVA and/or WGS-derived data and the identified cluster depended on the used method. According to WGS-derived typing results obtained by the EQA provider, four of the 12 test isolates formed a cluster of closely related isolates, whereas the remaining isolates clearly were genetically more distant. If PFGE or MLVA was used as the single typing method, 7 and 5 isolates respectively were indistinguishable, whereas the profiles of the remaining isolates had small or large differences to the cluster profile.

Compared to MLVA, the discriminatory power of PFGE is often too low for cluster detection, but overall, PFGE and analysis based on WGS data showed the same picture of relatedness between the test isolates, where clearly unrelated isolates by PFGE (REF8 and REF5) also had the highest differences by WGS.

The results by MLVA did not show exactly the same as one isolate (REF8, ST183) was more related to the predefined WGS cluster than the other ST11 test isolates by MLVA, which highlights the challenge of MLVA only measuring a few specific regions of the genome and sometimes showing incorrect phylogenetic relatedness.

The allele difference and SNP distances calculated of the cluster defined by PFGE or MLVA were much higher than in the 'true' WGS defined cluster. The SNP distance within the extended cluster defined by PFGE was 83 (provider result) and allele differences were up to 35 (cgMLST by provider). If the isolate with one band difference was included, the SNP distance within the cluster increased to 163 and the allele differences to 97.

The identification of closely related isolates by MLVA included one additional isolate with an SNP distance within the MLVA cluster of 61 (provider result) and allele differences up to 33 (cgMLST by provider). If the two isolates with

variation in one locus (REF6 and REF8) were included, the genetic distance within the cluster would increase noticeably. The distance from the closely related cluster to REF6 was 83 SNPs and 35 alleles. For REF8 (ST183), the distance was 1742 SNPs and 660 allele differences. However, the variation in the MLVA profile was connected to the highly conserved locus SE-3 and a one-repeat unit difference in this locus is usually not considered as a closely related MLVA profile.

This shows the difficulties of inter-laboratory comparability between Member States with regards to surveillance and outbreak investigation when different methods are used. Despite the increasing use of WGS as a typing tool for large outbreaks, some laboratories still use PFGE for their primary surveillance and outbreak investigation, which complicates communication regarding outbreaks, e.g. in ECDC's Epidemic Intelligence Information System (EPIS).

Acceptance by the Member States of the addition of cluster analysis in this EQA for *Salmonella* typing seems unclear (Annex 2). Only 13 of the 24 laboratories (54%) participated in the cluster part using PFGE-, MLVA- and/or WGS-derived data. No more than four participated in cluster identification using PFGE even though 17 laboratories participated in the PFGE part of the EQA and two of them also participated in cluster identification using WGS. Four laboratories participated in cluster identification using MLVA and all four also participated in cluster identification using WGS. Four laboratories participating in gel quality and gel analysis (PFGE part) in EQA-7 switched methods completely and participated in cluster analysis using only WGS-derived data and did not perform PFGE at all.

All laboratories participating in the cluster part by either PFGE or MLVA also participated in gel quality/gel analysis (PFGE part) or the individual MLVA part of EQA-8. However, remarkably few laboratories (two) in the cluster part chose to use PFGE or MLVA alone and not in combination with WGS. The further scheduled adjustment of the EQA scheme, where gel quality and analysis (PFGE part) is removed, may decrease the overall number of participants as laboratories no longer receive an EQA of their PFGE performance.

4.3.1 PFGE-derived data

Of the 24 laboratories, four (17%) performed cluster analysis using PFGE-derived data. Three laboratories (75%) correctly identified the cluster of seven closely related isolates designed by the EQA provider using *Xba*I. Laboratory 92 did not identify the correct cluster and reported three additional isolates in the cluster of closely related isolates with up to three band differences. This laboratory did not perform MLVA or WGS on the test isolates. *S.* Enteritidis is normally a very genetically related serovar and the discriminatory power of PFGE can be insufficient for cluster detection [12]. It is advisable to use very conservative criteria for interpreting the relatedness by PFGE for this serovar and only to consider isolates with indistinguishable PFGE profiles as closely related. Very few differences in total number of bands and in shared bands with the cluster PFGE-profile were observed for the four laboratories compared with the EQA provider.

4.3.2 MLVA-derived data

In the European *S.* Enteritidis outbreak from 2016 to 2018 linked to eggs, MLVA profiles were used in the definition of probable cases [7], but out of the 24 EQA participants, only four (17%) performed cluster analysis using MLVA-derived data . Performance was very high as all (100%) correctly identified the cluster of five closely related isolates. None of the laboratories included or suggested the inclusion of single-locus variants in the cluster, but all four laboratories also performed cluster analysis by WGS.

4.3.3 WGS-derived data

Eleven of 24 laboratories (46%) performed cluster analysis using WGS-derived data. This was a slightly lower participation compared to the corresponding EQA of *Listeria monocytogenes*, where 12 of 20 laboratories (60%) performed cluster analysis using WGS-derived data. Performance was high, as 10 (91%) correctly identified the cluster of closely related isolates, although one of these laboratories was unable to obtain WGS data for all test isolates and therefore missed one of the cluster isolates. Another of the 10 laboratories also omitted to report one of the cluster isolates, as it reported the need for epidemiological information to make a conclusion.

The majority of laboratories (11/12) reported the use of an Illumina platform and three laboratories reported the use of external assistance for sequencing. All reported using commercial kits for library preparation. Out of 11 laboratories, seven (64%) reported using an allele-based method as the main analysis and four (36%) reported using SNP analysis. If only evaluating the main analysis of the laboratories reporting the correct cluster, the distances reported using SNP-based analyses were 0–7 inside the cluster and the number of allele differences using cgMLST were 0–3 inside the cluster.

The two approaches to analyse WGS-derived data (allele- and SNP-based analysis) showed comparable results. In an evaluation of a European *Salmonella* serovar Enteritidis outbreak, cgMLST analysis was also congruent with the original SNP-based analysis [13]. One exception in EQA-8 was the results from the laboratory not identifying the

correct cluster. This laboratory used an SNP-based approach as the main analysis and identified a large number of SNPs between cluster isolates, including between the technical replicates, making the cluster identification unclear. If the laboratory had instead identified the cluster using cgMLST results, the correct cluster could have been identified more easily. Laboratory 106 reported the correct cluster by two SNP analyses with different mappers and variant callers. Neither of the analysis results were directly comparable to the other participants, likely due to the inclusion of the remote *S.* Dublin isolate in the analyses causing a large decrease of the core genome. However, it is likely that the BWA/GATK analysis would show the best correspondence since it did not show differences between the technical replicates. Furthermore, the analysis steps are more similar to those reported by other laboratories.

High similarity was seen for the reported cgMLST results based on Enterobase (0–3 allele differences within the cluster). Only one laboratory reported higher allele differences using this scheme (0–9 allele differences within the cluster). Other schemes used for allele-based analysis (wgMLST) showed similar results, with up to 6 allele differences within the cluster. Outside the cluster, the reported allelic differences were 31–1 793 based on cgMLST/Enterobase and up to 2 364 using wgMLST. This highlights the potential of cgMLST for standardisation and improved inter-laboratory comparability for cluster definitions.

Cluster data were based on a real international outbreak linked to eggs in 2017. The distances within the cluster were calculated by the EQA provider to be 0–6 SNP distances and 0–3 cgMLST differences. Most of the participants correctly identified the cluster, but only one laboratory stated the need for additional epidemiological data before including the fourth isolate (distance 7 SNPs) into the cluster. The EQA provider acknowledged that epidemiological information is important for any outbreak investigation and neither microbiological nor epidemiological data can stand on its own. Nevertheless, epidemiological data were not included in this EQA. This also shows that a variable 'cut-off' is needed for different outbreak situations.

Of the reported SNP results, all but one were largely comparable to allele-based results as far as cluster isolates are concerned. Longer distances were reported to the non-cluster isolates, which is likely caused by SNPs in regions not included in allele schemes or multiple SNPs in the same locus. The inclusion of the remote *S.* Dublin isolate negates this effect due to a smaller core genome. For most intents and purposes, the reported SNP and allele distances are sufficiently similar for inter-laboratory comparability and shared cluster definitions. The reported results give no clear indication on the influence the choice of analysis tools (assembler, allele calling method and software) has on the number of allele differences. According to results from laboratory 106, there may be an influence on the choice of mapper and variant caller when calculating SNP distances, but no clear recommendations can be made. The choice of scheme (cgMLST or wgMLST) has an influence on reported allele differences, particularly outside the cluster. Likewise, the inclusion of remote isolates has an influence on the distances reported in SNP analysis.

Participants reported the QC checks (quantitative and qualitative) used on their data before analysis and submission. Coverage was reported by the majority of laboratories (91%) and allele calling for cgMLST was reported by six (55%). Assembly length and quality, as well as species confirmation, were reported by all six laboratories (55%). In order to compare the quality of raw data, the EQA provider analysed the submitted raw reads to obtain selected QC measures. The three discarded sequences all had very low coverage, but many of the remaining submitted sequences had depths that vastly exceeded QC thresholds for coverage reported by the participants.

5 Conclusions

Twenty-four laboratories participated in the EQA-8 scheme: 17 laboratories (71%) performed PFGE and 13 (54%) performed MLVA. In the new part with cluster identification using molecular typing methods (PFGE, MLVA or/and WGS), 13 laboratories (54%) participated. Five laboratories (21%) completed all three parts of the EQA.

One new participant enrolled in the PFGE part. Seven laboratories who previously participated in PFGE (EQA-7) no longer participated in the PFGE part. Three of them did not participate in the EQA scheme at all and four participated only in the MLVA or cluster parts using WGS.

One new participant was enrolled in the MLVA part after MLVA for *S.* Enteritidis was included in the EQA. Two laboratories who previously participated in MLVA (EQA-7) no longer participated in the MLVA part. One did not participate in the EQA-scheme at all, while the other participated only in the cluster part using WGS.

The majority of participants (14/17; 82%) were able to produce a PFGE gel of sufficiently high quality to allow for inter-laboratory comparison of profiles. The comparability of profiles between laboratories primarily relies on the use of correct running conditions, good quality image acquisition and distinct bands. The subsequent normalisation and interpretation of profiles in BN were performed by 11 of participants (64%) in the PFGE part, a decrease compared with EQA-7. All but one analysed their gels in accordance with guidelines for producing inter-laboratory-comparable gels. Despite the new design of the gel analysis (of a provided gel), which revealed differences in the ability to assign correct double bands, all laboratories scored 2 or above in the parameter band assignment.

In EQA-8, participation in the MLVA part was possible for both *S.* Typhimurium and *S.* Enteritidis. The performance level was high for both schemes: nine laboratories (75%) reported correct allelic profiles for all test isolates in each. Except for one laboratory, all errors were caused by missing the presence of a locus fragment or reporting a fragment that was absent.

Incorporating a molecular typing-based cluster analysis in this EQA is up to date with the development of surveillance methods used by PH NRLs in Europe. This adjustment of the EQA seems in some degree to be accepted by the Member States, but an overall decrease in the numbers of participants was seen and only 13 of the 24 laboratories participating in EQA-8 (54%) performed cluster analysis using either PFGE, MLVA and/or WGS-derived data. Only one laboratory did not identify the correct cluster using PFGE, caused by including too many band differences in the cluster and one laboratory using WGS. During the next scheduled adjustment of the EQA scheme, where gel quality and analysis (PFGE part) is removed, the overall number of participants may possibly decrease further.

In total, 11 laboratories performed cluster analysis using WGS-derived data. Performance was high, with 10 of the participants (91%) correctly identifying the cluster of closely related isolates, but two laboratories only identified a cluster of three isolates: one due to data quality not meeting the laboratory's own QC limits for one of the cluster isolates and the other specifying the need for additional epidemiological investigation before inclusion of the isolate.

Most laboratories preferred an allele-based method, as 64% (7/11) used cgMLST and 36% (4/11) used SNP as the main reported cluster analysis. The use of a standard cgMLST scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results, but both SNP- and allele-based methods seem to be useful for inter-laboratory comparability and communication about cluster definitions. More challenges can appear if a non-standardised SNP analysis is used for comparison and communication between laboratories. Another difficulty is illustrated by data from one laboratory, where including a remote isolate (*S.* Dublin) in SNP analysis resulted in a reduced core genome and consequently fewer SNPs.

The current EQA scheme for typing of *Salmonella enterica* subsp. *enterica* is the eighth organised for laboratories in FWD-Net. The molecular surveillance system implemented as part of TESSy relies on the capacity of FWD-Net laboratories to produce analysable and comparable typing results in a central database. WGS-based typing for surveillance is increasingly used in EU. In 2018, it is planned to allow WGS variables for *L. monocytogenes* to be submitted to the TESSy database. It is anticipated that member states will also be able to upload WGS variables for *Salmonella* to the TESSy database in the near future.

6 Recommendations

6.1 Laboratories

The PFGE gel quality is directly dependent on the quality of the laboratory procedure. Therefore, it is strongly recommended that the protocol be followed strictly. The parameters 'Bands' and 'Image acquisition and running conditions' caused the most problems in both this and the previous EQA. It should therefore be emphasised that individual laboratories should use the EQA results and feedback for a thorough evaluation of their laboratory procedures and equipment. Laboratories are encouraged to use the possibility of rerunning the test isolates after adjustment of the procedures and submitting the new PFGE gel for assessment.

Although the vast majority of the participant's profiles were correct in the MLVA part, a few laboratories with repeated errors could use the possibility of repeating the MLVA analysis and submit the results for troubleshooting.

S. Enteritidis and *S.* Typhimurium are the two most common serovars in Europe and MLVA typing provides high discrimination within isolates of both serovars. Only half of the participants (54%) performed either MLVA for *S.* Typhimurium and/or *S.* Enteritidis. Some of the laboratories, who are not moving towards the use of WGS at this stage, could benefit from implementing MLVA because of its low-cost, easy analysis and interpretation compared to WGS.

One participant in the WGS based cluster analysis experienced that the generated sequences did not meet their own QC criteria. Participants are encouraged to assign sufficient resources to repeat failed analysis if necessary.

For one laboratory, a more conservative SNP calling would facilitate better cluster delineation.

Despite good consistency in the results, further standardization of analysis parameters could be relevant for improving inter-laboratory comparability.

The laboratories are encouraged to submit their high quality typing data to TESSy as close to real time as possible.

The laboratories should try to submit results for all methods and parts of the EQA as signing up for.

6.2 ECDC and FWD-Net

ECDC will encourage more participants to take part in the new molecular typing-based cluster analysis, also participants who have not previously participated in the PFGE gel analysis or MLVA part.

ECDC will continuously aim to include and assist new participants in getting even better, potentially with training or workshops.

ECDC plans to standardise the TESSy system for use of MLST and cgMLST nomenclature.

6.3 EQA provider

This year, the EQA provider changed the invitation letter to also contain recommended methods and a short description of the molecular typing-based cluster analysis. The requirements for submission and evaluation criteria were also listed. The submission protocol was short and precise, but some laboratories did not follow protocol regarding the labelling of FASTQ files. In the next round, the participants will be asked to rename their files.

The link to the online registration and submission were sent to the national focal point of FWD-Net and the subsequent need to circulate this to the relevant person within the institute occasionally caused misunderstandings and delays. The participants will be made aware of this issue in the next round.

As PFGE will remain in the cluster analysis, a short guide on when to assign double bands and perform cluster analysis using PFGE could possibly increase participant comfort in participating in cluster analysis.

In the next round of EQAs, laboratories will also have the possibility to submit the ST of isolates in cluster analysis. They will also be asked to report the number of loci in the used allelic schemes and the name of the used SNP pipeline if publicly available.

The EQA provider will try to give the participants more time to test and report the results.

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Annex 1. List of participants

Country	Laboratory	National institute
Austria	NRC Salmonella Austria	Institute for Medical Microbiology and Hygiene Graz, AGES
Belgium	National Reference Centre Salmonella	Scientific Institute Public Health
Czech Republic	National Reference Laboratory for Salmonella	The National Institute of Public Health
Denmark	Foodborne Infections	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board
Finland	Expert Microbiology	National Institute for Health and Welfare
France	CNR *Escherichia coli*, *Shigella* and *Salmonella*	Institut Pasteur
Germany	National Reference Centre for Salmonella and Other Bacterial Enteric Pathogens	Robert Koch Institute
Greece	National Reference Laboratory for Salmonella	National School of Public Health
Hungary	Department of Phage-Typing and Molecular Epidemiology	National Public Health Institute
Ireland	National Salmonella, Shigella and Listeria Reference Laboratory	University Hospital Galway
Italy	Department of Infectious Diseases	Istituto Superiore di Sanità
Latvia	National Microbiology Reference Laboratory	Infectology Centre of Latvia, Riga East University Hospital
Lithuania	National Public Health Surveillance Laboratory	Nacionaline Visuomenes Sveikatos Prieziuros Laboratorija
Luxembourg	Epidémiologie et Génomique Microbienne	Laboratoire National de Santé
Netherlands	Centre for Infectious Diseases Research, Diagnostics and Screening	National Institute for Public Health and the Environment
Norway	National Reference Laboratory for Enteropathogenic Bacteria	Norwegian Institute of Public Health
Romania	Molecular Epidemiology Laboratory	Cantacuzino National Institute of Research
Serbia	Laboratory for Molecular Microbiology	Public Health Institute Of Serbia, Center for Microbiology
Slovakia	NRC for Salmonelloses	Public Health Authority of the Slovak Republic
Slovenia	Department for Public Health Microbiology	National Laboratory of Health, Environment and Food, Centre for Medical Microbiology
Spain	Unit of Enterobacteriaceae	Instituto de Salud Carlos III, Centro Nacional de Microbiología
Sweden	MI-LB	Folkhälsomyndigheten
UK (England)	Gastrointestinal Bacterial Reference Unit	Public Health England

Annex 2. Participation overview EQA-7 and 8

	2016 to 2017 (EQA-7)				2017 to 2018 (EQA-8)							
	Total	PFGE		MLVA	Total	PFGE		MLVA		Cluster		
Laboratory	All	Gel quality	Gel analysis	STm	All	Gel quality	Gel analysis	STm	SE	PFGE	MLVA	WGS
19	X	X	X	X	X	X	X	X	X	X	X	X
36	X	X	X	X	X	X	X	X				X
49	X	X	X	X	X							X
55	X	X	X		X	X	X		X	X		
88	X			X								
92	X	X	X		X	X	X			X		
96	X	X			X	X						
100	X	X	X	X	X	X	X	X	X			
106	X	X	X		X	X	X					X
108	X	X	X	X	X			X	X		X	X
114	X	X										
127					X	X						
128	X	X	X		X	X	X					
129	X	X	X	X	X			X	X			X
130	X	X	X		X	X	X					
132	X	X	X									
134	X	X	X	X	X	X	X	X	X		X	X
135*	X	X	X	X	X			X	X			
138	X	X			X	X						
140	X	X			X	X						
142	X	X	X	X	X	X	X	X	X	X	X	X
144	X	X		X	X	X		X	X			
145	X	X			X	X						
147	X	X	X	X	X	X	X	X	X			X
148	X	X	X	X	X			X	X			X
149	X			X	X			X	X			
150					X							X
180	X	X										
Number of participants	26	24	17	14	24	17	11	12	12	4	4	11

**: previously laboratory 77*

Annex 3. TIFF quality grading guidelines EQA-8

	Grade [score in points]			
Parameter	**Poor [1]**	**Fair [2]**	**Good [3]**	**Excellent [4]**
Image acquisition and running conditions	-Gel does not fill whole TIFF and band finding is highly affected -Bottom band of standard not 1–1.5 cm from the base of the gel and analysis is strongly affected -Band spacing of standards does not match global standard and analysis is strongly affected -Too few reference lanes included	-Gel does not fill whole TIFF and band finding is slightly affected -Wells not included on TIFF -Bottom band of standard not 1–1.5 cm from the base of the gel and analysis is slightly affected -Band spacing of standards does not match global standard and analysis is slightly affected	-Gel does not fill whole TIFF, but band finding is not affected -Bottom band of standard not 1–1.5 cm from the base of the gel, but analysis is not affected	By protocol, for example: -Gel fills whole TIFF -Wells included on TIFF -Bottom band of standard1–1.5 cm from the base of the gel
Cell suspensions	Cell concentrations are uneven from lane to lane, making analysis impossible	-More than two lanes contain darker or lighter bands than the other lanes. -At least one lane is much darker or lighter than the other lanes, making the gel difficult to analyse.	One or two lanes contain darker or lighter bands than the other lanes	Cell concentration is approximately the same in each lane
Bands	-Band distortion making analysis difficult -Very fuzzy bands -Many bands too thick to distinguish -Bands at the base of the gel too light to distinguish	- Some band distortion (i.e. nicks) in two or three lanes, but still analysable -Fuzzy bands -Some bands (four or five) are too thick -Bands at the bottom or top of the gel are light but still analysable	-Slight band distortion in one lane, but analysis is not affected -Bands are slightly fuzzy and/or slanted -A few bands (three or less) are difficult to see clearly (i.e. DNA overload) especially at the bottom of the gel	Clear and distinct all the way to the bottom of the gel
Lanes	'Smiling' or curving affecting analysis	-Significant 'smiling' -Slight curves on the outside lanes, but still analysable	-Slight 'smiling' (higher bands in outside lanes than inside) -Slight curving -Lanes gradually run longer towards the right or left, but still analysable	Straight
Restriction	-More than one lane with several shadow bands -Lots of shadow bands over the whole gel	-One lane with many shadow bands -A few shadow bands spread out over several lanes	One or two faint shadow bands	Complete restriction in all lanes
Gel background	Lots of debris present, making analysis impossible	-Some debris present that may or may not make analysis difficult (i.e. auto band search finds too many bands) -Background caused by photographing a gel with very light bands (image contrast was enhanced making the image look grainy)	-Mostly clear background -Minor debris not affecting analysis	Clear
DNA degradation (smearing in lanes)	Smearing making several lanes unanalysable	-Significant smearing in one or two lanes that may or may not make analysis difficult -Minor background (smearing) in many lanes	Minor background (smearing) in a few lanes, but bands are clear	Not present

Annex 4. BioNumerics gel analysis quality guidelines EQA-8

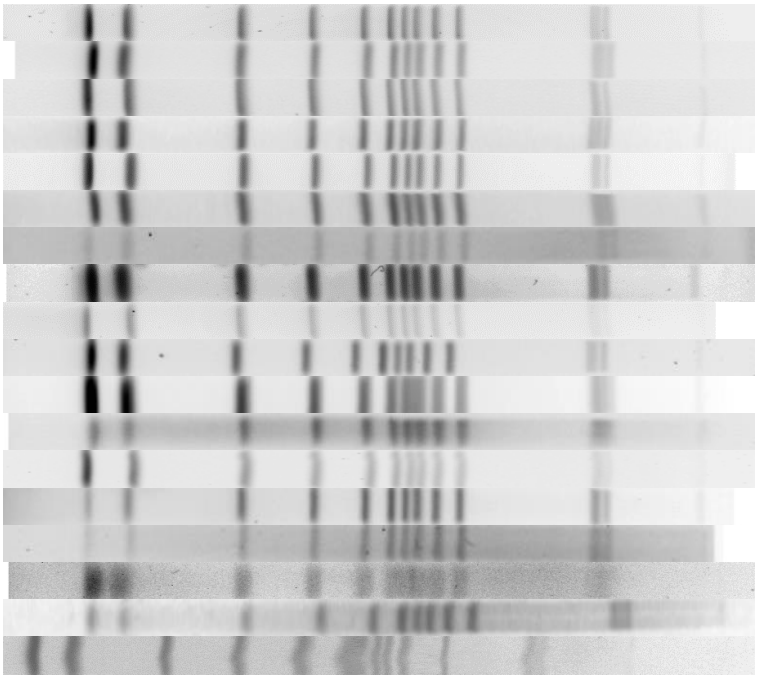
Parameter	Grade [score in points]		
	Poor [1]	Fair [2]	Excellent [3]
Position of gel frame	-Wells wrongly included when placing the frame -Gel not inverted	-Fframe is positioned too low -Too much space framed at the bottom of the gel -Too much space framed on the sides of the gel	Excellent placement of frame and gel is inverted
Strips	Lanes incorrectly defined	-Lanes are defined too narrowly (or widely) -Lanes are defined outside profile -A single lane is not correctly defined	All lanes correctly defined
Curves	Curve set so that artefacts will cause wrong band assignment	-Curve extraction is defined either too narrowly or including almost the whole lane	1/3 or more of the lane is used for averaging curve extraction
Normalisation	-Many bands not assigned in reference lanes -References were not included when submitting the data -Assignment of band(s) in reference lane(s) to incorrect size(s)	-Bottom bands <33kb are not assigned in some or all of the reference lanes -Some bands wrongly assigned in reference lane(s)	All bands correctly assigned in all reference lanes
Band assignment	-Incorrect band assignment making inter-laboratory comparison impossible -Few shadow bands assigned* -Few bands not assigned*	-Few double bands assigned as single bands or single bands assigned as double bands -Few bands are not assigned -Bands below <33kb assigned*	Excellent band assignment

**: Compared with 2016 guidelines, sentences were added to 'Band assignment' parameter.*
Compared with previous EQAs, in the gel analysis part, participants analysed a provided gel instead of their own.

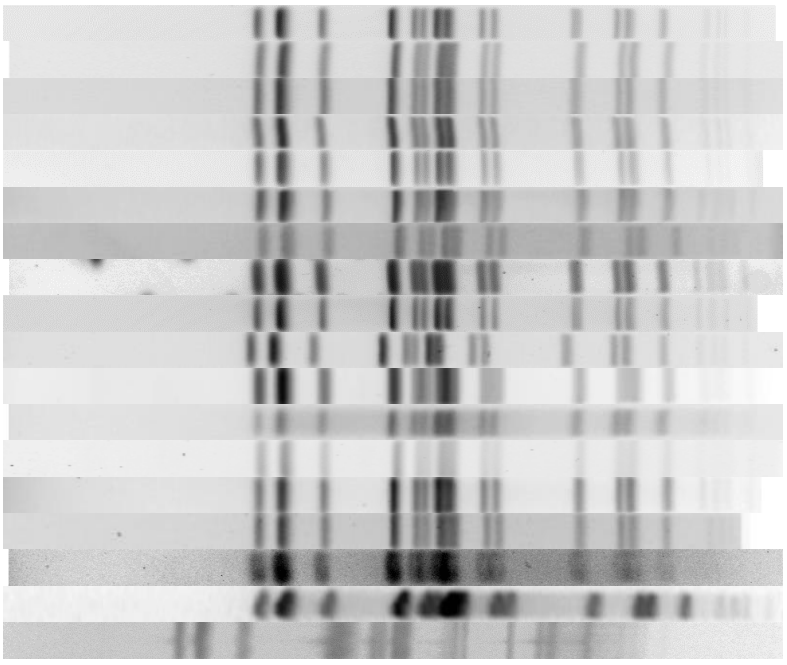
Annex 5. PFGE profiles of two test isolates

Eighteen PFGE profiles: 17 produced by participants and PFGE C by the EQA provider.

Isolate PFGE C (top) and PFGE H (bottom) cut with *Xba*I.



PFGE C	Total score	Parameter(s) with scores of 'poor' [1]
147	28	
92	27	
36	26	
106	26	
134	26	
142	24	
127	24	
19	24	
128	23	
55	23	
145	23	
140	23	
100	23	
144	22	
96	20	Band
130	19	Band
138	12	Image acquisition, Restriction, DNA degradation



PFGE H	Total score	Parameter(s) with scores of 'poor' [1]
147	28	
92	27	
36	26	
106	26	
134	26	
142	24	
127	24	
19	24	
128	23	
55	23	
145	23	
140	23	
100	23	
144	22	
96	20	Bands
130	19	Bands
138	12	Image acquisition, Restriction, DNA degradation

Annex 6. Scores of PFGE results

Gel quality

	Laboratory ID																
Parameters	**19**	**36**	**55**	**92**	**96**	**100**	**106**	**128**	**129**	**130**	**134**	**138**	**140**	**142**	**144**	**145**	**147**
Image acquisition and running conditions	3	4	3	4	3	4	3	3	4	4	4	1	3	3	3	3	4
Cell suspension	3	4	4	4	4	3	4	3	3	2	4	2	4	4	3	4	4
Bands	4	4	2	4	1	3	4	3	3	1	4	2	4	3	3	3	4
Lanes	3	3	3	3	4	3	4	4	3	4	3	3	3	4	4	3	4
Restriction	4	4	4	4	4	4	4	4	4	4	4	1	2	4	3	4	4
Gel background	4	3	4	4	2	3	3	3	4	2	4	2	3	4	3	4	4
DNA degradation	3	4	3	4	2	3	4	3	3	2	3	1	4	2	3	2	4
Total quality	24	26	23	27	20	23	26	23	24	19	26	12	23	24	22	23	28

Participant scores 1–4 (Poor, Fair, Good, Excellent) obtained for each of the seven TIFF quality grading guidelines parameters and the total score.

BN analysis

	Laboratory ID																
Parameters	**19**	**36**	**55**	**92**	**96**	**100**	**106**	**128**	**129**	**130**	**134**	**138**	**140**	**142**	**144**	**145**	**147**
Position of the gel	3	3	3	2	-	3	3	1	-	2	3	-	-	3	-	-	3
Strips	3	3	3	2	-	3	3	2	-	3	3	-	-	3	-	-	3
Curves	3	3	3	3	-	3	2	2	-	3	3	-	-	3	-	-	3
Normalisation	3	3	3	3	-	3	3	1	-	3	3	-	-	3	-	-	3
Band assignment	3	3	2	2	-	2	2	2	-	2	2	-	-	3	-	-	3
Total quality	15	15	14	12	-	14	13	8	-	13	14	-	-	14	-	-	14

Participant scores 1–3 (Poor, Fair, Excellent) obtained for each of the five BioNumerics Gel Analysis Quality Guidelines parameters and the total score.

Annex 7. Scores of MLVA results *S*. Typhimurium

Test isolates no. /allel

	STm1					STm2					STm3					STm4					STm5				
Lab. no.	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3
Ori.	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
19	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
36	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
100	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
108	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
129	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
134	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	4	13	212
135	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
142	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
144	3	15	9	-2	-2	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
147	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
148	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212
149	3	15	9	-2	211	2	15	6	-2	112	3	13	11	12	311	2	20	12	9	212	2	9	-2	13	212

	STm6					STm7					STm8					STm9					STm10				
Lab. no.	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3	STTR9	STTR5	STTR6	STTR10	STTR3
Ori.	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
19	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
36	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
100	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
108	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
129	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
134	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
135	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
142	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
144	3	12	9	-2	-2	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
147	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	19	211	3	13	-2	-2	211	5	15	7	-2	111
148	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111
149	3	12	9	-2	211	3	12	8	-2	211	6	11	-2	-2	211	3	13	-2	-2	211	5	15	7	-2	111

Purple: repeat strains in EQA-4 to 7
Pink: incorrect.

Annex 8. Scores of MLVA results *S.* Enteritidis

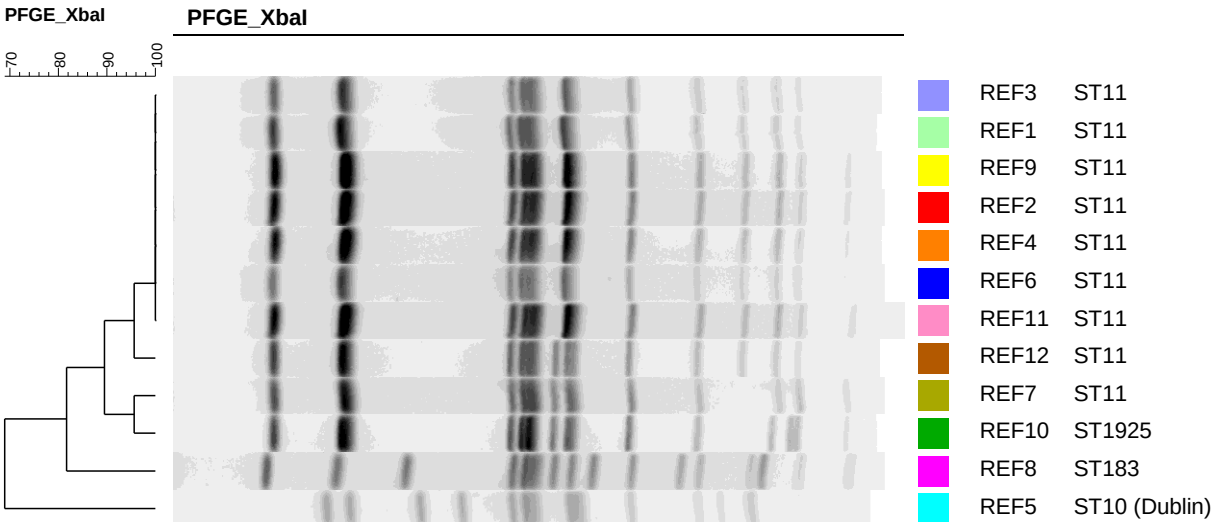
Test isolates no. /allel

	SE1					SE2					SE3					SE4					SE5				
Lab. no.	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3
Ori.	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
19	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
55	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
100	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
108	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
129	2	10	-2	5	2	3	11	-2	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
134	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
135	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
142	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
144	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
147	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2
148	2	10	11	5	2	3	11	11	4	1	2	9	11	3	2	2	11	11	3	2	2	10	7	3	2
149	2	10	8	5	2	3	11	4	4	1	2	9	7	3	2	2	11	7	3	2	2	10	7	3	2

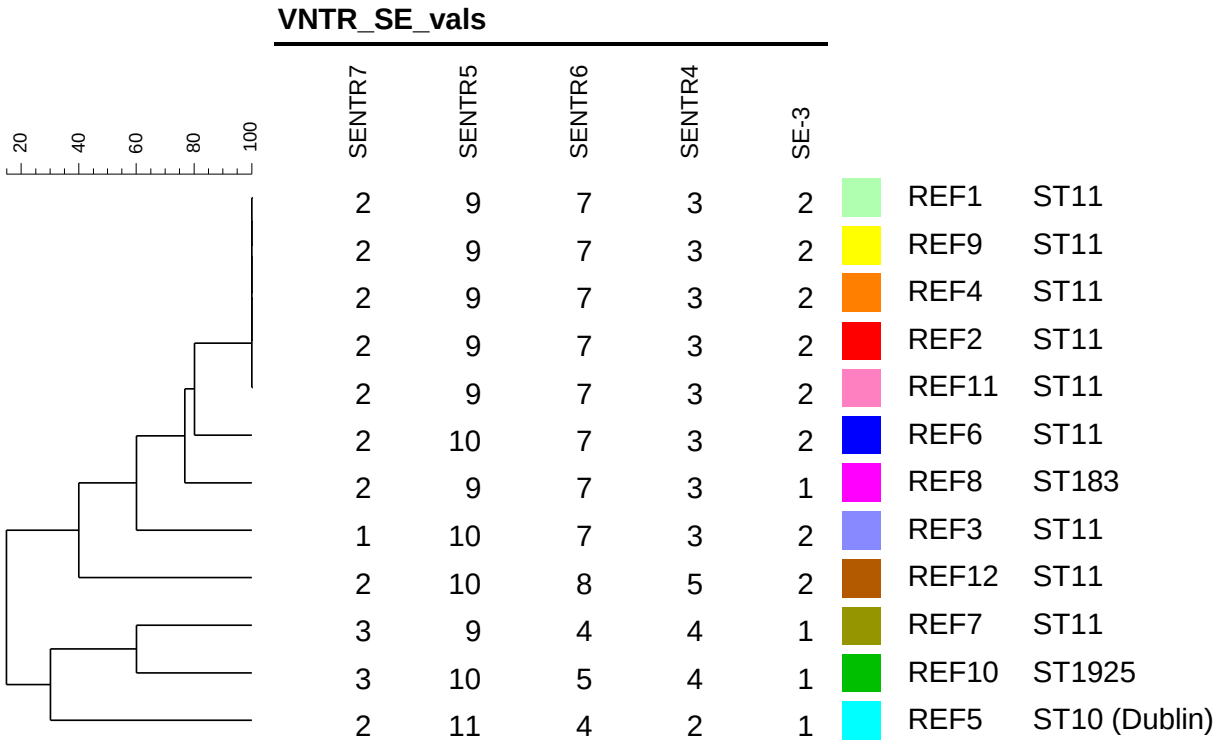
	SE6					SE7					SE8					SE9					SE10				
Lab. no.	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3	SENTR7	SENTR5	SENTR6	SENTR4	SE-3
Ori.	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
19	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
55	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
100	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
108	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
129	3	11	-2	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
134	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
135	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
142	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
144	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1
147	3	11	5	4	1	3	10	5	4	1	-2	10	7	3	2	2	10	10	5	2	3	10	4	4	1
148	3	11	5	4	1	3	10	11	4	1	1	10	-2	3	1	2	10	10	5	2	3	10	4	4	1
149	3	11	5	4	1	3	10	5	4	1	1	10	7	3	2	2	10	10	5	2	3	10	4	4	1

Pink: incorrect.

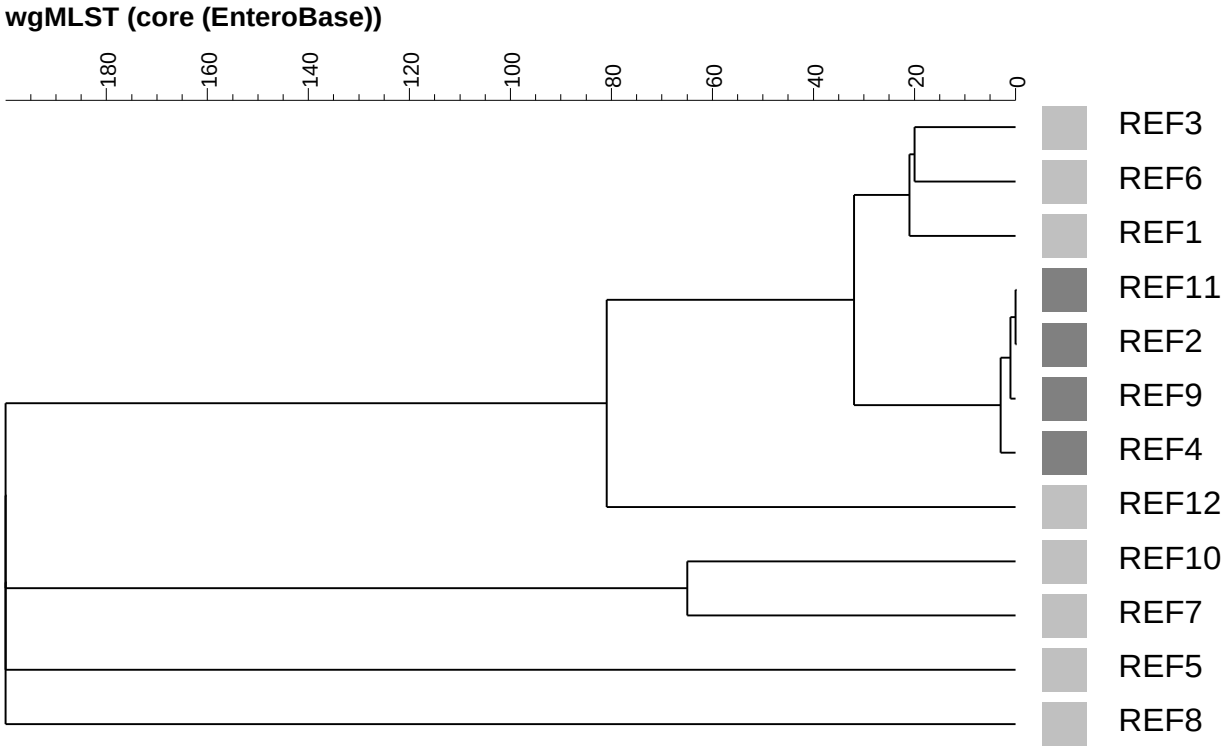
Annex 9. EQA provider cluster analysis based on PFGE-derived data



Annex 10. EQA provider cluster analysis based on MLVA-derived data



Annex 11. EQA provider cluster analysis based on WGS-derived data



Single linked dendrogram of core genome multi-locus sequence typing (cgMLST) profiles of Salmonella EQA-8 isolates (cgMLST, EnteroBase, https://enterobase.warwick.ac.uk).
Analysed in BioNumerics: maximum distance of 200 exceeded; results clipped.
Dark grey: cluster isolates
Light grey: outside cluster isolates.

Annex 12. Reported cluster of closely related isolates based on PFGE-derived data

Laboratory	Reported cluster	Corresponding REF isolates	Correct
Provider	REF1, REF2, REF3, REF4, REF6, REF9, REF11		
19	6196, 6508, 6613, 6884, 6934, 6075, 6453	REF1, REF3, REF6, REF11, REF9, REF4, REF2	Yes
55	6030, 6201, 6437, 6498, 6700, 6923, 6941	REF11, REF1, REF9, REF2 REF6, REF4, REF3	Yes
92	6047, 6070, 6120, 6171, 6298, 6301, 6333, 6165, 6355, 6589	REF3, REF4, REF11, REF1, REF2, REF8, REF6, REF12, REF10, REF7	No
142	6103, 6166, 6182, 6239, 6329, 6426, 6672	REF6, REF2, REF3, REF1, REF4, REF9, REF11	Yes

Annex 13. Reported PFGE band differences

			Laboratory ID			
Isolate number	**ST**	**Expected *Xba*I bands**	**92**	**55**	**142**	**19**
REF1‡	11	11	11	11	11	11
REF2‡#	11	11	11	11	11	11
REF3‡	11	11	11	11	11	11
REF4‡	11	11	11	11	11	11
REF5	10	Clearly unrelated profile	15	14	15	15
REF6‡	11	11	11	11	11	11
REF7	11	11	11	11	11	11
REF8	183	Clearly unrelated profile	15	15	15	15
REF9‡	11	11	11	11	11	11
REF10	1925	12	12	12	11	12
REF11‡#	11	11	11	11	11	11
REF12	11	12	12	12	12	12

			Laboratory ID			
Isolate number	**ST**	**Bands with shared *Xba*I**	**92**	**55**	**142**	**19**
REF1‡	11	11	11	11	11	11
REF2‡#	11	11	11	11	11	11
REF3‡	11	11	11	11	11	11
REF4‡	11	11	11	11	11	11
REF5	10	Clearly unrelated profile	7	9	7	7
REF6‡	11	11	11	11	11	11
REF7	11	10	9	10	10	9
REF8	183	Clearly unrelated profile	11	10	8	8
REF9‡	11	11	11	11	11	11
REF10	1925	9	9	9	10	8
REF11‡#	11	11	11	11	11	11
REF12	11	11	11	11	11	11

‡: cluster identification of closely related isolates (based on PFGE-derived data)
#: technical duplet
ST: sequence type.

Annex 14. Reported sequencing details

Sequencing performed	Protocol (library preparation)	Commercial kit	Sequencing platform
In own laboratory	Commercial kits	Illumina Nextera XT Library preparation kit, Illumina TruSeq rapid SBS kit*	HiSeq 2500
Externally	Commercial kits	Nextera XT	MiSeq
Externally	Commercial kits	Nextera XT DNA Library Preparation Kit	MiSeq
In own laboratory	Commercial kits	Illumina Nextera XT	MiSeq
Externally	Commercial kits	Nextera XT	NextSeq
In own laboratory	Commercial kits	Nextera XT	NextSeq
In own laboratory	Commercial kits	Nextera	MiSeq
In own laboratory	Commercial kits	Qiagen blood and tissue**	NextSeq
In own laboratory	Commercial kits	Nextera XT**	MiniSeq, Illumina
In own laboratory	Commercial kits	Ion Xpress TM Plus Fragment Library Kit	IonTorent S5XL
In own laboratory	Commercial kits	Illumina Nextera XT	MiSeq

**: 5ng input DNA (as opposed to 1ng)*
Altered PCR protocol to favour longer fragment sizes - adjustment of extension temperature (and final extension) from 72°C to 65°C.
'Manual' normalisation using library concentration and fragment size (as opposed to bead based normalisation).
***: 1/2 volume for all reagents.*

Annex 15. Reported cluster of closely related isolates based on MLVA-derived data

Laboratory	Reported cluster	Corresponding to REF isolates	Correct
Provider	REF1, REF2, REF4, REF9, REF11		
19	6075, 6196, 6453, 6884, 6934	REF4, REF1, REF2, REF11, REF9	Yes
108	6219, 6356, 6386, 6403, 6632	REF1, REF2, REF11, REF9, REF4	Yes
134	6053, 6363, 6599, 6607, 6814	REF9, REF4, REF1, REF2, REF11	Yes
142	6166, 6239, 6329, 6426, 6672	REF2, REF1, REF4, REF9, REF11	Yes

Annex 16. Reported MLVA profile data

				Laboratory ID			
Isolate number	**ST**	**MLVA scheme**	**Provider**	**19**	**108**	**138**	**142**
REF1‡	11	*S.* Enteritidis	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2
REF2‡#	11	*S.* Enteritidis	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2
REF3	11	*S.* Enteritidis	1-10-7-3-2	1-10-7-3-2	1-10-7-3-2	1-10-7-3-2	1-10-7-3-2
REF4‡	11	*S.* Enteritidis	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2
REF5	10	*S.* Enteritidis	2-11-4-2-1	2-11-4-2-1	2-11-4-2-1	2-11-4-2-1	2-11-4-2-1
REF6	11	*S.* Enteritidis	2-10-7-3-2	2-10-7-3-2	2-10-7-3-2	2-10-7-3-2	2-10-7-3-2
REF7	11	*S.* Enteritidis	3-9-4-4-1	3-9-4-4-1	3-9-4-4-1	3-9-4-4-1	3-9-4-4-1
REF8	183	*S.* Enteritidis	2-9-7-3-1	2-9-7-3-1	2-9-7-3-1	2-9-7-3-1	2-9-7-3-1
REF9‡	11	*S.* Enteritidis	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2
REF10	1925	*S.* Enteritidis	3-10-5-4-1	3-10-5-4-1	3-10-5-4-1	3-10-5-4-1	3-10-5-4-1
REF11‡#	11	*S.* Enteritidis	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2	2-9-7-3-2
REF12	11	*S.* Enteritidis	2-10-8-5-2	2-10-8-5-2	2-10-8-5-2	2-10-8-5-2	2-10-8-5-2

‡: cluster identification of closely related isolates (based on MLVA-derived data)
#: technical duplet
ST: sequence type.

Annex 17. Reported cluster of closely related isolates based on WGS-derived data

Laboratory	Reported cluster	Corresponding to REF isolates	Correct
Provider	REF2, REF4, REF9, REF11		
19	6884, 6453, 6934, 6075	REF11, REF2, REF9, REF4	Yes
36	6777, 6446, 6181, 6789	REF11, REF9, REF2, REF4	Yes
49	6193, 6302, 6360, 6397	REF11, REF2, REF 4, REF 9	Yes
106	6391, 6509, 6900#	REF11, REF2, REF4	Yes
108	6386, 6356, 6403¤	REF11, REF2, REF9	Yes
129	6206, 6568, 6691, 6852	REF11, REF2, REF9, REF4	Yes
134	6053, 6363, 6607, 6814	REF9, REF4, REF2, REF11	Yes
142	6166, 6672, 6426, 6329	REF2, REF11, REF9, REF4	Yes
147	6154, 6646, 6743, 6887	REF11, REF2, REF9, REF4	Yes
148	6008, 6023, 6440, 6742, 6823, 6914, 6925	REF2, REF4, REF3, REF9, REF1, REF11 RFE6	No
150	6041, 6225, 6522, 6774	REF7, REF11, REF2, REF9	Yes

#: Only 10 FASTQ files were used for laboratory 106 due to data quality not meeting the laboratory's own QC limits.
¤: Three isolates reported that they needed further investigation and epidemiological information to make a conclusion to include the fourth isolate.

Annex 18. Reported SNP distance and allelic differences

SNP distances

			Laboratory ID						
Isolate number	ST	Provider	19*	49*	106	106*	108	148	150
REF1	11	61	78	65	18	45	65	89	68
REF2‡#	11	0¤	0	0¤	0	3	0	0¤	0¤
REF3	11	63	80	66	15	43	67	92	70
REF4‡	11	6	7	6	3	9	7	22	7
REF5	10	9999	9999	9999	699	1415	9999	9999	9999
REF6	11	83	83	77	16	45	71	97	91
REF7	11	462	525	488	117	300	510	9999	493
REF8	183	1742	9999	1816	388	898	9999	9999	1582
REF9‡	11	1	1	1	9999	9999	1	19	1
REF10	1925	483	9999	511	124	329	9999	9999	522
REF11‡#	11	0	0¤	0	0¤	0¤	0¤	19	0
REF12	11	163	185	183	9999	9999	171	9999	177

Allelic differences

			Laboratory ID										
Isolate number	ST	Provider	19	36	49	49*	106*	129	134	142	147	148*	150*
REF1	11	33	33	54	33	55	33	37	32	33	33	33	33
REF2‡#	11	0	0	0	0¤	0¤	0	0	0¤	0¤	0	0¤	0¤
REF3	11	34	34	53	33	57	31	36	32	35	32	33	34
REF4‡	11	3	3	6	2	5	3	4	3	3	3	2	3
REF5	10	1779	1774	2310	1727	2364	1741	1804	1701	1793	1745	9999	9999
REF6	11	35	35	57	35	61	35	44	35	35	35	36	36
REF7	11	240	239	336	227	348	233	252	233	240	234	206	9999
REF8	183	660	659	910	662	994	636	692	637	667	664	639	9999
REF9‡	11	1	1	1	1	1	9999	2	1	1	1	1	3
REF10	1925	256	254	357	240	370	247	266	247	256	248	231	256
REF11‡#	11	0¤	0¤	0¤	0	0	0¤	0¤	0	0	0¤	1	9
REF12	11	97	98	130	98	138	9999	98	94	99	94	96	99

**: additional analysis*
‡: closely related isolates
#: technical duplicate isolates
¤: isolate used as cluster representative by the participant
9999: isolates not included in the analysis by the participant
ST: sequence type.

Annex 19. Reported QC parameters

	QC parameters	Thresholds
B	Average read coverage	>30
B	Number BAF multiple	<=10
C	Coverage	>20
D	Coverage	50 x
E	Average coverage	>29
F	Depth of coverage	50x
H	Coverage	>40
I	Minimum per site coverage of assembly	25
I	Difference of sum of lengths of contigs with average coverage >0 and >25	250 000
I	Difference of number of contigs with average coverage >0 and >25	1 000
J	Coverage	>50
K	Coverage	20x
L	Average coverage	30x
A	Confirmation of organism by similarity	Most similar reference organism must be *Salmonella enterica* Failure consequence: Sample processing stops
D	Confirmation of genus (JSpecies)	-
F	Genus confirmation	-
F	Contamination	-
G	KmerFinder species confirmation	-
I	Confirmation of genus	Main genus match in kraken must match supplied genus
I	Contamination check	Only one genus >5% on mini kraken
K	Confirmation of genous/species	-
B	N50	>100 000
B	Number of contigs	<150
C	Number of contigs	-
D	Number of contigs	200 bases (contigs shorter than 200 bases have to be ignored)
D	N50	-
F	N50	-
F	Number of contig	<120
G	Contig length	200 bp
I	Number of contigs	< 1 000
B	Genome length	4–5.5 MB
D	Consensus base count assembled	Approximate size of genome (for *Salmonella* ~ 4.7 million)
F	Number of base	between 4,2 Mb and 5,1Mb
I	Assembly length	>4.5 million & <5.3 million
J	Length of contig assembly	<reference genome+10%
K	Genome size	+/-20%
B	Core percent	>=95%
D	Number of good cgMLST targets	min. 90 %
E	Percentage of good targets	90%
H	% loci cgMLST found	>95%
J	cgMLST alleles found	>95%
L	Percent good cgMLST targets	95%
A	Length of read after trimming	50 bases
C	Length of sequences in fastQC	150
D	Fast QC: per sequence length distribution	-
G	Reads QC: minimum length	50bp
L	Average read length	180bp
A	Quality of bases at the start/end of each read	Phred score =>30
B	Average Quality	>30
D	Fast QC: per base sequence quality	-
D	Fast QC: per base sequence content	-
D	Fast QC: per sequence quality score	-
D	Q30	70–80%
H	PHRED score	>28
D	Target QC procedure	Length of consensus equals reference sequence area(s) length+/-3 triplets
L	Required identitiy to reference locus	90%
L	Required percentage aligned to ref. locus	100%
A	Quality of bases in the remainder of each read	Average Phred score=>20 across a 10-nucleotide sliding window Failure consequence: Read is trimmed at that point
G	Reads QC: Trimming (Trimmomatic)	4nt window Q20 threshold
A	Minimum number of raw reads received from sequencing	10 000
A	Minimum number of processed reads remaining after quality trimming	10 000
D	PHiX control (internal control for run performance)	-
D	Cluster density	~1 200–1 400 K/mm3
C	%GC	38
G	SNP cluster filter	3 SNPs in 1 000 nt window

Annex 20. Calculated qualitative/quantitative parameters

		Laboratory 19											
Parameters	Ranges*	6196	6735	6613	6075	6453	6784	6299	6508	6868	6884	6934	6932
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		2.44	2.7	1.97	1.79	2.07	2.5	2.85	2.28	2.35	2.45	2.21	2.43
Length at 25 x min. coverage (Mbp)	{>4.5 ∧<5.3}	4.7	4.9	4.7	4.7	4.7	4.9	4.7	4.7	4.7	4.7	4.7	4.7
Length [0–25] x min. coverage (Mbp)	{<0.25}	0	0	0	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	62	66	63	54	57	81	46	43	69	53	67	42
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	0	0	1	0	0	1	0	0	0
Average coverage	{>50}	145	136	124	207	149	130	173	183	153	164	154	184
Number of reads (x1 000)		2564	2500	2161	3656	2620	2397	3124	3249	2715	2919	2729	3312
Number of trimmed reads (x1 000)		2529	2467	2134	3610	2584	2366	3081	3206	2679	2883	2692	3268
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		139	139	141	141	140	139	138	139	139	138	139	139
Read insert size		312	300	311	299	314	299	311	299	299	290	304	300
Insert size StdDev		132	129	125	116	126	121	133	126	124	123	125	125
N50 (kbp)		154	134	129	167	149	125	229	229	128	162	120	220
N75 (kbp)		89	91	88	92	94	75	129	117	80	105	88	123

		Laboratory 36											
Parameters	Ranges*	6093	6181	6174	6425	6197	6334	6446	6777	6990	6789	6770	6961
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		0.54	0.38	5.32	1.44	4.11	3.89	0.41	0.47	0.36	4.71	3.65	1.75
Length at 25 x min. coverage (Mbp)	{>4.5 ∧<5.3}	4.7	4.7	4.9	4.8	4.7	4.7	4.7	4.7	4.7	4.7	4.9	4.7
Length [0–25] x min. coverage (Mbp)	{<0.25}	0	0	0	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum. coverage	{>0}	24	35	26	29	23	25	38	41	49	25	29	24
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	48	65	67	119	109	125	110	113	96	116	120	103
Number of reads (x1 000)		479	615	812	1328	1383	1544	1071	1146	909	1540	1523	1236
Number of trimmed reads (x1 000)		469	601	801	1303	1363	1519	1052	1115	892	1511	1501	1207
Maximum read length		301	301	301	301	301	301	301	301	301	301	301	301
Mean read length		243	258	208	221	193	197	249	243	257	184	201	203
Read insert size		372	405	332	328	318	329	372	351	400	301	328	312
Insert size StdDev		185	173	180	172	170	177	176	156	179	154	175	158
N50 (kbp)		494	289	479	401	492	422	180	196	171	479	299	442
N75 (kbp)		180	172	234	180	227	180	124	124	89	228	229	228

		Laboratory 49											
Parameters	**Ranges***	**6193**	**6217**	**6291**	**6302**	**6360**	**6320**	**6362**	**6397**	**6828**	**6526**	**6564**	**6888**
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.77	2.33	1.78	0.81	3	3.92	2.1	1	1.53	1.86	1.91	1.26
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.6	4.7	4.7	4.7	4.7	4.8	4.7	4.7	4.6	4.7	4.7	4.9
Length [0–25] x minimum coverage (Mbp)	{<0.25}	0.1	0	0	0	0	0	0	0	0.1	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	118	96	69	33	78	93	74	34	96	70	53	45
Number of contigs [0–25] x minimum coverage	{<1 000}	14	10	1	0	2	5	1	0	10	2	0	0
Average coverage	{>50}	34	33	41	44	40	38	41	41	34	42	43	75
Number of reads (x1 000)		846	838	1017	1067	1027	980	1036	986	849	1058	1092	1908
Number of trimmed reads (x1 000)		846	838	1017	1067	1027	980	1036	986	849	1058	1092	1908
Maximum read length		101	101	101	101	101	101	101	101	101	101	101	101
Mean read length		95	96	95	99	93	96	95	98	96	95	95	99
Read insert size		194	206	193	343	177	192	186	341	201	191	194	323
Insert size StdDev		84	83	84	191	86	82	84	195	84	83	83	176
N50 (kbp)		63	83	122	284	123	111	106	345	71	135	166	228
N75 (kbp)		37	40	75	144	60	59	67	138	42	67	93	117

		Laboratory 106									
Parameters	**Ranges***	**6900**	**6786**	**6841**	**6960**	**6966**	**6198**	**6391**	**6248**	**6937**	**6509**
Number of genera detected	{1}	1	1	1	1	2	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		3.08	1.81	1.07	1.36	11.55	1.26	1.01	1.09	0.95	1.28
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.1	4.9	4.7	4.7	4.9	4.7	4.8	4.7	4.7	4.7
Length [0–25] x minimum. coverage (Mbp)	{<0.25}	1.3	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	50	39	32	38	34	35	43	38	40	31
Number of contigs [0–25] x minimum coverage	{<1 000}	1	0	0	0	0	3	0	0	0	0
Average coverage	{>50}	41	193	278	249	183	333	236	240	242	323
Number of reads (x1 000)		883	3591	5001	4614	4766	6117	4830	5036	4872	5854
Number of trimmed reads (x1 000)		874	3545	4941	4556	4713	6039	4773	4971	4815	5778
Maximum read length		151	151	151	151	151	151	151	151	151	151
Mean read length		134	142	146	146	146	144	146	146	146	145
Read insert size		257	318	397	412	369	318	342	409	357	428
Insert size StdDev		142	162	153	154	149	149	146	152	142	177
N50 (kbp)		229	220	283	283	297	229	229	229	250	284
N75 (kbp)		95	122	135	153	154	135	123	123	123	153

		Laboratory 108										
Parameters	**Ranges***	**6219**	**6268**	**6386**	**6403**	**6632**	**6399**	**6356**	**6649**	**6601**	**6671**	**6769**
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.57	1.04	1.08	0.9	1.76	1.25	0.78	1.69	1.07	0.74	0.81
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.7	4.9	4.7	4.7	4.7	4.7	4.7	4.7	4.8	4.7	4.7
Length [0–25] x minimum coverage (Mbp)	{<0.25}	0	0	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	155	196	414	375	204	221	167	157	461	203	258
Number of contigs [0–25] x minimum coverage	{<1 000}	8	0	0	0	0	0	0	1	0	0	0
Average coverage	{>50}	31	120	67	92	39	68	106	35	96	80	102
Number of reads (x1 000)		506	1696	944	1284	654	874	1447	551	1391	1057	1349
Number of trimmed reads (x1 000)		465	1554	874	1188	604	817	1316	506	1292	991	1263
Maximum read length		193	304	292	297	196	303	304	204	294	306	302
Mean read length		175	228	210	216	168	222	224	178	211	223	219
Read insert size		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Insert size StdDev		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
N50 (kbp)		47	40	17	20	39	34	46	58	16	38	31
N75 (kbp)		28	25	10	11	21	22	29	31	9	24	17

		Laboratory 129											
Parameters	Ranges*	6003	6051	6324	6124	6568	6206	6469	6947	6852	6691	6763	6958
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.2	0.7	0.42	1.2	0.59	0.62	0.77	1.14	0.73	0.45	0.56	1.15
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	4.7	4.7	4.7	4.7	4.7	4.7	4.1	4.7	4.7	4.7	4.9
Length [0–25] x minimum coverage (Mbp)	{<0.25}	0	0	0	0	0	0	0	0.5	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	72	60	31	39	52	31	61	123	54	37	43	59
Number of contigs [0–25] x minimum coverage	{<1 000}	10	3	3	0	2	0	1	64	2	0	0	0
Average coverage	{>50}	45	81	55	77	69	84	81	29	72	93	94	77
Number of reads (x1 000)		794	1362	925	1313	1145	1406	1350	494	1211	1543	1544	1335
Number of trimmed reads (x1 000)		777	1340	909	1284	1126	1388	1338	483	1191	1524	1534	1312
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		142	144	144	141	144	143	145	143	144	145	145	145
Read insert size		557	516	572	498	535	471	424	483	499	472	380	429
Insert size StdDev		235	200	197	211	197	177	159	196	191	173	147	169
N50 (kbp)		115	123	399	245	196	345	159	47	171	258	181	152
N75 (kbp)		62	77	171	135	110	167	85	27	93	129	117	93

		Laboratory 134											
Parameters	Ranges*	6797	6802	6053	6936	6363	6607	6332	6238	6599	6814	6486	6971
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		2.81	1.84	3.86	1.44	1.39	2.51	1.93	2.35	1.6	1.68	1.88	1.51
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	1.3	4.7	4.7	4.6	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.9
Length [0–25] x minimum coverage (Mbp)	{<0.25}	3.5	0	0	0.1	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	19	42	34	61	35	34	37	42	37	41	36	45
Number of contigs [0–25] x minimum coverage	{<1 000}	44	0	0	5	0	0	0	0	0	0	0	0
Average coverage	{>50}	24	41	58	30	61	50	66	68	79	67	79	66
Number of reads (x1 000)		416	686	1037	492	1002	854	1110	1150	1309	1108	1326	1138
Number of trimmed reads(x1 000)		411	680	1024	487	993	844	1100	1139	1295	1097	1313	1130
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		144	142	135	145	146	142	144	143	145	145	143	146
Read insert size		418	337	338	310	391	384	367	352	361	383	346	376
Insert size StdDev		165	153	173	111	136	157	151	147	128	141	146	131
N50 (kbp)		127	226	226	137	283	283	322	225	220	226	283	191
N75 (kbp)		93	123	171	97	135	153	135	117	117	117	123	106

		Laboratory 142											
Parameters	Ranges*	6166	6182	6103	6239	6251	6285	6329	6286	6426	6790	6344	6672
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		0.77	0.27	0.83	0.86	1.87	1.02	1	2.09	0.87	0.87	1.44	0.93
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.7	4.7	4.7	4.7	4.9	4.7	4.7	4.9	4.7	4.7	4.8	4.7
Length [0–25] x minimum coverage (Mbp)	{<0.25}	0	0	0	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	23	23	23	25	22	21	24	28	22	23	29	24
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	0	0	0	2	0	0	0	0	0
Average coverage	{>50}	49	34	68	58	63	63	53	66	56	63	61	65
Number of reads (x1 000)		523	366	742	626	723	692	586	851	617	700	683	722
Number of trimmed reads (x1 000)		510	353	727	612	708	682	573	841	607	681	670	703
Maximum read length		251	251	251	251	251	251	251	251	251	251	251	251
Mean read length		231	226	224	226	221	221	221	196	220	224	220	222
Read insert size		457	437	380	383	367	358	366	327	372	391	369	372
Insert size StdDev		172	176	174	168	173	166	167	171	175	178	172	169
N50 (kbp)		493	490	490	418	494	492	479	299	492	482	404	479
N75 (kbp)		228	283	284	228	406	401	228	240	283	228	180	228

		Laboratory 147											
Parameters	**Ranges***	**6226**	**6646**	**6154**	**6545**	**6396**	**6679**	**6602**	**6709**	**6723**	**6743**	**6768**	**6887**
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.1	1.36	1.08	0.62	0.75	2.56	0.91	0.81	2.07	0.73	0.83	0.73
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	0	4.7	4.7	4.7	4.7	4.9	4.7	4.7	4.8	4.7	4.7	4.7
Length [0–25] x minimum coverage (Mbp)	{<0.25}	4.5	0	0	0	0	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	1	24	29	123	27	136	70	26	111	154	115	184
Number of contigs [0–25] x minimum coverage	{<1 000}	1019	0	0	0	0	0	0	0	0	0	0	1
Average coverage	{>50}	13	50	95	89	95	76	82	80	92	77	66	91
Number of reads (x1 000)		150	544	1054	979	1071	868	955	891	1036	878	707	1014
Number of trimmed reads (x1 000)		144	532	1030	947	1041	838	916	852	1013	850	681	986
Maximum read length		301	301	301	301	301	301	300	300	301	301	301	301
Mean read length		219	222	222	228	220	231	218	224	224	221	235	227
Read insert size		310	358	315	327	307	341	300	311	315	311	381	323
Insert size StdDev		122	139	128	133	131	140	122	122	135	121	146	134
N50 (kbp)		6	439	372	66	492	61	115	492	80	55	92	46
N75 (kbp)		3	230	230	40	153	35	74	180	41	29	37	26

		Laboratory 148											
Parameters	**Ranges***	**6338**	**6023**	**6440**	**6630**	**6816**	**6481**	**6742**	**6683**	**6008**	**6823**	**6914**	**6925**
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		1.48	0.87	0.93	0.95	0.73	1.27	0.98	0.83	0.8	0.71	0.86	0.66
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	4.5	4.6	4.5	4.8	4.6	4.8	4.6	4.6	4.6	4.5	4.5	4.6
Length [0–25] x minimum coverage (Mbp)	{<0.25}	0.1	0	0.1	0.1	0.1	0	0.1	0.1	0	0.1	0.1	0.1
Number of contigs at 25 x minimum coverage	{>0}	66	67	62	102	162	67	120	134	90	100	100	68
Number of contigs [0–25] x minimum coverage	{<1 000}	27	13	31	19	32	11	25	23	11	27	31	13
Average coverage	{>50}	52	74	78.8	72	88.8	76.8	112.2	96.9	82.4	83.7	74.2	116.7
Number of reads (x1 000)		903	1287	1372	1304	1564	1396	2026	1718	1438	1455	1290	2085
Number of trimmed reads (x1 000)		890	1268	1349	1287	1540	1376	1989	1690	1414	1436	1272	2055
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		143	146	145	146	146	145	145	146	146	146	146	146
Read insert size		328	348	346	336	378	333	392	379	372	365	358	360
Insert size StdDev		101	92	95	88	94	96	95	91	93	93	95	93
N50 (kbp)		128	119	117	77	46	117	75	65	82	75	76	116
N75 (kbp)		66	71	77	47	26	71	42	32	49	45	40	78

		Laboratory 150											
Parameters	**Ranges***	**6401**	**6225**	**6041**	**6232**	**6665**	**6487**	**6454**	**6394**	**6522**	**6880**	**6774**	**6922**
Number of genera detected	{1}	NA	1	1	1	1	1	1	1	1	1	1	1
Detected species	*{Se}*	NA	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*	*Se*
Unclassified reads (%)		NA	1.99	1.74	1.6	6.44	2.02	1.92	1.35	2.65	1.63	1.85	1.28
Length at 25 x minimum coverage (Mbp)	{>4.5 ∧ <5.3}	NA	4.7	4.7	4.7	4.4	4.8	4.7	4.7	4.7	4.7	4.7	4.7
Length [0–25] x minimum coverage (Mbp)	{<0.25}	NA	0	0	0	0.4	0	0	0	0	0	0	0
Number of contigs at 25 x minimum coverage	{>0}	NA	81	54	55	103	101	57	48	46	44	47	49
Number of contigs [0–25] x minimum coverage	{<1 000}	NA	2	2	2	40	12	2	0	0	1	0	2
Average coverage	{>50}	NA	45.3	60.3	57.1	33.4	40.2	53.5	60.8	72.5	67.6	74.4	50.1
Number of reads (x 1 000)		24	1156	1538	1451	890	1066	1365	1542	1875	1734	1908	1267
Number of trimmed reads (x1 000)		24	1156	1538	1451	890	1066	1365	1542	1875	1734	1908	1267
Maximum read length		101	101	101	101	101	101	101	101	101	101	101	101
Mean read length		87	95	95	95	95	94	95	95	94	95	95	95
Read insert size		NA	179	193	188	232	191	199	204	175	193	191	205
Insert size StdDev		NA	73	78	72	83	70	75	77	79	74	80	72
N50 (kbp)		NA	122	181	171	65	106	162	175	196	217	186	171
N75 (kbp)		NA	49	105	105	35	44	101	128	117	124	117	108

Se: Salmonella enterica
NA: not analysed.

Annex 21. EQA-8 laboratory questionnaire

This is a preview of all the fields and questions available.

Please keep in mind that, depending on your answers in the questionnaire, you will not necessarily have to answer all the questions.

1. Salmonella EQA-8 2017

Dear Participant,

Welcome to the eight External Quality Assessment (EQA-8) scheme for typing of Salmonella in 2017-2018. Please note that most of the fields are required to be filled in before the submission can be completed. Any comments can be written at the end of the form. You are always welcome to contact us at salm.eqa@ssi.dk.

Please start by filling in your country, your Laboratory name and your LAB_ID.

Available options in this submission form include:

- - Click "Options" and "Pause" to save your results and finish at a later time (using the same link)
- - Click "Options" and "Print" to print your answers. This can be done at any time, but before pressing "Submit results"
- - Click "Previous" to go back to the questions you have already answered
- - Click "Options" and "Go to.." to go back to a specific page number

Note: After pressing "Submit results" you will not be able to review your results.

2. Country

- Australia
- Austria
- Belgium
- Czech Republic
- Denmark
- Estonia
- Finland
- France
- Germany
- Greece
- Hungary
- Ireland
- Italy
- Latvia
- Lithuania
- Luxembourg
- New Zealand
- Norway
- Portugal
- Romania
- Scotland
- Serbia
- Slovak Republic
- Slovenia
- Spain
- Sweden
- Netherlands
- Turkey
- UK

3. Laboratory name

4. Laboratory ID

5. E-mail

6. Pulsed field gel electrophoresis (PFGE)

7. Submitting results

- TIFF (please fill in the strain ID's in the following section)
- BioNumerics analysis XML exports (please upload to ftp site)
- Did not participate in the PFGE part

8. Position of strain ID's on gel

Please enter the PFGE strain ID (4 digits) or '9999' (*S.* Braenderup H9812) in the position lanes.

	XbaI
Lane 1	___
Lane 2	___
Lane 3	___
Lane 4	___
Lane 5	___
Lane 6	___
Lane 7	___
Lane 8	___
Lane 9	___
Lane 10	___
Lane 11	___
Lane 12	___
Lane 13	___
Lane 14	___
Lane 15	___

9. Multiple-locus variable number of tandem repeats analysis (MLVA)

10. Submitting results

- Online here (please fill in the strain ID´s in the following section)
- Did not participate in the MLVA part

11. Select method

- *S.* Typhimurium and *S.* Enteritidis
- Only *S.* Typhimurium
- Only *S.* Enteritidis

12. MLVA strain ID's

Please enter the MLVA strain ID (4 digits)

S. Typhimurium

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___

13. Results for MLVA S. Typhimurium - Allele profile

Please use '-2' for not detected

Strain	STTR9	STTR5	STTR6	STTR10	STTR3
Strain 1					
Strain 2					
Strain 3					
Strain 4					
Strain 5					
Strain 6					
Strain 7					
Strain 8					
Strain 9					
Strain 10					

14. Submitting MLVA *S.* Enteritidis results

- Online here (please fill in the strain ID´s in the following section)
- Did not participate in the MLVA *S.* Enteritidis

15. MLVA strain ID's

Please enter the MLVA strain ID (4 digits)

S. Enteritidis

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___

16. Results for MLVA *S.* Enteritidis - Allele profile

Please use '-2' for not detected

	SENTR7	SENTR5	SENTR6	SENTR4	SE-3
Strain 1					
Strain 2					
Strain 3					
Strain 4					
Strain 5					
Strain 6					
Strain 7					
Strain 8					
Strain 9					
Strain 10					

17. Submitting cluster results

- Cluster analyses based on PFGE/MLVA/WGS
- Did not participate in the cluster part

18. Cluster strain ID's

Please enter the cluster strain ID (4 digits)

Cluster strain ID

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___
Strain 11 ___
Strain 12 ___

19. Submitting cluster results

- Cluster analysis based on PFGE
- Do not wish to submit any cluster results based on PFGE analysis

20. Cluster analysis based on PFGE data

21. Please list the ID for the strains included in the cluster detected by PFGE:

Please use semicolon (;) to separate the ID's

22. Select a representative strain with the cluster profile detected by PFGE:

Indicate the strain ID

23. Total number of bands (>33kb) in the selected representative cluster strain

24. Results for cluster analysis - PFGE (XbaI)

Please use '9999' for not analysed

	Total number of bands (>33kb)	Number of bands with same/shared position as the profile of the selected cluster strain (>33kb)
Strain 1		
Strain 2		
Strain 3		
Strain 4		
Strain 5		
Strain 6		
Strain 7		
Strain 8		
Strain 9		
Strain 10		
Strain 11		
Strain 12		

25. Submitting cluster results

- Cluster analysis based on MLVA
- Do not wish to submit any cluster results based on MLVA analysis

26. Cluster analysis based on MLVA data

27. Please list the ID for the strains included in the cluster by MLVA detection:

Please use semicolon (;) to separate the ID's

28. MLVA scheme used:

Please indicate serovar and/or protocol

29. Please list the loci in scheme used

30. Locus 1:

31. Locus 2:

32. Locus 3:

33. Locus 4:

34. Locus 5:

35. Results for cluster analysis (MLVA) - allele profile

Please use '-2' for not detected and '9999' for not analysed

	Locus 1	Locus 2	Locus 3	Locus 4	Locus 5
Strain 1					
Strain 2					
Strain 3					
Strain 4					
Strain 5					
Strain 6					
Strain 7					
Strain 8					
Strain 9					
Strain 10					
Strain 11					
Strain 12					

36. Submitting cluster results

- Cluster analysis based on MLVA
- Do not wish to submit any cluster results based on MLVA analysis

37. Cluster analysis based on WGS data

38. Please select the analysis used to detect the cluster on data derived from WGS

As basis for the cluster detection only one data analysis can be reported. If more than one analysis is performed please report later in this submission

- SNP-based
- Allele-based
- Other

39. If another analysis is used, please describe your approach:

40. Please select the approach used for the SNP analysis

- Reference-based
- Assembly-based

41. Reference genome used

Please indicate multilocus sequence type (e.g. ST34) and strain ID (e.g. one of the strains from the current cluster, ID of a public reference strain or an in-house strain)

42. Please indicate the read mapper used

(e.g. BWA, Bowtie 2)

43. Please indicate the variant caller used

44. Please indicate the assembler used

(e.g. SPAdes, Velvet)

45. Please specify the variant caller used

46. Please select tools used for the allele analysis

- BioNumerics
- SeqPhere
- Enterobase
- Assembly-based

47. If another tool is used, please enter here:

48. Please indicate allele calling method:

- Assembly based and mapping-based
- Only assembly-based
- Only mapping-based

49. Please indicate the assembler used (e.g. SPAdes, Velvet)

50. Please select scheme used for the allele analysis

- Applied Math (wgMLST)
- Applied Math (cgMLST/Enterobase)
- Enterobase (cgMLST)
- Other

51. If another scheme (e.g. in-house) is used, please give a short description

52. Cluster detected by analysis on data derived from WGS

On this page, you have to report the results for the cluster detected by the selected analysis (e.g. SNP-based). If another additional analysis (e.g. allele based or another SNP based analysis) is performed, please report results later, but you will not be asked to submit the ID's for strains in the cluster detected with the additional analysis.

53. Please list the ID's for the strains included in the cluster

Please use semicolon (;) to separate the strain ID's

54. Select a representative strain in the cluster indicate the strain ID

55. Results for cluster analysis (e.g. SNP- or allele-based)

Please use '9999' for not analysed

Distance (e.g. SNP) to the selected cluster strain

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___
Strain 11 ___
Strain 12 ___

56. Would you like to add results performed with another additional analysis on the data derived from the WGS?

E.g. if SNP-based results are submitted, you can also report allele-based results or results from a second SNP analysis

- Yes
- No

57. Please select the additional analysis used on data derived from WGS

- SNP-based
- Allele-based
- Other

58. If another analysis is used, please describe your approach:

59. Please select the approach used for the SNP analysis

- Reference-based
- Assembly-based

60. Reference genome used

Please indicate multilocus sequence type (e.g. ST34) and strain ID (e.g. one of the strains from the current cluster, ID of a public reference strain or an in-house strain)

61. Please indicate the read mapper used

(e.g. BWA, Bowtie 2)

62. Please indicate the variant caller used

(e.g. SAMtools, GATK)

63. Please indicate the assembler used

64. Please specify the variant caller used

(e.g. NUCMER)

65. Please select tool used for the allele analysis

- BioNumerics
- SeqPhere
- Enterobase
- Other

66. If another tool is used, please list here:

67. Please indicate allele calling method:

- BioNumerics
- SeqPhere
- Enterobase
- Other

68. Please indicate the assembler used

(e.g. SPAdes, Velvet)

69. Please select scheme used for the allele analysis

- Applied Math (wgMLST)
- Applied Math (cgMLST/Enterobase)
- Enterobase (cgMLST)
- Other

70. If another scheme (e.g. inhouse) is used, please give a short description

71. Additional analysis on data derived from WGS

72. Select a representative strain in the cluster detected by the additional analysis

Indicate the strain ID

73. Results for the additional cluster analysis (e.g. SNP- or allele-based)

Please use '9999' for not analysed

Distance (e.g. SNP) to the selected cluster strain

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___
Strain 11 ___
Strain 12 ___

74. Would you like to add results performed with a third analysis on the data derived from the WGS?

E.g. if SNP based results are submitted, you can also report allele-based results or results from a second SNP analysis

- Yes
- No

75. Please select the third analysis used on data derived from WGS

- SNP-based
- Allele-based
- Other

76. If another analysis is used, please describe your approach:

77. Please select the approach used for the SNP analysis

- Reference-based
- Assembly-based

78. Reference genome used

Please indicate multilocus sequence type (e.g. ST34) and strain ID (e.g. one of the strains from the current cluster, ID of a public reference strain or an in-house strain)

79. Please indicate the read mapper used

(e.g. BWA, Bowtie 2)

80. Please indicate the variant caller used

(e.g. SAMtools, GATK)

81. Please indicate the assembler used

(e.g. SPAdes, Velvet)

82. Please specify the variant caller used

(e.g. NUCMER)

83. Please select tool used for the allele analysis

- SNP-based
- Allele-based
- Allele-based
- Other

84. If another tool is used, please enter here:

85. Please indicate allele calling method:

- Assembly- and mapping-based
- Only assembly-based
- Only mapping-based

86. Please indicate the assembler used

(e.g. SPAdes, Velvet)

87. Please select scheme used for the allele analysis

- Applied Math (wgMLST)
- Applied Math (cgMLST/Enterobase)
- Enterobase (cgMLST)
- Other

88. If another scheme (e.g. in-house) is used, please give a short description

89. Third analysis on data derived from WGS

90. Select a representative strain in the cluster detected by the third analysis

91. Results for the third cluster analysis (e.g. SNP- or allele-based)

Distance (e.g. SNP) to the selected cluster strain

Strain 1 ___
Strain 2 ___
Strain 3 ___
Strain 4 ___
Strain 5 ___
Strain 6 ___
Strain 7 ___
Strain 8 ___
Strain 9 ___
Strain 10 ___
Strain 11 ___
Strain 12 ___

92. Additional questions to the WGS part

93. Where was the sequencing performed

- In own laboratory
- Externally

94. Protocol used to prepare the library for sequencing:

- In own laboratory
- Externally

95. Please indicate name of commercial kit:

96. If relevant, please list deviation from commercial kit shortly in few bullets:

97. For non-commercial kit please indicate a short summary of the protocol:

98. The sequencing platform used

- Ion Torrent PGM
- Ion Torrent Proton
- Genome Sequencer Junior System (454)
- Genome Sequencer FLX System (454)
- Genome Sequencer FLX+ System (454)
- PacBio RS
- PacBio RS II
- HiScanSQ
- HiSeq 1000
- HiSeq 1500
- HiSeq 2000
- HiSeq 2500
- HiSeq 4000
- Genome Analyzer Iix
- MiSeq
- MiSeq Dx
- MiSeq FGx
- ABI SOLiD
- NextSeq
- MinION (ONT)
- Other

99. If another platform is used, please list here:

100. Quantitative criteria used to evaluate the quality of sequence data.

Please list up to 10 different criteria (e.g. coverage, N50, number of contigs)

101. Quantitative criteria 1:

102. Threshold used for quantitative criteria 1:

103. Quantitative criteria 2:

104. Threshold used for quantitative criteria 2:

105. Quantitative criteria 3:

106. Threshold used for quantitative criteria 3:

107. Quantitative criteria 4:

108. Threshold used for quantitative criteria 4:

109. Quantitative criteria 5:

110. Threshold used for quantitative criteria 5:

111. Quantitative criteria 6:

112. Threshold used for quantitative criteria 6:

113. Quantitative criteria 7:

114. Threshold used for quantitative criteria 7:

115. Quantitative criteria 8:

116. Threshold used for quantitative criteria 8:

117. Quantitative criteria 9

118. Threshold used for quantitative criteria 9:

119. Quantitative criteria 10:

120. Threshold used for quantitative criteria 10:

121. Qualitative criteria used to evaluate the quality of sequence data

Please list up to 10 different criteria (e.g. contamination, confirmation of genus)

122. Qualitative criteria 1:

123. If relevant, threshold used for qualitative criteria 1:

124. Qualitative criteria 2:

125. If relevant, threshold used for qualitative criteria 2:

126. Qualitative criteria 3:

127. If relevant, threshold used for qualitative criteria 3:

128. Qualitative criteria 4:

129. If relevant, threshold used for qualitative criteria 4:

130. Qualitative criteria 5:

131. If relevant threshold used for qualitative criteria 5:

132. Qualitative criteria 6:

133. If relevant threshold used for qualitative criteria 6:

134. Qualitative criteria 7:

135. If relevant, threshold used for qualitative criteria 7:

136. Qualitative criteria 8:

137. If relevant, threshold used for qualitative criteria 8:

139. If relevant, threshold used for qualitative criteria 9:

140. Qualitative criteria 10:

141. If relevant, threshold used for qualitative criteria 10:

142. Comment(s):

E.g. remarks to the submission, the data analyses or the laboratory methods

143. Thank you for your participation

Thank you for filling out the Submission form for the Salmonella EQA-8.
For questions, please contact salm.eqa@ssi.dk or phone +45 3268 8341 +45 3268 8372.
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