TECHNICAL REPORT

Ninth external quality assessment scheme for *Salmonella* typing

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Abbreviations

BN	BioNumerics
cgMLST	Core genome multilocus sequence typing
wgMLST	Whole genome multilocus sequence typing
EQA	External Quality Assessment
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 sequencing

Executive summary

This report presents the results of the ninth round of the external quality assessment (EQA-9) 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-9 scheme was arranged by the Section for Foodborne Infections at the Statens Serum Institut (SSI) in Denmark.

Salmonellosis was the second-most commonly reported zoonotic disease in EU in 2017, with a notification rate of 19.7 cases per 100 000 population. From 2008 to 2017, a decreasing trend of confirmed salmonellosis cases was observed for 25 countries that consistently reported during the period. However, during the last five years (2013–2017), the overall EU/EEA trend did not show any significant increase or decrease [3]. Since 2007, ECDC's Food- and Waterborne Diseases and Zoonoses 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 effective molecular typing-enhanced surveillance relies on the capacity of PH NRLs in the FWD-Net to produce comparable typing results. Currently, molecular typing 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-8 included assessment of the PFGE typing methods for all *Salmonella* serovars and MLVA for *Salmonella* Typhimurium (STm). In the present scheme, the PFGE part was modified to address the cluster detection. This round (EQA-9) contained, as in EQA-8, an assessment of the ability of the laboratories to perform MLVA for *S.* Enteritidis (SE) and their 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-9 scheme were 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. Three sets of 10 to 12 isolates were selected, including *S.* Typhimurium and *S.* Enteritidis isolates for the two MLVA methods and a mixture of different sequence types (ST) in the cluster analysis.

Twenty-six laboratories signed up and 23 completed the exercise despite some only completing part of the methods for which the laboratory had signed up for. This is a minor decrease compared with EQA-8 (N=24), but a larger decrease of 12% in overall participation compared with EQA-7. It is unknown if the removal of the PFGE part (gel quality and analysis) was the cause of this. A minority (35%) of participants completed the full EQA scheme (MLVA and molecular typing-based cluster analysis). In total, 10 (43%) participated in both MLVA methods (STm and SE) and 21 (91%) in the molecular typing-based cluster analysis. Twelve (52%) laboratories submitted WGS-based typing results for cluster analysis.

For the STm MLVA schemes, a lower performance was obtained compared with previous years, as only two laboratories out of 10 (20%) reported correct allelic profiles for all test isolates. In the MLVA part for *S.* Enteritidis, a higher performance was seen compared with last year (EQA-9, 98% vs. EQA-8, 92%). Only three participants reported incorrect results for one isolate each.

Out of the 23 laboratories participating in EQA-9, 21 (91%) 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 monophasic *S.* Typhimurium ST34 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 a minimum of four and up to nine isolates based on PFGE, six isolates by MLVA and four if the identification was based on WGS-derived data. All four cluster isolates had been part of a national outbreak linked to meatloaf [7].

Thirteen laboratories used PFGE for cluster analysis and for nine, PFGE was the only cluster identification method. Despite an extended cluster, four laboratories (31%) did not identify the correct cluster using PFGE. Two laboratories used MLVA for cluster analysis and both also performed cluster analysis based on WGS data. None of the laboratories identified the correct cluster using MLVA, however one laboratory included one isolate with only one-locus variation based on a routine procedure for cluster identification.

Performance was high using WGS-derived data for cluster analysis, with 10/12 (83%) of the participants correctly identifying the cluster of four closely related isolates. The participants were free to choose their preferred analytical method for WGS-based cluster identification. The majority 10/12 (83%) of participants preferred an allele-based method, using cgMLST or wgMLST and only 2/12 (17%) 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 and useful for inter-laboratory comparability and communication. A very high degree of homogeneity in the results were shown using a cgMLST standard scheme (e.g. Enterobase).

Comparison and communication between laboratories is challenging because many laboratories still use PFGE and will probably not switch to WGS in the near future, however laboratories seem to be moving towards replacing *S.* Typhimurium MLVA with WGS.

1. Introduction

1.1 Background

The European Centre for Disease Prevention and Control is an 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 shall foster the development of sufficient capacity within the European Community's network for diagnosis, detection, identification and characterisation of infectious agents that may threaten public health. ECDC shall maintain and extend such cooperation and support the implementation of quality assurance schemes [1].

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

ECDC's disease 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
- 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 pathogens. The contracted EQA-9 scheme for *Salmonella* covers MLVA typing of both *S.* Typhimurium and *S.* Enteritidis and molecular typing-based cluster analysis. This report presents the results of the *Salmonella* EQA-9.

1.2 Surveillance of non-typhoidal salmonellosis

In 2017, non-typhoidal salmonellosis (later 'salmonellosis') was the second-most commonly reported zoonotic disease in the EU, with a total of 91 662 cases reported by 28 EU Member States (EU notification rate of 19.7 cases per 100 000 population), the lowest number of cases since 2013 (87 753). As in previous years, the most commonly reported *Salmonella* serovars were *S.* Enteritidis, *S.* Typhimurium, monophasic *S.* Typhimurium, and *S.* Infantis. In contrast to 2016, when *S.* Derby was among the top five, *S.* Newport was reported in fifth place this year [3].

Since 2007, ECDC's Food- and Waterborne Diseases and Zoonoses 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 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. Three priority food- and waterborne 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
- 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 or who are implementing it to their surveillance at the national level.

1.3 Objectives

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

The *Salmonella* EQA-9 aimed to determine and support the assessment of analytical results quality (reproducibility) and comparability of *S. enterica* subsp. *enterica* serovar Typhimurium and serovar Enteritidis MLVA results in the participating laboratories. 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]).

1.3.2 Molecular typing-based cluster analysis

The objective of the molecular typing-based cluster analysis of *Salmonella* EQA-9 was to assess the ability of laboratories to detect a cluster of genetically 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-9 was funded by ECDC and arranged by SSI following the requirements in ISO/IEC 17043:2010 [6]. The EQA-9 included MLVA of *S.* Typhimurium and *S.* Enteritidis and molecular typing-based cluster analysis using either PFGE and/or WGS-derived data. From EQA-8 to EQA-99 a change was made to exclude the quality assessment part with PFGE. EQA-9 was conducted between June 2018 and October 2018.

Invitations were emailed to ECDC contact points in FWD-Net (26 countries, which nominated laboratories to participate in the EQA rounds 2017-2020) by 31 May 2018, with a deadline to respond of 8 June 2018. In addition, invitations were sent to EU candidate and potential candidate countries Serbia, Turkey, and Kosovo[i], which signed up to the *Salmonella* EQA rounds in 2017-2020. Each laboratory was asked to fill in the reason for participating or non-participating.

Twenty-six PH NRLs in the EU/EEA and EU candidate countries accepted the invitation to participate, but only 23 submitted results (Annex 1). In Annex 2, details of participation in EQA-8 and EQA-9 are listed to give an overview of the trend in the number of participants.

EQA test isolates were sent to the laboratories from 26–29 June 2018. The participants were asked to submit their results to an SSH File Transfer Protocol (SFTP) site and complete the online form by 1 October 2018 (Annex 19).

EQA submission protocol, Excel sheets for the MLVA reference isolates and MLVA allele calling were available at the online site.

2.2 Selection of test isolates

Seventy *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 9
- include closely related isolates.

The 70 selected isolates were analysed using the methods in the EQA before and after having been re-cultured. All candidate isolates remained stable using these methods and the final test isolates were selected. For the MLVA part, 10 *S.* Typhimurium and 10 *S.* Enteritidis were selected to cover common and various MLVA profiles (Annexes 5-6). The 12 isolates for cluster analysis were selected to include isolates with different (or varying) relatedness and comprised different multilocus sequence types (ST19, 34 and 2212). The cluster contained six isolates if based on PFGE derived data at the time for selection, six if based on MLVA derived data and four isolates (one technical duplicate) if based on WGS-derived data. After reviewing the submitted PFGE data the cluster definition was changed and subsequently the cluster contained a minimum four and up to nine isolates based on PFGE derived data (see 3.3.1 and Annex 6). The characteristics of the test isolates and reported results are listed in Annexes 4-18.

Table 1. Serovars of test isolates

Method	Number of test isolates	Serovars	Annex
MLVA *S.* Typhimurium	10	Typhimurium/monophasic Typhimurium *STm4 (3-13-NA-NA-211), *STm8 (3-12-9-NA-211)	4
MLVA *S.* Enteritidis	10	Enteritidis **SE3 (3-11-4-4-1), *SE10 (1-10-7-3-2)	5
Cluster identification	12	*S.* Typhimurium (ST19 and ST2212) monophasic *S.* Typhimurium (ST34)	6–18

**: repeat isolates included in EQA-4 to 9.*
***: repeat isolates included in EQA-9.*
NA: designates a locus not present (-2 by submission, Annex 4 and 5).

[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 26 June 2018. 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 26 June as an extra precaution. Sixteen participants received their dispatched isolates within one day, nine within three days and only one participant received the isolates late (13 days) after shipment. 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 5 July 2018, 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 could download 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 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 among 12 test isolates. The cluster test isolates were pre-categorised by the EQA provider.

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 19). If PFGE analysis was conducted, the participant reported the total number of bands in each isolate and 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.

Based on PFGE (*Xba*I profiles), the EQA provider defined a cluster of a minimum four and up to nine monophasic *S.* Typhimurium ST34 isolates: REF1, REF2, REF5, REF6, REF7, REF9, REF10, REF11 and REF12 (REF2 and REF7 were technical duplicates). The nine isolates grouped into three categories: A, B and C (Annex 6-7).

Based on MLVA-derived data, the cluster consisted of six monophasic *S.* Typhimurium ST34 isolates, REF1, REF2, REF5, REF6, REF7 and REF12 with the MLVA profile 3-11-11-NA-211 (Annex 8). One isolate, REF8 (3-13-11-NA-211), differed from the cluster MLVA-profile with two repeats in one locus (STTR5). The last five test isolates had variation in two to four loci (Annex 8).

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 a 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). In this EQA, the laboratories had the possibility to submit the sequence type (ST) of isolates in the cluster analysis and were also asked to report the number of loci in the used allelic scheme and the name of the used SNP pipeline.

Based on WGS-derived data, the cluster consisted of four monophasic *S.* Typhimurium ST34 isolates: REF2, REF5, REF7 and REF12 (Annex 9). The analysis for categorisation was an allele-based cgMLST [9] and an SNP analysis (NASP [10]). The EQA provider found 0–1 allele differences and a distance of 0–2 SNPs between any two isolates in the cluster. The rest of the cluster test isolates were additional ST34 (6), ST19 (1) and ST2212 (1).

2.5 Data analysis

As the participating laboratories submitted their results, the MLVA and cluster analysis results, as well as the participants' uploaded raw reads, were imported to a dedicated *Salmonella* EQA-9 BN database.

No errors were identified in the submission process, however one participant needed BioNumerics software support. In addition, few participants were reminded to upload the raw reads.

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

The 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 after expansion of up to nine (section 3. 3.1 and annex 6) of the 12 test isolates (REF1, REF2, REF5, REF6, REF7, REF9, REF10, REF11 and REF12; REF2 and REF7 were technical duplicates). The EQA provider's MLVA results were based on the *S.* Typhimurium scheme [5] and included six monophasic *S.* Typhimurium ST34 isolates: REF1, REF2, REF5, REF6, REF7 and REF12 with the MLVA profile 3-11-11-NA-211. The EQA provider's cluster analysis on WGS-derived data was based on an allele-based (cgMLST, [9]) and SNP analysis (NASP, [10]). The correct number of closely related monophasic *S.* Typhimurium isolates were four out of the 12 isolates (REF2, REF5, REF7 and REF12) and all four were part of a national *S.* Typhimurium outbreak [7]. The EQA provider found at most a 0–1 allele difference or 0–2 SNP distances between any two isolates in the cluster. The rest of the cluster test isolates were an additional six ST34 isolates, one ST19, and one ST2212.

Individual evaluation reports were distributed to the participants in the beginning of February 2019 and certificates of attendance in March 2019. 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 (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 26 participants who signed up for the EQA, 23 managed to complete and submit their results. Only one of three laboratories who did not sign up at all gave a reason (lack of laboratory capacity) for not participating.

Eight laboratories completed MLVA (STm and SE) and molecular typing-based cluster analysis (PFGE, MLVA and/or WGS). Ten (43%) laboratories participated in the MLVA part, in both *S.* Typimurium and *S.* Enteritidis. Twenty-one laboratories (91%) participated in the cluster analysis part and most of them (13, 62%) reported cluster analysis based by PFGE and 12 laboratories (57%) reported based on WGS-derived data. Two participants (10%) among these reported cluster identification using both PFGE and WGS derived data and two participants reported the cluster using all three methods (Table 2). Laboratories mainly reported 'Laboratory policy to enhance the typing quality' as the reason for participating, but also accreditation needs, and institute/national policy were reported (See Annex 3 for details).

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

	MLVA		Cluster					All
	STm and SE	Total	PFGE only	WGS only	PFGE + WGS	PFEG + MLVA + WGS	Total	Total
Number of participants	10	10	9	8	2	2	21	23
Percentage of participants	100%	43*	43%	38%	10%	10%	91%*	-

**: Percentage of the total number of participating laboratories (23)*
STm: S. Typhimurium
SE: S. Enteritidis.

3.2 Multiple locus variable number of tandem repeats analysis

For the second time in an ECDC EQA, MLVA for *S.* Enteritidis was included. In total, 10 laboratories (43%) participated in the MLVA part of the EQA and all in both MLVA for *S.* Typhimurium and for *S.* Enteritidis (Annex 4 and 5).

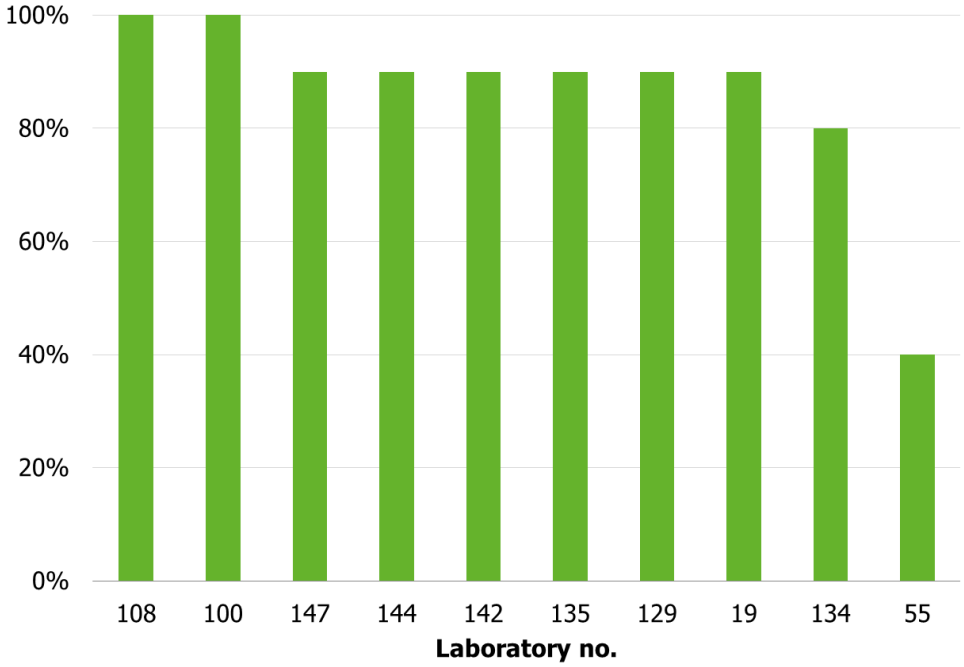
3.2.1 MLVA for *S.* Typhimurium

Ten out of the 23 participants in EQA-9 (43%) performed the MLVA typing of *S.* Typhimurium, however only two (20%) of these reported the correct allelic profiles for all ten test isolates (Figure 1).

Laboratory 55 had the most errors, reporting a fragment in an absent loci (STTR10) in five of the test isolates (STm4, 7, 8, 9 and 10) and reported incorrect all five times the allele number of 28. For two isolates (STm4 and 9), laboratory 55 assigned an incorrect allele number in STTR3 and for STm1 a wrong allele number in STTR6 was assigned too.

Laboratory 134 had two errors, reporting a fragment in absent loci (STTR6 and STTR10) in two different test isolates (STm4 and 9). Six other laboratories had reported an incorrect allelic profile for only one test isolate (Annex 4). The errors were various and in four different isolates. Laboratory 19 reported the same profile for STm1 and 7, but only the result for STm1 was correct.

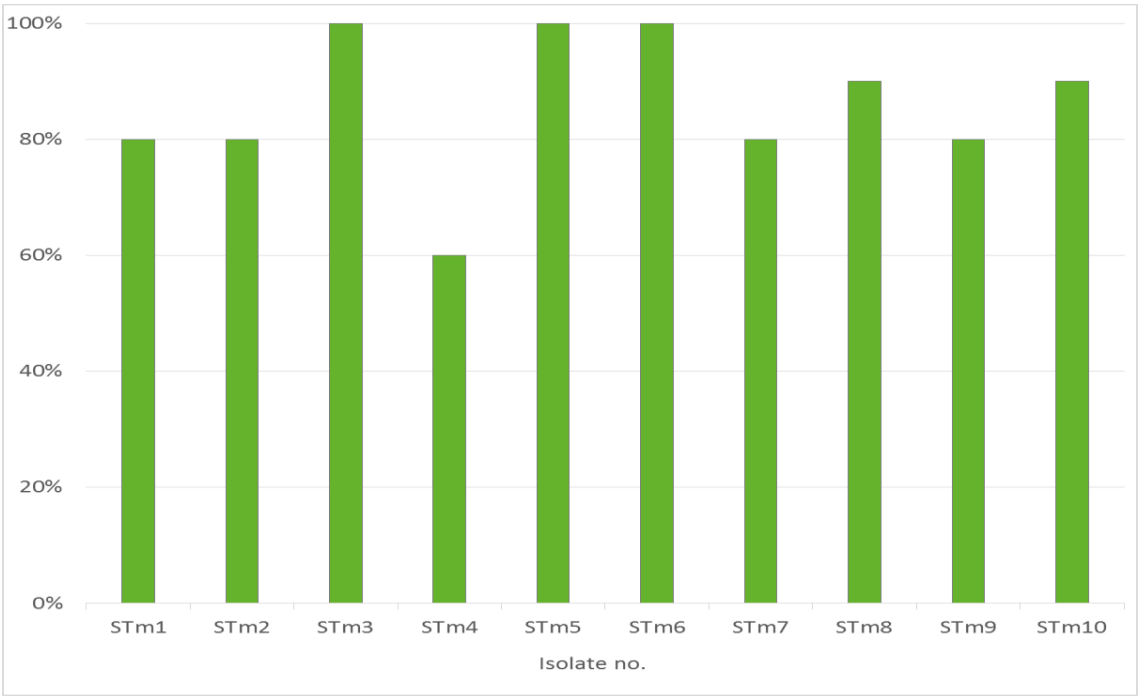
Figure 1. Participant scores for MLVA typing of the 10 *S.* Typhimurium test isolates



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 2. The correct MLVA profile was reported for three of the 10 *S.* Typhimurium test isolates by all participants. No common isolate characteristics caused the problems (Annex 4) as the 14 incorrect MLVA profiles concerned seven different isolates (STm1, 2, 4, 7, 8, 9 and 10). Only one identical error occurred as both laboratory 142 and 147 did not report a present fragment (STTR5) for STm2.

Figure 2. Average percentage scores of the 10 *S.* Typhimurium test isolates



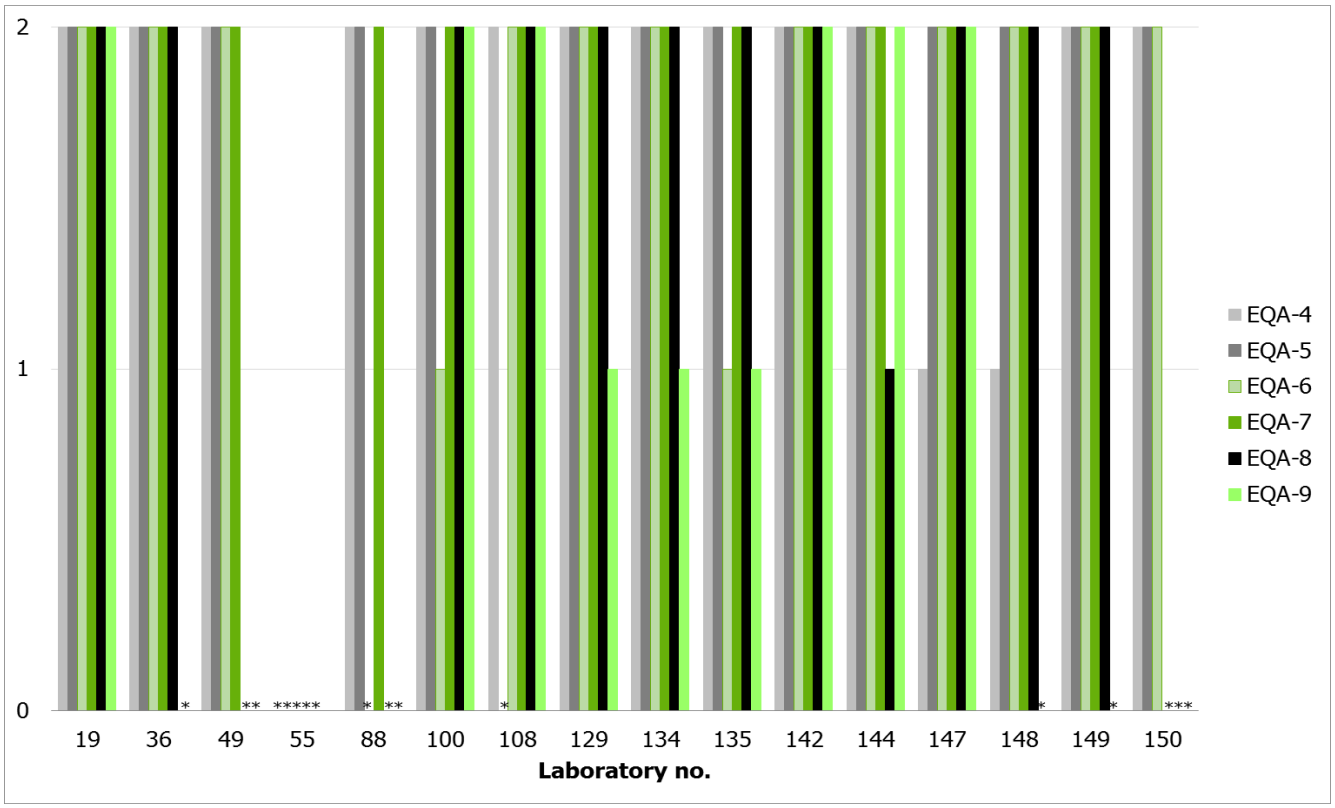
Bars represent the percentage of MLVA profiles correctly assigned by the participants.
#: repeat isolates (STm4 and STm8) in EQA-4 to 9.

To follow the development of individual laboratory performance, two isolates with different allelic profiles were included in EQA-4 through 9: isolate STm4 (3-13-NA-NA-211) and STm8 (3-12-9-NA-211). Figure 3 shows the individual performance by the laboratories of these two repeated isolates during the six EQAs. The majority of participants (6/10; 60%) performed at the same or a better level than the last time they participated. Four of the errors in this EQA were in repeat isolate STm4 (Figure 2) and most caused by identifying an absent fragment in STTR6 or STTR10.

Two laboratories 129 and 134, which obtained incorrect results in EQA-9, had not previously generated errors on the repeated isolates. Laboratory 135 reported incorrect result for the same isolate in EQA-6, however not the same loci. Laboratory 55 had error in both repeat isolates and was the only laboratory reporting incorrect for STm8 (Figure 2).

The MLVA results on the repeated isolates show a decreased performance among the participants compared with previous years.

Figure 3. Correct MLVA typing of two repeated *S.* Typhimurium isolates from EQA-4 to 9

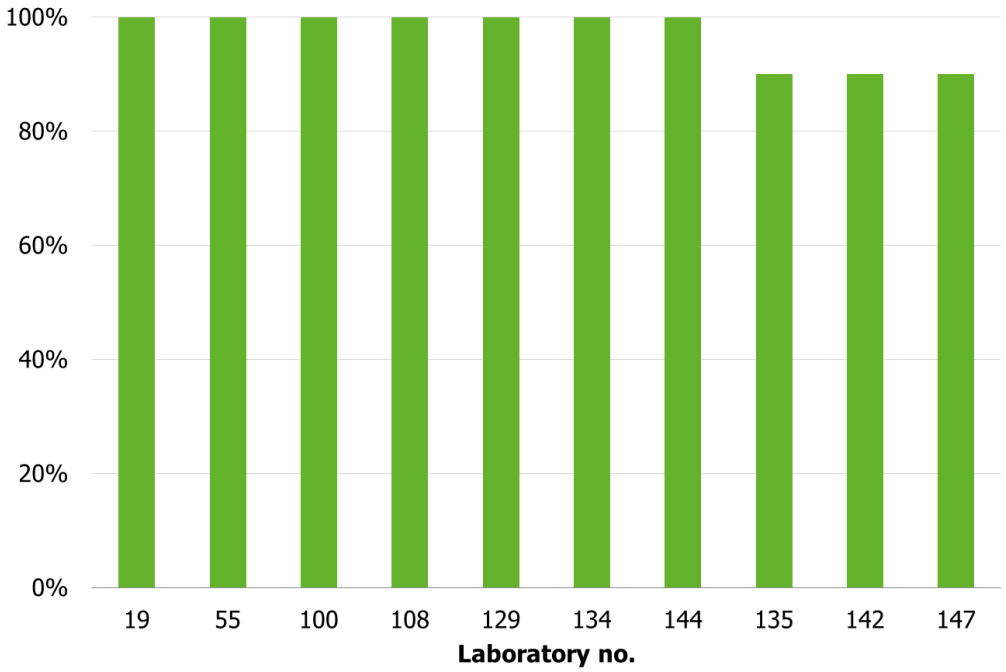


Arbitrary numbers represent the participating laboratories. Bars represent the number of correctly assigned allelic profiles for the two repeated isolates (STm4 and STm8).
**: laboratory not participating in this round of EQA.*

3.2.2 MLVA for *S.* Enteritidis

Ten out of the 23 participants (43%) in EQA-9 performed the MLVA typing of *S.* Enteritidis and seven (70%) of these reported the correct allelic profiles for all ten test isolates (Figure 4).

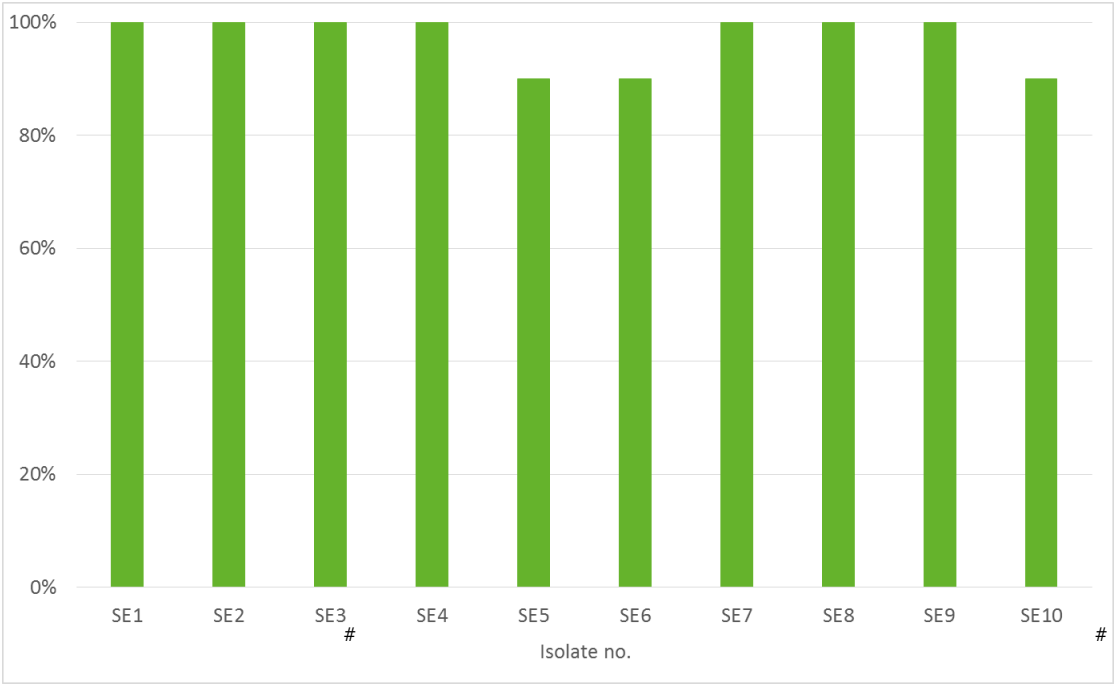
Figure 4. Participant scores for MLVA typing of the 10 *S.* Enteritidis test isolates



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

Three laboratories 135, 142 and 147 had one error each in different isolates (Annex 5). Laboratory 135 and 147 missed a present fragment in SENTR5 (isolate SE5) and in SENTR7 (isolate SE10), respectively, and laboratory 142 reported an incorrect allele number in SENTR7 for isolate SE6.

Figure 5. Average percentage score of the 10 MLVA *S.* Enteritidis test isolates

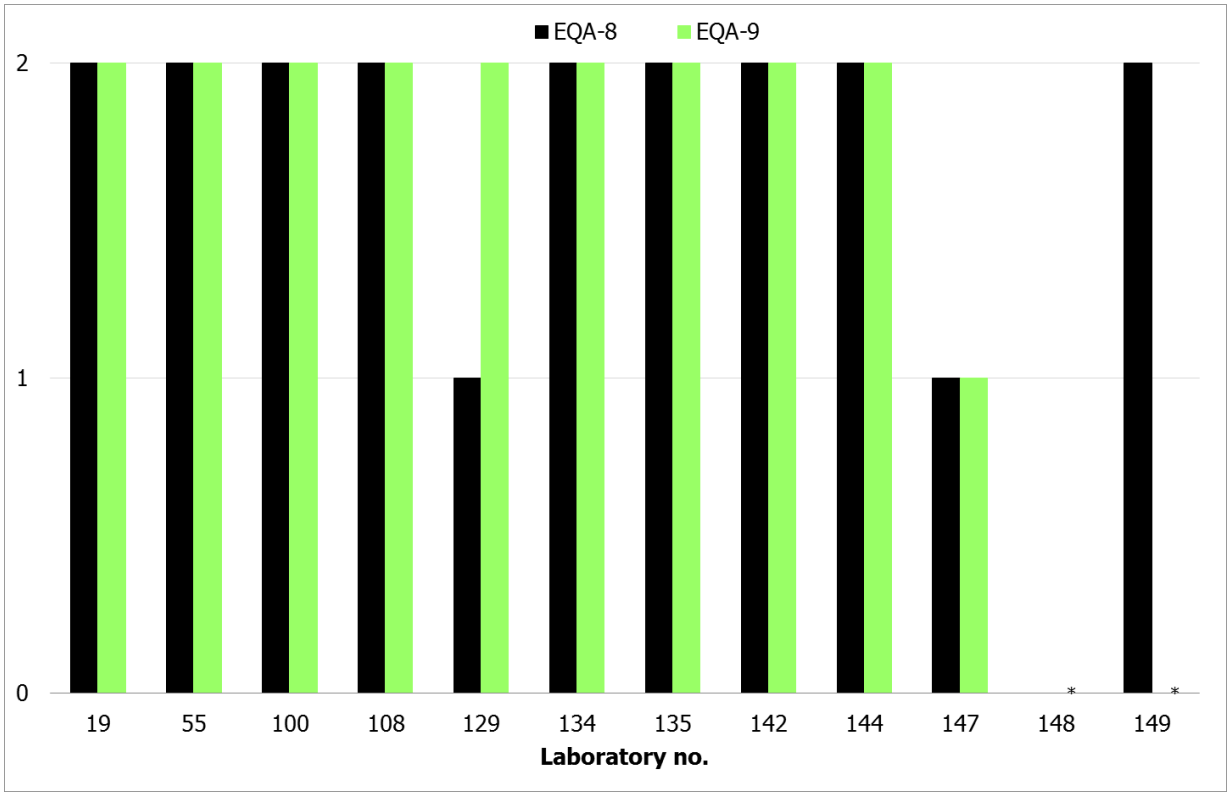


Bars represent the percentage of MLVA profiles correctly assigned by the participants.
#: repeat isolates (SE3 and SE10) in EQA-8 and 9.

To follow the development of individual laboratory performance, two isolates with different allelic profiles were included in EQA-8 and EQA-9: isolate SE3 (3-11-4-4-1) and SE10 (1-10-7-3-2). Figure 6 shows the individual performance by the laboratories of these two repeated isolates during the two EQAs. The MLVA results on the repeated isolates show stability and high performance among the participants.

All participants performed at the same or a better level than the last time they participated. Laboratory 147 missed both in EQA-8 and EQA-9 a fragment present in locus SENTR7 for repeat isolate SE10. Laboratory 148, which obtained incorrect results in EQA-8, did not participate in EQA-9.

Figure 6. Correct MLVA typing of two repeated *S.* Enteritidis isolates from EQA-8 to 9



Arbitrary numbers represent the participating laboratories. Bars represent the number of correctly assigned allelic profiles for the two repeated isolates (SE3 and SE10).
**: laboratory not participating in this round of EQA.*

3.3 Molecular typing-based cluster analysis

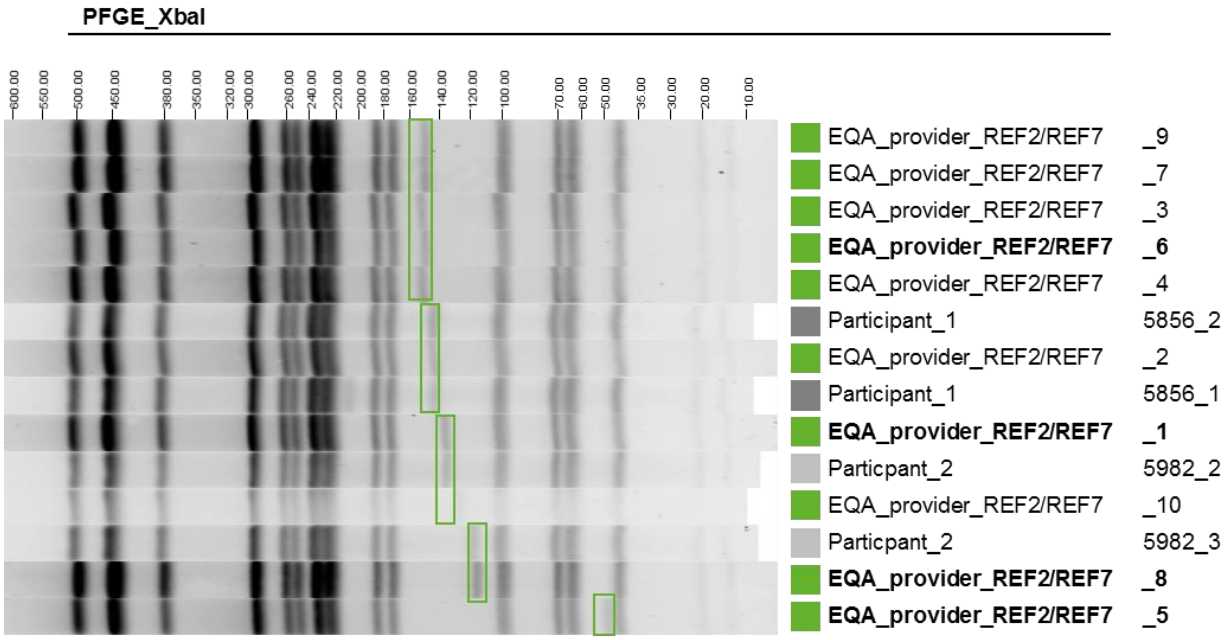
In this part of the EQA, the participants should correctly identify a cluster of closely related isolates among 12 test isolates by using either PFGE, MLVA and/or WGS-derived data. The cluster test isolates were pre-categorised by the EQA provider.

3.3.1 PFGE-derived data

After reviewing the submitted data, the EQA provider noticed large difference among the participant number of closely related isolates and especially divergent results for the two technical duplicates (REF2 and REF7). All data are available in Annexes 6, 7 and 10-11.

The EQA provider asked the participants to share their TIFF files with the EQA provider, not to evaluate the TIFF quality but in order to elucidate the issues of the technical duplicates. In addition, two participants were asked to return their stored isolates of REF2 and REF7 to the EQA provider. Despite the fact that all EQA isolates during the stability testing at the EQA provider, were sub-cultured before storage, the EQA provider could easily find five different PFGE profiles when repeating the PFGE analysis with 10 single colonies from the storage tube of REF2/REF7 (see Figure 7, marked in green). Also, the isolates returned by the participants showed different PFGE profiles (See Figure 7, marked in grey). The band variation was between 110-160kb and one profile suggested a band size of 40kb.

Figure 7. Different colonies of REF2/REF7



Green box indicates the band with variation.

The EQA provider sent four of the isolates/colonies (Figure 7, marked in bold) to be sequenced by Nanopore MinION and by Illumina NextSeq (both at SSI). Nanopore and Illumina data were combined in a hybrid assembly using Unicycler (v0.4.7). Visualisation of the hybrid assembly in Bandage (v.0.8.1) showed that each isolate contained a chromosome of approx. 4957-4989kb and an additional genetic element of approx. 8kb (Annex 6). Bandage shows this element as a circular element; however this is incorrect and caused by a duplicated sequence of 820bp that Unicycler erroneously assumes is an overlap. Visual analysis in CLC genomics workbench (v10.1.1) by BLAST-mapping of nanopore reads longer than 10kb to the assembly revealed that the 'circular' element is in fact a tandem repeat (Annex 6 for details).

Based on these findings the cluster definition of closely related isolates based on PFGE (*XbaI* profiles), was extended with three profiles (REF1, REF6 and REF11) and divided into three categories (A, B and C).

A: REF5, REF9, REF10 and REF12 with indistinguishable PFGE-profile as a minimum
B: REF2 and REF7 with 0-1 band difference to the PFGE-profile in A
C: REF1, REF6 and REF11 with up to 2 band differences to the PFGE-profile in A.

Thirteen (57%) participants performed the cluster analysis using PFGE-derived data. Nine (69%) of the participants correctly identified the cluster of closely related isolates. Table 3 provides an overview of the isolates each participant included (Yes) or excluded (No) in their cluster identification. Laboratory 128 did not report REF12 as one of the four minimum required. REF10 was also part of the four in category A, but laboratory 144 and 145 did not included REF10 in the PFGE cluster. One laboratory (140) identified incorrectly REF8 as part of the cluster based on PFGE and included all ST34 in the cluster (Annex 10).

Table 3. Results of cluster identification based on PFGE-derived data

Isolate no.		Laboratory												
Isolate number	ST	19	55	92	96	127	128	132	138	140	142	144	145	147
REF1‡ C	34	No	No	No	No	No	No	Yes	Yes	Yes	No	No	No	Yes
REF2‡# B	34	Yes	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	Yes
REF3	2212	No	No	No	No	No	No	No	No	No	No	No	No	No
REF4	19	No	No	No	No	No	No	No	No	No	No	No	No	No
REF5‡ A	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF6‡ C	34	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
REF7‡# B	34	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF8	34	No	No	No	No	No	No	No	No	Yes	No	No	No	No
REF9 A	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF10 A	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes
REF11‡ C	34	No	No	No	No	No	No	Yes	Yes	Yes	No	No	No	Yes
REF12‡ A	34	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cluster-identified		Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	No	Yes

‡: closely related isolates (in grey)
#: technical duplicate isolates (in bold)
A: REF5, REF9, REF10 and REF12 with indistinguishable PFGE-profile as a minimum
B: REF2 and REF7 with 0-1 band difference to the PFGE-profile in A
C: REF1, REF6 and REF11 with up to 2 band differences to the PFGE-profile in A

3.3.2 MLVA-derived data

Two participants (10%) performed cluster analysis using MLVA-derived data and both selected the *S.* Typhimurium scheme and reported the loci in the correct order: STTR9, STTR5, STTR6, STTR10 and STTR3.

Performance was low, with both participants not identifying the correct cluster of closely related isolates (MLVA profile: 3-11-11-NA-211) defined by a pre-categorisation by the EQA provider among the 12 cluster test isolates. Table 4 shows the overview of the isolates each participant included (Yes) and excluded (No) in their cluster analysis. Figure 8 shows a dendrogram of the reported MLVA results. All data are available in Annexes 8, 13 and 14.

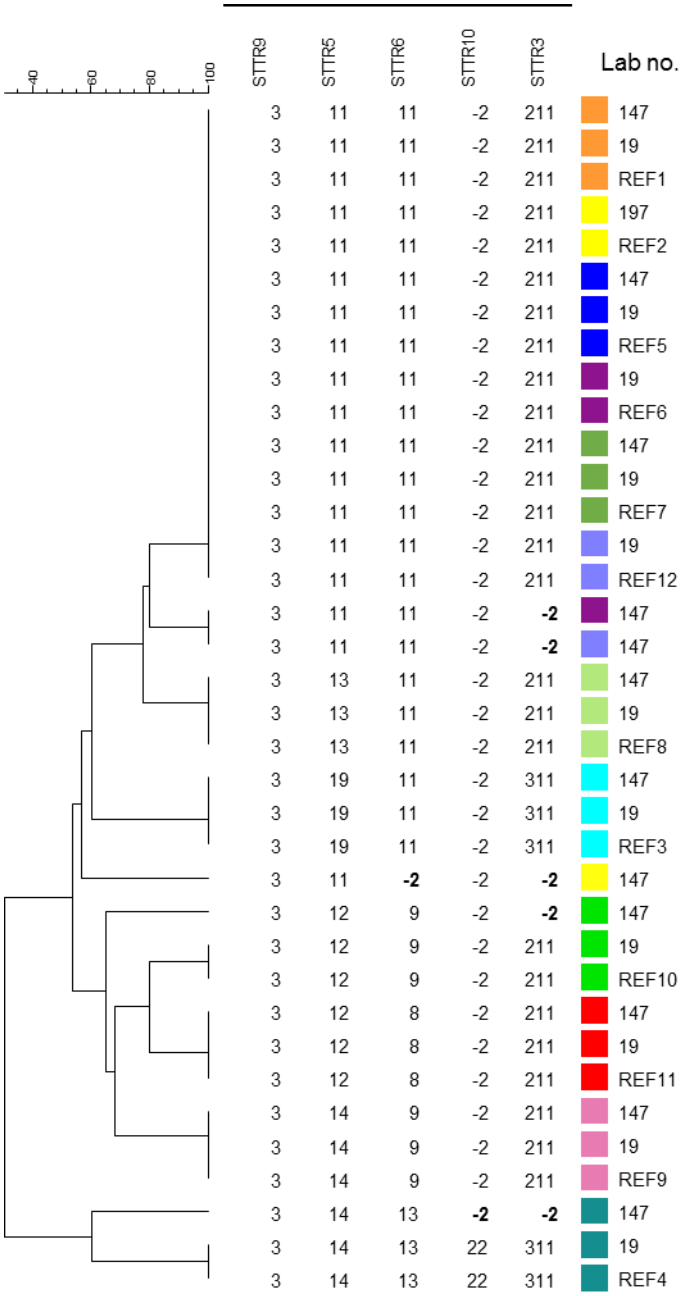
Table 4. Results of cluster identification based on MLVA-derived data

			Laboratory ID	
Isolate number	ST	MLVA-profile	19	147
REF1‡	34	3 - 11 - 11 - NA - 211	Yes	Yes
REF2‡#	34	3 - 11 - 11 - NA - 211	Yes	No
REF3	2212	3 - 19 - 11 - NA - 311	No	No
REF4	19	3 - 14 - 13 - 22 - 311	No	No
REF5‡	34	3 - 11 - 11 - NA - 211	Yes	Yes
REF6‡	34	3 - 11 - 11 - NA - 211	Yes	No
REF7‡#	34	3 - 11 - 11 - NA - 211	Yes	Yes
REF8	34	3 - 13 - 11 - NA - 211	Yes	No
REF9	34	3 - 14 - 9 - NA - 211	No	No
REF10	34	3 - 12 - 9 - NA - 211	No	No
REF11	34	3 - 12 - 8 - NA - 211	No	No
REF12‡	34	3 - 11 - 11 - NA - 211	Yes	No
Cluster-identified		3 - 11 - 11 - NA - 211	No	No

‡: closely related isolates (in grey)
#: technical duplicate isolates (in bold)
NA: designates a locus not present (-2 by submission, Annex 14).

Laboratory 19 reported the correct MLVA profile for all 12 test isolates but included one additional isolate (REF8) with one locus variation (STTR-5) in the cluster based on MLVA-derived data. Laboratory 147 included only three isolates (REF1, REF5 and REF7) in the cluster and missed three isolates. For REF6 and REF12 laboratory, 147 did not identify a fragment in locus STTR3, and for REF2, the laboratory missed a fragment in both STTR6 and STTR3. Furthermore, laboratory 147 reported missing fragments (Figure 8) for two isolates outside the MLVA cluster (REF4 and REF10).

Figure 8. Reported MLVA results of each test isolate



Dendrogram from BioNumerics of MLVA profiles reported by laboratories 19 and 147. Each of the 12 test isolates has a different colour.
REF1 to REF12: results from EQA provider.

3.3.3 WGS-derived data

Reported results from participants

Twelve participants (52%) performed cluster analysis using WGS-derived data. Only one laboratory reported using external assistance for sequencing. Different sequencing platforms were listed among the participants: one MiniSeq, seven MiSeq, one HiSeq, two NextSeq and one Ion Torrent. All reported using commercial kits for library preparation. Of the 12 participants, nine (75%) used Illumina's Nextera kit. Two reported volume changes from the manufacturer's protocol (Annex 14).

Performance was high in cluster analysis with WGS-derived data, with 10 (83%) participants correctly identifying the cluster of closely related isolates defined by a pre-categorisation by the EQA provider among the 12 test isolates.

Ten laboratories correctly reported ST of all 12 isolates and only two laboratories (148 and 142) used the submission field for Enterobase [5] level cluster codes or did not report at all.

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

		Laboratory ID											
Isolate number	ST	19	36	49	108	129	134	142	144	147	148	149	150
REF1	34	No	No	No	No	Yes	No	No	No	No	No	No	No
REF2‡#	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF3	2212	No	No	No	No	No	No	No	No	No	No	No	No
REF4	19	No	No	No	No	No	No	No	No	No	No	No	No
REF5‡	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF6	34	No	No	No	No	Yes	No	Yes	No	No	No	No	No
REF7‡#	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF8	34	No	No	No	No	Yes	No	No	No	No	No	No	No
REF9	34	No	No	No	No	Yes	No	No	No	No	No	No	No
REF10	34	No	No	No	No	Yes	No	Yes	No	No	No	No	No
REF11	34	No	No	No	No	Yes	No	No	No	No	No	No	No
REF12‡	34	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Main analysis		Allele (cgMLST)	Allele ("cgMLST")	Allele (cgMLST)	SNP	Allele ("wgMLST")	Allele (cgMLST)	Allele (cgMLST)	Allele (cgMLST)	Allele (cgMLST)	Allele (cgMLST)	Allele (cgMLST)	SNP
Additional analysis 1		Allele (wgMLST	Allele ("wgMLST")	SNP							SNP		
Additional analysis 2		SNP	SNP										
Identified cluster		Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes

‡: closely related isolates (in grey)
#: technical duplicate isolates (in bold)
"cgMLST" / "wgMLST": assigned by provider based on reported information (Table 7)
ST: sequence type
Allele: allele-based analysis
SNP: single-nucleotide polymorphism analysis.

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. Laboratories 19, 36, 49 and 148 reported additional analyses.

Of the six participants using SNP analysis, two (laboratory 108 and 150) used SNP as the main analysis for cluster detection, two (laboratory 49 and 148) reported SNP as an additional analysis and laboratory 19 and 36 reported SNP-based analyses as a third analysis. All used a reference-based approach with different *S.* Typhimurium isolates as reference. Three used an in-house pipeline to the SNP analysis and three reported use of NASP, BioNumerics and PHE-NIX, respectively. As read mapper, three used Burrows-Wheeler Aligner (BWA), two used Bowtie and one used CLC. Two laboratories reported the use of GATK as variant caller, SAMtools (two laboratories), VarScan and CLC were also used.

Tables 6 and 7 show the overview of the submitted data. For laboratory reported SNP distance/allelic differences by isolate, see Annex 16.

Table 6. Reported results of SNP-based cluster analysis

	SNP-based						
Laboratory	SNP pipeline	Approach	Reference	Read mapper	Variant caller	Distance within cluster	Distance outside cluster ¤
Provider	NASP	Reference-based	REF2	BWA	GATK	0-2	27–269 (795–1 170)
Provider	NASP + recombination filter	Reference-based	REF2	BWA	GATK	0-2	25–105 (614–750)
19*	NASP	Reference based	ST34 SSI_AA530	BWA	GATK	0-2	23–248 (782–1 153)
36*	in-house	Reference based	STM LT2 (NC_003197.2)	BWA-MEM	Varscan	0-2	22–67 (548–746)
49*	BioNumerics	Reference based	ST34 and isolate ID 5327	Bowtie	SAMtools	0-2	25–192 (888–893)
108	in-house pipeline	Reference based	in-house strain resp ST	CLC assembly cell v4.4.2	CLC assembly cell v4.4.2	0-2	25–270 (not reported)
148*	In-house	Reference based	ST34 TW-Stm6 CP019649	Bowtie2	SAMtools	0-9	31–72 (599–670)
150	PHE-NIX	Reference based	gi|16445344|gb|AE006468.1| Salmonella enterica subsp. enterica serovar Typhimurium str. LT2, complete genome	BWA	GATK	0-2	24–73 (675–844)

**: additional analysis*
¤: reported differences to ST34 (non-ST34). For detailed data, see Annex 16.

Of the 10 participants using an allele-based analysis, all selected the method as the main analysis for cluster detection. Seven of 10 (70%) used only an assembly-based allele calling method and two (20%) used both assembly- and mapping-based allele calling methods. Five used SPAdes as the assembler and four used Velvet. The remaining laboratory (10%) used only a mapping-based allele calling method.

Eight of 10 laboratories (80%) reported using Enterobase (cgMLST) as the scheme for analysis. One laboratory (36) reported the use of cgMLST in an ad hoc scheme for *Salmonella* enterica based on 2.143 core loci and laboratory 129 used wgMLST (1.423 core and 2.055 accessory loci). Furthermore, two laboratories reported an additional analysis by wgMLST, laboratory 19 using Enterobase/Applied Math (15.867 loci) and laboratory 36 using in-house scheme (2.143 core and 2.201 accessory loci).

Table 7. Reported results of allele-based cluster analysis

	Allele-based analysis						
Laboratory	Approach	Allelic calling method	Assembler	Scheme	Number of loci	Difference within cluster	Difference outside cluster¤
Provider	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Maths (cgMLST/Enterobase)		0–1	15–38 (292–341)
19	BioNumerics	Assembly based and mapping based	Spades	Applied Math (cgMLST/Enterobase)	3 002	0–1	15–37 (293–340)
19*	BioNumerics	Assembly based and mapping based	Spades	Applied Math (wgMLST/Enterobase)	15 867	0-2	21–62 (450–515)
36	SeqSPhere	Mapping based only	-	Other	§ In house scheme based on 2.143 core loci	0	10–28 (197–241)
36*	SeqSPhere	Mapping based only	-	Other	2.143 core + 2.201 accessory loci	0-2	21–58 (440–533)
49	BioNumerics	Assembly based and mapping based	SPAdes	Applied Math (cgMLST/Enterobase)	3 002	0–1	15–38 (294–342)
129	SeqSPhere	Mapping based only	Velvet	Other	§ Ad hoc scheme for Salmonella enterica based on 1.423 core and 2.055 accessory loci "(wgMLST")	0-17	(137–168)
134	SeqSPhere	Mapping based only	Velvet	Enterobase (cgMLST)	3 002	0–1	14–59 (285–337)
142	Enterobase	Mapping based only	SPAdes	Enterobase (cgMLST)	3 018	0–5	8–37 (286–333)
144	SeqSPhere	Mapping based only	Velvet	Enterobase (cgMLST)	3 002	0-2	14–37 (286–339)
147	SeqSPhere	Mapping based only	SPAdes 3.11.1	Enterobase (cgMLST)	3 002	0–1	15–38 (288–340)
148	Enterobase	Mapping based only	SPAdes	Enterobase (cgMLST)	3 000	Not reported	
149	SeqSPhere	Mapping based only	Velvet	Enterobase (cgMLST)	3 002	0–1	14–37 (288–335)

**: additional analysis*
§: modified from submitted information
¤: reported differences to ST34 (non-ST34). For detailed data, see Annex 16.

Eight of the ten laboratories (80%) using an allele-based analysis as the main method could identify the correct cluster of four closely related ST34 isolates (Figure 9). All these eight laboratories performed cgMLST and they reported an allele difference within the cluster at 0–2. However, one laboratory (148) did not report allelic differences between a selected isolate and each test isolate included in the analysis as described in the protocol, and instead laboratory 148 reported Enterobase [5] level cluster codes in the field for ST submission. The lack of reported distances affected the data presented in this report and both the discussion and conclusion, as the reported [5] level cluster codes complicated the comparison.

Furthermore, the two laboratories (19 and 36) performing an additional allele-based analysis reported 0-2 allele difference using wgMLST.

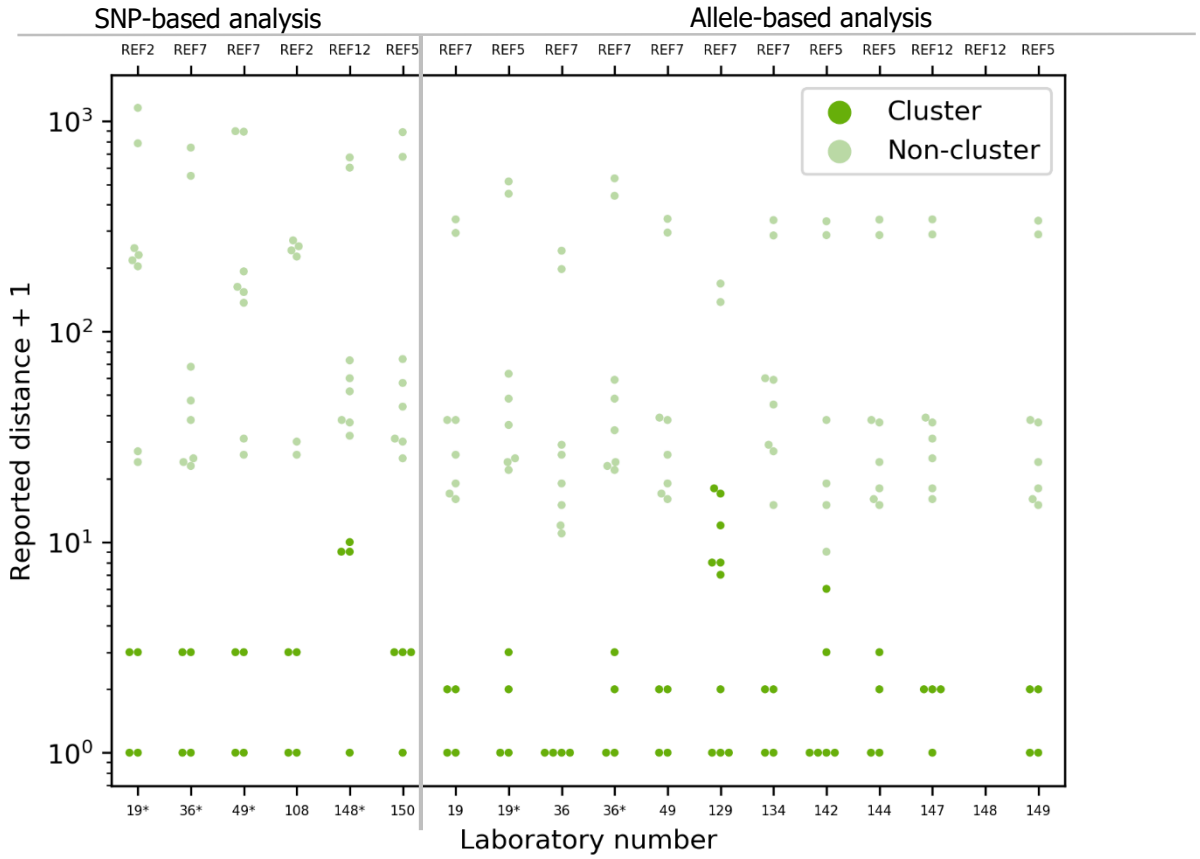
Six other test isolates (REF1, REF6, REF8, REF9, REF10 and REF11) were also ST34, but not predefined by the EQA provider as part of the cluster. The eight laboratories performing cgMLST with correct cluster identification reported allele differences to the selected cluster isolate at 10-59 for this group of isolates (difference outside cluster), though laboratory 148 did not report as instructed for these isolates neither.

Laboratory 129 did not identify the correct cluster and used wgMLST 'in house' scheme of 1423 core and 2055 accessory loci. The laboratory reported 0–17 allele difference within the identified cluster and included all six additional ST34 isolates along with the four correct isolates. For the four isolates defined by the EQA provider as the correct cluster the laboratory reported 0-1 allele difference and 6-17 allele differences were reported for the additional six ST34 isolates.

Laboratory 142 did not identify the correct cluster using Enterobase (cgMLST) for the allele analysis. The laboratory reported a cluster of six isolates with 0–5 allele difference but reported non allele difference within the correct cluster of four isolates defined by the EQA provider. The two additional isolates had a reported allele difference of 2 (REF10) and 5 (REF6). For the last four ST34 isolates the reported allele differences were 8-37 and these isolates were not including in the cluster.

Of the eight laboratories using the cgMLST scheme by Enterobase, laboratories 142 and 148 were the only two reporting directly analysing data in Enterobase (Table 7), while the other six laboratories analysed in BioNumerics or SeqSPhere.

Figure 9. Reported SNP distances or allele differences for each test isolate to selected cluster representative isolate

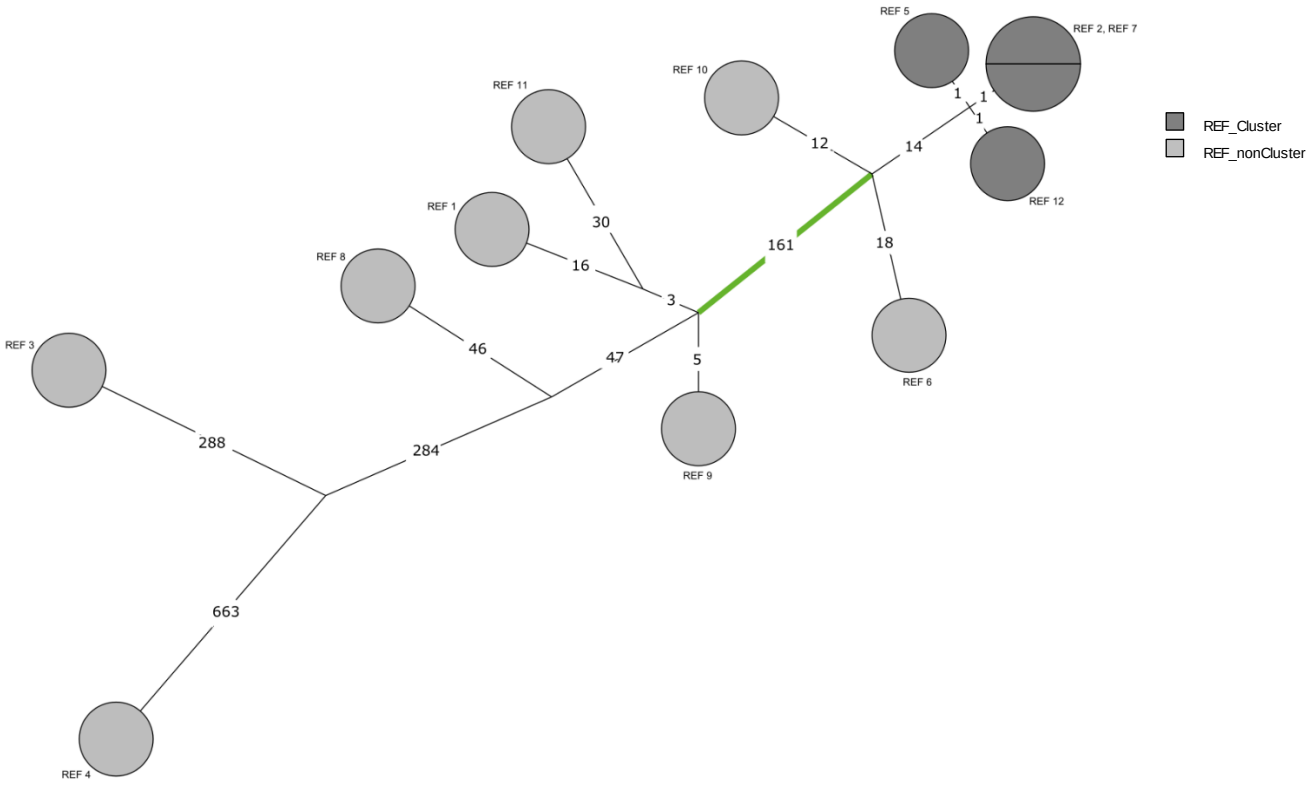


**: additional analysis*
SNP: single-nucleotide polymorphism analysis
Selected cluster representative marked as REF
Dark green: reported cluster of closely related isolates
Light green: reported not part of cluster.
Allele based results from 148 laboratory not shown (not reported as instructed)

The two laboratories (108 and 150) performing SNP analysis as main analysis both identified the correct cluster of closely related isolates (Figure 9). Four other laboratories (19, 36, 49 and 148) that identified the correct cluster of closely related isolates by cgMLST (main analysis) also performed SNP analysis as additional or third analysis.

The reported SNP distances within the cluster were 0-2 for laboratories 19, 36, 49, 108 and 150. Laboratory 148 reported the SNP distances within the cluster as 0–9. High variation in the SNP distance was reported outside the cluster and the reported distance was 22-270 for the non-cluster ST34 isolates. The EQA provider performed two SNP analyses both with and without recombination filter (Table 6) and identified mainly one region of 154 SNP linked to recombination. The highlighted recombination branch in Figure 10, were reduced from 161 to 7 SNP when applying the recombination filter.

Figure 10. Maximum parsimony tree of provider data based on SNP distances



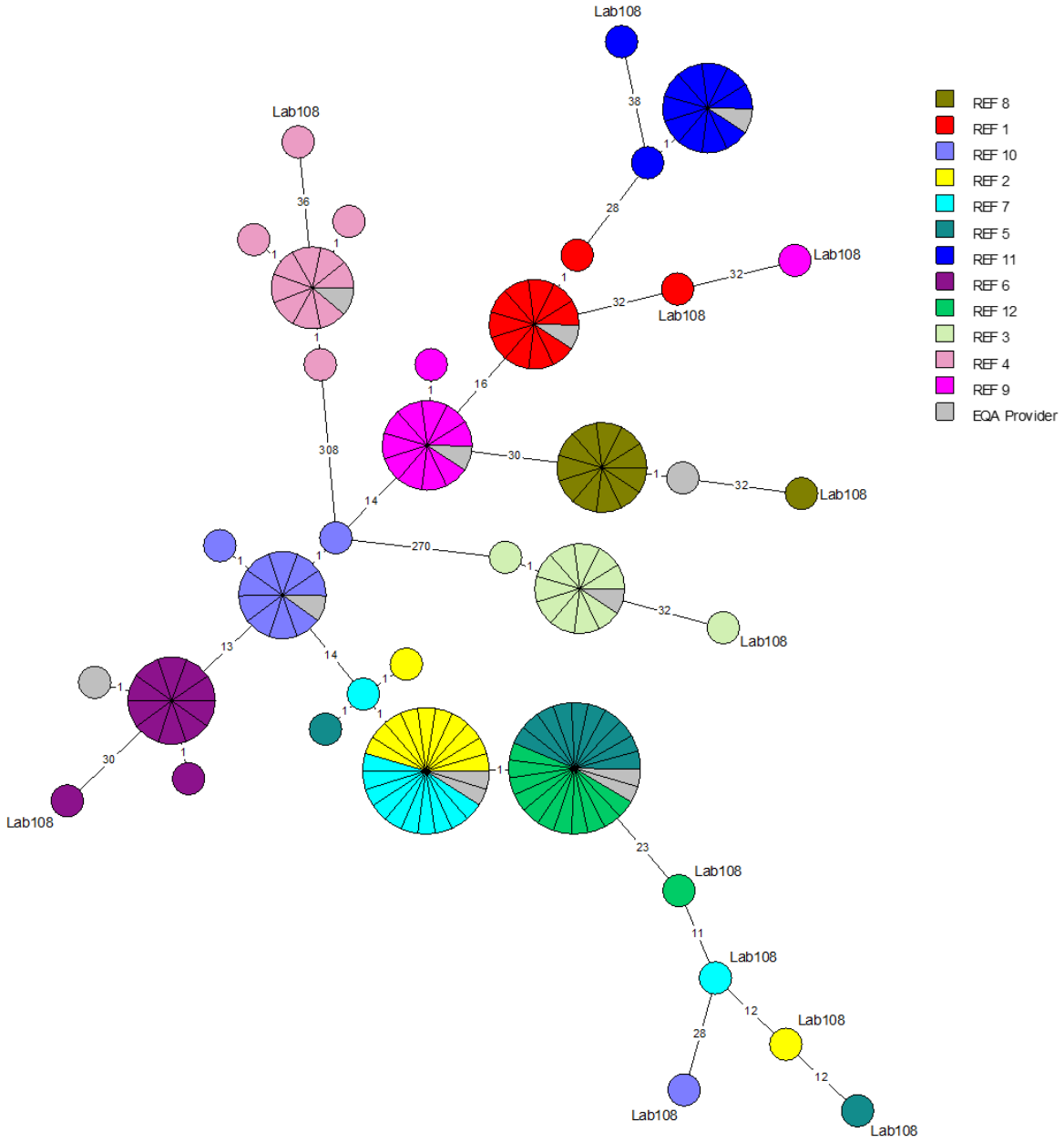
Recombinant branch highlighted in green.

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 were 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]).

The overall cgMLST analysis by the provider, shown in the minimum spanning tree (MST, Figure 11) and based on submitted raw reads from 12 laboratories, shows clear clustering of the results for each test isolate. Only data from laboratory 108 are separated (or removed) from the other results.

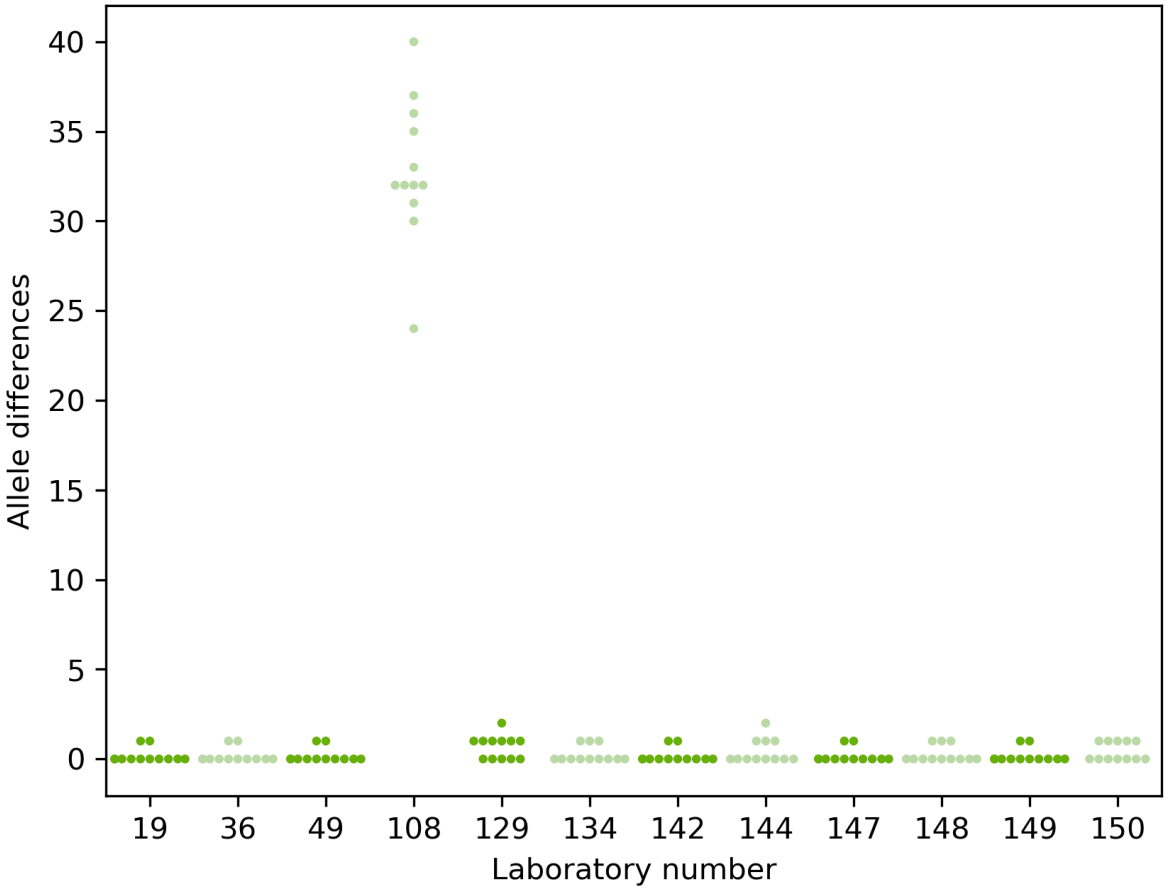
Figure 11. Minimum spanning tree of core genome multilocus sequence typing, participant FASTQ files



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.

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 12 shows the allele differences between each submitted sequence and the corresponding reference. As seen in Figure 11, the provider isolates REF6 and REF8 are both one allele removed from most of the participant isolates.

Figure 12. 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).

For 130 of 144 results (90%), 0-1 differences were identified (Figure 12). For two results, a difference of two alleles from the REF isolate was calculated. For 12 results (8%), a difference of 24-40 alleles was seen, all by laboratory 108.

Separately, the laboratories responded to QC parameters used to evaluate their data. As seen in Table 8, both coverage and confirmation of genus was the most widely used QC parameter with 91% and 82% of the laboratories using this parameter. Different thresholds of coverage ranging between 20-60X coverage were used. Many different programs were used for the contamination cheek. Number of good cgMLST loci was used by 73% of laboratories with a threshold ranging between 95-99%. Q score and genomic size were used by 55% and 45%, respectively. A few laboratories reported additional parameters (Annex 17) and the full QC evaluation of all isolates can be seen in Annex 18.

Table 8. Summary of selected QC parameters reported by participants

Laboratory	Confirmation of genus	Coverage	Q score (Phred)	Genome size	No. of good cgMLST loci
19	Kraken database	Minimum per site coverage of assembly >25	no of contigs < 200	Assembly length as expected for Salmonella >4.500.000	BioNumerics QC character set
36	KRAKEN	20x	No	No	% good targets
49	No	>= 30	N50	4.5 to 6.5 MB	Core percent
108	No	20x	In-house	Dynamic core genome >97%	No
129	SISTR	Avg. Coverage (Assembled) >40	Perc. Good Targets >95%	No	No
134	MLST	50	No	length of contigs assembled< ref size genome +10%	cgMLST alleles found and alleles called>95%
142	Kraken	30	No	No	>95%
144	PathogenFinder (DTU)	>40	SeqSphere+	No	percentage of good cgMLST targets >99%
147	JSpecies	60x	No	No	% good targets (98% min)
148	BLAST	50x	N50 + contigs number + Genome length	4,5-5,1 MB	No
149	KRAKEN	No	No	No	% good cgMLST loci >95%
% of laboratories using the QC parameter	**82%**	**91%**	**55%**	**45%**	**73%**

For each laboratory, the submitted raw reads (FASTQ files) were evaluated by the EQA provider's in-house quality control pipeline [11]. Table 9 shows the QC parameters and range of QC values per laboratory. For the full QC evaluation of all isolates, see Annex 18. Overall, the coverage of the raw data was high when evaluated by the EQA provider´s QC pipeline.

Table 9. Results of participants' raw sequence data evaluated by EQA provider's QC pipeline

		Laboratory No.											
Parameters	Ranges	19	36	49	108	129	134	142	144	147	148	149	150
Number of genera detected	{1}	1	1	1	1	1	1	1	1	1-3	1	1	1
Detected species	{*Se*}	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se
Unclassified reads (%)		1,4-3,1	1,3-25,4	0,5-6,1	1,8-3,8	0,2-7,1	0,6-8,2	0,5-3,9	0,4-4,4	1,4-36,2	0,5-2,5	1,1-3,6	0,4-4,0
Length at 25 x minimum coverage (Mbp)	{>4.5 ^ <5.3}	4,8-5	4,9-5,1	4,9-5,1	4,8-5	4,9-5,1	0,1-5	4,2-5,1	4,9-5,1	0-5,1	4,7-5	4,9-5,1	3,8-5
Length [0–25] x minimum. coverage (Mbp)	{<0.25}	0	0-0,1	0	0	0	0-4,8	0-0,6	0	0-4,7	0-0,1	0	0-1,1
Number of contigs at 25 x minimum coverage	{>0}	75-149	60-115	48-124	285-492	63-109	15-97	52-106	53-68	7-208	74-168	51-66	54-87
Number of contigs [0–25] x minimum coverage	{<1 000}	2-5	0-46	0	0	0-3	0-156	0-38	0-4	0-515	0-30	0-4	0-14
Average coverage	{>50}	77-125	56-142	42-129	66-136	54-118	22-79	33-73	39-127	15-81	60-144	102-174	32-82
Number of reads (x1 000)		1 371-2 282	682-1 778	427-1 598	1 038-2 136	929-2 060	391-1 397	372-802	463-1 482	194-1 112	1 090-2 997	1 130-2 005	800-2 055
No. of trimmed reads (x1 000)		1 353-2 258	660-1 711	412-1 555	955-1 998	914-2 023	388-1 387	352-785	457-1 453	176-1 077	1081-2 962	1078-1 932	800-2 055
Maximum read length		151	301	301	30-359	151	151	251	251	301	151	301	101
Mean read length		140-144	200-216	214-255	202-226	144-147	144-148	230-238	216-227	189-227	146-148	226-252	99-100
Read insert size		322-367	293-316	294-530	NA	410-483	324-444	424-629	301-374	263-418	320-405	316-380	346-401
Insert size StdDev		128-146	137-142	121-185	NA	158-172	128-171	169-209	112-147	109-189	77-106	136-159	173-198
N50 (kbp)		61-167	91-271	75-283	17-32	90-232	58-247	61-283	181-283	15-150	50-174	192-283	129-239
N75 (kbp)		33-87	53-102	42-178	9-18	53-102	28-102	36-135	85-175	8-59	26-81	91-150	82-104

**: indicative QC range*
Se: Salmonella enterica
NA: not analysed.

4. Discussion

The total number of participants decreased from 26 in EQA-7, 24 in EQA-8 to 23 in EQA-9. Among the 23 participants, one (laboratory 132) was participating again after a break last year. Two other laboratories (106 and 130) participated in both EQA-7 and EQA-8 but not in EQA-9. Laboratory 106 participated in both the cluster part by WGS and in the quality assessment of PFGE, while laboratory 130 only participated in the PFGE part of EQA-8.

4.1 Multiple-locus variable number of tandem repeats analysis

Ten laboratories (43%) participated in the MLVA part of both *S.* Typhimurium and for *S.* Enteritidis. MLVA for *S.* Enteritidis was included in the EQA for the second time and the relatively high number of participants confirms that it was relevant to include this method, but again this year the number of participants in MLVA for *S.* Typhimurium was lower than in previous years, decreasing from 15 participants in EQA-4 to 10 participants in this EQA-9. This can reflect a trend, where more laboratories are switching to WGS-based surveillance and outbreak detection using WGS instead of MLVA.

Only two laboratories (20%) obtained a total score of 100% for *S.* Typhimurium and reported the correct MLVA types for all 10 test isolates. The overall performance in this round was 86%, which was lower compared with previous years. From EQA-4 to EQA-9, the overall performance in each round was 92%, 96%, 96%, 96%, 97% and 86%, respectively. There were no obvious reasons for the decrease in this round, however one laboratory (55) participated for the first time in MLVA for *S.* Typhimurium and caused 43% of the incorrect results. The overall performance without the results from laboratory 55 was 92% and still lower compared with the previous four years.

The MLVA results of the two repeated *S.* Typhimurium isolates from EQA-4 through EQA-9 showed good performance by the participants. The majority of participants (60%; 6/10) performed at the same level as the last time they participated, however several of the laboratories had unusual incorrect result for one of the repeat isolates leading to general lower performance in EQA-9.

Mistakes in the MLVA for *S.* Typhimurium were mainly caused by reporting alleles in a locus with no fragment present, but also by assigning an incorrect allele in a present fragment. No common characteristics of the isolates caused problems among the participants, however laboratory 55 seemed to make a systematic error reporting allele number 28 for *S.* Typhimurium in locus STTR10 several times and the error by laboratory 19 was probably caused by analysing or reporting the same isolate twice.

For MLVA of *S.* Enteritidis, seven laboratories (70%) obtained a total score of 100% and the overall performance was 98% which was higher compared with EQA-8 (92%). The few mistakes in the MLVA for *S.* Enteritidis were twice reporting absent alleles where fragments were present and once an incorrect allele assigning in a present fragment.

The reasons for identifying the presence of an absent locus or vice versa (false negative allele number for a present locus) could be from not using a freshly prepared primer mix. An unbalanced primer mix could result in very different peak heights and background noise could be identified as a signal or a correct signal could mistakenly be recognised as background noise. Furthermore, 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. The lower performance in general may suggest a decreased use of the MLVA method combined with reduced maintenance of quality and skills while implementing WGS as routine method.

4.2 Molecular typing-based cluster analysis

In the present EQA scheme, a molecular typing-based cluster analysis was included for the second time. Participants were free to choose their preferred method between PFGE, MLVA and/or WGS-derived data and the identified cluster depended on the method used. Four of the 12 test isolates formed a cluster of closely related isolates according to WGS-derived typing results obtained by the EQA provider, whereas the remaining isolates clearly were genetically more distant. If MLVA was used as the single typing method, six isolates were indistinguishable, whereas the profiles of the remaining isolates had small or large differences to the cluster profile, and by PFGE a broad cluster definition was used in the evaluation of PFGE derived data and up to nine isolates could be included in the PFGE cluster.

The adjustment of the EQA scheme by adding cluster identification seem much more accepted by Member States compared with last year (Annex 2). Twenty-one of the 23 laboratories (91%) participated in the cluster part using PFGE-, MLVA- and/or WGS-derived data which was higher comparable with EQA-8 (54%). This year's adjustment, where the PFGE part with gel quality and analysis assessment was excluded, increased the number of laboratories participating in cluster identification using PFGE from 4 to 13. Five of the laboratories participating in the PFGE part last year did not switch to perform PFGE in the cluster part. Two of these five laboratories participated in the cluster part with only WGS, while three of these laboratories did not participate in the cluster part or in the EQA-9 at all.

Two new laboratories participated in the cluster part using WGS and only one laboratory performing WGS last year did not report WGS based results this year, thus the number of participants only performing WGS-based cluster analysis was almost the same in EQA-8 and EQA-9 (respectively 8 and 7 laboratories).

Only two laboratories participated in cluster identification using MLVA and both also participated in cluster identification using PFGE and WGS. The number of laboratories only performing PFGE-based cluster analysis increased markedly from two to nine and the high number of laboratories only performing PFGE shows that the method is apparently still an important and needed routine method for many laboratories.

The allele difference and SNP distances calculated from the cluster defined by PFGE or MLVA were much higher than in the 'true' WGS defined cluster. The allele differences within the cluster defined by PFGE were up to 18 (cgMLST by provider) using the minimum PFGE cluster definition of four isolates (category A), or up to 38 allele differences using the extended PFGE cluster definition of nine isolates. The SNP distance within the cluster defined by PFGE was 27 (provider result with recombination filter) for category A isolates and up to 55 using the extended PFGE cluster definition of nine isolates.

The identification of closely related isolates by MLVA included two additional isolates and the allele differences within the MLVA cluster were up to 24 (cgMLST by provider). If the isolate (REF8) with variation in one locus (STTR5) was included, the genetic distance within the cluster would increase to 38 allele differences. The SNP distance within the cluster defined by MLVA was 41 (provider result with recombination filter) and 105 if the one locus variation was included.

This shows the difficulties of inter-laboratory comparability between Member States regarding surveillance and outbreak investigation when different methods are used. Despite the increasing use of WGS as a typing tool for large outbreaks, many laboratories still use PFGE for their primary surveillance and outbreak investigation. PFGE can still have a value for investigating outbreaks at the national level and it can support bridging the historical national databases from human and veterinary sector for case finding and hypothesis generation by WGS.

4.2.1 PFGE-derived data

Of the 23 laboratories, 13 (57%) performed cluster analysis using PFGE-derived data. During the evaluation of the data from the participants, the EQA provider obtained additional knowledge regarding the chromosomal composition of the isolate REF2/REF7 and band variation was seen inside the cluster of four isolates defined on WGS. The PFGE cluster was extended from six up to nine isolates and differences up to two bands inside the PFGE-cluster were accepted.

Nine laboratories (69%) correctly identified the cluster. Despite the very broad PGFE cluster definition four laboratories (31%) did not identify the cluster of up to nine isolates. Three laboratories did not include all four isolates with indistinguishable PFGE-profile. Two of the laboratories probably incorrectly included a band below 33kb and one laboratory accepted a clearly unrelated profile as being part of the cluster. One laboratory that failed in the PFGE cluster identification managed to identify the correct cluster by WGS, while the additional three laboratories with incorrect result did not performed WGS or MLVA.

The results highlight one of the challenges of using PFGE for cluster identification, as small biological events like the genetic element shown in REF2/REF7 can influence the PFGE output and conclusions. This genetic element was not detectable by the normal WGS analysis.

4.2.2 MLVA-derived data

Performance was low as none of the two participating laboratories correctly identified the cluster of six closely related isolates using MLVA-derived data, but both laboratories identified the correct cluster by PFGE and WGS.

One of the laboratories (19) included a single-locus variant in fast-changing loci (STTR5). In routine cluster analysis and in some outbreak situations it can be relevant to accept changes in these loci [14], therefore the EQA provider acknowledged a MLVA cluster definition including a one-locus variant (REF8; 3-13-11-NA-211). However, the true genetic distance within the cluster increased markedly if REF8 was included.

The other laboratory (147) reported several incorrect MLVA profiles and the results were not useful to make a correct cluster identification. However, in the MLVA part of this EQA, laboratory 147 identified correct MLVA profiles for three isolates which had the same profile as reported incorrect in the cluster part using MLVA analysis.

As in EQA-8, no laboratories were only using MLVA for the cluster analysis and the number of laboratories performing MLVA decreased from four to two. This could, as in the *S.* Typhimurum MLVA part of this EQA, indicate an ongoing decrease in the use of the method.

4.2.3 WGS-derived data

Twelve of 23 laboratories (52%) performed cluster analysis using WGS-derived data. This was a slightly higher participation compared with EQA-8, where 11 of 24 laboratories (46%) performed cluster analysis using WGS-derived data. Performance was again high, as 10 (83%) correctly identified the cluster of closely related isolates.

The majority of laboratories (11/12) reported the use of an Illumina platform and all reported using commercial kits for library preparation. Only one laboratory reported the use of external assistance for sequencing, which is a change compered with EQA-8, where three laboratories had sequencing performed externally.

Ten laboratories (83%) reported using an allele-based method as the main analysis and two (17%) reported using SNP analysis. Compared with EQA-8 this is an increase in the use of allele-based analysis, where 64% reported using an allele-based method for the main analysis and 36% reported using SNP analysis.

If only evaluating the main analysis of the laboratories reporting the correct cluster, the number of allele differences reported using cgMLST were 0–2 inside the cluster and using SNP-based analyses, the distances reported were 0–2 inside the cluster.

As seen in EQA-8, the two approaches to analyse WGS-derived data (allele- and SNP-based analysis) showed comparable results, however in EQA-9, more incorrect results were observed using allele-based analysis, where two laboratories did not identify the correct cluster. Last year, all laboratories identified the right cluster using allele-based analysis and the two laboratories not identifying the correct cluster this year reported use of the same method/scheme in EQA-8 as in EQA-9. Results from laboratory 148 are not included in the discussion, as distances are not reported.

High similarity was seen for the reported results using cgMLST/Enterobase (3002 loci) as the scheme for analysis, when analysed in BioNumerics or SeqSPhere. One of the laboratories (142) with incorrect result also used the cgMLST/Enterobase scheme but reported analysing directly in Enterobase and use of 3018 loci. Laboratory 142 had less allele differences for some of the ST34 isolates outside the cluster, making the cluster identification unclear. The laboratory accepted an isolate with five allele differences as part of the cluster, while an isolate with eight allele differences was not included. The EQA provider analysed by cgMLST the raw reads from laboratory 142 which showed expected results (Figure 11). In addition, raw reads from the EQA provider were submitted for an analysis directly in Enterobase, and viewed in Grapetree (data not shown), which showed similar results as obtained from the analysis by the provider in BioNumerics. The reason for the incorrect results from laboratory 142 remains unclear.

A similar result with markedly lover number of allele differences between the ST34 isolates was seen for the other laboratory (129) not identifying the correct cluster. This laboratory used an in-house wgMLST scheme and used a 'cut-off' at 17 allele differences for the cluster definition. In general, monophasic *Salmonella* Typhimurium is highly related and therefore low cut-off values are recommended for assigning clusters in ST34 [15]. The allelic differences are affected by the loci included in the scheme, the number of loci and analysing approach.

Laboratory 19 and 36 also used wgMLST as additional analysis and had a similar result as seen for the laboratories with correct result using cgMLST/Enterobase. The wgMLST schemes used by laboratory 19 and 36 had higher number of loci (15.867 and 4.344 respectively) compared with laboratory 129, which only reported 3.478 loci in the used wgMLST scheme. The results highlight the advantage and need for standardisation of schemes used for inter-laboratory comparisons.

The reported SNP results were largely comparable to allele-based results. Only one laboratory (148) reported a higher SNP distance than 0-2 within the cluster (as additional analysis), however compared with last year, the cluster identification for laboratory 148 was clear with many SNPs between the isolates inside and outside the cluster.

In general, the SNP distances were very variable for the ST34 isolates outside the cluster, but for all laboratories the distance between the cluster isolates and the non-cluster ST34 were large, which made the cluster identification clear. The variation in the reported SNP distances outside the cluster was probably caused by an area with recombination and depending on whether the analyses were performed with or without filtration.

The main reported QC parameters were coverage, cgMLST allele calls and genus/species confirmation, which are all essential for the end use of the data.

The EQA provider's analysis of the submitted raw data showed that when using a standardised cgMLST analysis a very high concordance was obtained (Figure 11). Only laboratory 108 had allele differences ranging from 24-40 for all isolates. This laboratory provided Ion Torrent data for which the EQA provider's analysis is not optimised, making correct assembly difficult. Thus, the observed allele differences may be method artefacts, however the use of Ion Torrent data can complicate the communication and investigation of multi country outbreaks if only allelic method is used.

5. Conclusions

Twenty-three laboratories participated in the EQA-9 scheme: 10 (43%) performed MLVA and 21 (91%) cluster identification. Eight laboratories (35%) completed both parts of the EQA.

In the EQA-9, a change was made excluding quality assessment of PFGE. The molecular typing-based cluster analysis using either PFGE, MLVA and/or WGS-derived data which was for the first time included in EQA-8, also continued in EQA-9. Incorporating molecular typing-based cluster analysis by WGS is up to date with the development of surveillance methods used by PH NRLs in Europe.

This adjustment of the EQA seemed better accepted by Member States as the number of laboratories which participated in the cluster identification increased in EQA-9. In particular, the number of laboratories performing PFGE increased, probably because the PFGE quality assessment part was removed and only three of laboratories participating in the PFGE part of EQA-8 did not switch to the cluster part using PFGE and/or WGS.

Again, in EQA-9, participation in the MLVA part was possible for both *S.* Typhimurium and *S.* Enteritidis, but the overall number of participants decreased. The performance level was high for *S.* Enteritidis: seven laboratories (70%) reported correct allelic profiles for all test isolates. In the *S.* Typhimurium MLVA, the performance was lower and only two laboratories (20%) reported correct allelic profiles for all test isolates, the errors were various except for one laboratory reporting the same fragment incorrect several times.

Thirteen laboratories participated using PFGE for cluster analysis and nine participated solely using PFGE-derived data for analysis. Despite broad cluster definition, four did not identify the correct cluster using PFGE either missing one of the isolates with indistinguishable PFGE profile (three laboratories) or including non-cluster isolate (one laboratory). The several incorrect results together with the challenges of the PFGE method, highlight the problem that many laboratories still use and probably will use the PFGE method for several years. The added value of PFGE is to bridge the historical PFGE databases with WGS data. This offers a good tool for selecting isolates for WGS analysis on the national level and offers a chance to identify relevant non-human isolates for a joint WGS analysis to develop a hypothesis of the vehicle/source of infection.

Twelve laboratories performed cluster analysis using WGS-derived data. The performance was very high, 10 (83%) of the participants correctly identified the cluster of closely related isolates. Ten of 12 (83%) preferred an allele-based method and only 37% (2/12) used SNP as the main reported cluster analysis. This was a decrease of SNP analyses compared with the previous EQA-8. The two laboratories with incorrect result used both an allele-based method.

The use of a standard cgMLST scheme (e.g. Enterobase) showed a high degree of homogeneity in the results, despite different approaches for analysing. Although different methods were probably used (different thresholds for allele calling, including or not including missing alleles in the analysis, assembly based and/or mapping based allele calling etc.), the results show that the use of a standardised cgMLST scheme leave little room for error, resulting in good performance. However, one of the laboratories (142) using Enterobase (cgMLST) did not identify the correct cluster without an obvious reason for the incorrect result.

The other laboratory (129) with incorrect cluster identification, did the analyses using an in-house wgMLST scheme and the result highlighted challenges for comparison and communication between laboratories, if different schemes and different number of loci are applied. Furthermore, the current EQA also showed deviation comparing Illumina and Ion Torrent data using cgMLST, leading to lower inter-laboratory comparability across sequencing platforms.

SNP analyses can provide valid cluster detection at the national level and can be used for communication about cluster definitions, however, few laboratories performed SNP analysis in EQA-9. The recombination area of 154 SNP had no importance for the cluster identification and only showed variations in the SNP distances outside the cluster depending of the SNP analysis performed.

The current EQA scheme for typing of *Salmonella enterica* subsp. *enterica* is the ninth organised for laboratories in FWD-Net. The molecular typing-enhanced surveillance system implemented as part of TESSy relies on the capacity of FWD-Net laboratories to produce analysable and comparable typing results into a centralised database. WGS-based typing for surveillance is increasingly used in EU. In 2019, ECDC has opened the possibility to submit WGS data for *Salmonella* to TESSy to be used for EU-wide surveillance and cross-sector comparison.

6. Recommendations

6.1 Laboratories

Laboratories with repeated or several errors in the MLVA part 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. Less than half of the participants (43%) performed MLVA for *S.* Typhimurium and *S.* Enteritidis. Laboratories, not moving towards the use of WGS at this stage, can benefit from using MLVA because of its low-cost and easy analysis. However, the results of this EQA-9 showed that it is important to continuously maintain the routine and expertise in the laboratory, e.g. the calibration needed by the use of standardisation strains [4].

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

The laboratories are recommended to use the EQA provided data and isolates to validate their analysis methods when incorrect results (e.g. EQA) are obtained or when implementing new methods and procedures.

6.2 ECDC and FWD-Net

ECDC is working actively with FWD-Net to improve the quality of sequence data generation and analysis through appropriate means like EQA schemes, expert exchange visits and workshops.

ECDC is encouraging 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 to conduct an EQA feedback survey among participants.

6.3 EQA provider

On the coming EQA round the EQA provider will evaluate the possibility to modify the cluster analysis to mimic a more realistic microbiological investigation by including genome sequences for the WGS analysis. This part is designed to be a simulation of an outbreak situation in a country to detect genetically closely related isolates and to compare the original cluster with genomes produced in other laboratories, which might be using different procedures and equipment.

References

1. Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European centre for disease prevention and control, Article 5.3. Available from: http://ecdc.europa.eu/en/aboutus/Key%20Documents/0404_KD_Regulation_establishing_ECDC.pdf.
2. Decision No 1082/2013/EU of the European Parliament and the Council 22 October 2013 on serious cross-border threats to health and repealing Decision No 2119/98/EC (Text with EEA relevance). Available from: http://ec.europa.eu/health/preparedness_response/docs/decision_serious_crossborder_threats_22102013_en.pdf.
3. European Food Safety Authority and European Centre for Disease Prevention and Control. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal. 2018;16(12):5500. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5500.
4. European Centre for Disease Prevention and Control. Laboratory standard operating procedure for MLVA of *Salmonella enterica* serotype Typhimurium. Stockholm: ECDC; 2011. Available from: http://ecdc.europa.eu/publications-data/laboratory-standard-operating-procedure-mlva-salmonella-enterica-serotype.
5. European Centre for Disease Prevention and Control. Laboratory standard operating procedure for multiple-locus variable-number tandem repeat analysis of *Salmonella enterica* serotype Enteritidis. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/publications-data/laboratory-standard-operating-procedure-multiple-locus-variable-number-tandem.
6. International Organisation for Standardization. ISO/IEC 17043:2010: Conformity assessment -- General requirements for proficiency testing. ISO: Geneva, 2010. Available from: http://www.iso.org/iso/catalogue_detail.htm?csnumber=29366.
7. Danish outbreak description, https://www.ssi.dk/aktuelt/nyheder/2017/2017-3-salmonella.
8. PulseNet International. Standard operating procedure for PulseNet of *Escherichia coli* O157:H7, *Salmonella* serotypes, *Shigella sonnei* and *Shigella flexneri*. Wellington: Institute of Environment Science and Research; 2013. Available from: http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL05_Ec-Sal-ShigPFGEprotocol.pdf.
9. Warwick Medical School. EnteroBase [Internet]. Coventry: University of Warwick; 2018 [cited 21 August 2018]. Available from: http://enterobase.warwick.ac.uk.
10. Sahl JW, Lemmer D, Travis J, Schupp JM, Gillece JD, Aziz M, et al. NASP: an accurate, rapid method for the identification of SNPs in WGS datasets that supports flexible input and output formats. Microb Genom. 2016 Aug 25;2(8):e000074.
11. Statens Serum Institut. SerumQC [Internet]. Copenhagen: SSI; 2018 [cited 21 August 2018]. Available from: http://www.github.com/ssi-dk/SerumQC.
12. Deng X, Shariat N, Driebe EM, Roe CC, Tolar B, Trees E, et al. Comparative Analysis of Subtyping Methods against a Whole-Genome-Sequencing Standard for *Salmonella enterica* Serotype Enteritidis. J Clin Microbiol. 2015 Jan;53(1):212-8.
13. Pearce ME, Alikhan N, Dallman TJ, Zhou Z, Grant K, Maiden MCJ. 2018. Comparative analysis of core genome MLST and SNP typing within a European *Salmonella* serovar Enteritidis outbreak. Int J Food Microbiol. 2018 Jun 2:274:1-11.
14. Tandem Repeat Analysis for Surveillance of Human Salmonella Typhimurium Infections. Mia Torpdahl, Gitte Møller Sørensen, Bjørn-Arne Lindstedt, E M Nielsen. Published in Emerging infectious diseases 2007 DOI:10.3201/eid1303.060460
15 Gymoese G, Sørensen G, Litrup E, Olsen JE, Nielsen EM and Torpdahl M., 2017, Emerging Infectious Diseases, Vol. 23, No. 10. Available at: https://wwwnc.cdc.gov/eid/article/23/10/pdfs/16-1248.pdf

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	Diagnostic and Typing of Gastrointestinal Bacteria	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
Hungary	Department of Phage-Typing and Molecular Epidemiology	National Public Health Institute
Iceland	Landspitali University Hospital	Micro- and virology
Ireland	National Salmonella, Shigella and Listeria Reference Laboratory	University Hospital Galway
Italy	Department of Food Safety, Nutrition and Veterinary Public Health	Istituto Superiore di Sanità
Latvia	National Microbiology Reference laboratory	Infectology Centre of Latvia
Lithuania	National Public Health Surveillance Laboratory	Nacionaline Visuomenes Sveikatos Prieziuros Laboratorija
Luxembourg	Epidemiology and Microbial Genomics	Laboratoire National de Sante
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
Slovak Republic	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
Sweden	Microbiology	Folkhälsomyndigheten
the Netherlands	Centre for Infectious Diseases Research, Diagnostics and Screening	National Institute for Public Health and the Environment
UK	Gastrointestinal Bacterial Reference Unit	Public Health England

Annex 2. Participation overview EQA-8 and 9

	2017 to 2018 (EQA-8)								2018 to 2019 (EQA-9)					
	Participation (min. 1 part)	PFGE		MLVA		Cluster			Participation (min. 1 part)	MLVA		Cluster		
Laboratory		Gel quality	Gel analysis	STm	SE	PFGE	MLVA	WGS		STm	SE	PFGE	MLVA	WGS
19	X	X	X	X	X	X	X	X	X	X	X	X	X	X
36	X	X	X	X				X	X					X
49	X							X	X					X
55	X	X	X		X	X			X	X	X	X		
88														
92	X	X	X			X			X			X		
96	X	X							X			X		
100	X	X	X	X	X				X	X	X			
106	X	X	X					X						
108	X			X	X		X	X	X	X	X			X
127	X	X							X			X		
128	X	X	X						X			X		
129	X			X	X			X	X	X	X			X
130	X	X	X											
132									X			X		
134	X	X	X	X	X		X	X	X	X	X			X
135*	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		X
144	X	X		X	X				X	X	X	X		X
145	X	X							X			X		
147	X	X	X	X	X			X	X	X	X	X	X	X
148	X			X	X			X	X					X
149	X			X	X				X					X
150	X							X	X					X
Total number of participants	**24**	**17**	**11**	**12**	**12**	**4**	**4**	**11**	**23**	**10**	**10**	**13**	**2**	**12**

**: previously laboratory 77*

Annex 3. Reason(s) for participating in EQA

	MLVA (STm and SE)				Cluster			
LAB ID	**Accreditation needs**	**Institute policy**	**National policy**	**Enhance typing quality**	**Accreditation needs**	**Institute policy**	**National policy**	**Enhance typing quality**
19	X	X			X	X		X
36	*Not relevant to our laboratory				X	X		X
49	*Not relevant to our laboratory				X			X
55				X				X
92	*Not routinely used as a typing method/ lack of financial means/no experience					X		X
96	*Don't perform MLVA method				X			X
100		X	X	X	*Lack of laboratory capacity			
108				X				X
127	*Lack of laboratory capacity							X
128	*Lack of laboratory capacity							X
129		X		X		X		X
132	*Lack of laboratory capacity							X
134				X				X
135#	X	X			X	X		
138	*Lack of laboratory capacity							X
140	*Lack of laboratory capacity							X
142	X				X			
144	X	X	X	X	X	X	X	X
145	*Lack of laboratory capacity/ Lack of financial means						X	X
147	X	X		X	X	X		X
148	*Not relevant to our laboratory					X	X	X
149	*Lack of financial means				X	X		X
150	*Not relevant to our laboratory				X			X
Number of participants	5	6	2	7	10	9	3	20

**: Reasons given when not participating.*
#: Laboratory 135 did not submit results for the cluster analysis despite the laboratory had signed up.

Annex 4. Scores of MLVA results *S*. Typhimurium

	Test isolates no./allel																																																	
	STm1					STm2					STm3					STm4					STm5					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	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
Provider	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
19	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	2	11	6	9	212	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
55	2	11	18	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	28	409	3	19	11	-2	311	3	14	13	22	311	3	12	17	28	211	3	12	9	28	211	3	13	11	28	309	3	11	11	28	211
100	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
108	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
129	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	4	13	208	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
134	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	6	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	11	211	3	11	11	-2	211
135	2	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	11	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
142	2	11	6	9	212	4	-2	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
144	3	11	6	9	212	4	4	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211
147	2	11	6	9	212	4	-2	12	8	211	2	22	17	11	212	3	13	-2	-2	211	3	19	11	-2	311	3	14	13	22	311	3	12	17	-2	211	3	12	9	-2	211	3	13	11	-2	211	3	11	11	-2	211

Purple: repeat strains in EQA-4 to -9
Pink: incorrect.

Annex 5. Scores of MLVA results *S.* Enteritidis

	Test isolates no. /allel																																																	
	SE1					SE2					SE3					SE4					SE5					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	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
Provider	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
19	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
55	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
100	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
108	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
129	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
134	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
135	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	-2	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
142	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	1	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
144	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	1	10	7	3	2
147	2	11	11	5	1	2	11	12	6	1	3	11	4	4	1	3	9	5	4	1	3	16	5	5	2	3	5	5	3	1	2	7	12	7	1	2	10	8	3	2	2	14	7	3	2	-2	10	7	3	2

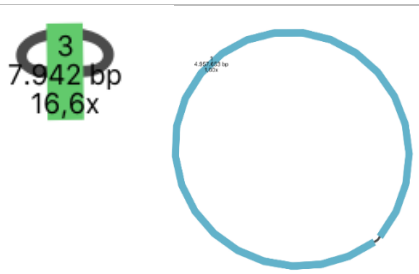
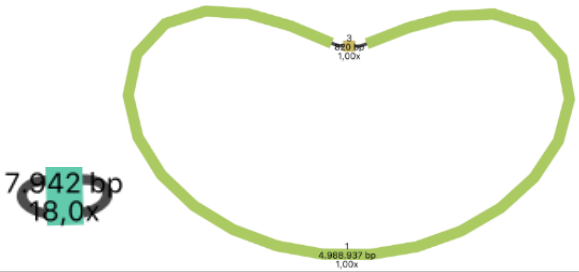
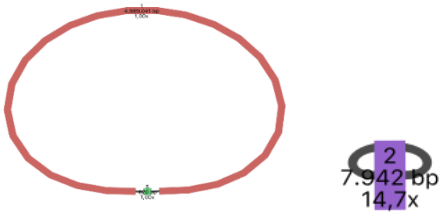
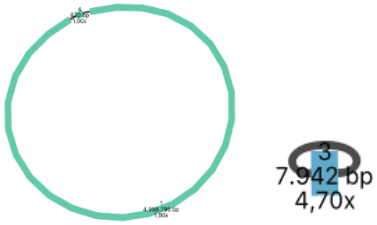
Purple: repeat strains in EQA-8 and -9

Pink: incorrect.

Annex 6. Genetic element analysis

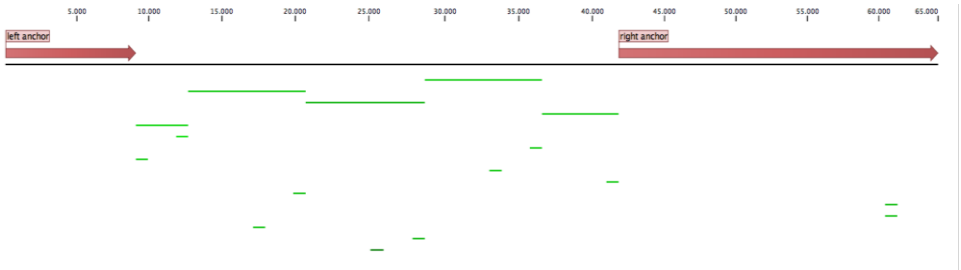
Each isolate had a different copy number of this genetic element, from 18 copies down to 4.7 copies. When comparing the band size from Figure 8 and the calculated size of the fragment, it seems to be comparable. Isolate '_1' that had a large band at around 130-140kb, had repeats of the 8kb element in 18 copies as shown in the Figure 8. 7942bp x 18.0 copies = 143kb. Isolate "_8" which had a band at around 110-120kb, had repeats of the 8kb element in 14.7 copies which gives 7942bp x 14.7 copies = 117kb. Isolate "_5" which had a band at 40-50kb, had repeats of the 8kb element in 4.7 copies which gives 7942bp x 4.7 copies = 37kb. The 140-160kb size of the band of "Isolate _6" does not match well with the calculated size of the sequence 7942bp x 16.6 copies = 132kb. However, in each DNA purification more than one colony is purified, which if different affects the calculation of the coverage and thereby the copy number.

Figure A. Assembly of the sequences (Nanopore and colonies of REF2/REF7

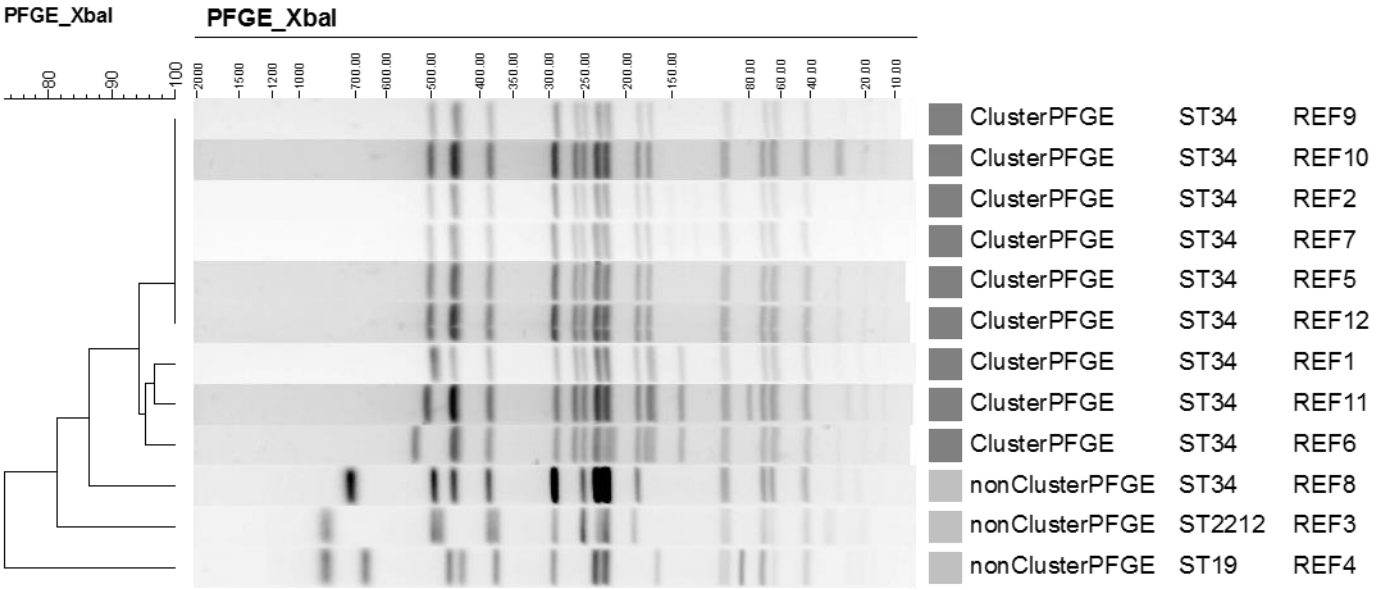
EQA_provider_REF2/REF7_6	**EQA_provider_REF2/REF7_1**
	
Genetic element: 7942kb x 16.6 = 132kb	Genetic element: 7942kb x 18=143kb
Band size: 140-160kb	Band size: 130-140kb
EQA_provider_REF2/REF7_8	**EQA_provider_REF2/REF7_5**
	
Genetic element: 7942kb x 14.7=117kb	Genetic element: 7942kb x 4.7 = 37kb
Band size: 110-120kb	Band size: 40-50kb

In addition, the genetic element of Isolate '_5' was blasted against the nanopore reads, which showed that the genetic element was placed in the chromosome in multiple copies. Figure 9 shows the nanopore long-reads, marked with the anchoring part of the chromosome, and the sequence of the genetic element found in this between 4-5 times. In each side of the genetic element 820bp repeats (transposase) are positioned.

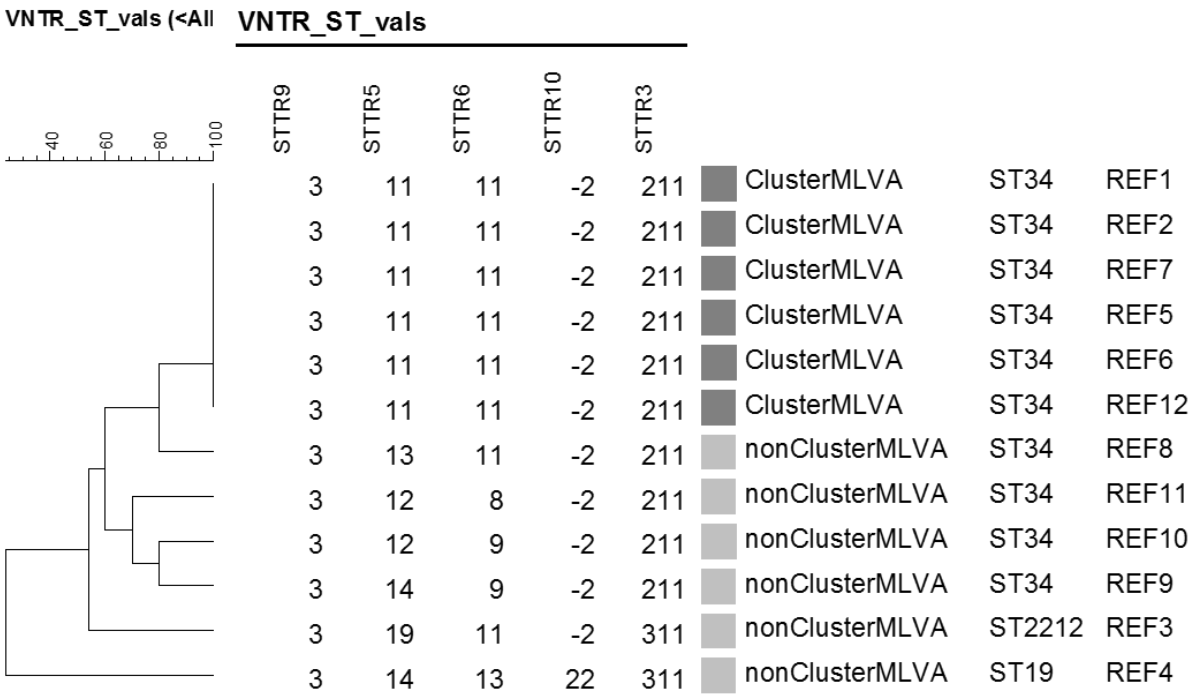
Figure B. Blast of genetic element against nanopore long reads (_5)



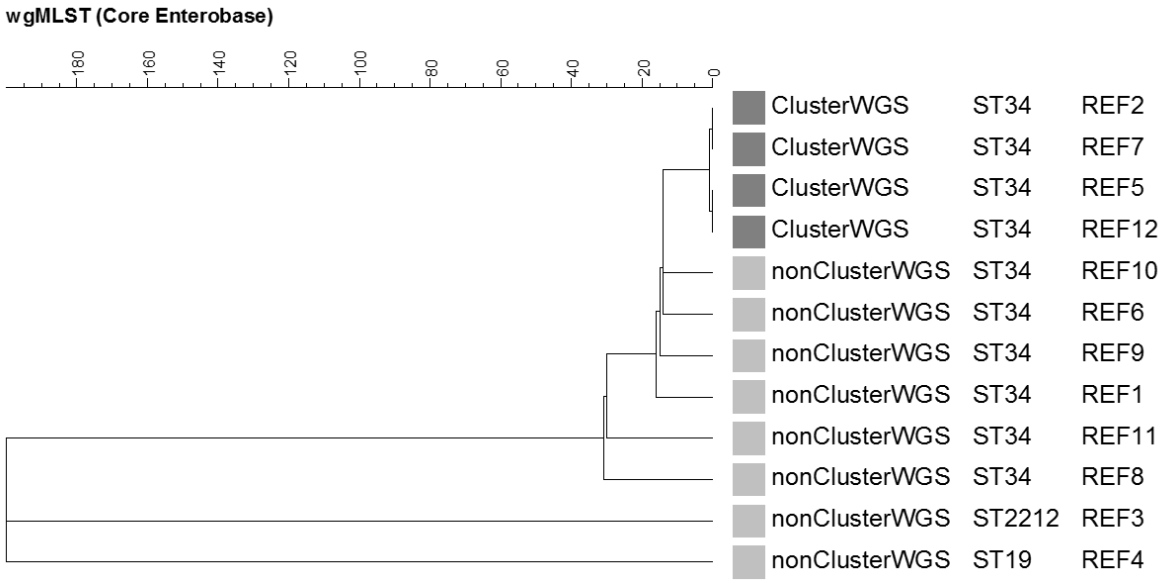
Annex 7. EQA provider cluster analysis based on PFGE-derived data



Annex 8. EQA provider cluster analysis based on MLVA-derived data



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



Single linked dendrogram of core genome multi-locus sequence typing (cgMLST) profiles of Salmonella EQA-9 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 10. Reported cluster of closely related isolates based on PFGE-derived data

Laboratory	Reported cluster	Corresponding REF isolates	Correct
Provider		A: REF5, REF9, REF10, REF12 B: REF2, REF7 C: REF1, REF6, REF11	
19	5080, 5785, 5033, 5982, 5365, 5164	REF9; REF5; REF10; REF2; REF12; REF7	Yes
55	5073, 5398, 5423, 5595, 5892	REF9; REF5; REF7; REF12; REF10	Yes
92	5388, 5401, 5457, 5784	REF12; REF5; REF9; REF10;	Yes
96	5010, 5071, 5093, 5205, 5651	REF9; REF10; REF12; REF5; REF7	Yes
127	5251, 5775, 5920, 5965	REF10; REF5; REF12; REF9	Yes
128	5352, 5624, 5684, 5754, 5847	REF5; REF10; REF9; REF2; REF7	No
132	5233, 5266, 5413, 5416, 5621, 5823, 5864, 5791	REF5; REF7; REF9; REF12; REF10; REF2; REF1; REF11	Yes
138	5109, 5159, 5166, 5192, 5207, 5631, 5644, 5807, 5857	REF2; REF11; REF6; REF12; REF10; REF9; REF5; REF1; REF7	Yes
140	5090, 5165, 5336, 5377, 5512, 5548, 5896, 5919, 5950, 5976	REF12; REF5; REF10; REF6; REF1; REF11; REF9; REF7; REF2; REF8	No
142	5146, 5342, 5552, 5596, 5626	REF5; REF12; REF9; REF7; REF10	Yes
144	5143, 5045, 5955, 5389	REF9; REF5; REF12; REF7	No
145	5128, 5232, 5374, 5773	REF12; REF9; REF5; REF7	No
147	5308, 5313, 5346, 5456, 5668, 5728, 5776, 5843	REF5; REF10; REF7; REF12; REF1; REF11; REF2; REF9	Yes

A.: REF5, REF9, REF10 and REF12 with indistinguishable PFGE-profile as a minimum
B.: REF2 and REF7 with 0-1 band difference to the PFGE-profile in A
C.: REF1, REF6 and REF11 with up to 2 band differences to the PFGE-profile in A

Annex 11. Reported PFGE band differences

			Laboratory ID												
Isolate number	ST	Expected *Xba*I bands	19	55	92	96	127	128	132	138	140	142	144	145	147
REF1‡	34	15	16	15	15	15	15		14	15	15	15	13	15	15
REF2‡#	34	14/15	14	15	15	15	15		13	15	15	15	13	15	15
REF3	2212	Clearly unrelated profile	9999	14	14	10	11		12	14	11	14	11	14	14
REF4	19	Clearly unrelated profile	15	13	15	14	14		14	14	14	14	12	13	15
REF5‡	34	14	14	14	14	14	14		13	14	14	14	12	14	14
REF6‡	34	15	16	15	15	15	15		15	15	15	15	13	15	15
REF7‡#	34	14/15	15	14	15	14	15		13	15	15	14	12	14	15
REF8	34	13	13	13	13	13	13		12	13	13	13	13	13	13
REF9‡	34	14	14	14	14	14	14		13	14	14	14	12	14	14
REF10‡	34	14	14	14	14	14	14		13	15	14	14	13	15	14
REF11‡	34	16	16	16	16	16	16		15	16	16	16	14	16	16
REF12‡	34	14	14	14	14	14	14		13	14	14	14	12	14	14

			Laboratory ID												
Isolate number	ST	Bands with shared *Xba*I	19	55	92	96	127	128	132	138	140	142	144	145	147
REF1‡	34	14	14	12	14	14	14	13	13	14	13	14	12	14	12
REF2‡#	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14
REF3	2212	Clearly unrelated profile	9999	8	10	9	10	11	10	9	7	9	8	9	999
REF4	19	Clearly unrelated profile	6	6	6	14	8	13	8	7	7	9	9	4	999
REF5‡	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14
REF6‡	34	13	13	13	14	13	13	14	7	13	13	12	9	13	999
REF7‡#	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14
REF8	34	12	12	12	10	12	12	13	10	12	12	12	10	12	999
REF9‡	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14
REF10‡	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14
REF11‡	34	14	14	14	14	14	14	13	11	14	14	14	12	14	11
REF12‡	34	14	14	14	14	14	14	12	13	14	14	14	12	14	14

‡: cluster identification of closely related isolates (based on PFGE-derived data)
#: technical duplet
ST: sequence type.
: not reported

Annex 12. Reported sequencing details

Sequencing performed	Protocol (library preparation)	Commercial kit	Sequencing platform
In own laboratory	Commercial kits	Nextera XT DNA Sample Preparation Kit (Illumina)*	HiSeq 2500
In own laboratory	Commercial kits	Nextera XT	MiSeq
In own laboratory	Commercial kits	Nextera XT DNA Library Preparation Kit	MiSeq
In own laboratory	Commercial kits	Illumina Nextera XT	MiSeq
In own laboratory	Commercial kits	Nextera	MiSeq
In own laboratory	Commercial kits	NexteraXT (Illumina)	NextSeq
In own laboratory	Commercial kits	SureSelect QXT Library Prep Kit (Agilent)	MiSeq
In own laboratory	Commercial kits	Nextera-XT kit Illumina**	MiniSeq Illumina
In own laboratory	Commercial kits	Ion Xpress TM Plus Fragment Library Kit	Ion Torrent S5XL
Externally	Commercial kits	Illumina Nextera XT	MiSeq
In own laboratory	Commercial kits	Kapa HyperPlus (KapaBiosystems)	MiSeq
In own laboratory	Commercial kits	NexteraXT	NextSeq

**: In term of deviation, library prep has been optimised on the robotics for half volume Nextera XT sample Prep reagents instead of the full volume.*
***: We use half the volume of reagents for each step of the protocol*

Annex 13. Reported cluster of closely related isolates based on MLVA-derived data

Laboratory	Reported cluster	Corresponding to REF isolates	Correct
Provider		REF1, REF2, REF5, REF6, REF7, REF12	
19	5785 5164 5982 5599 5365 5326 5470	REF5, REF7, REF2, REF1, REF12, REF6, REF8	No
147	5308 5346 5668	REF5, REF7, REF1	No

Annex 14. Reported MLVA profile data

				Laboratory ID	
Isolate number	ST	MLVA scheme	Provider	19	147
REF1‡	34	*S.* Typhimurium	3-11-11- NA-211	3-11-11- NA -211	3-11-11- NA -211
REF2#	34	*S.* Typhimurium	3-11-11- NA -211	3-11-11- NA -211	3-11- NA - NA - NA
REF3	2212	*S.* Typhimurium	3-19-11- NA -311	3-19-11- NA -311	3-19-11- NA -311
REF4	19	*S.* Typhimurium	3-14-13-22-311	3-14-13-22-311	3-14-13- NA - NA
REF5‡	34	*S.* Typhimurium	3-11-11- NA -211	3-11-11- NA -211	3-11-11- NA -211
REF6‡	34	*S.* Typhimurium	3-11-11- NA -211	3-11-11- NA -211	3-11-11- NA - NA
REF7#	34	*S.* Typhimurium	3-11-11- NA -211	3-11-11- NA -211	3-11-11- NA -211
REF8	34	*S.* Typhimurium	3-13-11- NA -211	3-13-11- NA -211	3-13-11- NA -211
REF9	34	*S.* Typhimurium	3-14-9- NA -211	3-14-9- NA -211	3-14-9- NA -211
REF10	34	*S.* Typhimurium	3-12-9- NA -211	3-12-9- NA -211	3-12-9- NA - NA
REF11	34	*S.* Typhimurium	3-12-8- NA -211	3-12-8- NA -211	3-12-8- NA -211
REF12‡	34	*S.* Typhimurium	3-11-11- NA -211	3-11-11- NA -211	3-11-11- NA - NA

‡: cluster identification of closely related isolates (based on MLVA-derived data)
#: technical duplet
ST: sequence type
NA: designates a locus not present (-2 submitted by participants).

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

Laboratory	Reported cluster	Corresponding to REF isolates	Correct
Provider		REF2, REF5, REF7, REF12	
19	5164, 5982, 5785, 5365	REF7, REF2, REF5, REF12	Yes
36	5040, 5122, 5334, 5581	REF7, REF2, REF12, REF5	Yes
49	5327, 5461, 5679, 5860	REF7, REF2, REF5, REF12	Yes
108	5672, 5727, 5838, 5495	REF2, REF7, REF12, REF5	Yes
129	5052, 5088, 5127, 5153, 5418, 5426, 5478, 5571, 5844, 5871	REF8, REF9, REF1, REF6, REF7, REF5, REF12, REF10, REF11, REF2	No
134	5072, 5079, 5237, 5430	REF2, REF12, REF5, REF7	Yes
142	5146, 5342, 5596, 5804, 5626, 5689	REF5, REF12, REF7, REF2, REF10, REF6	No
144	5045, 5116, 5389, 5955	REF5, REF2, REF7, REF12	Yes
147	5308, 5346, 5456, 5776	REF5, REF7, REF12, REF2	Yes
148	5021, 5142, 5578, 5710	REF12, REF2, REF7, REF5	Yes
149	5095, 5226, 5211, 5654	REF2, REF7, REF5, REF12	Yes
150	5341, 5669, 5719, 5874	REF12, REF7, REF5, REF2	Yes

Annex 16. Reported SNP distance and allelic differences

SNP distances

				Laboratory ID					
Isolate number	ST	Provider + Recombination filter	Provider	19*	36*	49*	108	148*	150
REF1	34	41	195	217	37	153	242	51	43
REF2‡#	34	0¤	0¤	0¤	0	0	0¤	9	2
REF3	2212	614	795	782	548	888	9999	599	675
REF4	19	750	1170	1153	746	893	9999	670	884
REF5‡	34	2	2	2	2	2	2	8	0¤
REF6	34	25	33	23	22	25	25	37	24
REF7‡#	34	0	0	0	0¤	0¤	0	8	2
REF8	34	105	269	248	67	192	270	72	73
REF9	34	27	181	203	23	136	226	31	30
REF10	34	27	27	26	24	30	29	36	29
REF11	34	55	209	230	46	162	253	59	56
REF12‡	34	2	2	2	2	2	2	0¤	2

Allelic differences

			Laboratory ID											
Isolate number	ST	Provider	19	19*	36	36*	49	129	134	142	144	147	148	149
REF1	34	24	25	35	18	33	25	11	44	14	23	24	9999	23
REF2‡#	34	0	0	1	0	0	0	0	0	0	1	1	9999	1
REF3	2212	292	293	450	197	440	294	137	285	286	286	288	9999	288
REF4	19	341	340	515	241	533	342	168	337	333	339	340	9999	335
REF5‡	34	1	1	0¤	0	1	1	0	1	0¤	0¤	1	9999	0¤
REF6	34	17	16	21	10	21	16	6	26	5	15	30	9999	15
REF7‡#	34	0¤	0¤	2	0¤	0¤	0¤	0¤	0¤	0	2	1	9999	1
REF8	34	38	37	62	28	58	37	17	58	37	36	36	9999	36
REF9	34	18	18	23	14	22	18	7	28	8	17	17	9999	17
REF10	34	15	15	24	11	23	15	7	14	2	14	15	9999	14
REF11	34	38	37	47	25	47	38	16	59	18	37	38	9999	37
REF12‡	34	1	1	0	0	2	1	1	1	0	0	0¤	9999	0

**: additional analysis*
‡: closely related isolates
#: technical duplicate isolates
¤: isolate used as cluster representative by the participant
9999: results not reported by the participant, however instead of reporting the 7 loci MLST the participant reported Enterobase b[5] level cluster codes. Cluster isolates had 134479.
ST: sequence type.

Annex 17. Additional reported QC parameters

Lab ID	1		2		3	
	Parameter	Threshold	Parameter	Threshold	Parameter	Threshold
49	N50	No threshold- Usually >100,000 for Salmonella	Avg Quality	No threshold but always aim for >=30	Number of multiple alleles (NrBAF multiple)	No threshold but normally be <10
108	Genome size	+/- 20%				
144	N50	>130000	Avg. Read Length (Processed, Unassembled)	>225		
147	FastQC	per base sequence quality, per base sequence content, per sequence quality score				
148	Contamination	Kraken (<3%)				
150	Minimum Read Length	>50 after trimming with trimmomatic	Minimum Read count	>10,000	Assessment of bacterial contamination	Kmer ID, look at similarity and reference genome, it is rejected if there is >10% unexplained similarity

Annex 18. Calculated qualitative/quantitative parameters

		Laboratory 19											
Parameters	Ranges*	5080	5033	5326	5458	5164	5365	5470	5785	5755	5599	5799	5982
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 (%)		3.11	3.14	1.81	1.44	2.0	1.98	2.82	2.25	1.86	2.82	2.59	1.83
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	5.0	5.0	4.9	4.9	4.9	4.8	4.9	4.9	4.9	5.0	4.9
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum coverage	{>0}	95	106	125	144	87	112	138	75	87	149	125	100
Number of contigs [0–25] x minimum coverage	{<1 000}	4	3	4	3	2	4	4	2	5	4	5	2
Average coverage	{>50}	97.1	125.1	76.5	93.6	117.5	105.6	100.2	124.1	115.4	117.5	100.8	105.4
Number of reads (x1 000)		1747	2282	1371	1684	2111	1884	1787	2236	2058	2173	1867	1882
Number of trimmed reads (x1 000)		1726	2258	1353	1662	2087	1860	1764	2211	2036	2150	1845	1857
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		142.4	142.2	144.1	143.3	142.8	143.5	141.9	142.5	142.0	140.1	141.9	144.2
Read insert size		357.7	323.1	362.1	367.0	323.9	364.5	362.8	337.8	345.2	322.1	349.9	363.6
Insert size StdDev		142.1	129.9	128.4	138.8	141.8	140.1	145.8	145.1	142.7	142.0	145.7	135.2
N50 (kbp)		106.8	88.8	81.7	61.0	133.5	88.1	61.2	167.1	92.6	65.4	80.2	100.9
N75 (kbp)		56.3	51.8	43.8	34.6	72.9	47.0	34.4	86.9	56.5	32.7	47.0	50.7

		Laboratory 36											
Parameters	Ranges*	5040	5064	5334	5550	5122	5581	5414	5873	5269	5637	5912	5933
Number of genera detected	{1}	1	1	1	1	1	1	1	1	2	1	1	1
Detected species	*{Se}*	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se
Unclassified reads (%)		2.22	3.23	2.32	7.23	1.83	1.92	1.34	25.35	10.08	2.03	1.63	2.52
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	5.0	4.9	5.0	4.9	4.9	5.0	5.0	4.9	5.0	4.9	5.1
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	63	115	74	82	82	72	70	74	61	68	60	78
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	1	0	0	0	0	46	0	8	0
Average coverage	{>50}	72.6	108.0	62.5	56.4	106.2	60.9	70.5	68.2	121.0	142.2	126.6	128.0
Number of reads (x1 000)		920.7	1342.8	810.5	681.7	1293.1	774.1	915.1	1155.9	1745.7	1777.5	1579.6	1682.4
Number of trimmed reads (x1 000)		890.3	1289.4	781.7	660.3	1252.7	743.0	884.7	1114.5	1690.0	1711.0	1528.3	1623.0
Maximum read length		301	301	301	301	301	301	301	301	301	301	301	301
Mean read length		204.1	215.5	199.6	215.9	213.7	204.7	201.0	206.3	211.1	211.7	208.9	203.7
Read insert size		295.7	311.9	293.8	316.2	306.3	298.6	292.6	301.0	305.2	300.2	299.8	295.6
Insert size StdDev		137.4	139.8	139.7	141.6	138.7	138.1	138.9	138.8	141.1	137.1	139.7	141.5
N50 (kbp)		270.6	91.1	192.4	135.2	135.3	153.1	172.4	169.0	226.4	181.2	191.7	223.3
N75 (kbp)		102.4	52.6	81.6	69.2	72.8	88.6	85.6	86.8	90.7	86.8	86.9	82.3

		Laboratory 49											
Parameters	**Ranges***	**5193**	**5086**	**5327**	**5311**	**5679**	**5378**	**5461**	**5671**	**5437**	**5888**	**5860**	**5992**
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 (%)		0.51	2.02	1.3	3.73	0.97	6.1	1.33	2.88	1.9	0.5	1.0	1.17
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	5.0	5.0	4.9	5.0	4.9	4.9	4.9	4.9	5.1	4.9	4.9	5.0
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	72	92	78	77	124	72	67	68	78	52	48	65
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}	93.8	97.4	92.9	122.0	55.3	78.9	79.8	75.6	128.9	42.1	43.5	59.2
Number of reads (x1 000)		1076.0	1177.7	1066.2	1422.3	564.0	909.6	918.7	829.4	1598.2	427.3	459.7	601.3
Number of trimmed reads (x1 000)		1038.3	1107.9	1023.0	1376.9	550.5	878.0	869.7	800.0	1554.8	412.1	436.9	585.5
Maximum read length		301	301	301	301	301	301	301	301	301	301	301	301
Mean read length		228.3	223.5	227.5	225.4	252.3	228.3	229.7	234.9	214.4	251.5	247.3	254.9
Read insert size		337.9	332.6	341.0	325.6	429.0	335.3	351.2	363.2	293.7	518.0	530.1	455.9
Insert size StdDev		125.5	121.7	124.9	121.5	164.6	128.3	127.3	132.7	120.7	178.8	185.4	171.7
N50 (kbp)		166.5	126.5	149.9	176.7	75.3	224.8	174.9	223.3	270.6	184.5	282.8	229.1
N75 (kbp)		77.8	69.2	81.6	75.6	41.7	75.2	87.0	89.5	82.3	99.8	177.7	90.9

		Laboratory 108											
Parameters	**Ranges***	**5187**	**5434**	**5495**	**5538**	**5554**	**5653**	**5664**	**5727**	**5939**	**5672**	**5838**	**5187**
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 (%)		2.5	2.14	2.12	2.74	3.45	1.81	2.78	2.1	3.51	2.3	2.1	2.5
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	4.9	4.9	4.8	4.9	4.9	5.0	4.9	5.0	4.9	4.9	4.9
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	431	415	320	332	434	295	365	285	381	338	293	431
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}	66.4	86.5	114.8	89.9	88.6	129.5	89.1	93.4	98.8	135.6	121.1	66.4
Number of reads (x1 000)		1037.7	1398.5	1721.0	1375.5	1408.0	1958.2	1384.1	1546.8	1615.7	2136.1	1888.2	1037.7
Number of trimmed reads (x1 000)		955.0	1296.7	1613.5	1267.4	1299.6	1825.5	1284.5	1437.1	1488.2	1998.2	1767.0	955.0
Maximum read length		30	356	355	358	359	358	353	279	282	279	282	30
Mean read length		220.7	213.5	226.1	221.4	220.4	224.0	219.9	204.3	213.7	222.4	216.8	220.7
Read insert size		NA	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	NA
N50 (kbp)		18.9	19.5	24.8	24.5	18.9	31.1	23.5	32.1	20.2	23.5	27.9	18.9
N75 (kbp)		10.0	10.6	13.3	14.4	10.2	14.7	12.2	17.6	11.7	13.3	15.7	10.0

		Laboratory 129											
Parameters	Ranges*	5088	5127	5153	5052	5418	5426	5478	5571	5871	5852	5932	5844
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 (%)		7.09	1.92	1.66	2.5	1.18	1.24	1.17	3.73	0.99	0.57	0.22	1.28
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	4.9	5.0	4.9	4.9	4.9	4.9	5.0	4.9	4.9	5.0	5.1
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	87	109	88	75	70	67	63	79	73	70	67	73
Number of contigs [0–25] x minimum coverage	{<1 000}	2	3	0	2	0	0	0	2	1	1	0	0
Average coverage	{>50}	70.4	67.4	70.9	83.7	118.2	114.2	108.9	83.0	53.8	93.0	82.9	96.1
Number of reads (x1 000)		1275.4	1224.9	1286.7	1533.6	2059.9	1982.0	1892.6	1453.6	929.3	1618.8	1444.1	1725.6
Number of trimmed reads (x1 000)		1233.0	1175.1	1235.8	1442.6	2022.8	1951.9	1861.9	1429.4	914.1	1581.0	1423.5	1688.9
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		145.1	144.3	144.7	143.7	146.5	146.7	146.5	146.7	146.7	145.9	146.8	146.2
Read insert size		461.2	446.6	430.9	483.4	434.7	409.7	425.0	471.8	439.3	450.7	436.2	426.1
Insert size StdDev		164.2	158.6	157.9	169.9	166.2	164.0	163.9	166.7	171.4	164.7	172.0	160.9
N50 (kbp)		113.6	90.3	111.8	161.3	221.4	199.7	232.0	132.1	164.9	130.1	149.4	184.8
N75 (kbp)		72.9	53.0	75.5	90.6	90.7	82.2	102.0	75.4	82.2	81.3	78.0	82.2

		Laboratory 134											
Parameters	Ranges*	5072	5079	5098	5237	5145	5161	5594	5427	5430	5440	5278	5608
Number of genera detected	{1}	1	1	1	1	1	1	1	2	1	1	1	1
Detected species	*{Se}*	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se
Unclassified reads (%)		1.36	1.44	1.81	1.44	2.7	6.97	4.24	8.18	1.42	0.61	1.72	1.53
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	4.9	5.0	4.9	5.0	4.9	0.1	4.9	4.9	4.9	5.0	4.9
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	97	95	91	67	90	68	15	74	83	94	95	70
Number of contigs [0–25] x minimum coverage	{<1 000}	2	1	0	0	0	0	156	0	1	1	0	0
Average coverage	{>50}	58.8	57.4	64.9	45.5	73.7	75.7	22.3	62.0	73.8	64.4	79.1	46.9
Number of reads (x1 000)		1003.8	978.9	1124.9	779.0	1288.2	1339.8	391.2	1124.6	1262.4	1105.9	1396.5	799.5
Number of trimmed reads (x1 000)		996.1	971.6	1118.7	773.9	1279.5	1331.7	387.5	1116.5	1254.0	1099.7	1386.9	793.2
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		147.7	147.6	147.0	147.6	147.2	146.5	143.9	147.5	147.6	147.6	147.2	146.5
Read insert size		428.9	436.0	373.6	363.0	365.1	323.8	443.9	369.3	380.7	387.4	351.2	364.5
Insert size StdDev		140.9	142.2	135.8	135.1	129.3	133.5	171.0	136.0	130.0	128.0	127.9	132.2
N50 (kbp)		96.5	104.6	108.8	223.6	134.3	247.1	57.9	174.2	164.1	102.2	154.4	130.1
N75 (kbp)		51.5	52.9	69.0	90.8	69.0	102.0	28.2	86.7	82.0	58.5	75.5	75.5

		Laboratory 142											
Parameters	**Ranges***	**5263**	**5146**	**5342**	**5552**	**5596**	**5557**	**5810**	**5689**	**5804**	**5862**	**5626**	**5991**
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 (%)		1.6	0.93	0.86	2.16	0.9	1.13	0.45	1.24	1.01	0.58	3.85	1.25
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.2	4.9	4.9	4.9	4.9	5.1	4.3	5.0	4.9	4.9	5.0	5.0
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.6	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	106	52	66	54	52	78	94	61	54	60	53	70
Number of contigs [0–25] x minimum coverage	{<1 000}	38	0	1	0	0	4	27	0	0	1	1	0
Average coverage	{>50}	33.3	49.6	60.9	60.9	49.7	51.4	33.1	67.1	63.1	46.3	73.1	48.3
Number of reads (x1 000)		371.5	532.7	654.3	658.0	534.2	567.9	382.8	728.4	674.9	504.5	802.1	530.9
Number of trimmed reads (x1 000)		351.7	518.6	636.7	640.1	519.5	551.8	359.2	712.9	663.3	484.4	784.6	513.1
Maximum read length		251	251	251	251	251	251	251	251	251	251	251	251
Mean read length		231.9	238.1	238.2	237.6	238.2	237.9	230.0	237.5	237.1	235.3	235.3	236.9
Read insert size		610.8	524.8	488.9	468.9	513.1	528.5	629.4	453.3	451.4	555.4	424.1	510.6
Insert size StdDev		203.9	181.0	175.8	181.5	181.3	182.7	208.7	177.1	174.2	186.7	168.7	184.4
N50 (kbp)		61.2	282.8	223.7	270.6	270.6	170.1	70.4	182.3	276.2	163.7	276.0	174.6
N75 (kbp)		36.2	135.2	90.8	135.2	135.2	74.3	42.5	90.7	100.0	86.9	135.2	108.4

		Laboratory 144											
Parameters	**Ranges***	**5143**	**5116**	**5148**	**5389**	**5307**	**5045**	**5462**	**5483**	**5579**	**5569**	**5806**	**5955**
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 (%)		2.94	1.09	1.45	1.07	4.37	1.03	1.38	1.8	1.86	0.41	0.87	1.29
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	5.0	4.9	5.1	4.9	5.0	4.9	5.0	4.9	5.0	5.0	4.9	4.9
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	61	56	73	59	60	55	67	58	68	60	53	60
Number of contigs [0–25] x minimum coverage	{<1 000}	4	4	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	56.3	60.6	48.4	56.7	45.6	127.4	62.4	57.2	38.9	55.3	62.3	48.4
Number of reads (x1 000)		676.7	700.9	572.5	645.8	542.5	1482.3	714.3	652.7	462.7	651.4	723.8	573.2
Number of trimmed reads (x1 000)		659.4	690.6	566.7	636.8	535.0	1452.5	704.9	642.9	457.4	643.1	706.7	566.7
Maximum read length		251	251	251	251	251	251	251	251	251	251	251	251
Mean read length		219.5	224.8	221.5	224.9	217.7	225.6	226.8	222.7	216.6	219.2	221.4	215.9
Read insert size		329.1	349.8	323.5	358.2	306.2	374.0	363.0	347.9	314.8	318.2	333.5	301.1
Insert size StdDev		126.5	138.1	125.4	139.4	112.1	146.5	139.0	138.0	117.8	122.0	121.2	111.5
N50 (kbp)		239.1	241.6	223.3	270.6	241.6	282.9	217.1	282.8	181.2	204.7	186.0	270.6
N75 (kbp)		135.2	135.2	88.2	135.2	90.7	149.9	91.2	174.9	85.2	93.1	101.3	135.2

		Laboratory 147											
Parameters	Ranges*	5248	5456	5308	5607	5524	5313	5346	5668	5728	5776	5778	5248
Number of genera detected	{1}	1	1	1	1	1	1	1	3	1	1	2	1
Detected species	*{Se}*	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se	Se
Unclassified reads (%)		3.32	1.65	2.09	2.33	1.35	3.52	2.08	13.26	2.12	2.17	10.57	3.32
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	0.0	4.9	4.8	4.9	5.0	5.0	4.9	5.0	5.1	4.9	4.9	0.0
Length [0–25] x min. coverage (Mbp)	{<0.25}	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.7
Number of contigs at 25 x minimum. coverage	{>0}	7	126	208	88	104	149	156	152	122	88	139	7
Number of contigs [0–25] x minimum coverage	{<1 000}	515	3	17	0	1	4	1	1	1	1	2	515
Average coverage	{>50}	14.8	36.4	39.2	67.5	66.0	57.2	54.9	55.5	78.7	81.2	60.6	14.8
Number of reads (x1 000)		194.4	413.0	451.0	852.5	841.3	673.0	637.3	919.6	984.3	1007.8	938.4	194.4
Number of trimmed reads (x1 000)		176.1	401.4	427.2	824.9	806.5	650.6	611.7	890.1	917.3	969.9	906.4	176.1
Maximum read length		301	301	301	301	301	301	301	301	301	301	301	301
Mean read length		204.7	225.6	227.0	203.4	206.5	221.6	224.0	213.5	221.6	210.7	188.8	204.7
Read insert size		418.3	323.9	394.2	279.9	284.6	317.1	336.8	310.9	297.8	289.0	262.5	418.3
Insert size StdDev		189.2	130.7	148.2	118.7	115.5	122.3	126.5	124.8	111.9	114.0	109.4	189.2
N50 (kbp)		14.6	79.2	35.6	113.2	99.0	58.7	52.2	72.1	82.1	149.8	72.0	14.6
N75 (kbp)		8.0	38.2	22.2	56.8	58.6	34.2	29.9	32.8	48.1	57.1	34.2	8.0

		Laboratory 148											
Parameters	Ranges*	5021	5017	5235	5486	5406	5555	5578	5710	5794	5835	5420	5142
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 (%)		1.28	2.01	1.8	1.74	1.58	0.76	1.24	1.03	0.5	1.06	2.49	1.32
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	5.0	4.9	5.0	4.9	4.9	4.9	4.7	4.8	4.9	4.9	4.9
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	126	76	102	98	117	74	90	165	144	168	108	86
Number of contigs [0–25] x minimum coverage	{<1 000}	7	1	1	2	4	1	2	27	30	22	0	2
Average coverage	{>50}	74.0	144.3	138.7	81.0	101.8	122.7	112.6	59.5	64.5	61.3	100.1	126.2
Number of reads (x1 000)		1395.8	2996.6	2758.7	1580.9	2038.1	2377.7	2212.7	1089.9	1219.5	1162.6	1954.6	2560.1
Number of trimmed reads (x1 000)		1381.8	2962.0	2728.6	1564.7	2014.1	2352.3	2189.0	1080.9	1208.8	1152.0	1934.3	2532.6
Maximum read length		151	151	151	151	151	151	151	151	151	151	151	151
Mean read length		147.0	146.3	146.8	146.4	146.5	147.2	147.1	147.9	148.0	148.0	147.2	147.1
Read insert size		341.7	320.0	366.1	323.4	352.4	337.2	340.7	380.8	399.7	405.1	335.4	352.7
Insert size StdDev		84.0	76.7	91.7	80.0	88.5	79.9	81.4	102.0	105.6	105.9	80.6	86.7
N50 (kbp)		76.8	174.4	92.8	108.8	89.1	124.3	131.2	49.8	56.7	54.6	96.4	114.3
N75 (kbp)		42.0	81.4	55.4	68.1	49.5	77.3	75.5	26.5	28.5	25.9	54.2	72.9

		Laboratory 149											
Parameters	**Ranges***	**5095**	**5167**	**5214**	**5514**	**5226**	**5587**	**5211**	**5610**	**5654**	**5676**	**5999**	**5667**
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 (%)		1.57	1.72	3.59	1.33	1.79	1.13	1.56	1.63	1.15	2.03	2.05	2.04
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	5.0	5.0	4.9	4.9	5.0	4.9	5.1	4.9	4.9	5.0	5.0
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of contigs at 25 x minimum. coverage	{>0}	55	65	56	51	60	58	57	71	55	57	60	66
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	0	0	0	4	0	0	0	0	0
Average coverage	{>50}	110.6	101.9	111.2	108.1	102.3	111.6	174.4	102.6	131.7	131.7	143.8	131.9
Number of reads (x1 000)		1222.6	1129.6	1235.6	1188.5	1142.0	1262.7	2005.1	1173.1	1362.4	1358.7	1546.1	1419.9
Number of trimmed reads (x1 000)		1171.3	1077.8	1180.4	1134.8	1092.2	1201.7	1932.3	1124.6	1302.1	1297.2	1473.2	1356.1
Maximum read length		301	301	301	301	301	301	301	301	301	301	301	301
Mean read length		234.8	237.9	234.6	234.3	232.6	232.6	225.7	232.9	251.5	249.8	245.3	244.3
Read insert size		352.3	358.9	351.9	352.3	351.6	348.5	316.4	345.6	379.4	376.0	368.7	367.6
Insert size StdDev		142.7	146.1	144.5	144.1	143.9	141.4	136.4	138.8	159.0	154.8	154.4	152.5
N50 (kbp)		270.6	223.3	270.6	191.7	270.6	222.8	282.8	270.6	270.6	270.6	270.6	223.3
N75 (kbp)		149.9	91.8	135.2	101.4	105.0	105.0	149.7	90.9	149.9	135.2	135.2	108.4

		Laboratory 150											
Parameters	**Ranges***	**5341**	**5368**	**5415**	**5442**	**5625**	**5666**	**5669**	**5706**	**5874**	**5719**	**5981**	**5907**
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 (%)		1.27	2.05	1.84	1.07	1.75	1.69	1.3	2.59	1.5	1.29	0.4	4.04
Length at 25 x min. coverage (Mbp)	{>4.5 ∧ <5.3}	4.9	4.9	4.9	4.9	5.0	5.0	4.9	4.9	4.9	4.9	3.8	5.0
Length [0–25] x min. coverage (Mbp)	{<0.25}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0
Number of contigs at 25 x minimum. coverage	{>0}	63	66	82	66	75	87	70	70	67	66	54	70
Number of contigs [0–25] x minimum coverage	{<1 000}	0	0	0	0	0	0	0	0	0	0	14	0
Average coverage	{>50}	68.5	70.0	71.8	62.8	55.4	47.8	58.9	57.3	53.4	81.6	31.7	55.5
Number of reads (x1 000)		1721.5	1745.9	1830.0	1567.9	1405.5	1229.9	1478.2	1444.0	1345.1	2055.4	799.8	1411.4
Number of trimmed reads (x1 000)		1721.5	1745.9	1830.0	1567.9	1405.5	1229.9	1478.2	1444.0	1345.1	2055.3	799.8	1411.4
Maximum read length		101	101	101	101	101	101	101	101	101	101	101	101
Mean read length		99.7	99.6	99.5	99.3	99.6	99.6	99.7	99.7	99.4	99.7	99.6	99.5
Read insert size		396.1	388.0	348.6	346.3	362.2	382.4	401.3	392.5	353.3	384.7	360.2	353.5
Insert size StdDev		179.8	192.5	182.2	197.9	186.1	173.4	180.6	179.2	193.5	177.9	187.2	184.6
N50 (kbp)		239.1	220.3	154.0	149.9	129.4	164.5	223.1	232.8	223.0	223.3	157.9	223.3
N75 (kbp)		104.4	101.4	86.6	82.2	82.2	82.9	90.5	101.6	90.5	90.5	83.2	90.5

Se: Salmonella enterica
NA: not analysed.

Annex 19. EQA-9 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-9 2018

Dear Participant
Welcome to the ninth External Quality Assessment (EQA-9) scheme for typing of Salmonella in 2018-2019.
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

(State one answer only)

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

3. Institute name

4. Laboratory name

5. Laboratory ID

Consisting of country code (two letters) Lab ID on the vial e.g. DK_SSI

6. E-mail

7. Multiple-Locus Variable number of tandem repeats Analysis (MLVA)

8. Submitting results

(State one answer only)

- Online here (please fill in the isolate ID´s in the following section) - Go to 9
- Did not participate in the MLVA part - Go to 14

9. Select method

(State one answer only)

- S. Typhimurium and S. Enteritidis - Go to 10
- Only S. Typhimurium - Go to 10
- Only S. Enteritidis - Go to 12

10. MLVA isolate ID's

Please enter the MLVA isolate ID (4 digits)

	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
S. Typhimurium										

11. Results for MLVA S. Typhimurium - Allele profile

Please use -2 for not detected

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

12. MLVA isolate ID´s

Please enter the MLVA isolate ID (4 digits)

	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
S. Enteritidis										

13. Results for MLVA S. Enteritidis - Allele profile

Please use -2 for not detected

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

14. Submitting Cluster results

(State one answer only)

- Cluster analyses based on PFGE / MLVA / WGS - Go to 15
- Did not participate in the Cluster part - Go to 125

15. Cluster isolate ID´s

Please enter the cluster isolate ID (4 digits)

	Cluster isolate ID
Isolate 1	
Isolate 2	
Isolate 3	
Isolate 4	
Isolate 5	
Isolate 6	
Isolate 7	
Isolate 8	
Isolate 9	
Isolate 10	
Isolate 11	
Isolate 12	

16. Submitting Cluster results

(State one answer only)

- Cluster analysis based on PFGE - Go to 17
- Do not wish to submit any cluster results based on PFGE analysis - Go to 22

17. Cluster analysis based on PFGE data

18. Please list the ID for the isolates included in the cluster of closely related isolates detected by PFGE (bands >33kb used):

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

19. Select a representative isolate with the cluster profile detected by PFGE:

Indicate the isolate ID

20. Total number of bands (>33kb) in the selected representative cluster isolate

21. 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 isolate (>33kb)
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		
Isolate 11		
Isolate 12		

22. Submitting cluster results

(State one answer only)

- Cluster analysis based on MLVA – Go to 23
- Do not wish to submit any cluster results based on MLVA analysis – Go to 33

23. Cluster analysis based on MLVA data

24. Please list the ID for the isolates included in the cluster of closely related isolates detected by MLVA:

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

25. MLVA scheme used:

Please indicate serovar and/or protocol

26. Please list the loci in scheme used

27. Locus 1:

28. Locus 2:

29. Locus 3:

30. Locus 4:

31. Locus 5:

32. 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					

33. Submitting cluster results

(State one answer only)

- Cluster analysis based on WGS data – Go to 34
- Do not wish to submit any cluster results based on WGS data - Go to 125

34. Cluster analysis based on WGS data

35. 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

(State one answer only)

- SNP based – Go to 37
- Allele based – Go to 44
- Other – Go to 36

36. If another analysis is used please describe your approach: - Go to 51

37. Please report the used SNP-pipeline

(reference if publicly available or in-house pipeline)

38. Please select the approach used for the SNP analysis

(State one answer only)

- Reference based – Go to 39
- Assembly based – Go to 42

39. Reference genome used

Please indicate Multil-locus Sequence Type (e.g. ST34) and isolate ID (e.g. one of the isolates from the current cluster, ID of a public reference strain or an in-house isolate)

40. Please indicate the read mapper used

(e.g. BWA, Bowtie2)

41. Please indicate the variant caller used

(e.g. SAMtools, GATK)

42. Please indicate the assembler used

(e.g. SPAdes, Velvet)

43. Please specify the variant caller used

(e.g. NUCMER)

44. Please select tools used for the allele analysis

(State one answer only)

- BioNumerics – Go to 46
- SeqSPhere – Go to 46
- Enterobase – Go to 46
- Other – Go to 45

45. If another tool is used please enter here:

46. Please indicate allele calling method:

- Assembly based and mapping based – Go to 47
- Only assembly based – Go to 47
- Only mapping based – Go to 48

47. Please indicate the assembler used

(e.g. SPAdes, Velvet)

48. Please select scheme used for the allele analysis

(State one answer only)

- Applied Math (wgMLST) – Go to 50
- Applied Math (cgMLST/Enterobase) – Go to 50
- Enterobase (cgMLST) – Go to 50
- Other – Go to 49

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

50. Please report the number of loci in the used allelic scheme

51. 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 isolates in the cluster detected with the additional analysis.

52. Please list the ID´s for the isolates included in the cluster of closely related isolates:

Please use semicolon (;) to separate ID´s

53. Select a representative isolate in the cluster

Indicate the isolate ID

54. Results for cluster analysis (e.g. SNP or Allele based)

Please use 9999 for not analysed

	ST	Distance (e.g. SNP) to the selected cluster isolate
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		
Isolate 11		
Isolate 12		

55. 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

(State one answer only)

- Yes – Go to 56
- No – Go to 95

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

(State one answer only)

- SNP based – Go to 58
- Allele based – Go to 65
- Other – Go to 57

57. If another analysis is used please describe your approach: - Go to 72

58. Please report the used SNP-pipeline

(reference if publicly available or in-house pipeline)

59. Please select the approach used for the SNP analysis

- Reference based – Go to 60
- Assembly based – Go to 63

60. Reference genome used

Please indicate Multi-locus Sequence Type (e.g. ST34) and isolate ID (e.g. one of the isolates from the current cluster, ID of a public reference strain or an in-house isolate)

61. Please indicate the read mapper used

(e.g. BWA, Bowtie2)

62. Please indicate the variant caller used

(e.g. SAMtools, GATK)

63. Please indicate the assembler used

(e.g. SPAdes, Velvet)

64. Please specify the variant caller used

(e.g. NUCMER)

65. Please select tool used for the allele analysis

- BioNumerics – Go to 67
- SeqSPhere – Go to 67
- Enterobase – Go to 67
- Other – Go to 66

66. If another tool is used please list here:

67. Please indicate allele calling method:

(State one answer only)

- Assembly based and mapping based - Go to 68
- Only assembly based - Go to 68
- Only mapping based - Go to 69

68. Please indicate the assembler used

(e.g. SPAdes, Velvet)

69. Please select scheme used for the allele analysis

(State one answer only)

- Applied Math (wgMLST) – Go to 71
- Applied Math (cgMLST/Enterobase) – Go to 71
- Enterobase (cgMLST) – Go to 71
- Other – Go to 70

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

71. Please report the number of loci in the used allelic scheme

72. Additional analysis on data derived from WGS

73. Select a representative isolate in the cluster detected by the additional analysis'

Indicate the isolate ID

74. Results for the additional cluster analysis (e.g. SNP or Allele based)

Please use 9999 for not analysed

	ST	Distance (e.g. SNP) to the selected cluster isolate
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		
Isolate 11		
Isolate 12		

75. 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

(State one answer only)

- Yes – Go to 76
- No – Go to 95

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

- SNP based – Go to 78
- Allele based – Go to 85
- Other – Go to 77

77. If another analysis is used please describe your approach: - Go to 92

78. Please report the used SNP-pipeline

79. Please select the approach used for the SNP analysis

- Reference based – Go to 80
- Assembly based – Go to 83

80. Reference genome used

Please indicate Multi-locus Sequence Type (e.g. ST34) and isolate ID (e.g. one of the isolates from the current cluster, ID of a public reference strain or an in-house isolate)

81. Please indicate the read mapper used

(e.g. BWA, Bowtie2)

82. Please indicate the variant caller used

(e.g. SAMtools, GATK)

83. Please indicate the assembler used

(e.g. SPAdes, Velvet)

84. Please specify the variant caller used

(e.g. NUCMER)

85. Please select tool used for the allele analysis

(State one answer only)

- BioNumerics - Go to 87
- SeqSPhere - Go to 87
- Enterobase - Go to 87
- Other - Go to 86

86. If another tool is used please enter here:

87. Please indicate allele calling method:

- Assembly based and mapping based - Go to 88
- Only assembly based - Go to 88
- Only mapping based - Go to 89

88. Please indicate the assembler used

(e.g. SPAdes, Velvet)

89. Please select scheme used for the allele analysis

- Applied Math (wgMLST) - Go to 91
- Applied Math (cgMLST/Enterobase) - Go to 91
- Enterobase (cgMLST) - Go to 91
- Other - Go to 90

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

91. Please report the number of loci in the used allelic scheme

92. Third analysis on data derived from WGS

93. Select a representative isolate in the cluster detected by the third analysis

Indicate the isolate ID

94. Results for the third cluster analysis (e.g. SNP or Allele based)

Please use 9999 for not analysed

	ST	Distance (e.g. SNP) to the selected cluster isolate
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		
Isolate 11		
Isolate 12		

95. Additional questions to the WGS part

96. Where was the sequencing performed

- In own laboratory
- Externally

97. Protocol used to prepare the library for sequencing:

(State one answer only)

- Commercial kits - Go to 98
- Commercial kits - Go to 98

98. Please indicate name of commercial kit:

99. If relevant please list deviation from commercial kit shortly in few bullets: - Go to 101

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

101. The sequencing platform used

(State one answer only)

- Ion Torrent PGM - Go to 103
- Ion Torrent Proton - Go to 103
- Genome Sequencer Junior System (454) - Go to 103
- Genome Sequencer FLX System (454) - Go to 103
- Genome Sequencer FLX+ System (454) - Go to 103
- PacBio RS - Go to 103
- PacBio RS II - Go to 103
- HiScanSQ - Go to 103
- HiSeq 1000 - Go to 103
- HiSeq 1500 - Go to 103
- HiSeq 2000 - Go to 103
- HiSeq 2500 - Go to 103
- HiSeq 4000 - Go to 103
- Genome Analyzer Iix - Go to 103
- MiSeq - Go to 103
- MiSeq Dx - Go to 103
- MiSeq FGx - Go to 103
- ABI SOLiD - Go to 103
- NextSeq - Go to 103
- MinION (ONT) - Go to 103
- Other - Go to 102

102. If another platform is used please list here:

103. Criteria used to evaluate the quality of sequence data

In this section you can report criteria used to evaluate the quality of sequence data.

Please first reply on the use of 5 selected criteria, which were the most frequently reported by the participants in the Salmonella EQA-8 scheme, 2017.

Next you will be asked to report 5 additional criteria of your own choice.

For each criteria please also report the threshold or procedure used to evaluate the current criteria.

104. Did you use confirmation of organism to evaluate the quality of sequence data?

(State one answer only)

- Yes
- No – Go to 106

105. Procedure used to evaluate confirmation of organism:

106. Did you use coverage to evaluate the quality of sequence data?

(State one answer only)

- Yes
- No – Go to 108

107. Procedure or threshold used for coverage:

108. Did you evaluate assembly quality?

(State one answer only)

- Yes
- No – Go to 110

109. Procedure used to evaluate assembly quality:

110. Did you use assembly length to evaluate the quality of sequence data?

(State one answer only)

- Yes
- No – Go to 112

111. Procedure or threshold used for assembly length:

112. Did you evaluate allele calling result?

(State one answer only)

- Yes
- No – Go to 114

113. Procedure used to evaluate allele calling:

114. Other criteria used to evaluate the quality of sequence data

Please list up to 5 additional criteria (e.g. N50, read length, contamination)

115. Other criteria used to evaluate the quality of sequence data - additional criteria 1:

116. Threshold or procedure used to evaluate the additional criteria 1:

117. Other criteria used to evaluate the quality of sequence data - additional criteria 2:

118. Threshold or procedure used to evaluate the additional criteria 2:

119. Other criteria used to evaluate the quality of sequence data - additional criteria 3:

120. Threshold or procedure used to evaluate the additional criteria 3:

121. Other criteria used to evaluate the quality of sequence data - additional criteria 4:

122. Threshold or procedure used to evaluate the additional criteria 4:

123. Other criteria used to evaluate the quality of sequence data - additional criteria 5 :

124. Threshold or procedure used to evaluate the additional criteria 5:

125. Comment(s):

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

126. Thank you for your participation

Thank you for filling out the Submission form for the Salmonella EQA-9.

We highly recommend to document this Submission form by printing it. You will find the Print option after pressing the "Options" button.

Important: After pressing "Submit results" you will no longer be able to edit or print your information.

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