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TECHNICAL REPORT

Tenth external quality assessment scheme for typing of Shiga toxin-producing *Escherichia coli*

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Taina Niskanen (ECDC, Emerging, Food- and Vector-borne Diseases Programme) and produced by Susanne Schjørring, Louise Gade Dahl, Gitte Sørensen, Kristoffer Kiil, Flemming Scheutz, and Eva Møller Nielsen of the Foodborne Infections Unit at Statens Serum Institut, Copenhagen, Denmark.

Suggested citation: European Centre for Disease Prevention and Control. External quality assessment scheme for typing of Shiga toxin-producing *Escherichia coli*. Stockholm: ECDC; 2021.

Stockholm, November 2021

ISBN 978-92-9498-557-6
DOI 10.2900/581918
Catalogue number TQ-01-21-438-EN-N

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Abbreviations

aaiC	*aai* chromosomal Type VI Secretion System
AEEC	Attaching and effacing *E. coli*
aggR	Gene encoding the master regulator in enteroaggregative *E. coli*
BN	BioNumerics
bp	Base pair
cgMLST	Core genome multilocus sequence typing
eae	The intimin gene
EAEC	Enteroaggregative *E. coli*
EFSA	European Food Safety Authority
EQA	External quality assessment
EURL	European Union Reference Laboratory
FWD	Food- and waterborne diseases
FWD-Net	Food- and Waterborne Diseases and Zoonoses Network
GFN	Global Foodborne Infections Network, Food Safety
HUS	Haemolytic uraemic syndrome
ND	Not done
NSF	Non-sorbitol fermenter
NT	Non-typeable
PFGE	Pulsed-field gel electrophoresis
NPHRL	National public health reference laboratory
QC	Qualitative control
SF	Sorbitol fermenting
SKESA	Strategic k-mere extension for scrupulous assemblies
SNP	Single nucleotide polymorphism
SOP	Standard operating procedure
SPAdes	St. Petersburg genome assembler
SSI	Statens Serum Institut
ST	Sequence type
STEC	Shiga toxin-producing *E. coli* (synonymous with verocytotoxin-producing *E. coli;* VTEC)
Stx1	Shiga toxin 1
stx1	Gene encoding Shiga toxin 1
Stx2	Shiga toxin 2
stx2	Gene encoding Shiga toxin 2
TESSy	The European Surveillance System
wgMLST	Whole genome multilocus sequence typing
WGS	Whole genome sequencing

Executive summary

This report presents the results of the 10th round of the external quality assessment (EQA-10) scheme for typing of Shiga toxin-producing *Escherichia coli* (STEC). This EQA was organised for national public health reference laboratories (NPHRLs) providing data to the Food- and Waterborne Diseases and Zoonoses Network (FWD-Net) managed by the European Centre for Disease Prevention and Control (ECDC). Since 2012, the unit of Foodborne Infections at Statens Serum Institut (SSI) in Denmark has arranged this EQA under a framework contract with ECDC. EQA-10 contains serotyping, detection of virulence genes, and molecular typing-based cluster analysis.

Human STEC infection is a zoonotic disease. In 2019, the disease had an EU notification rate of 2.2 cases per 100 000 population, with a similar rate in 2018. The most commonly reported STEC O group was O157 (26.6% of cases with known O group).

Since 2007, ECDC has been responsible for the EU-wide surveillance of STEC, including facilitating the detection and investigation of food-borne outbreaks. Surveillance data, including basic typing parameters and molecular typing data for the isolated pathogen, are reported by Member States to The European Surveillance System (TESSy). The surveillance system relies on the capacity of NPHRLs in FWD-Net providing data to produce comparable typing results. To ensure that the EQA is linked to the development of surveillance methods used by NPHRLs, a molecular typing-based cluster analysis using either pulsed-field gel electrophoresis (PFGE) and/or whole genome sequencing (WGS)-derived data has been included since EQA-8. The quality assessment of PFGE as performed in EQA-4 to-7 has been excluded.

The objectives of the EQA are to assess the quality and comparability of typing data reported by NPHRLs participating in FWD-Net. Test isolates for the EQA were selected to cover strains currently relevant to public health in Europe and represent a broad range of clinically relevant types of STEC. Two separate sets of 10 test isolates were selected for serotyping/virulence profile determination and molecular typing-based cluster analysis respectively.

Twenty-seven laboratories registered and 26 completed the exercise, representing a small increase in participation of 10% from the EQA-9.

The full O:H serotyping was performed by 62% (16/26) of participating laboratories, with an average score of 94%. In general, the more common European serotypes generated the highest scores, e.g. 100% for O157:H7 isolates, while the less frequent O5:H19 and O80:H2 obtained an average score of 88%. Notably, not all laboratories demonstrated the capacity to determine all O groups and H types and participation in H typing was low (16/26). A shift towards the WGS-based method was observed for O grouping, 52% (11/21) compared to 50% in EQA-9 and only 26% EQA-8.

The quality of the virulence profile determination results was generally good, with high average scores of 98%, 100%, and 99% for *eae, stx1*, and *stx2*, respectively, similar to previous EQAs. The participants identified the true enteroaggregative *E. coli* (EAEC) isolate by correctly reporting the presence of the *aaiC* and/or *aggR* for 19/21 laboratories (90%). However, the additional isolate with an *aaiC* variant was identified by 10/21 laboratories, as the variant is only detectable by WGS (virulence finder) or dot blot hybridisation but not by PCR using Boisen et al. 2008 primers (see section 3.4.3 and Annex 23 in ECDC's report of STEC EQA-6 [20]). Correct subtyping of *stx1* and *stx2* obtained a combined average score of 97%, the highest score since EQA-4.

Out of the 26 laboratories participating in the EQA-10, 16 (62%) performed molecular typing-based cluster analysis using various methods. The purpose of the cluster analysis part of the EQA was to assess the NPHRL's ability to identify a cluster of genetically closely related isolates i.e. to correctly categorise the cluster test isolates regardless of the method used, not to follow a specific procedure.

The EQA-10 included one cluster of closely related isolates. The cluster contained four ST21 isolates, and the expected cluster was based on a predefined categorisation by the organiser using WGS. Fifteen laboratories used WGS as the only method, one laboratory participated using PFGE, and one laboratory participated using both PFGE and WGS derived data for cluster analysis. This was a decrease from eight participants in EQA-9 to two using PFGE in this EQA.

Performance was high among the 15 laboratories using WGS-derived data, with 14 (93%) participants correctly identifying the cluster of closely related isolates. In this EQA, participants were free to choose their preferred analytical method for the WGS-based cluster identification. An allele-based method was most frequently used; 87% (13/15) used core genome MultiLlocus Sequence Type (cgMLST) compared to 13% (2/15) using single nucleotide polymorphism (SNP) for the reported cluster analysis as the main analysis.

In general, for cgMLST the reported results were at a comparable level despite analysis with different approaches and allelic calling methods. Only two laboratories reported SNP results. They showed similar SNP distances, but the conclusion of isolates within the cluster (cut-off) were different and one of the laboratories did not identify the correct cluster. For inter-laboratory comparability and communication about cluster definitions, cgMLST using a

standard scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results, while the use of non-standardised SNP analysis may be more challenging. There are two main challenges: difficulty in comparing SNP with cgMLST results, and variations between SNP analyses in general, as demonstrated in this EQA, which makes the comparison and communication between laboratories difficult.

In this EQA, the EQA provider introduced an additional part to the molecular typing-based cluster analysis: an assessment of five EQA provided genomes. In an urgent outbreak situation, the sequence data available are not always of high quality, so this EQA-part was designed to mimic this situation. The participants assessed additional genomes, some of which were modified by the EQA provider in order to give a realistic view of different quality issues. Most of the participants successfully identified the genomes of high quality as a cluster isolate or a non-cluster isolate. The 10% contamination with *Klebsiella pneumonia* was identified by 9/15 and the inter-species contamination with a different *E. coli* was only detected by 4/15. The poor quality for one genome was observed by 13/15 laboratories.

1. Introduction

1.1 Background

ECDC is a European Union (EU) agency with a mandate to operate dedicated surveillance networks. The agency's mission is to identify, assess, and communicate about current and emerging threats to human health from communicable diseases. ECDC's founding regulation outlines its mandate as fostering the development of sufficient capacity within the EU/European Economic Area (EEA) network for diagnosis, detection, identification, and characterisation of infectious agents that may threaten public health. ECDC maintains 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 and uses an external organiser to assess the performance of laboratories on test samples supplied specifically for the quality assessment purpose.

ECDC has outsourced the organisation of EQA schemes for EU/ EEA countries in the disease networks. EQAs aim to identify areas for improvement in laboratory diagnostic capacities relevant for 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 purposes of EQA schemes are to:

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

Since 2012, the unit of Foodborne Infections at Statens Serum Institut (SSI), Denmark, has been the EQA provider for the three EQA schemes covering typing of *Salmonella enterica* ssp. *enterica*, Shiga toxin/verocytotoxin-producing *Escherichia coli (E. coli;* STEC/VTEC) and *L. monocytogenes*. In 2016, SSI was granted the new round of tenders (2017–2020) for all three schemes. For STEC, the EQA scheme no longer covers assessment of PFGE quality as was done in EQA-8 and EQA-9. However, it still covers serotyping, virulence profile determination, and molecular typing-based cluster analysis. This report presents the results of the 10th EQA scheme (STEC EQA-10).

1.2 Surveillance of STEC infections

STEC is a group of *E. coli* characterised by the ability to produce Shiga toxins (Stxs).

Human pathogenic STEC often harbour additional virulence factors important to the pathogenesis of the disease. A large number of serotypes of *E. coli* have been recognised as Stx producers. Notably, the majority of reported human STEC infections are sporadic cases. Symptoms associated with STEC infection in humans vary from mild diarrhoea to life-threatening haemolytic uraemic syndrome (HUS), which is clinically defined as a combination of haemolytic anaemia, thrombocytopenia and acute renal failure.

In 2019, the EU notification rate of STEC infections was 2.2 cases per 100 000 population, which was similar to 2018 (2.3 cases per 100,000 population). The total number of confirmed STEC infection cases was 7 775, a slight decrease from 2018 (n=8 161). Ten deaths due to STEC infection were reported, resulting in an EU case fatality of 0.21%. As in previous years, the most commonly reported STEC O group was O157 (26.6% of cases with known O group), followed by O26 [3].

One of ECDC's key objectives is to improve and harmonise the surveillance system in the EU to increase scientific knowledge of aetiology, risk factors, and burden of FWDs and zoonoses. Surveillance data, including some basic typing parameters for the isolated pathogen, are reported by Member States to TESSy. In addition to the basic characterisation of the pathogens isolated from human infections, there is public health value in using more discriminatory typing techniques for pathogen characterisation in the surveillance of food-borne infections. Since 2012, ECDC has enhanced EU surveillance by incorporating molecular typing data through isolate-based reporting. Three selected FWD pathogens were included: *Salmonella enterica* ssp. *enterica, L. monocytogenes*, and STEC/VTEC. The overall aims of integrating molecular typing into EU level surveillance are to:

- foster the rapid detection of dispersed international clusters/outbreaks;
- facilitate the detection and investigation of transmission chains and relatedness of isolates across Member States and contribution to global investigations;
- detect the emergence of new evolving pathogenic isolates;

- support investigations to trace the source of an outbreak and identify new risk factors; and
- aid the study of a particular pathogen's characteristics and behaviour in a community of hosts.

Molecular typing-enhanced surveillance gives Member State users access to EU-wide molecular typing data for the pathogens included. It also gives users 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 are part of a multinational cluster that may require cross-border response collaboration.

1.3 STEC characterisation

State-of-the-art characterisation of STEC includes O:H serotyping in combination with a few selected virulence genes, i.e. the two genes for production of Shiga toxin Stx1 (*stx1*) and Stx2 (*stx2*) and the intimin *(eae)* gene associated with attaching and effacing lesion of enterocytes, also seen in attaching and effacing non-STEC *E. coli* (AEEC), including enteropathogenic *E. coli* (EPEC). The combination of virulence genes and subtypes of toxin genes is clinically relevant. The *stx2a* in *eae*-positive STEC and the activatable *stx2d* subtype in *eae*-negative STEC appear to be highly associated with the serious sequela HUS [4–6]. In the recent Scientific Opinion by EFSA, analysis of the confirmed reported human STEC infections in the EU/EEA (2012–2017) reveals that all Stx toxin subtypes may be associated with some cases of severe illness defined as bloody diarrhoea, HUS and/or hospitalisation [7]. Understanding the epidemiology of stx subtypes is therefore important to prevent the risk of STEC infection and for the surveillance of STEC.

The recommended method for stx subtyping is a specific PCR [8]. STEC serotype O157:[H7] may be divided into two groups: non-sorbitol fermenters (NSF) and a highly virulent sorbitol fermenting (SF) variant of O157. STEC EQA-10 included O:H serotyping, detection of virulence genes (*eae, stx1* and *stx2*, including subtyping of *stx* genes) and *aaiC* and *aggR* genes specific for enteroaggregative *E. coli* (EAEC), and molecular typing-based cluster analysis.

1.4 Objectives

EQA schemes offer quality support for those NPHRLs that are performing molecular typing-enhanced surveillance and those implementing it in their surveillance system at national level.

1.4.1 Serotyping

The objectives of STEC serotyping in EQA-10 were to assess the ability to assign correct O groups and H types by using either serological (detection of somatic 'O' and flagellar 'H' antigens) or molecular typing methods (PCR or WGS).

1.4.2 Virulence profile determination

The objectives of the virulence gene determination of STEC EQA-10 were to assess the ability to assign the correct virulence profile; the presence/absence of *stx1*, *stx2*, *eae, aaiC* and *aggR* genes and subtyping of *stx* genes (*stx1a, stx1c* and *stx1d* and *stx2a* to *stx2g*).

1.4.3 Molecular typing-based cluster analysis

The objective of the molecular typing-based cluster analysis of STEC EQA-10 was to assess the ability to detect a cluster of closely related isolates. Laboratories could perform the analyses using PFGE or derived data from WGS. In addition, the participant was offered to assess extra genomes and determine whether the genomes were part of the defined cluster and describe their observations and considerations leading to the decision. The genomes were manipulated by the EQA provider. In the individual reports, this analysis was not evaluated and therefore not directly commented on, but will be summarised in this report.

2. Study design

2.1 Organisation

STEC EQA-10 was funded by ECDC and arranged by SSI following ISO/IEC 17043:2010 [9]. EQA-10 included serotyping, virulence gene determination, and a molecular typing-based cluster analysis, and was carried out between December 2019 and July 2020.

Invitations were emailed to ECDC contact points in FWD-Net (27 countries, which nominated laboratories to participate in the EQA rounds 2017-2020) by 6 December 2019 with a deadline to respond by 3 January 2020. In addition, invitations were sent to EU candidate and potential candidate countries Turkey and North Macedonia, which signed up to the STEC EQA rounds in 2017-2020. Each laboratory was asked to fill in the reason for participation or non-participation.

Twenty-seven NPHRLs in EU/EEA and EU candidate countries accepted the invitation to participate, and 26 submitted results (Annex 1). EQA test isolates were sent to participants from 26 February to 15 March 2019. In Annex 2, participation details in EQA-9 and EQA-10 are listed to give an overview of the trend in the number of participants. In addition, Annex 3 contains each laboratory's reason for participating or not participating. Nineteen self-funded laboratories were invited to participate in the EQA. Participants were asked to submit their results to an SFTP-site and complete the online form before the extended deadline (because of the COVID-19 pandemic) of 17 June 2020 (Annex 14). Two laboratories asked for the submission protocol to be sent again, two other laboratories were asked to submit the missing raw reads, and two laboratories were asked to remember to complete data online; one never submitted any data.

The EQA submission protocol, invitation letter, and an empty submission form were available on the website.

2.2 Selection of test isolates/genomes

Thirty-five test isolates were selected to fulfil the following criteria:

- represent commonly reported strains in Europe;
- remain stable during the preliminary test period at the organising laboratory; and
- include genetically closely related isolates.

The 35 selected isolates were analysed with the methods used in the EQA (serotyping and virulence profile determination or WGS) before and after having been re-cultured 10 times. All candidate isolates remained stable using these methods and the final test isolates and additional genomes were selected. The 10 test isolates (Table 1) for serotyping/detection of virulence gene were selected to cover different serotypes and *stx* subtypes relevant for the current epidemiological situation in Europe (Annexes 4-5). The 10 test isolates for cluster analysis were selected to include isolates with different or varying relatedness and different sequence types (ST21 and ST29). A set of technical duplicates was included in the cluster test isolates. (Annexes 6–7, 9-10). The characteristics of all the STEC test isolates are listed as 'Original/REF' in Annexes 4–10. For the additional five genomes, three were altered, one with reduced coverage and two mixed with either 10% different *Escherichia coli* or 10% *Klebsiella pneumonia*. Two of the five genomes were cluster isolates, one genome of acceptable quality and the other with reduced coverage as listed above.

Table 1. Characterisation of test isolates/genomes

Method	Number of test isolates/genomes	Characterisation	Annexes
Serotyping	10 isolates[#] REF1-REF10	O5:H19/H-, O27:H30, O80:H2, O91:H21, O104:H4/H-, O104:H7/H-, O111;H8, O145:H34, O154:H31, O157:H7/H-	4
Virulence profile determination	10 isolates[#] REF1-REF10	*stx1c stx2b, stx2b, eae stx2d, stx2d, stx1c, aaiC aggR* (x1), *eae stx1a aaiC, stx2a*, *eae stx2f, stx1d, eae stx1a*	5
Cluster analysis	10 isolates REF11-REF20	9 x ST21 (O26:H11 *stx1a*) and 1 x ST29 (O177:H11)	6–7, 9-10
	5 genomes REF19*, REF21-REF24	5 x ST21, O26:H11: The four modified: one with reduced coverage, one contaminated with 10% *E. coli*, one contaminated with 10% *Klebsiella pneumonia* and one without *stx* genes	13

[#]: same 10 isolates.
[*]: genome is modified by the EQA provider compared to the original REF.

2.3 Distribution of isolates/genomes

All test isolates were blinded and shipped on 26 February 2020 (23/27 packages). Three were sent on 5 March and one was delayed shipment until 4 May. The delay was caused by sending the package with UN2814 during the of COVID-19 pandemic. Letters stating the unique isolate IDs were included in the packages and distributed individually to the participants by email on the day of shipment as an extra precaution. Thirteen participants received the isolates within one to two days, 10 within five to eight days and four within 10 to 20 days after shipment, respectively. No participants reported damage to the shipment or errors in the unique isolate IDs.

On 6 March 2020, instructions for the submission of results procedure were emailed to the participants. This included the links to the online site for uploading sequences and downloading the additional genomes and the empty submission form.

2.4 Testing

In the serotyping part, 10 STEC isolates were tested to assess the participants' ability to obtain the correct serotype. The participants could perform conventional serological methods according to suggested protocol [10] or molecular-based serotyping (PCR or WGS). The results of serotypes were submitted in the online form.

The same set of isolates as used for serotyping analysis was used for the virulence profile. The analyses were designed to assess the participants' ability to obtain the correct virulence profile. The participants could choose to perform detection of *aaiC* and *aggR* (two EAEC associated genes), *eae* and *stx1* and *stx2,* as well as subtyping of *stx* genes *stx1 (stx1a, stx1c or stx1d)* and *stx2 (stx2a - stx2g)* according to suggested protocol [11]. The results were submitted in the online form.

In the molecular typing-based cluster analysis part, participants could perform the laboratory part using PFGE [12] or WGS-derived data. Participants were instructed to report the IDs of isolates included in the cluster of closely related isolates by method. If PFGE analysis was conducted, the participant reported the total number of bands and number of shared bands with a selected cluster representative isolate.

Laboratories performing WGS could use their own analysis pipeline for cluster analysis, e.g. single nucleotide polymorphism analysis (SNP-based) or whole/core genome Multi Locus Sequence Typing (wgMLST/cgMLST) (allele-based) and were asked to submit the isolates identified as a cluster of closely related isolates based on the analysis used. Laboratories could report results from up to three analyses (one main and up to two additional), but the detected cluster were required 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) to an SFTP site.

In addition, each participant could assess extra genomes (manipulated by the EQA provider) and determine whether the genomes were part of the defined cluster (Yes/No) and describe their observations and considerations leading to the decision.

2.5 Data analysis

As the participating laboratories submitted their results, the serotype, virulence profile, and cluster analysis results, as well as the participants' uploaded raw reads, were imported to a dedicated STEC EQA-10 BioNumerics (BN) database. If errors in the submission process were identified, the EQA provider reported this to participants, thereby obtaining analysable results. The EQA provider was in contact with two participants in order to ensure sequences were uploaded to the SFTP site.

Serotyping results were evaluated according to the percentage of correct results, generating a score from 0–100% for O group, H type and O:H serotype.

The virulence profile determination results were evaluated according to the percentage of correct results, generating a score from 0–100% for *eae*, *aaiC*, *aggR*, *stx1*, *stx2*, subtyping of *stx1* and *stx2* and combined subtype (Table 1).

Molecular typing-based cluster analysis was evaluated according to correct or incorrect identification of the expected cluster of closely related isolates based on a pre-defined categorisation by the organiser. The EQA provider's WGS-derived cluster analysis was based on allele-based cgMLST [13] and SNP analysis (NASP) [14]. The cluster categorisation is based on WGS data and the correct cluster delineation might be difficult to obtain by the use of less discriminatory methods, e.g. PFGE. The cluster contained four ST21 isolates: REF11, REF15, REF19 and REF20 (REF11 and REF15 were technical duplicates). The EQA provider found at most two allele differences or three SNPs between any two isolates in the cluster. The rest of the cluster test isolates were additional five ST21s and one ST29.

The participants' descriptions of the manipulated genomes are listed in Annex 13. This analysis was not commented on in the individual reports but will be summarised in this report.

Individual evaluation reports were distributed to participants in September 2020 and certificates of attendance in October 2020. 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

Laboratories could either participate in the full EQA scheme or one part only (serotyping, virulence profile determination or molecular typing-based cluster analysis). Of the 27 participants who signed up, 26 completed and submitted their results. Just over half of the participants (57%; 15/26) completed the EQA with at least one analysis in each of the three parts. In total, 21 (81%) participants participated in serotyping, 25 (96%) participated in the detection of one or more of the virulence genes and 16 (62%) in cluster analysis. (Table 2).

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

	Serotyping[1]	Virulence profile determination[2]	Cluster analysis[3]
Number of participants	21	25	16
% of participants	81*	96*	62*

[1]: O grouping and/or H typing.
[2]: detection of at least one gene (aaiC, aggR, eae, stx1 and stx2) and/or subtyping of stx1 and stx2.
[3]: molecular typing-based cluster analyses based on PFGE or WGS-derived data.
**: percentage of the total number (26) of participating laboratories.*

O grouping results were provided by 21 participants (81%) and H typing results were provided by 16 (62%). Just over half 11/21 (52%) used molecular-based serotyping (only one reported PCR-based method) (Annex 4). Almost all participants (96%, 25/26) participated in the detection of virulence genes *stx1* and *stx2*. Slightly fewer 92% (24/26) participated in the detection of *eae*. Detection of enteroaggregative genes *aaiC* and *aggR* were reported by 81% (21/26), and 85% (22/26) participated in the *stx* subtyping (Annex 5). The majority participants in the cluster analyses (58%, 15/26) used WGS-derived data and one of these used both WGS and PFGE, while one (4%) reported using PFGE as the only method for cluster analyses. (Table 3). 'Laboratory policy to enhance the typing quality' was indicated as the main reason for participating (for all the parts of the EQA), but also accreditation needs, and institute/national policy were reported (See Annex 3 for details).

Table 3. Detailed participation information for the parts of serotyping, virulence profile determination and molecular typing-based cluster analysis

	Serotyping n=21		Virulence profile determination n=25					Cluster analysis n=16		
	O group	H type	*aaiC*	*aggR*	*eae*	*stx1* and *stx2*	*stx* subtyping	PFGE	WGS	Both
Number of participants	21#	16Δ	21	21	24	25	22	1	14	1
Percentage of participants^	100%	76%	84%	84%	96%	100%	88%	6%	88%	6%
Percentage of participants *	81%	62%	81%	81%	92%	96%	85%	4%	54%	4%

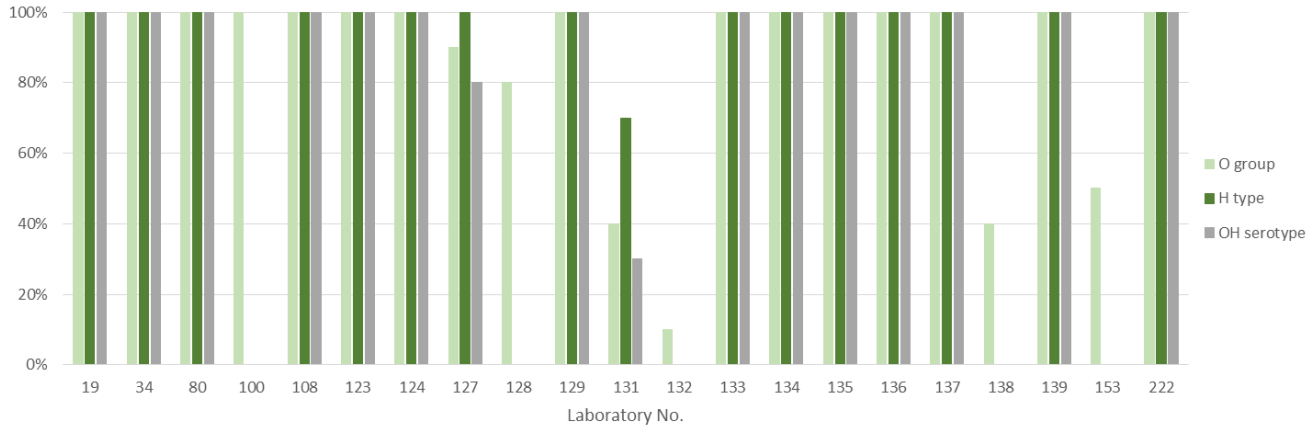
^: percentage of participants in respective part of EQA.
**: percentage of total number of participating laboratories (26).*
#: phenotypic (n=10)/PCR-based (n=1)/WGS-based (n=10).
Δ: phenotypic (n=2)/PCR-based (n=2)/WGS-based (n=12).

3.2 Serotyping

Twenty-one (81%) laboratories performed O grouping and 15 (71%) of the 21 were able to type all 10 test isolates correctly, and only four laboratories had a score of ≤ 50%, giving an average score of 86% (Figure 1). Fifteen laboratories (71%) reported the correct O group for the rare O group O5 (isolate REF3) and 16 (76%) correctly reported O80 (isolate REF5) (Figure 2). The highest performances were displayed for the O157 (100%), O145 (95%) and O104 (95%) isolates, (Figure 2), which are included in the minimum requirements of ECDC NRLs [15]. One laboratory (132) detected O157 only, generating incorrect (non-O157) results for the 9 other isolates (Annex 4, Figure 1).

Sixteen (62%) laboratories performed H typing. Of the 21 laboratories participating in O grouping, 76% (16/21) also reported H type. The general performance for H typing was higher than O grouping, with the majority (94%; 15/16) of participants correctly H typing all 10 test isolates, resulting in an average score of 98% (Figure 1). Only one laboratory reported two incorrect H types (H8 instead of H21 and H26 instead of H30) and H- for four isolates where all other participants could assign the H type. (Annex 4).

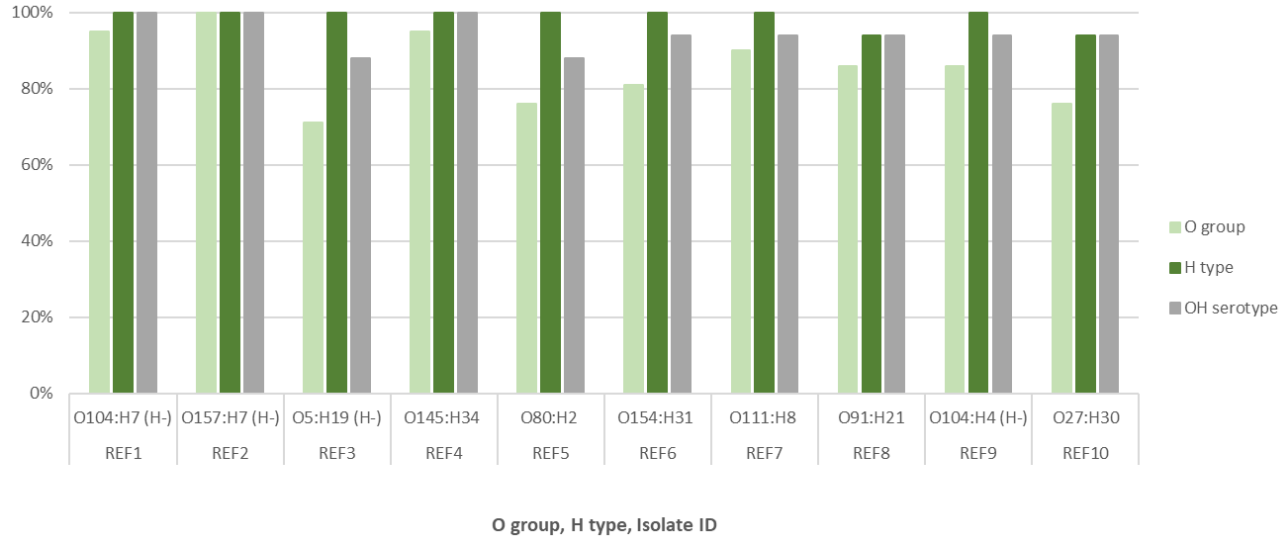
Figure 1. Participant percentage scores for O grouping and H typing



Arbitrary numbers represent participating laboratories.
Bars represent the percentage of correctly assigning O groups (light green), n= 21 participants, H types (dark green), n=16 participants, Combined O:H serotypes (grey), n=16 participants.

Complete O:H serotyping was performed by 16 (62%) participants with an average score of 94%, and for each isolate the score was ranging from 88% (14/16) for O5:H19(H$^-$) (REF3) and O80:H2 (REF5) to 100% (16/16) for isolate O104:H7 (REF1), O157:H7(H$^-$) (REF2) and O145:H34 (REF4) of the participants reporting the correct serotype (Figure 2).

Figure 2. Average percentage test isolate score for serotyping of O and H



Bars represent the percentage of laboratories correctly assigning O groups (light green): n=21 participants.
H types (dark green): n=16 participants. Combined O:H serotypes (grey): n=16 participants.
Average scores: O group, 86%; H type, 98% and combined O:H serotype, 94%.

3.3 Virulence profile determination

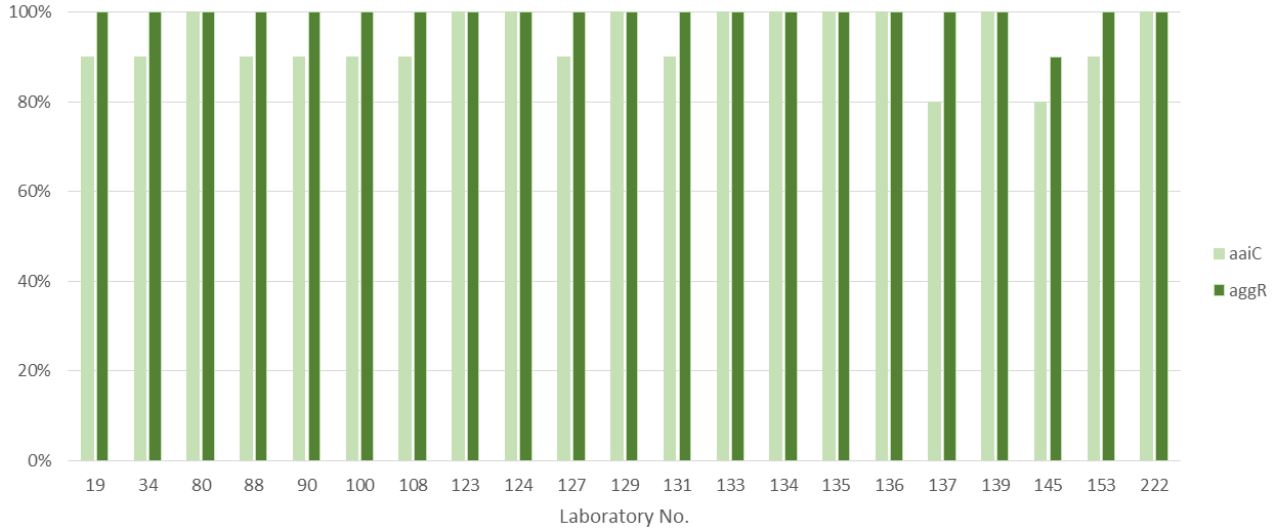
Between 21 and 25 laboratories submitted results for each of the virulence genes, consisting of detection of EAEC *(aaiC* and *aggR*) and virulence genes (*eae*, *stx1* and *stx2*) and subtyping of *stx1* and *stx2* genes. Twenty-four participants submitted results for *eae* and 25 *stx* genes. Twenty-two laboratories submitted subtyping results of *stx1* and *stx2* genes and 21 the EAEC genes *aggR* and *aaiC.*

3.3.1 Detection of EAEC genes (*aaiC* and *aggR*)

Among the isolates in this EQA, REF9 was an EAEC isolate containing both *aggR* and *aaiC*. The isolate of O104:H7 from EQA-6 was also included. This isolate REF1 contains a variant of *aaiC*, which has a 66% amino acid identity compared with the FN554766 (EAEC 042, serotype O44:H18). The variant is only detectable by WGS

(virulence finder) or dot blot hybridisation but not by PCR using Boisen et al. 2008 [16] primers. See section 3.4.3 and Annex 23 in ECDC's report of STEC EQA-6 [17]. As a result, the performance of the laboratories reporting correct genotyping results for all *aaiC* was lower than previously (48%; 10/21) with an average score of 94% for *aai*C. (Figure 3). The performance for *agg*R was much higher (95%, 20/21) with an average score of 99.5% (*agg*R) (Figure 3, Annex 5).

Figure 3. Participant percentage scores for genotyping of *aaiC* and *aggR*

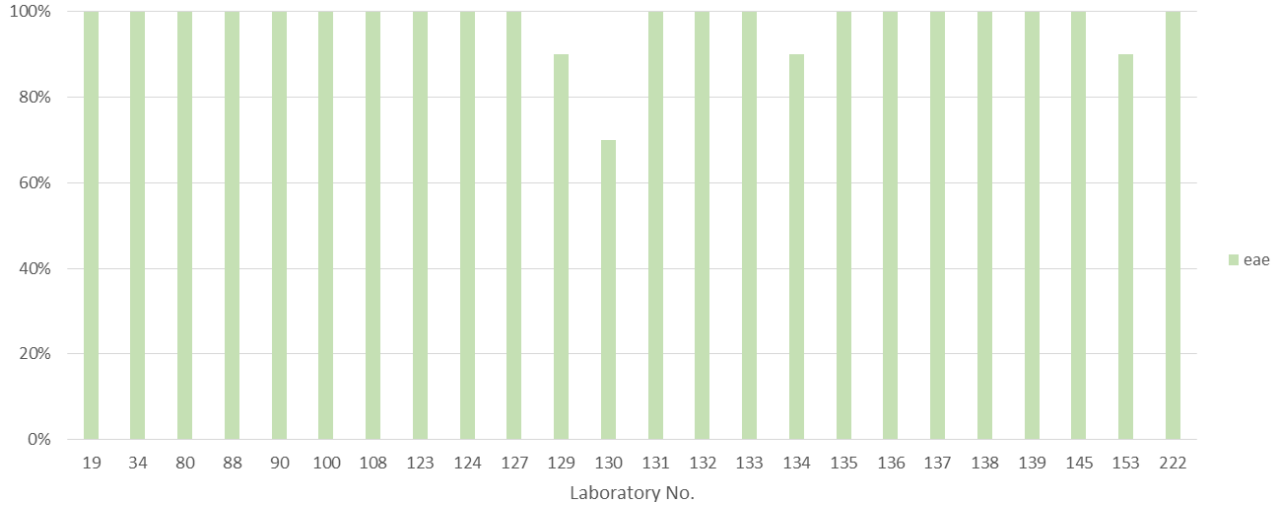


Arbitrary numbers represent participating laboratories.
Bars represent percentage of correct genotyping of aaiC *(light green) n=21 participants and* aggR *(dark green): n=21 participants.*

3.3.2 Detection of virulence genes *eae, stx1* and *stx2*

Detection of virulence genes *eae*, *stx1* and *stx2* was performed by 24-25 (92-96%) laboratories with a generally high performance (Figures 4–5). For *eae* detection, 20 (83%) laboratories obtained a 100% score (Figure 4). Four laboratories (129, 130, 134 and 222) reported incorrect *eae* results. Three laboratories reported incorrect results for the same isolate (REF4). In total, *eae* was misidentified as three false negatives (REF4) and one false negative for REF7 and two false positives, one for REF6 and one for REF8 respectively (Annex 5).

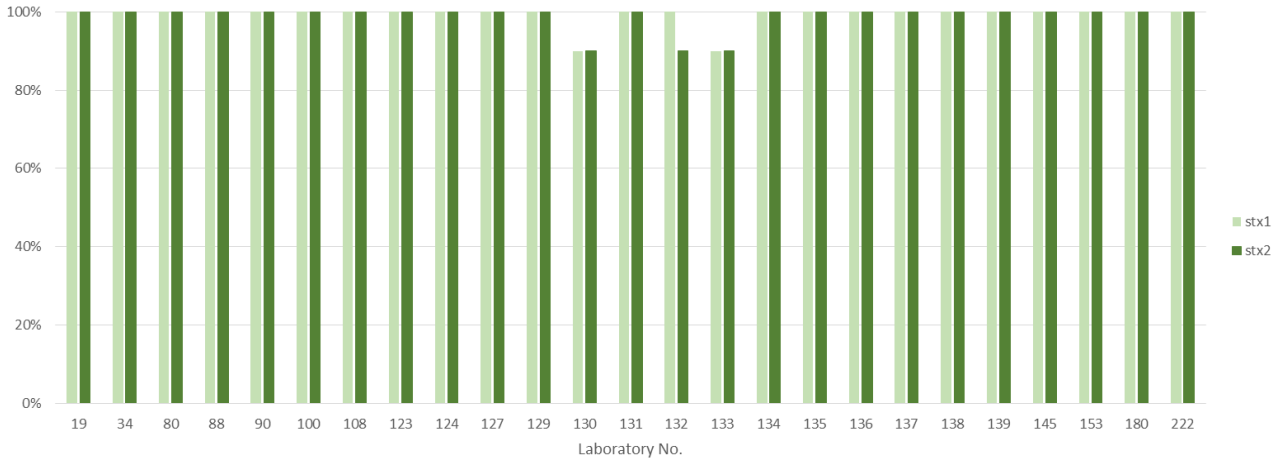
Figure 4. Participant percentage scores for genotyping of *eae*



Arbitrary numbers represent participating laboratories.
Bars represent percentage of correct genotyping of eae *(light green): n=24 participants.*

The performance of detection of *stx*1 and *stx*2 genes was high; 23 (92%) laboratories reported 100% correct *stx1* results and 22 (88%) laboratories reported 100% correct *stx2* results (Figure 5). The three incorrect *stx2* results were reported by three different laboratories in two different isolates (REF4 and REF6). (Annex 5).

Figure 5. Participant percentage scores for detection of *stx1* and *stx2*



Arbitrary numbers represent participating laboratories.
Bars represent percentage of correct genotyping of stx1 *(light green) and* stx2 *(dark green): n=25 participants.*
Average scores: stx1, *99%;* stx2, *99%.*

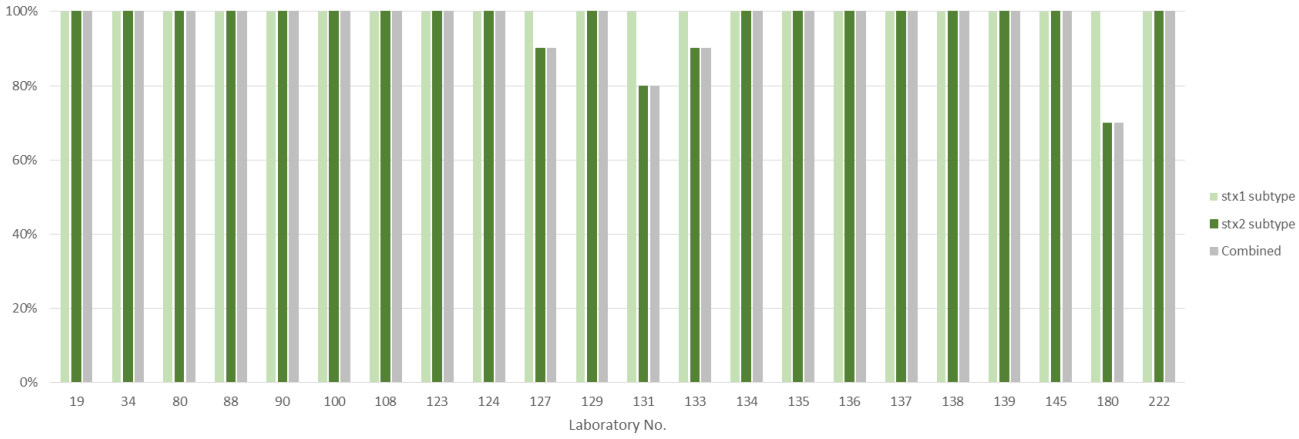
3.3.3 Subtyping of stx1 and stx2

Subtyping of *stx1* and *stx2* was performed by 22 laboratories. All 22 (100%) subtyped *stx1* correctly and 18 (82%; 18/22) reported correct *stx2* subtype for all 10 test isolates (Figure 6; Annex 5).

Laboratories were not allowed to only report results for selected test isolates for a particular test, so reporting ND was considered as an incorrect result if the laboratory reported results of other isolates for that test.

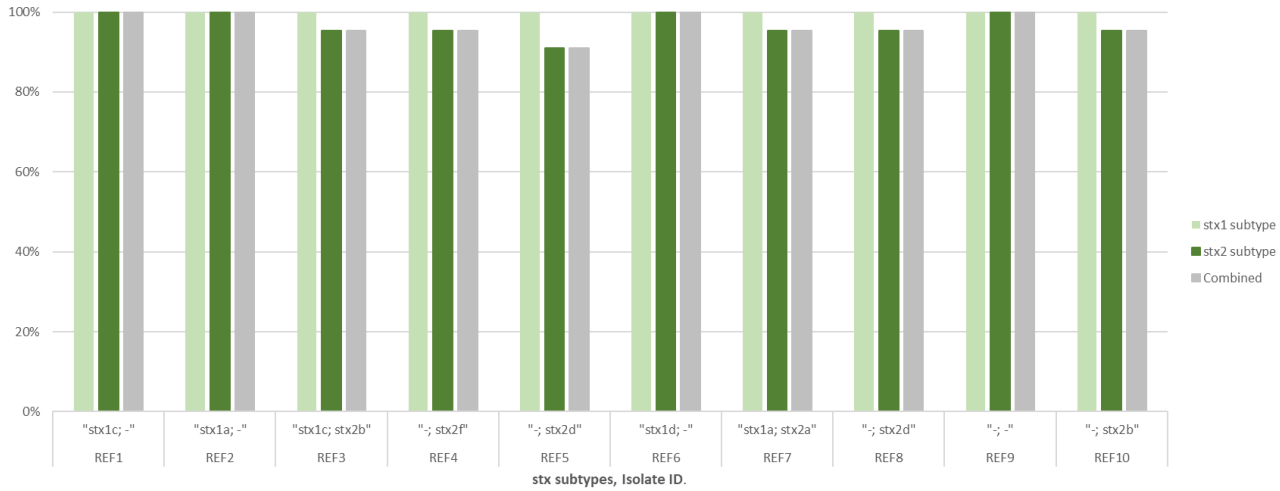
Only four laboratories (18%) reported an incorrect subtyping of *stx2* for one or more isolates, primarily by reporting incorrect *stx* subtypes for the isolates and not ND. In total, the average score was 97% (Figure 6). The number of instances of mis-subtyping *stx2* was seven. Laboratory 180 reported three of the seven incorrect *stx2* subtypes. Laboratory 131 reported two incorrect *stx2* subtypes and laboratories 127 and 133 reported one incorrect result each.

Figure 6. Participant percentage scores for subtyping of *stx1* and *stx2*



Arbitrary numbers represent participating laboratories.
Bars represent percentage of correct subtyping of stx1 (light green), stx2 (dark green), combined stx1 and stx2 (grey), n=22 participants. Reporting ND (not done) evaluated as incorrect.

Figure 7. Average percentage test isolate score for subtyping of *stx1* and *stx2*



Bars represent percentage of laboratories correctly subtyping stx1 (light green), stx2 (dark green) and combined stx1 and stx2 (grey), n=22.
Average scores: stx1, 100%; stx2, 97% and combined stx1 and stx2, 97%.

Most incorrect results are no longer due to reporting ND instead of negative result, as in EQA-8. The incorrect results of *stx2* subtyping shown in Table 4 are divided into three categories: false negatives, incorrect subtype of *stx2* (6/7) or ND (1/7).

Table 4. Incorrect *stx2* subtype results

		Incorrect subtype results			
Isolate ID	**EQA provider**	**False negative**	**Incorrect**	**Total true errors**	**Errors by reporting ND#**
REF1	-				
REF2	-				
REF3	*stx2b*		*stx2a* (1)	1	
REF4	*stx2f*		*stx2c* (1)	1	
REF5	*stx2d*		*stx2a* (1)	1	1
REF6	-				
REF7	*stx2a*		*stx2b* (1)	1	
REF8	*stx2d*		*stx2f* (1)	1	
REF9	-				
REF10	*stx2b*		*stx2a* (1)	1	
Total				**6**	**1**

ND#: not done.

3.4 Molecular typing-based cluster analysis

In this part of the EQA, participants were asked to identify a cluster of closely related isolates among 10 test isolates by using either PFGE and/or WGS-derived data. The cluster test isolates were pre-categorised by the EQA provider.

The expected cluster of closely related STEC ST21 (O26:H11 *stx1a*) isolates contained four isolates based on WGS derived data. The characteristics of the test isolates and reported results are listed in Annexes 6-7 and 9-10.

3.4.1 PFGE-derived data

Of the 26 participants in the EQA, two (8%) performed cluster analysis using PFGE-derived data. Laboratory 127 correctly identified the cluster of closely related isolates defined by a pre-categorisation from the EQA provider among the 10 cluster test isolates. Laboratory 90 only identified two (REF15 and REF20) of the four cluster isolates. Table 5 shows the overview of the isolates each participant included or excluded in cluster identification. Laboratory 90 missed one of the technical duplicate isolates (REF11) and REF19 in the cluster of closely related isolates.

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

		Laboratory number	
Isolate ID	**ST**	**90**	**127**
REF11ǂ#	21	No	Yes
REF12	21	No	No
REF13	21	No	No
REF14	29	No	No
REF15ǂ#	21	Yes	Yes
REF16	21	No	No
REF17	21	No	No
REF18	21	No	No
REF19ǂ	21	No	Yes
REF20ǂ	21	Yes	Yes
Cluster-identified		**No**	**Yes**

ǂ: closely related isolates (in grey).
#: technical duplicates isolates (in bold).
(Annex 7)

3.4.2 WGS-derived data

3.4.2.1 Reported results from participants

Fifteen participants (58%) performed cluster analysis using WGS-derived data. Two laboratories reported using external assistance for sequencing. Different sequencing platforms were listed among the participants: 2 MiniSeq, 5 MiSeq, 1 HiSeq, 4 NextSeq, 1 Novaseq, 2 Ion Torrent (Ion GeneStudio S5 System and Ion Torrent S5XL). All laboratories reported using commercial kits for library preparation. Of the 15 participants, 11 (73%) used Illumina's Nextera kit. Three participants reported changes from the manufacturer protocol, two in the volume, and one in the shearing time (Annex 8).

Performance was high in cluster analysis with WGS-derived data. Fourteen participants (93%) correctly identified the cluster of closely related isolates defined by pre-categorisation from the EQA provider among the 10 test isolates (Table 6). One laboratory did not include isolate REF19 (ST21) in the cluster of closely related isolates.

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

		Laboratory number														
Isolate ID	**ST**	**19**	**34**	**80**	**100**	**108**	**123**	**124**	**127**	**133**	**134**	**135**	**136**	**137**	**139**	**222**
REF11ǂ#	21	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF12	21	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF13	21	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF14	29	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF15ǂ#	21	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
REF16	21	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF17	21	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF18	21	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
REF19ǂ	21	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
REF20ǂ	21	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Main analysis		Allele	Allele	Allele	Allele	SNP	Allele	Allele	Allele	Allele	Allele	Allele	Allele	SNP	Allele	Allele
Additional analysis		SNP			SNV			Allele	SNP							
Additional analysis 2					SNP				Allele							
Cluster-identified		**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**Yes**	**Yes**

ǂ: closely related isolates (in grey).
#: technical duplicates isolates (in bold).
ST: sequence type.
Allele: allele-based analysis.
SNP: single-nucleotide polymorphism.
SNV: single-nucleotide variant.
(Annex 9)

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 up to two additional), but the detected cluster had to be based on results from the main analysis.

Two participants used SNP as their main analyses and three laboratories reported SNP as an additional analysis. All used a reference-based approach with different in-house isolates or one of the EQA isolate as reference. Four used Burrows-Wheeler Aligner (BWA) and one used CLC as the read mapper, and selected to use different variant callers (Table 7).

Table 7. Results of SNP-based cluster analysis

Lab	SNP-based					
	Approach	Reference	Read mapper	Variant caller	Distance within cluster	Distance outside cluster¤
Provider	Reference-based NASP [14]	ST21 (REF20)	BWA	GATK	0–3	120-746 (3366)
Provider	Reference-based NASP [14] + recombination filter [19]	ST21 (REF20)	BWA	GATK	0–3	117-626 (951)
19*	Reference-based	Isolate 9470	BWA	GATK	0-3	106-582(921)
100#	Reference-based	ST29, 97-3250 NZ_CP027599	BWA	-	0-3	115-747 (2042)
108	Reference-based	Reference in-house	CLC assembly cell v4.4.2	CLC assembly cell v4.4.2	0–6	124-1065 (not reported)
127*	Reference-based	ST21, Isolate ID = 9128	Enterobase SNP pipeline BWA§	Enterobase SNP pipeline	0-4	106-598 (3527)
137	Reference-based	In House	BWA	GATK	0–2	6-839 (3989)

¤: reported distance to ST21 (non-ST21) isolates (Annex 10).
**: additional analysis 1.*
#: additional analysis 2.
§: data added by the EQA provider.

Thirteen participants used allele-based analysis as the main analysis for cluster detection – three reported additional analysis (one SNV, one wgMLST and one cgMLST with a different approach, but same scheme) (Table 8). Just over half (7/13; 54%), used an assembly-based allele calling method and the rest (six) laboratories used both mapping- and assembly-based allele calling (Table 8).

Table 8. Results of allele-based cluster analysis

Lab	Allele-based analysis						
	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)	2513	0–2	40-112 (283)
19	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Enterobase)	2513	0-1	40-110 (282)
34	SeqPhere	Assembly-based and mapping-based	SKESA	Enterobase (cgMLST)	2513	0-5	44-204 (308)
80	SeqPhere	Only assembly-based	Velvet	Enterobase (cgMLST)	2513	0-1	37-113 (278)
100	SeqPhere	Only assembly-based	Velvet	SeqSphere (cgMLST)	3152	0-2	54-286 (518)
100*	SNV					0-4	58-216 (1275)
123	SeqPhere	Only assembly-based	SPAdes	Enterobase (cgMLST)	2513	0-1	38-114 (279)
124	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (cgMLST/Enterobase)	2513	0-1	40-110 (280)
124*	BioNumerics	Assembly- and mapping-based	SPAdes	Applied Math (wgMLST/Enterobase)	17380	0-3	90-270 (610)
127	Enterobase	Only assembly-based	SPAdes	Enterobase (cgMLST)	2513	0-3	43-119 (287)
127#	BioNumerics	Only assembly-based	SPAdes	Applied Math (cgMLST/Enterobase)	2513	0-2	35-91 (213)
133	BioNumerics	Assembly- and mapping-based	Spades	Applied Math (cgMLST/Enterobase)	2513	0-2	41-112 (283)
134	SeqPhere	Assembly- and mapping-based	SKESA	Enterobase (cgMLST)	2513	0-1	38-114 (279)
135	SeqPhere	Only assembly-based	SPAdes	Enterobase (cgMLST)	2513	0-1	38-114 (279)
136	SeqPhere	Only assembly-based	SPAdes	Enterobase (cgMLST)	3054	0-2	52-152 (378)
139	Enterobase	Assembly- and mapping-based	SPAdes	Enterobase (cgMLST)	Unknown §2513	0-2	50-100 (200)
222	ChewBBACA on ARIES webserver	Only assembly-based	§SPADES version 3.12.0	Enterobase (cgMLST, Innuendo-curate)	2360	0-11	44-116 (275)

¤: reported differences to ST21 (non-ST21; Annex 10).
**: additional analysis 1.*
#: additional analysis 2.
§: data added by the EQA provider.

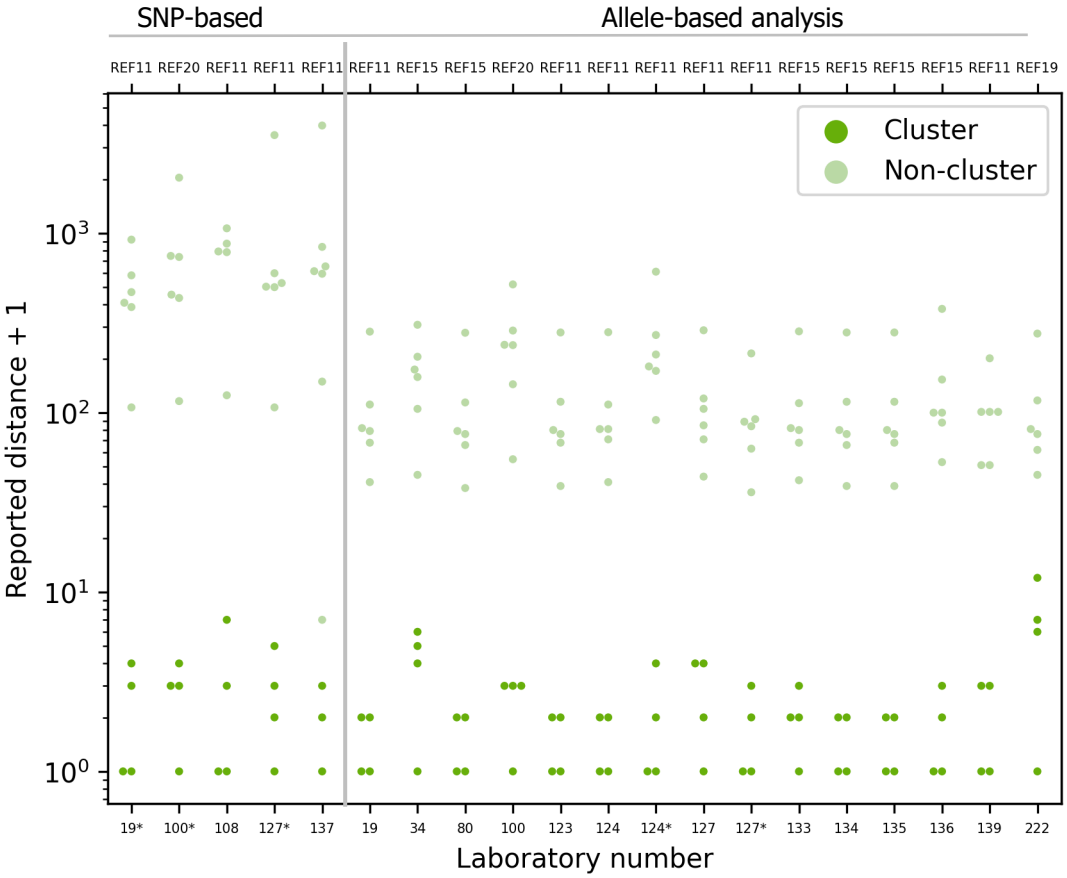
Of the 13 laboratories using allele-based methods (main analysis), all (100%) identified the correct cluster of four closely related isolates (Table 7 and Table 8). Ten laboratories performed cgMLST using the same scheme as the EQA provider (cgMLST/Enterobase [12]) with 2513 loci. Two laboratories (100 and 136) used a scheme with a slightly higher number of loci (3152/3054) and laboratory 222 used the INNUENDO cgMLST scheme with a slightly lower number of 2360 loci and one laboratory used wgMLST in the additional analysis and obtained allelic differences within the cluster (17380 loci).

Eleven of the 13 laboratories that identified the correct cluster reported allele differences of 0–3 within the cluster of closely related isolates in their main analysis (Figure 8, Table 8). Laboratory 34 reported a slightly higher number of alleles 0-5 within the cluster using the same scheme and approach for analysing as laboratory 134 which only got 0-1 allele difference. In addition, laboratory (222) reported a high number of allele difference 0-11 within the cluster when using the INNUENDO cgMLST scheme in combination with the Ion Torrent data. Both laboratory 34 and 222 did identify the correct cluster.

Three laboratories reported additional allelic analysis; all three showed allele differences of 0-4 or less within the cluster.

Five other test isolates (REF12, REF13, REF16, REF17 and REF18) were also ST21, but not pre-defined by the EQA provider as part of the cluster. Based on the main analysis of cgMLST, 13 laboratories reported allele differences to the selected cluster isolate at 37-286 for this group of isolates. Based on cgMLST, the reported differences were 200-518 for non-ST21 isolate (REF14). (Table 8, Annex 10).

Figure 8. Reported SNP distances or allelic differences for each test isolate to selected cluster representative isolate



SNP: single nucleotide polymorphism.
Selected cluster representative marked as REF in the top scale.
Dark green: reported cluster of closely related isolates, Light green: not reported as part of cluster.

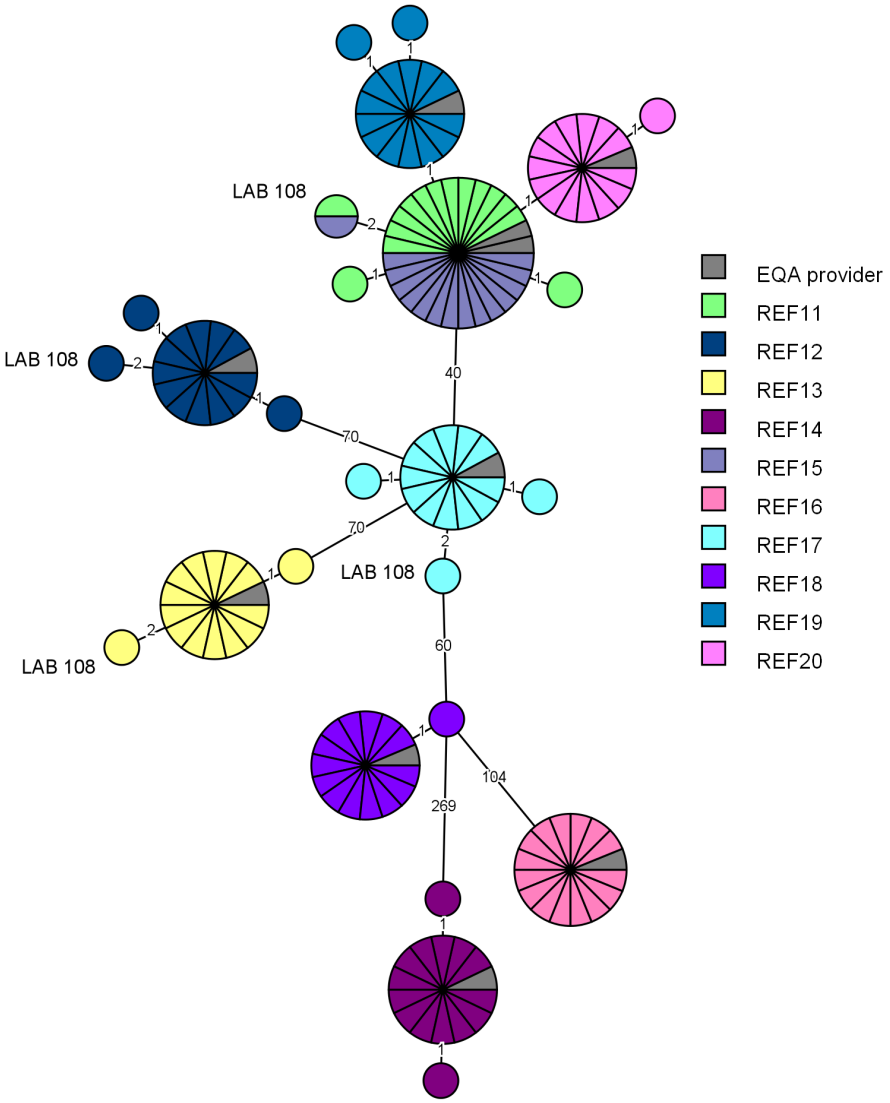
All laboratories performing SNP analyses, reported SNP distances within the cluster isolates (REF11, REF15, REF19 and REF20) with a maximum of 0–6 SNP distances (Table 7/Figure 8). Laboratory 137 did not identify the correct cluster of closely related isolates, as it excluded REF19 with a SNP distance of six from their selected cluster representative (REF11).

3.4.2.2 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 FASTQ files were uploaded to an Applied Maths calculation engine for allele calling (Enterobase) [12] and evaluated by the EQA provider's in-house quality control (QC) pipeline [18].

The overall cgMLST analysis, shown in the minimum spanning tree (MST) based on submitted raw reads (FASTQ files) from 12 laboratories reveals clear clustering of the results for each test isolate (Figure 9).

Figure 9. Minimum spanning tree of core genome multilocus sequence typing participant FASTQ files

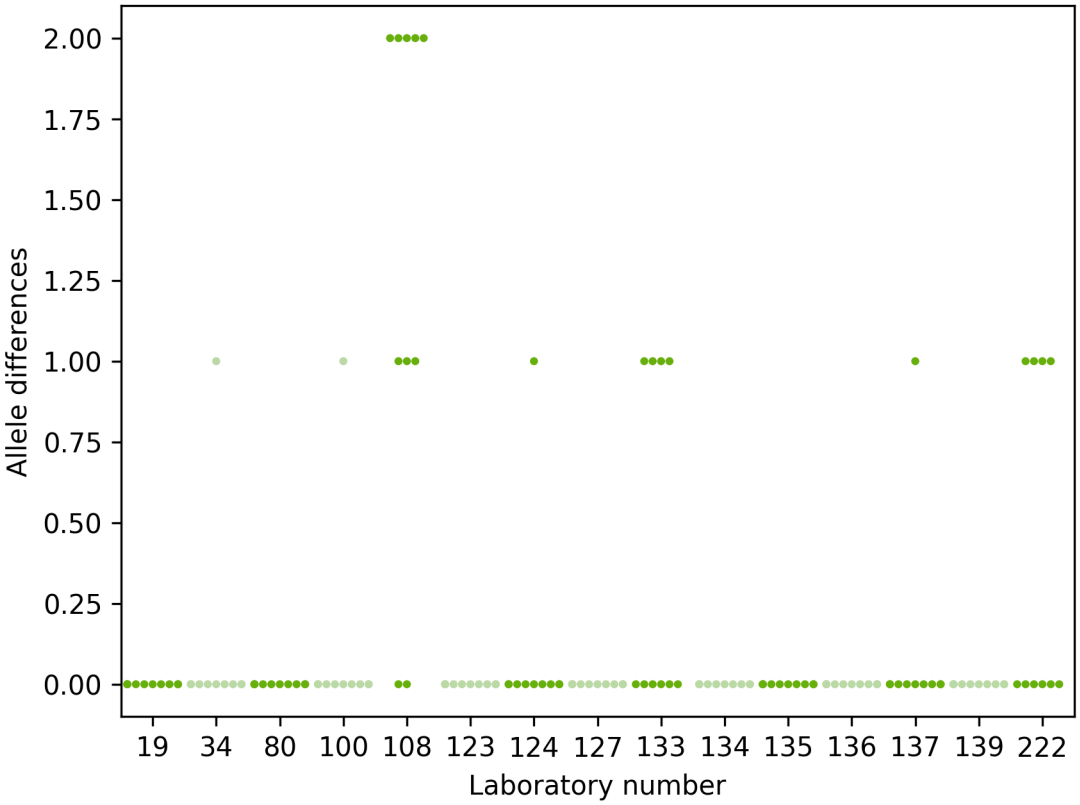


Minimum spanning tree (MST) in log scale of core genome multilocus sequence typing (cgMLST) [12] based on submitted raw reads (FASTQ files).
Each of the REF11–20 test isolates have a different colour. REF results from the EQA-provider are in grey and REF11 and REF15 were technical duplicates' isolates.
Results from laboratory 108 and 222 were run in CE (using Ion Torrent setup for allele calling).

The allele differences in Figure 9 do not exactly match those illustrated in the individual reports and consequently those in Figure 10, where the same data are used. This discrepancy is caused by *loci* being dropped if they did not pass QC for all isolates in the analysis. Joint analysis accordingly contains fewer *loci*.

For each laboratory, cgMLST was performed on the submitted raw reads (FASTQ files), applying Applied Maths allele calling with the Enterobase scheme [12]. A hierarchical single linkage clustering was performed on the submitted data for each laboratory along with the EQA provider's reference isolates. Figure 10 shows the allele differences between each submitted sequence and the corresponding reference.

Figure 10. Participant allele difference from reference result (EQA-provider) for each test isolate



Allele difference from corresponding REF isolates (EQA provider) based on submitted raw reads (FASTQ files) and analysed by EQA provider.

For 130 of 150 results (86%), no allele difference was identified. For 15 results (10%), a difference of one allele from the REF isolate was calculated, and for five results (3%) a difference of two alleles was observed, all five by laboratory 108.

Separately, the laboratories listed quantitative and qualitative QC parameters used to evaluate their data. As seen in Table 9, almost all laboratories have implemented QC threshold for accepting the data. Using different Q score parameters (Phred) was the most reported parameter, followed by confirmation of genus, and coverage with acceptance thresholds ranging from 20–50X were the most widely used QC parameters. Genome size and difference Q score parameters were also included. The number of good cgMLST *loci* was also listed as an important parameter for QC. Refer to the additional QC parameters reported by the participants in Annex 11.

Table 9. 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 and < 5% contamination with others	Min x25	N50 value and number of contigs - No threshold	4,64-5,56 Mbp	Core% and loci with multiple consensus - No actual threshold employed on regular basis for either
34	Kraken	RIDOM SeqSphere+	RIDOM SeqSphere+	RIDOM SeqSphere+	RIDOM SeqSphere+
80	-	-	N50, Contig Count, Read Count, Average Coverage, Perc. Good Targets	more than 4	-
100	KmerFinder 3.2 CGE	40x	FastQC	assembled genome app. 5,2 Mbp)	-
108	Species total match size average similarity	>20x	Similarity, length, CDS covered	1	All above, similarity, coverage >10 times
123	high percentage of cgMLST targets in E. coli scheme is required	>50	%cgMLST targets	> 5 000	>98%
124	Genome size GC% in silico PCR e coli det	>100	BioNumerics provides a quality statistics window for quality assessment. This is used for assembly QC.	BioNumerics provides a quality statistics window for quality assessment. This is used for de novo assembly QC.	BioNumerics provides a quality statistics window for quality assessment. This is used for allele call QC.
127	Kraken in Enterobase	>= 40	Number of contigs <= 800	between 4,2 - 6,5 Mbp	Bionumerics summary calls % of core present >=80
133	-	-	contig number, N50, average read quality > 30	>4,7MB, <5,7MB No an exact rule further analysis done if outside these parameters	NrAFPerfect, NrAFPresent, NrBAFPerfect, NrBAFPresent
134	match in SeqSphere	= 50x but if it's less the number of targets found should be >90%	-	length of contigs assembled < ref genome + 10%	cgMLST targets found and called > 90-95%
135	Kraken / Bracken, <4% contamination allowed	-	number of contigs (>=500nt) <650	resulting genome size between 4,6 and 5,8 Mb	>90% assigned alleles
136	k-mer	> 95%	N50, contig count, read count, average count	more than 4	-
137	Kmer ID	1	-	-	Average coverage of all alleles (Achtman 7 gene MLST)
139	-	>50x	Use of Enterobase website and quality check, no of contigs < 500 ¤N50 > 30 000 bp	Genome size 4.7 Mbp	-
222	No mismatches (7 MLST panel Warwick)	§Mentalist tool: 100% coverage in length for all 7 MLST genes >30x average depth of coverage	§ Mentalist tool: assessment of the depth of coverage, correct allele calling of 7 MLST genes, >80% of the 2360 total loci found. N50 >30 000.	-	At least 80% of the total 2360 loci of cgMLST scheme found.
% of laboratories using the QC parameter	**80%**	**80%**	**87%**	**80%**	**73%**

§*: Text adjusted for purpose of the table.*
¤*: 'N50 >30 000bp' reported by Lab 139 has been moved to the Q score column instead of Genome size column (details is added to the Annex 11).*

For each laboratory, the submitted raw reads (FASTQ files) were evaluated by the EQA provider's in-house quality control pipeline [18]. For the full QC evaluation of all isolates, see Annex 12.

According to the QC parameters, sequencing quality was uniformly good. Only two sequences from two different laboratories were flagged as a possible contamination (one with *Shigella sonnei* and one with *Pseudomonas tolaasii*). The coverage was overall sufficient.

Table 10. Results of raw reads submitted by participants evaluated by EQA provider QC pipeline summarised by laboratory

Parameters	Ranges*	19	34	80	100	108	123	124	127	133	134	135	136	137	139	222
Detected species	{Ec}, {Ss} or {Pt}	Ec	Ec	Ec/ Pt	Ec	Ec	Ec/ Ss	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
Species 1 (%)		89-94	80-93	81-89	93-97	96-97	85-94	97-98	92-96	86-96	90-95	86-94	93-97	82-94	89-98	93-98
Species 2 (%)		0.5-2.6	0.3-4.0	2.8-6.0	0.7-1.8	0.9-1.7	0.3-5.0	0.5-1.3	0.6-1.8	0.6-2.4	0.5-3.0	0.8-4.0	0.5-2.1	0.1-1.8	0.1-2.5	0.5-1.4
Unclassified reads (%)	{<100}	4.4-7.0	4.3-14.4	6.3-9.1	1.7-4.1	2.0-2.5	2.6-8.4	1.1-1.6	2.7-5.6	2.5-10.8	2.7-5.8	3.3-8.1	1.7-3.7	6.0-14.7	1.6-7.8	0.9-4.1
Length at >25 x min. coverage (Mbp))	{>45 ∧ <58}	5.2-5.5	0.6-5.6	5.3-5.6	5.4-5.7	5.2-5.6	5.2-5.7	5.4-5.7	5.3-5.6	5.2-5.6	4.4-5.6	5.3-5.6	5.3-5.7	1.0-5.5	4.7-5.7	4.7-5.6
Length [1-25] x min. coverage (kbp)	{<250}	44-90	0-4895	0-14	0-0.7	0-0.6	0-240	0	7-85	1-195	0-1061	0	0	0-4166	0	0-0.6
Number of contigs at 25 x min. coverage	{>0}	472-750	225-381	331-420	227-284	#723-2515	239-368	191-228	282-531	309-1270	232-417	252-364	242-373	79-368	66-306	285-1340
No. of contigs [1-25] x min. coverage	{<1000}	43-140	0-276	0-5	0-1	#0-3	0-82	0	6-94	1-231	0-61	0	0	0-286	0	0-2
Average coverage	{>50}	67-108	36-73	126-214	47-80	77-149	47-82	256-649	58-109	61-125	32-89	136-177	222-307	23-83	54-110	174-311
Number of reads (x1000)		2556-4374	910-1979	5585-9718	1147-1918	1668-3251	1070-1860	6000-14955	2270-4331	1451-2901	1215-3371	5524-6928	8549-11596	1224-4607	2018-3848	2925-5454
Average read length		135-143	202-243	151-151	235-240	256-267	228-258	251-251	144-149	227-275	151-151	149-151	146-149	95-100	151-151	309-349
Average insert size		188-228	231-314	171-252	320-377	NA	256-340	456-469	327-481	220-325	165-402	226-358	226-335	212-371	296-325	7-63
N50 (kbp)		19-33	32-118	83-112	95-118	£4-19	42-104	108-127	28-91	9-68	73-102	83-114	87-115	31-152	88-228	8-34

Quality assessment made by the EQA-provider in-house quality control pipeline.
**: indicative QC range.*
Ec: E. coli, Ss: Shigella sonnei, Pt: Pseudomonas tolaasii (listed if >5%).
NA: not analysed.
#: QC values unreliable due to assembly issues for Ion Torrent data.

Assessment of the provided genomes

The five provided genomes should individually be assessed and compared with the already produced data in the cluster analysis and the participants had to determine whether or not the genomes were part of the defined cluster.

The participants were instructed to describe their observations and considerations leading to the decision. The EQA-provider had manipulated the raw reads. The five genomes represented raw reads of two cluster isolates (one with high-quality raw reads an one with reduced coverage), and three non-cluster isolates (one with high-quality data in an assembly file, and two contaminated with *E. coli* and *Klebsiella pneumonia* respectively (table 11)). Raw data can be seen in Annex 13.

For genome 1, with good quality of reads but contaminated with a different *E. coli*, 100% correctly described the genome as a non-cluster isolate, but only 27% (4/15) correctly described contamination present of the same species in genome 1.

For genome 2, a strategic k-mere extension for scrupulous assembled (SKESA) file, 100% correctly described the genome as a non-cluster isolate. One laboratory (7%) incorrectly described too low a percentage of good targets for cgMLST cluster analysis.

For genome 3, with a good quality of reads but contaminated with *Klebsiella pneumonia*, 93% (14/15) correctly described the genome as a non-cluster isolate, but one laboratory did state that this was only as a possible case until the isolate could be re-sequenced. In addition, 60% (9/15) correctly described contamination present in genome 3.

For genome 4, a cluster isolate with good quality of reads, but lacking the *stx* gene, 100% accepted the quality of the genome and 93% (14/15) correctly described the genome as a cluster isolate. Three even described the absence of the *stx* gene.

For genome 5, with reduced coverage, 87% (13/15) correctly observed poor quality in genome 5. Four participants, despite the low coverage, described they would include the genome 5 in the cluster until a better sequence would be available, while 10 would exclude the sequences until a better sequence was available. One participant submitted inconclusive data.

Table 11. Results of the participants' assessment of the EQA provided genomes

Genome	Characteristics	Characteristics identified by participants	Yes	No	Not analysed
1	A non-cluster isolate (REF21) mixed with a different *Escherichia coli* (approx. 10%)	Contamination was observed	4	11	0
		Suggested to be a cluster isolate	0	15	0
2	A non-cluster isolate (REF22), good quality of reads, assembled with SKESA to a FASTA file (76 AD to cluster isolate REF1/REF5)	Quality accepted	14	1	0
		Suggested to be a cluster isolate	0	15	0
3	A non-cluster isolate (REF23) mixed with a *Klebsiella pneumonia (*approx. 10%)	Contamination was observed	9	6	0
		Suggested to be a cluster isolate	1	14	0
4	A cluster isolate (REF24), good quality sequence, but without stx genes (0 AD to cluster isoalte REF1/REF5/REF10	Quality accepted	15	0	0
		Suggested to be a cluster isolate	14	1	0
5	A cluster isolate (REF19) with altered coverage (reduced to 12x)	Poor quality was observed	13	2	0
		Suggested to be a cluster isolate	4	10	1

Raw data in Annex 13

4. Discussion

4.1 Serotyping

Twenty-one (81%) laboratories participated in the serotyping part of the EQA-10, of which 10 participants (48%) provided phenotypic serotyping results and 11 (52%) provided molecular serotyping results (one by PCR and 10 by WGS). An increase from six to 10 participants from EQA-8 to EQA-9 using WGS-based serotyping, remained at 10 participants in this EQA. Sixteen participants performed both O group and H typing and 12 correctly assigned all 10 test isolates for both O and H.

4.1.1 O group

From EQA-4 through EQA-10 (26/28; 26/29; 26/29; 27/30; 23/25; 20/24 to 21/26 [93%]), a decrease in participation in O grouping was observed.

The performance of O grouping was the same in EQA-10 as in EQA-9, but higher than in EQA-8. Fifteen participants (71%) reported the correct O group for all 10 test isolates compared to only 50% in EQA-9. All the incorrect O group results were reported by laboratories using phenotypic method. Eight of the 29 incorrect results were reported as an incorrect type, while the rest were reported as non-typable/rough or not done. Still, one laboratory only screens for O157, and is therefore responsible for nine of the 19 non-typable.

O group O5 is not so common in Europe and was reported as O8, O127 or non-typable by three laboratories. All six laboratories reporting incorrect result for O5 used phenotypic method for the O grouping. The EQA provider has no knowledge of cross-reaction of O5 with any of the mis-typed O groups (O8 and O127). Likewise, no cross-reactions between the other mis-typed O groups (O45/O103/O154 or O27/O45/O130) are known by the EQA provider.

Some of the more common O groups, also included in ECDC's minimum requirements, generated the highest performances (O157: 100%, O145: 95%, O111:90%, O104:95/86% and O91: 86%). The average score was slightly higher in the current EQA (86%) compared to EQA-9 (85%) and EQA-8 (79%). The shift from phenotypic serotyping towards WGS based analysis that were observed from EQA-8 (26%) to EQA-9 (50%) increased slightly in EQA-10 (52%).

4.1.2 H type

The decrease in H typing participation which was seen from EQA-4 (18 laboratories) to EQA-9 (13 laboratories) was not detected in EQA-10 as the number had increased to 16 laboratories. The general performance for H typing was higher than O grouping, but fewer performed H typing. Almost all participants (94%, 15/16) correctly H typed all 10 test isolates (Figure 5). One laboratory reported three errors. The EQA provider has no knowledge of cross-reaction between H8 and H21 or between H26 and H30. Compared to the previous EQAs, again this year the average score increased; 98% correct results compared to EQA-9 (94%), EQA-8 (92%) and EQA-7 (81%).

4.1.2 OH serotyping

The O:H serotyping results ranged from 100% for isolate O157:H7 to 88% (14/16) of the participants reporting correct serotype for O5:H19 (H^-) and O80:H2. The average percentage O:H serotyping in this EQA was again higher (94%) compared to EQA-9 (92%), EQA-8 (86%), EQA-7 (71%), and EQA-6 (78%). In general, the less common European serotypes generated were more difficult to identify.

In addition to O grouping, H typing is crucial for outbreak detection, epidemiological surveillance, taxonomic differentiation of *E. coli* and detection of pathogenic serotypes. As such, it remains a main challenge to enable more NPHRLs to perform complete and reliable O:H serotyping, particular H typing. However, with the use of WGS, this might be more feasible for some countries in future.

4.2 Virulence profile determination

Twenty-five laboratories participated in the detection of the virulence profile with the participation rate and performance varying substantially between the different tests. As in previous EQAs, the participation rate was highest for the genotypic detection of the *stx* genes (96%) and detection of *eae* (92%), and lowest for the detection of *aaiC/aggR* (81%) and subtyping of *stx* genes (85%).

4.2.1 Detection of *aaiC* and *aggR*

The performance of detection the EAEC *aggR* genes was high, with 94% of the participants detecting *aggR* correctly. The gene *aaiC* was detectable in two isolates, as the EQA provider included one isolate with both *aggR* and *aaiC* (REF9) and REF1 which only has an *aaiC* variant and which is only detectable by WGS (virulence finder)

or dot blot hybridisation but not by PCR using Boisen et al. 2008 primers. This *aaiC* variant has a 66% amino acid identify compared with the FN554766 (EAEC prototype 042, serotype O44:H18) and was described in detail in the EQA-6 [16]. Because of this variant, the performance of the laboratories reporting correct genotyping results for, *aaiC* was lower than previously (48%; 10/21) with an average score of 94%. Seven of the 10 using WGS-based method detected the variant *aaiC* gene. In addition, three of the 11 were able to detect the *aaiC* variant using a 'different' method. Only one laboratory missed the *aaiC* gene in the EAEC isolate REF9.

4.2.2 Detection of *eae*

Genotyping of *eae* had a high participation rate (92%) and performance; 20 (83%) laboratories obtained a 100% score, giving an average score of 98%. The average correct score has been fairly unchanged through the EQAs. (EQA-4 to EQA-9, 96%-99%).

4.2.3 Detection of *stx1* and *stx2*

Both the participation (96%) and performance rates were high for genotyping of *stx1* (99%) and *stx2* genes (99%), similar to previous EQAs.

4.2.4 Subtyping of *stx1* and *stx2*

The average scores of correct subtyping of *stx1* and *stx2* were 100% and 97% respectively, which is an increase compared to both EQA-9 (93% and 92%) and EQA-8 (84% and 87%) but also a higher performance compared to all previous EQAs. The unexpected reporting of 'not done' results, which was an issue in EQA-8, was only reported by one laboratory for one isolate. The EQA-provider specified in the invitation letter and in the submission protocol that when a participant signs up for a test and subsequently participates, all isolates must be analysed using this test.

In the current EQA, the true errors ('not done' results excluded) were six incorrect *stx2* subtyping results. All errors were reported by four laboratories for three different isolates. New subtypes of stx (from *stx2h-stx2l*) have been identified in the recent years by different groups and is discussed by the EFSA BIOHAZ Panel in the EFSA report. [7]. The EQA provider is working on developing a new protocol for detecting these new variants.

4.3 Molecular typing-based cluster analysis

Since EQA-8, the EQA scheme of STEC no longer covers PFGE as an independent part, but contains a cluster analysis using either PFGE and/or WGS-derived data. Fourteen laboratories participated in cluster identification using WGS-derived data, one laboratory participated using PFGE-derived data and one of the 16 laboratories participated in cluster identification using both methods. Two of the 'PFGE participants' from EQA-9 did not participate in EQA-10. In addition, one 'PFGE participant' from EQA-8, who did not participate in EQA-9, returned to participate with a WGS-based method in EQA-10.

4.3.1 PFGE-derived data

Of the 26 laboratories, two (8%) performed cluster analysis using PFGE-derived data, and one of the two participants (50%) correctly identified the cluster of closely related isolates. The participation using PFGE in the cluster analysis decreased clearly as eight laboratories performed PFGE in EQA-9. This indicates that PFGE is no longer a main method used by the laboratories for cluster identification.

Compared to PFGE analysis of *Salmonella* or *Listeria*, the PFGE profile of *E. coli* contains a large number of bands within the region of 200–350 kb, which makes the cluster analysis based on PFGE harder to interpret. The PFGE gel needs to be of a very good quality in order to correctly assign all bands in this region. The one laboratory (only using PFGE) that did not identify the correct cluster missed one of the technical duplicates (REF11) and RFE19 in the cluster. The other laboratory performed cluster analysis using both PFGE and WGS.

4.3.2 WGS-derived data

Fifteen of 26 laboratories (58%) performed cluster analysis using WGS-derived data. Two laboratories reported the use of external assistance for sequencing, and the majority (13/15) reported using an Illumina platform. All reported using commercial kits for preparing the library.

Performance was very high, with 14 (93%) laboratories correctly identifying the cluster of closely related isolates, which is comparable to last year (92%). Of 15 laboratories, 13 (87%) reported using an allele-based method as the main analysis and two (13%) reported using SNP analysis. The two laboratories that used SNP-based analysis reported the same SNP distances for the pre-determined cluster isolates, but one laboratory used a stricter cut-off (below 6 SNPs) and did therefor not identify the correct cluster. The distances reported using SNP-based analyses were 0–6 inside the cluster and the number of allele differences using cgMLST were 0–11 inside the cluster. However, 11/13 laboratories reported 0-3 allele differences within the cluster.

When assessing the reported allele difference or SNP distances, both approaches showed comparable results as both show a clear separation of the cluster and non-cluster isolates. One exception was the results from one laboratory using allelic-based analysis with a lower number of *loci* and Ion Torrent data. A higher number of allelic differences was observed by this laboratory (222) among the cluster isolates when using their scheme of a reduced number of *loci* (2360) compared to the Enterobase scheme (2513 *loci*). Laboratory 222 reported 0-11 allelic differences within the cluster compared to 0-3 by most of the other laboratories, whereas the allele difference among the rest of the ST21 isolates was very similar to the other laboratories. However, this year the results from laboratory 222 show a clear separation between the cluster isolates and the rest and reported the correct cluster.

When testing the submitted raw reads from laboratory 222 (IonTorret data) in BioNumerics (Enterobase) scheme, the allele differences observed were similar to the EQA provider's analysis (1 AD difference). Last year, the conclusion on the INNOENDO scheme and ChewBBACA pipeline was that the analysis only used assembly-based mapping, and the Ion Torrent data are not assembled correctly leading to the many incorrect allele calls.

High similarity was seen for the reported cgMLST results based on Enterobase (most had three allele differences or below three within the cluster). Only one laboratory (using Enterobase scheme in SeqPhere and SKESA as assembler) reported five allelic differences within the cluster, a slight increase compared to other laboratories using the same scheme and approach. Additionally, the laboratory (222) reported 0-11 alleles as described above.

SNP analyses can provide valid cluster detection at a national level and can be used for communication about cluster definitions, but the two laboratories using SNP as the main analysis reported six SNP to the cluster isolate REF19. One laboratory excluded the isolate (REF19) with six SNPs, the other laboratory accepted the distance of six SNPs. The EQA provider's analysis using NASP with standard settings gave three SNPs to this REF19. An additional NASP analysis with changed settings; allowing duplicated regions showed six SNPs difference of the same sequence. This emphasises the importance of understanding the pipeline and carefully evaluating the data. From the data visualised in Figure 8, there is a clear separation between the cluster isolates and the remaining isolates. The EQA provider highlights that a definitive cut-off in STEC WGS analysis has not been formally established as this is of course difficult to do. This REF19 was one isolate among 38 in this specific outbreak (0-3 AD, cgMLST), primarily among children in day-care centres across Denmark during September-November 2018.

Recombination had no significance for the cluster identification. However, some discrepancies were observed depending on which software was used. The highest variations in the SNP distances were outside the cluster depending on the SNP pipeline and additional analysis performed.

The main reported QC parameters were genome size, number of contigs, %cgMLST and confirmation of genus, which are all essential for the end use of the data. A higher number of laboratories report they use QC parameters and the genome size and number of *loci* in particular was frequently reported.

In order to compare the quality of the raw data, the EQA provider analysed the submitted raw reads to obtain selected QC parameters. All laboratories submitted sequences of fine quality, with only two sequences flagged as potentially contaminated with 5-6% of a different species.

The EQA provider's analysis of the submitted raw data showed that when using a standardised cgMLST analysis, it is not unlikely to observe a random variation of one allele, even with high coverage (Figure 10). However, one participant (108) deviated consistently. This is likely due to a combination of sequencing technology and allele-calling software.

In the assessment of the additional EQA-provided genomes, most of the participants successfully determined whether or not the genomes (of good quality) were part of the defined cluster sequences. Only one participant did not identify Genome 4 as a cluster isolate (without the *stx* gene) as part of the cluster. The analysis was done by SNPs, but the other participant known to use SNP did identify the genome 4 as a cluster isolate. Almost all laboratories (13/15) correctly reported low coverage for the cluster isolate (Genome 5). It is very important to identify low coverage issues because they higly affect the conclusions as less called loci resulting in missing data.

As expected, it was easier for the laboratories to identify the contamination with *Klebsiella pneumonia* (9/15) than for the genome contaminted with a different *E. coli* (4/15). Three participants used 'too large' genome size as a partial agument as well as too many contigs, high multiple consensus *loci* and/or many unidentified bases to discard the genome as not having sufficient quality. One laboratory detected two different alleles for three *loci* (*fumC*, *gyrB* and *icd*) of the MLST scheme.

The FASTA file of genome 2 was analysed by 14/15 and correctly identified as a non-cluster sequence. Only one reported that genome 2 had a too low percentage of good targets for cgMLST cluster analysis, but still identified it as non-cluster isolate.

Almost all the laboratories had in this EQA used more time to assess the modified genomes. In general, the participants had described in detail what they observed and not just as previous suggested the re-run of the isolate. It seems as if the participants accepted the challenge and used the time to try to analyse the more questionable data and suggest if it was a cluster isolate or not.

5. Conclusions

Twenty-six laboratories participated in the EQA-10 scheme, with 21 (81%) performing the serotyping part, 25 (96%) the virulence profile determination part, and 16 (62%) cluster identification. As in the EQA-9, this EQA contains the inclusion of molecular typing-based cluster analysis using either PFGE and/or WGS-derived data. This adjustment of the EQA seemed to be well accepted by most Member States, but a decrease in the number of participants was seen compared with previous years for the cluster analysis (one less than in EQA-9). In addition, a small increase was seen both for serotyping (one participant) and virulence profile determination (two participants) in EQA-10 compared to EQA-9.

The O:H serotyping was only performed by 62% (16/26) of the participants, with an average score of 94%. As in previous EQAs, participation in the O grouping was higher than in H typing. Notably, not all laboratories demonstrated the capacity to determine all O groups and H types. In general, the more common European serotypes generated the highest scores. Serotype O5:H19 generated the lowest scores, correctly reported by 14 laboratories.

Detection of the true EAEC isolate was frequent. However, the isolate with the variant of *aaiC* reduced the overall performance of detecting both *aaiC* isolates to 45%. The present EQA demonstrated a high performance for *aggR,* with 99.5% average scores.

Detection of *eae* had high participation rates and average scores through the EQAs has always been above 96% (EQA-4: 96%; EQA-5: 98%; EQA-6: 97%; EQA-7: 98%; EQA-8: 96%; EQA-9: 99% and EQA-10: 98%).

Similarly to previous EQAs, the participation and average scores for *stx1* and *stx2* gene detection were high, with a 99% average score for both *stx1* and *stx2.* Subtyping of *stx1* and *stx2* is valuable since specific subtypes (*stx*2a) have been associated with increased risk of HUS, hospitalisation, or bloody diarrhoea respectively [7]. The high participation rate of 85% is therefore very encouraging. The average score for subtyping of *stx1* (100%) and *stx2* (97%).

Incorporating the molecular typing-based cluster analysis in this EQA is up to date with the development of surveillance methods used by NPHRLs in Europe. Sixteen laboratories performed cluster analysis, while 15 used WGS-derived data (three more than in EQA-9). A clear decrease (from eight to two) was seen in the number of laboratories who used PFGE for cluster analysis. However, only two stopped completely participating in the cluster identification. Most of the laboratories continued using only WGS or switched to WGS instead of PFGE.

Performance was high among the participants using WGS, with 14 (93%) of participants correctly identifying the cluster of five closely related isolates. One laboratory missed one of the four cluster isolates. Only one of the two laboratories using PFGE for their cluster analysis identified the correct cluster using PFGE.

An allele-based method was preferred by most laboratories, as 87% (13/15) used cgMLST compared to 13% (2/15) using SNP as reported cluster analysis as the main analysis. The use of a standard cgMLST scheme (e.g. Enterobase) gives a very high degree of homogeneity in the results, and allele-based methods seem to be useful for inter-laboratory comparability and communication about cluster definitions. SNP analyses can also provide valid cluster detection at the national level and can be used for communication about cluster definitions. However, only the same two laboratories performed SNP analysis in this EQA and in EQA-9.

A new addition to the cluster analysis was introduced in EQA-10. Sequence data of five isolates were made accessible by the EQA provider, and the participants were asked to include these in the cluster analysis and report characteristics and quality issues. Contamination with a different species is easier to identify than inter-species contamination. All but two laboratories correctly concluded that the two genomes of high quality were part of the same cluster. For the genome with low coverage, most of the participants did identify the quality issues, and some suggested that the genome might be a part of the cluster, which was correct.

The current EQA scheme for typing STEC is the 10th EQA organised for laboratories in FWD-Net. The molecular surveillance system implemented as part of TESSy relies on the capacity of FWD-Net laboratories to produce analysable and comparable typing results into a central database. WGS-based typing for surveillance is increasingly used in EU. ECDC coordinates centralised analysis of WGS STEC data when needed to support multi-country outbreak investigations.

6. Recommendations

6.1 Laboratories

Participants are encouraged to assign sufficient resources to repeat failed analysis if required to meet the deadline of submission.

Laboratories are expected to employ each method as an individual test irrespective of results obtained in the screening and detection or any other test. Therefore, when a participant signs up for a test and subsequently participates, all isolates must be tested using this test, e.g. subtyping of *stx*.

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 encourages more participants to take part in the new molecular typing-based cluster analysis, as well as participants who have not previously participated in the PFGE gel analysis part.

6.3 EQA provider

The evaluation of the provided genome sequences was a success. Almost all participants performed the analysis and identified the modifications introduced by the EQA provider. For the following EQA rounds, the EQA provider will continue and expand this part of the EQA in order to challenge the participants in their assessment of poor-quality genomes as well as contaminated ones, putting emphasis on the importance of assessing the genomes despite a low-level contamination or other quality issues – but of course concluded with the utmost caution.

The EQA provider will for the next FWD-Network meeting suggest an open 'cut-off' discussion of STEC cluster.

The newest published recommendation defines EAEC isolates as harbouring *aggR* and a complete cluster of AAF-encoding genes (usher, chaperone, and both major and minor pilin subunit genes) or the ETEC colonisation factor (CF) CS22 gene. As a result, the EQA provider recommends that the *aaiC* gene should not be evaluated in the EQA [20].

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Annex 1. List of participants

Country	Laboratory	National institute
Austria	National Reference Center for Escherichia coli including VTEC	Institute for Medical Microbiology and Hygiene
Belgium	National Reference Laboratory STEC	Universitair Ziekenhuis Brussels
Czechia	NRL for E. coli and Shigella	National Institute of Public Health
Denmark	International Escherichia and Klebsiella Centre	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board
Finland	Expert Microbiology Unit	Finnish Institute for Health and Welfare
France	Laboratoire de Microbiologie - Centre de Référence Escherichia coli	Centre Hospitalo-Universitaire Robert-Debré
Germany	NRC Salmonella	Robert Koch Institute
Greece	National Reference Centre for Salmonella, Shigella, VTEC	Department of Public Health Policy, School of Public Helath
Hungary	Reference Laboratories, Department of Bacteriology	National Public Health Center
Iceland	Dept. of Clinical Microbiology	Landspítali University Hospital
Ireland	NRL-VTEC	Public Health Laboratory
Italy	Microbiological Food Safety and Foodborne Disease Unit	Istituto Superiore di Sanità
Latvia	National Microbiology Reference laboratory	Infectology Centre of Latvia
Lithuania	National Public Health Surveillance laboratory	National public health survellance labortaory
Luxembourg	Epidemiology and Microbial Genomics (EPIGEM)	Laboratoire National de Sante
North Macedonia	Food institute, Laboratory for molecular analysis of food and GMO	Faculty of veterinary medicine-Skopje
Norway	National Reference Laboratory for Enteropathogenic Bacteria	Norwegian Institute of Public Health
Poland	Department of Bacteriology and Biocontamination Control	National Institute of Public Health – National Institute of Hygiene
Portugal	LNR Infeções Gastrintestinais	Instituto Nacional de Saúde Dr. Ricardo Jorge
Romania	Molecular Epidemiology Laboratory	Cantacuzino National Medico-Military Institute of Research and Development
Slovenia	Department for Public Health Microbiology	National Laboratory of Health, Environment and Food
Spain	Unidad de Enterobacterias	Instituto de Salud Carlos III
Sweden	Mikrobiologi	Folkhäslomyndigheten
The Netherlands	Centre for Infectious Disease Research, Diagnostics and Laboratory Surveillance	RIVM
United Kingdom	Gastrointestinal Bacteria Reference Unit	Public Health England

Annex 2. Participation overview EQA-9/EQA-10

	2018-2019 (EQA-9)					2019-2020 (EQA-10)				
				Cluster					Cluster	
Laboratory number	Participation (min. 1 part)	Serotyping	Virulence	PFGE	WGS	Participation (min. 1 part)	Serotyping	Virulence	PFGE	WGS
19	x	x	x	x	x	x	x	x		x
34	x	x	x		x	x	x	x		x
80	x	x	x		x	x	x	x		x
88	x		x			x		x		
90	x		x	x		x		x	x	
100	x	x	x		x	x	x	x		x
108	x	x	x		x	x	x	x		x
123	x	x	x	x	x	x	x	x		x
124	x	x	x	x		x	x	x		x
127	x	x	x	x		x	x	x	x	x
128	x	x	x			x	x			
129	x	x	x			x	x	x		
130	x			x		x		x		
131						x	x	x		
132	x	x	x	x		x	x	x		
133	x	x	x		x	x	x	x		x
134	x	x	x		x	x	x	x		x
135	x	x	x		x	x	x	x		x
136						x	x	x		x
137	x	x	x		x	x	x	x		x
138	x	x	x			x	x	x		
139	x	x	x		x	x	x	x		x
145	x	x	x			x		x		
153	x	x	x			x	x	x		
180	x		x			x		x		
222	x	x	x	x	x	x	x	x		x
Number of participants	**24**	**20**	**23**	**8**	**12**	**26**	**21**	**25**	**2**	**15**

Annex 3. Reason(s) for participating in EQA

		Laboratory number																											
Part	Reason(s)	19	34	80	88	90	100	108	114	123	124	127	128	129	130	131	132	133	134	135	136	137	138	139	145	153	180	222	No.
Serotyping	Accreditation needs	x	x	x				x	x	x	x	x	x			x		x		x	x	x	x	x	x	x	x	x	20
	Institute policy		x		d	e	x	x	x					x	b					x		x			x		x		9
	National policy		x				x	x	x	x				x									x						7
	Laboratory policy to enhance the typing quality		x				x	x	x		x	x	x	x			x	x	x	x	x	x			x		x	x	17
Virulence profile (stx1/stx2)	Accreditation needs	x	x	x	x			x	x	x	x	x	x			x		x		x	x	x	x	x	x		x	x	20
	Institute policy		x				x	x	x					x	x					x		x			x		x		10
	National policy		x				x	x	x	x				x	x					x			x		x				10
	Laboratory policy to enhance the typing quality		x			x	x	x	x		x	x	x	x	x		x	x	x	x	x	x			x	x	x		19
Virulence profile (eae)	Accreditation needs	x	x	x	x			x	x	x	x	x	x		x	x		x		x	x	x	x	x	x		x	x	21
	Institute policy		x				x	x						x	x					x		x			x		x		9
	National policy		x				x	x	x	x				x	x					x			x		x				10
	Laboratory policy to enhance the typing quality		x			x	x	x	x		x	x	x	x	x		x	x	x	x	x	x			x	x	x		19
Virulence profile (aaiC/aggR)	Accreditation needs	x		x	x					x	x							x			x	x			x		x		10
	Institute policy						x	x	x				a	x	b	f	a			g		x	h		x		x		7
	National policy						x	x		x				x											x				5
	Laboratory policy to enhance the typing quality	x	x			x	x	x	x		x	x		x				x	x		x	x		x	x	x	x	x	18
Virulence profile (subtyping)	Accreditation needs	x		x	x					x	x							x			x	x	x		x		x		11
	Institute policy		x				x	x					a	x	b		a			g		x			x	d	x		7
	National policy						x	x	x	x				x									x			x				7
	Laboratory policy to enhance the typing quality	x	x			x	x	x	x		x	x		x		x		x	x		x	x		x	x		x	x	18
Cluster analysis (PFGE/WGS)	Accreditation needs	x		x					x	x	x							x		x	x	x							9
	Institute policy		x		d		x	x	x				a	a + b	x		a			x		x	d			d	a + c		7
	National policy		x				x	x	x	x		x			x															7
	Laboratory policy to enhance the typing quality	x	x			x	x	x	x		x	x			x	x		x	x	x	x	x		x	x			x	18

*Reasons for **not** participating in the EQA (in grey)*
a Lack of laboratory capacity.
b Lack of financial means.
c No national surveillance of STEC.
d Method not relevant to our laboratory.
e The laboratory in charge of E. coli serotyping did not develop an extensive protocol of STEC serotyping. The current approach is based on O grouping and targets the common EPEC/STEC O groups identified with common commercially available antisera.
f Lack of accreditation in this area.
g Experimental detection based on WGS, not applied for routine purposes at our laboratory.
h We use commercial RT PCR just to detect EAEC with aggR gene.

Annex 4. Serotyping result scores

O group

		Laboratory number																				
Isolate	**EQA**	**19**	**34**	**80**	**100**	**108**	**123**	**124**	**127**	**128**	**129**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**153**	**222**
REF1	O104	104	104	104	104	104	104	104	104	104	104	104	NT	104	104	104	104	104	104	104	104	104
REF2	O157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157	157
REF3	O5	5	5	5	5	5	5	5	Rough	8	5	NT	NT	5	5	5	5	5	127	5	NT	5
REF4	O145	145	145	145	145	145	145	145	145	145	145	145	NT	145	145	145	145	145	145	145	145	145
REF5	O80	80	80	80	80	80	80	80	NT	80	80	NT	NT	80	80	80	80	80	128	80	NT	80
REF6	O154	154	154	154	154	154	154	154	154	154	154	45	NT	154	154	154	154	154	103	154	NT	154
REF7	O111	111	111	111	111	111	111	111	111	111	111	11	NT	111	111	111	111	111	111	111	111	111
REF8	O91	91	91	91	91	91	91	91	91	91	91	91	NT	91	91	91	91	91	146	91	NT	91
REF9	O104	104	104	104	104	104	104	104	104	104	104	NT	NT	104	104	104	104	104	NT	104	104	104
REF10	O27	27	27	27	27	27	27	27	27	ND	27	130	NT	27	27	27	27	27	45	27	NT	27
Method		**C**	**C**	**C**	**A**	**C**	**C**	**A**	**A**	**A**	**C**	**A**	**A**	**C**	**C**	**C**	**B**	**C**	**A**	**C**	**A**	**A**

n=20 participants
Purple shading: incorrect result
A: phenotypic serotyping, B: PCR-based serotyping, C: WGS-based serotyping
NT: non-typable
ND: not done

H type

		Laboratory number														
Isolate	**EQA**	**19**	**34**	**80**	**108**	**123**	**124**	**127**	**129**	**131**	**133**	**134**	**135**	**136**	**137**	**139**	**222**
REF1	H7/H-	H-	7	7	7	7	7	7	7	H-	7	7	7	7	7	7	7
REF2	H7/H-	H-	7	7	7	7	7	7	7	H-	7	7	7	7	7	7	7
REF3	H19/H-	H-	19	19	19	19	19	19	19	H-	19	19	19	19	19	19	19
REF4	H34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
REF5	H2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
REF6	H31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
REF7	H8	8	8	8	8	8	8	8	8	H-	8	8	8	8	8	8	8
REF8	H21	21	21	21	21	21	21	21	21	8	21	21	21	21	21	21	21
REF9	H4/H-	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
REF10	H30	30	30	30	30	30	30	30	30	26	30	30	30	30	30	30	30
Method		**A**	**C**	**C**	**C**	**C**	**C**	**B**	**C**	**A**	**C**	**C**	**C**	**B**	**C**	**C**	**C**

n=16 participants.
Purple shading: incorrect results.
A: phenotypic serotyping, B: PCR-based serotyping, C: WGS-based serotyping.
Some H- results was accepted as correct results (REF1, 2, 3 and 9), when the EQA provider observed a tendency to be H- more than one during testing.

Annex 5. Virulence profiles result scores

Detection of *aaiC*

		Laboratory number																				
Isolate	**EQA**	**19**	**34**	**80**	**88**	**90**	**100**	**108**	**123**	**124**	**127**	**129**	**131**	**133**	**134**	**135**	**136**	**137**	**139**	**145**	**153**	**222**
REF1	+	-	-	+	-	-	-	-	+	+	-	+	-	+	+	+	+	-	+	-	-	+
REF2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+
REF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Method		**A**	**C**	**C**	**A**	**A**	**A**	**C**	**C**	**A**	**A**	**C**	**A**	**C**	**C**	**C**	**A**	**C**	**C**	**A**	**A**	**A**

n=21 participants.
Purple shading: incorrect results.
A: Other method than WGS, C: WGS-based serotyping.

Detection of *aggR*

		Laboratory number																				
Isolate	**EQA**	**19**	**34**	**80**	**88**	**90**	**100**	**108**	**123**	**124**	**127**	**129**	**131**	**133**	**134**	**135**	**136**	**137**	**139**	**145**	**153**	**222**
REF1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
REF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n=21 participants.
Purple shading: incorrect results.

Detection of *eae*

		Laboratory number																							
Isolate	**EQA**	**19**	**34**	**80**	**88**	**90**	**100**	**108**	**123**	**124**	**127**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
REF1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF4	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+
REF5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
REF7	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
REF8	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
REF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n=24 participants.
Purple shading: incorrect results.

Detection of *stx1*

		Laboratory number																								
Isolate	EQA	19	34	80	88	90	100	108	123	124	127	129	130	131	132	133	134	135	136	137	138	139	145	153	180	222
REF1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF6	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+
REF7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n=25 participants.

Detection of *stx2*

		Laboratory number																								
Isolate	EQA	19	34	80	88	90	100	108	123	124	127	129	130	131	132	133	134	135	136	137	138	139	145	153	180	222
REF1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF4	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
REF5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
REF7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
REF10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

n=25 participants.

stx subtyping

stx1

		Laboratory number																								
Isolate	EQA	19	34	80	88	90	100	108	123	124	127	129	131	133	134	135	136	137	138	139	145	180	222			
REF1	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*			
REF2	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*			
REF3	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*	*1c*			
REF4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF6	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*	*1d*			
REF7	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*	*1a*			
REF8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			

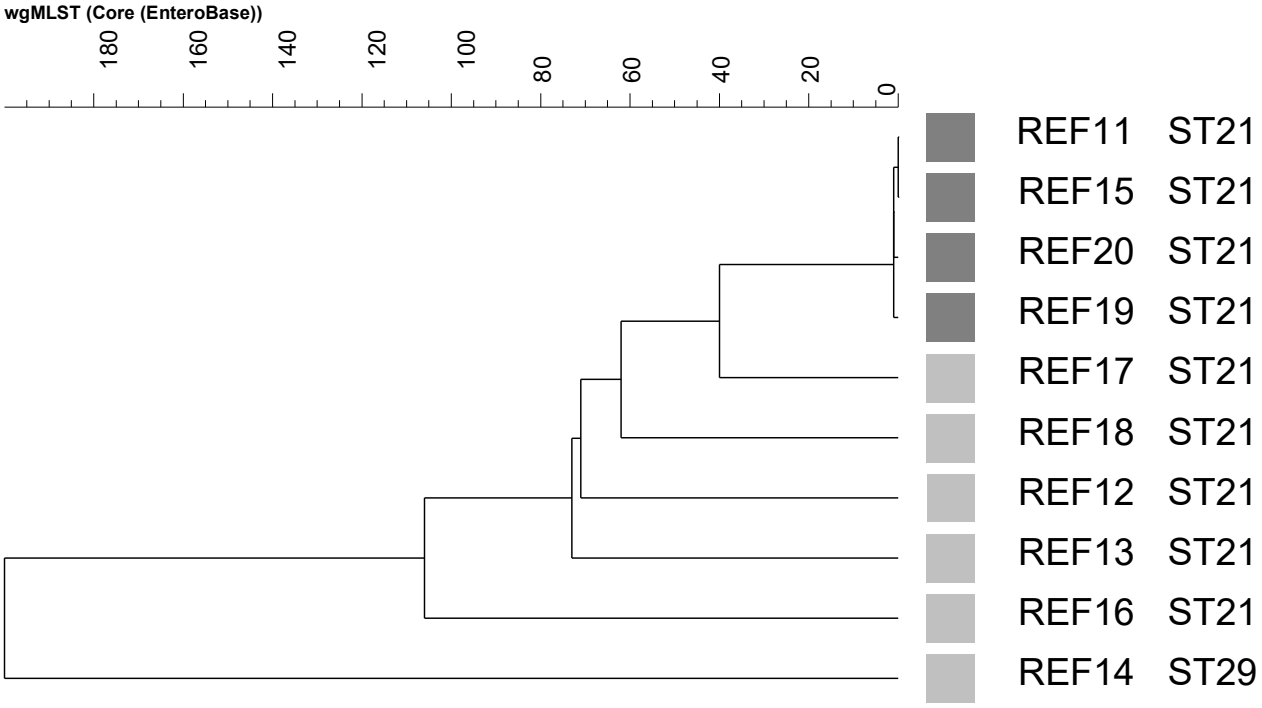
n=22 participants.

stx2

		Laboratory number																								
Isolate	EQA	19	34	80	88	90	100	108	123	124	127	129	131	133	134	135	136	137	138	139	145	180	222			
REF1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF3	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2a*	*2b*			
REF4	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2c*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*	*2f*			
REF5	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2a*	*2d*	*2d*	*ND*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*			
REF6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF7	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2a*	*2b*	*2a*			
REF8	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2f*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*	*2d*			
REF9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
REF10	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2b*	*2a*	*2b*			

n=22 participants, Purple shading: incorrect results, ND: not done.

Annex 6. EQA provider cluster analysis-based on WGS-derived data



Single linked dendrogram of core genome multilocus sequence typing (cgMLST) profiles of STEC EQA-10 isolates (cgMLST, EnteroBase, http://enterobase.warwick.ac.uk).
Analysed in BioNumerics: maximum distance of 200 exceeded, results clipped.
Cluster isolates: dark grey, outside cluster isolates: light grey.
REF11 and REF15 are technical duplicates.

Annex 7. Reported cluster of closely related isolates based on PFGE-derived data

Laboratory	Reported cluster	Corresponding REF isolates	Correct
Provider		REF11, REF15, REF19, REF20	Yes
90	9217, 9554	REF20, REF15	No
127	9128, 9180, 9378, 9603	REF11, REF19, REF15, REF20	Yes

REF11 and REF15 are technical duplicates.

Annex 8. Reported sequencing details

Sequencing performed	Protocol (library prep)	Commercial kit	Sequencing platform
In own laboratory	Commercial kits	Illumina NextersXTFlex	MiSeq
In own laboratory	Commercial kits	Illumina Nextera XT	MiSeq
In own laboratory	Commercial kits	NexteraXT	NextSeq
Externally	Commercial kits	Nextera XT DNA Library Preparation Kit	MiSeq
In own laboratory	Commercial kits	KAPA Library preparation kit, Roche	NextSeq
In own laboratory	Commercial kits	Nextera DNA flex (illumina)	MiniSeq
In own laboratory	Commercial kits	NEBNext® Fast DNA Fragmentation & Library Prep Set for Ion Torrent, New England Biolabs**	Ion GeneStudio S5 System
In own laboratory	Commercial kits	Ion express TM Plus Fragment Library kit	Ion Torrent S5XL
Externally	Commercial kits	KAPA HyperPlus Kit and Pippin prep size selection	NovaSeq 6000
In own laboratory	Commercial kits	Nextera Flex Illumina*	MiniSeq Illumina
In own laboratory	Commercial kits	Nextera XT Library Prep Kit (Illumina)*	MiSeq
In own laboratory	Commercial kits	Nextera	HiSeq 2500
In own laboratory	Commercial kits	Illumina Nextera Flex	MiSeq
In own laboratory	Commercial kits	Nextera XT	NextSeq
In own laboratory	Commercial kits	Nextera DNA Flex	NextSeq

**: adjusted volume of reagents.*
***: decreased shearing time.*

Annex 9. Reported cluster of closely related isolates based on WGS-derived data

Laboratory	Reported cluster	Corresponding to REF isolates	Correct
Provider		REF11, REF15, REF19, REF20	Yes
19	9155, 9287, 9470, 9994	REF11, REF15, REF19, REF20	Yes
34	9477, 9561, 9575, 9878	REF15, REF11, REF20, REF19	Yes
80	9065, 9274, 9284, 9406	REF20, REF11, REF19, REF15	Yes
100	9247, 9466, 9923, 9987	REF11, REF15, REF20, REF19	Yes
108	9471, 9608, 9515, 9401	REF11, REF15, REF20, REF19	Yes
123	9120, 9185, 9423, 9491	REF11, REF15, REF20, REF19	Yes
124	9100, 9392, 9631, 9929	REF11, REF19, REF15, REF20	Yes
127	9128, 9180, 9378, 9603	REF20, REF19, REF15, REF11	Yes
133	9077, 9357, 9368, 9555	REF19, REF15, REF11, REF20	Yes
134	9745, 9108, 9907, 9226	REF20, REF19, REF11, REF15	Yes
135	9080, 9276, 9453, 9986	REF19, REF20, REF15, REF11	Yes
136	9415, 9181, 9409, 9898	REF19, REF20, REF15, REF11	Yes
137	9482, 9519, 9539	REF11, REF15, REF20	No
139	9173, 9450, 9468, 9746	REF19, REF11, REF15, REF20	Yes
222	9706, 9729, 9914, 9990	REF20, REF19, REF11, REF15	Yes

REF11 and REF15 are technical duplicates.

Annex 10. Reported SNP distance and allelic differences

SNP distances

				Laboratory number				
Isolate ID	ST	Provider	Provider (recombination –deleted [19])	19*	100*	108	127*	137
REF11‡#	21	2	2	0¤	2	0¤	0¤	0¤
REF12	21	604	503	469	435	875	500	653
REF13	21	530	446	409	747	784	503	594
REF14	29	3366	951	921	2042	ND	3527	3989
REF15‡#	21	2	2	0	2	0	1	1
REF16	21	746	626	582	737	1065	598	839
REF17	21	120	117	106	115	124	106	148
REF18	21	538	413	387	454	791	527	614
REF19‡	21	3	3	3	3	6	4	6
REF20‡	21	0¤	0¤	2	0¤	2	2	2

Allelic differences

			Laboratory number														
Isolate ID	ST	Provider	19	34	80	100	100*	123	124	124*	127	127*	133	134	135	136	139	222
REF11‡#	21	1	0¤	4	0	2	2	0¤	0¤	0¤	0¤	0¤	1	0	0	0	0¤	5
REF12	21	78	78	173	75	237	165	75	80	210	84	62	79	75	75	99	100	75
REF13	21	80	81	157	78	238	157	79	80	170	104	91	81	79	79	99	100	80
REF14	29	283	282	308	278	518	1275	279	280	610	287	213	283	279	279	378	200	275
REF15‡#	21	1	0	0¤	0¤	2	2	0	0	1	3	0	0¤	0¤	0¤	0¤	0	6
REF16	21	112	110	204	113	286	216	114	110	270	119	83	112	114	114	152	100	116
REF17	21	40	40	44	37	54	58	38	40	90	43	35	41	38	38	52	50	44
REF18	21	67	67	104	65	143	156	67	70	180	70	88	67	65	67	87	50	61
REF19‡	21	2	1	5	1	2	4	1	1	0	3	2	1	1	1	2	2	0¤
REF20‡	21	0¤	1	3	1	0¤	0¤	1	1	3	1	1	2	1	1	1	2	11

ST: sequence type.
‡: closely related isolates (in grey).
#: technical triplicates isolates.
¤: isolate used as cluster representative by participant.
ND: isolates not included in analysis by participant.

Annex 11. Reported QC parameters

Lab no.	1		2		3	
	Parameter	**Threshold**	**Parameter**	**Threshold**	**Parameter**	**Threshold**
100	SAV	Cluster density, clusters passing filter and Q30 score were all according to Illumina recommendations	Contig count	Less than 35 contigs		
108	Contamination	Genome size, MLST all genes 100%				
123	N50	>50 000				
124	N50	Threshold set in the quality control window of BioNumerics >52100	non-ACGT bases	Scatterplot (length vs non-ACGT bases)	Nr BAFPerfect	Scatterplot (length vs Nr BAFPerfect)
127	No. of N bases	< 3%				
133	core percent	97	NrNonACGT	look at ration to total length and bases	N50	70000
135	N50	>20 000 bp	GC%	GC% between 49.5 and 51.0%	phred score	>30
137	(Achtman 7 gene MLST) Min. consensus depth Max. % non-consensus bases Average coverage of all alleles	 > 0 ≥ 15% 1	Variant ratio (SNP-typing)	≥ 0.9	Average depth coverage (SNP-typing)	≥ 30x
222	N50	N50>30000 used as threshold when assessing contigs for which no fastq files were available, used together with information on 7-loci MLST and number of loci of cgMLST scheme found				

Annex 12. Calculated qualitative/ quantitative parameters

Quality Assessment made by the SSI in-house quality control pipeline https://github.com/ssi-dk/bifrost [18]

		Laboratory 19									
Parameters	**Ranges***	**9155**	**9191**	**9211**	**9287**	**9470**	**9487**	**9523**	**9668**	**9759**	**9994**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		90.6	94.0	92.8	91.3	91.4	93.2	92.2	93.5	89.9	89.3
% Species 2		2.3	0.5	1.0	2.1	1.9	1.8	0.9	1.1	2.6	2.5
Unclassified reads (%)	{<100}	5.8	5.0	5.4	5.4	5.4	4.4	5.8	5.2	6.0	7.0
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.5	5.2	5.5	5.5	5.5	5.2	5.4	5.3	5.4	5.4
Length [1-25] x min. coverage (kbp)	{<250}	45.1	90.2	70.9	65.7	43.9	44.0	61.5	77.2	43.9	80.2
No. of contigs at 25 x min. coverage	{>0}	664	718	614	644	557	472	594	661	685	750
No. of contigs [1-25] x min. coverage	{<1 000}	56	140	100	81	43	43	68	101	53	101
Average coverage	{>50}	108	67	77	88	96	76	85	93	107	86
No. of reads (x 1 000)		4368	2556	3104	3546	3909	2906	3338	3712	4374	3661
Average read length		141	142	142	142	141	143	142	140	138	135
Average insert size		212	217	223	222	216	228	217	210	200	188
N50 (kbp)		25	19	24	23	32	33	27	24	25	21

		Laboratory 34									
Parameters	**Ranges***	**9062**	**9345**	**9477**	**9560**	**9561**	**9575**	**9820**	**9877**	**9878**	**9958**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		88.3	86.1	84.6	93.1	84.0	79.7	91.0	88.5	80.5	92.8
% Species 2		1.3	3.6	3.8	2.0	4.0	4.0	0.3	0.8	3.6	1.0
Unclassified reads (%)	{<100}	9.0	8.4	10.1	4.3	10.4	14.4	7.9	9.7	13.6	6.0
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.4	5.5	4.1	5.3	5.6	5.2	5.4	1.7	0.6	5.5
Length [1-25] x min. coverage (kbp)	{<250}	33.4	2.0	1451.2	0.5	3.9	368.2	0.0	3913.1	4895.2	0.7
No. of contigs at 25 x min. coverage	{>0}	298	310	294	250	307	381	320	254	225	332
No. of contigs [1-25] x min. coverage	{<1000}	14	3	51	1	6	52	0	159	276	2
Average coverage	{>50}	41	58	39	62	55	49	66	36	46	73
No. of reads (x 1000)		990	1580	1014	1623	1468	1455	1754	910	1129	1979
Average read length		243	215	233	216	224	202	215	235	238	216
Average insert size		314	256	302	254	278	231	251	303	302	250
N50 (kbp)		66	103	59	118	87	48	82	40	32	96

		Laboratory 80									
Parameters	**Ranges***	**9065**	**9072**	**9074**	**9274**	**9284**	**9385**	**9406**	**9507**	**9797**	**9826**
Detected species	{Ec} or {Pt}	Ec	Ec	Ec	Ec	Ec, Pt	Ec	Ec	Ec	Ec	Ec
% Species 1		85.5	86.9	85.9	83.8	80.9	83.9	86.8	87.7	87.5	88.5
% Species 2		4.4	4.2	5.0	4.6	6.0	4.6	3.3	3.3	3.3	2.8
Unclassified reads (%)	{<100}	7.3	6.6	6.8	8.4	9.1	7.7	6.7	7.2	6.7	6.3
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.6	5.5	5.3	5.6	5.6	5.5	5.6	5.4	5.6	5.5
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.0	13.6	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	420	355	331	366	337	346	392	356	369	333
No. of contigs [1-25] x min. coverage	{<1000}	0	0	0	0	0	0	5	0	0	0
Average coverage	{>50}	207	151	214	126	133	134	152	146	145	149
No. of reads (x 1000)		9429	6698	9718	5585	6124	5977	6721	6105	6365	6289
Average read length		151	151	151	151	151	151	151	151	151	151
Average insert size		192	224	171	243	252	242	207	235	227	233
N50 (kbp)		87	103	103	83	87	104	87	95	89	112

		Laboratory 100									
Parameters	**Ranges***	**9098**	**9208**	**9247**	**9283**	**9466**	**9819**	**9830**	**9879**	**9923**	**9987**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		96.3	95.7	94.3	95.6	94.8	94.8	96.6	95.3	93.9	92.8
% Species 2		1.1	1.5	1.8	0.7	1.7	1.2	1.4	0.7	1.7	1.8
Unclassified reads (%)	{<100}	2.0	2.1	2.6	2.5	2.2	2.5	1.7	3.3	3.3	4.1
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.7	5.4	5.6	5.5	5.7	5.6	5.6	5.4	5.7	5.7
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	275	227	284	245	278	263	255	247	267	258
No. of contigs [1-25] x min. coverage	{<1000}	0	0	1	0	0	0	0	0	0	0
Average coverage	{>50}	65	66	47	78	60	80	65	58	54	62
No. of reads (x 1000)		1600	1530	1147	1858	1470	1918	1536	1353	1332	1510
Average read length		237	237	236	238	235	238	239	240	237	238
Average insert size		335	338	330	340	320	338	367	377	360	369
N50 (kbp)		95	118	98	118	98	114	114	104	101	98

		Laboratory 108									
Parameters	**Ranges***	**9017**	**9031**	**9315**	**9390**	**9401**	**9430**	**9471**	**9515**	**9608**	**9735**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		95.7	96.2	96.5	96.0	95.5	96.2	96.0	95.7	95.7	96.1
% Species 2		1.7	0.9	1.2	1.2	1.0	1.0	1.1	1.0	1.0	1.0
Unclassified reads (%)	{<100}	2.0	2.2	2.0	2.2	2.5	2.2	2.1	2.2	2.3	2.0
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.3	5.4	5.4	5.6	5.6	5.2	5.4	5.4	5.4	5.4
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.6	0.0
No. of contigs at 25 x min. coverage	{>0}	#759	#723	#1182	#1019	#942	#1991	#2201	#2168	#2515	#1841
No. of contigs [1-25] x min. coverage	{<1000}	#0	#0	#0	#0	#0	#1	#0	#1	#3	#0
Average coverage	{>50}	121	149	140	127	121	91	95	98	77	97
No. of reads (x 1000)		2472	3251	2984	2800	2710	1897	2039	2142	1668	2077
Average read length		266	256	264	261	257	266	267	264	267	264
Average insert size		NA	NA	NA	NA	NA	NA	NA	NA	NA	
N50 (kbp)		#16	#19	#10	#12	#13	#5	#4	#4	#4	#5

		Laboratory 123									
Parameters	**Ranges***	**9067**	**9120**	**9159**	**9185**	**9275**	**9423**	**9491**	**9622**	**9770**	**9886**
Detected species	{Ec} or {Ss}	Ec	Ec	Ec	Ec	Ec	Ec, Ss	Ec	Ec	Ec	Ec
% Species 1		87.6	88.5	94.1	89.9	89.4	84.9	86.8	94.1	94.4	93.9
% Species 2		1.7	4.1	0.3	3.5	3.5	5.0	4.2	1.3	2.3	0.7
Unclassified reads (%)	{<100}	8.4	5.6	4.8	5.0	5.0	7.8	7.1	4.2	2.6	4.3
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.2	5.5	5.4	5.5	5.4	5.5	5.5	5.5	5.4	5.7
Length [1-25] x min. coverage (kbp)	{<250}	240.4	107.5	16.7	92.2	128.7	134.2	94.5	79.9	0.0	4.4
No. of contigs at 25 x min. coverage	{>0}	368	344	337	343	328	323	321	337	239	309
No. of contigs [1-25] x min. coverage	{<1000}	82	46	12	25	45	75	26	32	0	3
Average coverage	{>50}	47	56	58	65	53	51	67	51	82	78
No. of reads (x 1000)		1070	1336	1281	1659	1165	1128	1640	1136	1860	1815
Average read length		247	241	251	228	257	258	236	253	242	250
Average insert size		306	281	308	256	313	311	272	340	285	301
N50 (kbp)		42	60	60	84	61	70	91	52	104	82

		Laboratory 124									
Parameters	**Ranges***	**9038**	**9052**	**9100**	**9194**	**9296**	**9392**	**9631**	**9837**	**9929**	**9934**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		97.0	97.4	96.9	96.9	97.7	97.0	96.6	96.8	96.8	97.5
% Species 2		1.1	0.6	0.6	0.6	0.7	0.6	0.5	1.3	0.5	0.7
Unclassified reads (%)	{<100}	1.3	1.2	1.4	1.3	1.1	1.2	1.6	1.1	1.5	1.1
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.7	5.5	5.7	5.6	5.6	5.7	5.7	5.4	5.7	5.5
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	227	209	225	227	224	223	228	191	224	222
No. of contigs [1-25] x min. coverage	{<1000}	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	588	263	569	649	262	256	565	471	507	613
No. of reads (x 1000)		13773	6000	13355	14955	6000	6000	13268	10503	11889	13752
Average read length		251	251	251	251	251	251	251	251	251	251
Average insert size		468	468	465	466	464	456	458	457	466	469
N50 (kbp)		108	127	108	115	123	108	108	127	108	114

		Laboratory 127									
Parameters	Ranges*	9083	9128	9152	9180	9367	9378	9603	9704	9741	9793
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		91.8	93.0	93.9	94.3	95.3	95.0	94.1	92.9	95.9	95.0
% Species 2		1.4	1.8	0.6	1.1	1.7	0.6	0.7	0.7	0.8	1.3
Unclassified reads (%)	{<100}	5.1	4.0	4.9	3.5	2.7	3.5	4.2	5.6	3.1	3.3
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.4	5.6	5.4	5.5	5.3	5.6	5.6	5.5	5.5	5.5
Length [1-25] x min. coverage (kbp)	{<250}	54.7	7.0	12.9	85.2	28.8	23.4	19.6	13.9	8.7	59.5
No. of contigs at 25 x min. coverage	{>0}	354	313	314	531	334	419	435	292	282	380
No. of contigs [1-25] x min. coverage	{<1000}	32	6	13	94	24	30	24	15	7	47
Average coverage	{>50}	70	76	80	58	96	92	109	83	79	79
No. of reads (x 1000)		2729	2978	3025	2270	3561	3639	4331	3185	3089	3078
Average read length		145	148	149	147	146	144	144	148	145	146
Average insert size		481	408	400	327	380	335	371	424	414	473
N50 (kbp)		46	76	64	28	58	44	41	60	91	46

		Laboratory 133									
Parameters	Ranges*	9077	9088	9193	9281	9357	9368	9555	9571	9624	9950
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		90.4	93.2	93.4	93.9	86.3	90.9	94.2	95.1	95.1	95.7
% Species 2		1.8	0.7	1.2	2.4	1.5	0.9	0.9	1.4	0.6	0.7
Unclassified reads (%)	{<100}	6.4	5.2	3.6	3.1	10.8	6.7	3.7	3.1	3.1	2.5
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.4	5.3	5.5	5.2	5.5	5.3	5.6	5.5	5.5	5.6
Length [1-25] x min. coverage (kbp)	{<250}	72.7	19.4	2.1	52.6	1.6	194.9	2.5	1.0	1.3	4.0
No. of contigs at 25 x min. coverage	{>0}	1040	717	516	761	805	1270	394	492	309	508
No. of contigs [1-25] x min. coverage	{<1000}	61	26	3	62	4	231	4	1	3	8
Average coverage	{>50}	118	68	95	61	117	93	107	125	93	80
No. of reads (x 1000)		2663	1524	2115	1451	2901	2494	2322	2627	1983	1763
Average read length		258	258	262	239	253	227	270	275	269	268
Average insert size		296	278	296	245	266	220	309	325	307	302
N50 (kbp)		12	17	42	15	16	9	61	32	68	30

		Laboratory 134									
Parameters	Ranges*	9009	9108	9226	9272	9278	9647	9691	9745	9907	9921
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		95.4	90.3	89.5	94.6	91.4	93.2	91.8	90.9	91.2	95.4
% Species 2		0.5	2.6	3.0	1.7	1.7	1.2	1.9	2.5	2.6	1.0
Unclassified reads (%)	{<100}	3.2	5.5	5.8	2.7	4.9	4.5	3.5	5.1	4.6	3.2
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.4	5.5	5.6	5.3	4.4	5.1	5.4	5.6	5.6	5.5
Length [1-25] x min. coverage (kbp)	{<250}	0.0	80.5	0.0	0.0	1060.9	466.3	0.6	0.8	0.0	51.6
No. of contigs at 25 x min. coverage	{>0}	297	277	297	232	243	264	417	278	321	264
No. of contigs [1-25] x min. coverage	{<1000}	0	5	0	0	61	23	2	1	0	7
Average coverage	{>50}	89	48	58	57	32	37	54	63	87	37
No. of reads (x 1000)		3312	1840	2213	2091	1215	1456	2234	2429	3371	1380
Average read length		151	151	151	151	151	151	151	151	151	151
Average insert size		276	374	357	350	402	394	165	366	292	395
N50 (kbp)		102	87	90	93	73	88	98	95	89	92

		Laboratory 135									
Parameters	**Ranges***	**9021**	**9080**	**9276**	**9453**	**9552**	**9698**	**9714**	**9849**	**9883**	**9986**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		93.4	92.0	88.7	86.2	92.2	92.5	86.1	91.1	93.9	87.8
% Species 2		0.8	1.8	2.3	3.2	1.1	1.3	4.0	1.0	1.6	2.6
Unclassified reads (%)	{<100}	4.7	5.0	6.9	8.1	5.8	5.0	6.8	6.4	3.3	7.4
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.5	5.6	5.6	5.6	5.4	5.5	5.5	5.6	5.3	5.6
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	252	276	349	364	359	342	349	351	278	352
No. of contigs [1-25] x min. coverage	{<1000}	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	176	160	136	142	173	168	161	145	177	151
No. of reads (x 1000)		6626	6170	5524	5893	6928	6843	6587	5996	6834	6201
Average read length		149	149	151	151	151	151	151	151	151	151
Average insert size		358	350	246	231	226	227	229	232	236	238
N50 (kbp)		114	93	83	87	92	104	91	89	104	87

		Laboratory 136									
Parameters	**Ranges***	**9111**	**9130**	**9181**	**9245**	**9294**	**9391**	**9409**	**9415**	**9609**	**9898**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		95.9	95.1	94.3	95.7	96.8	97.0	93.9	93.2	96.1	94.3
% Species 2		0.7	0.8	1.4	0.5	1.2	0.9	1.8	2.1	1.1	1.7
Unclassified reads (%)	{<100}	2.3	3.2	3.2	3.3	1.7	1.9	3.3	3.7	2.5	2.9
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.5	5.5	5.6	5.4	5.3	5.5	5.6	5.6	5.7	5.6
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	286	272	373	295	242	285	305	306	298	297
No. of contigs [1-25] x min. coverage	{<1000}	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	307	245	270	301	297	255	236	275	231	222
No. of reads (x 1000)		11596	9181	10595	11121	10873	9581	9162	10624	8970	8549
Average read length		149	149	146	149	149	149	148	149	148	149
Average insert size		335	322	226	307	295	324	293	298	304	318
N50 (kbp)		102	115	87	102	111	108	95	95	92	92

		Laboratory 137									
Parameters	**Ranges***	**9058**	**9126**	**9306**	**9314**	**9482**	**9519**	**9539**	**9884**	**9976**	**9997**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		92.0	91.1	92.0	91.8	90.5	92.0	90.9	91.1	91.1	91.6
% Species 2		1.0	0.7	0.9	0.9	0.4	0.4	0.7	0.4	0.7	1.1
Unclassified reads (%)	{<100}	6.9	7.1	6.6	6.8	8.1	6.9	7.5	7.7	7.3	6.8
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.4	4.9	5.0	4.1	5.5	2.9	5.5	5.3	4.2	4.3
Length [1-25] x min. coverage (kbp)	{<250}	11.4	467.0	243.1	1199.4	2.8	2552.3	31.1	15.3	1266.7	1139.3
No. of contigs at 25 x min. coverage	{>0}	346	314	256	275	368	226	351	327	307	281
No. of contigs [1-25] x min. coverage	{<1000}	10	37	46	97	4	194	14	11	104	114
Average coverage	{>50}	52	40	38	33	78	28	52	48	36	33
No. of reads (x 1000)		2915	2250	2089	1813	4607	1607	2964	2730	2044	1913
Average read length		100	100	100	100	97	100	100	99	99	100
Average insert size		335	331	354	324	283	313	329	300	298	322
N50 (kbp)		69	61	90	61	76	50	77	77	57	56

		Laboratory 139									
Parameters	**Ranges***	**9171**	**9173**	**9450**	**9468**	**9476**	**9581**	**9632**	**9746**	**9900**	**9901**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		96.2	94.8	95.0	94.9	97.2	96.1	95.7	95.0	94.4	95.8
% Species 2		0.6	1.0	0.9	1.0	0.6	0.8	1.4	0.8	1.2	1.1
Unclassified reads (%)	{<100}	2.4	2.8	2.8	2.7	1.8	2.4	1.9	2.9	2.8	2.5
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.5	5.6	5.6	5.6	5.5	5.4	5.3	5.6	5.5	5.7
Length [1-25] x min. coverage (kbp)	{<250}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. of contigs at 25 x min. coverage	{>0}	290	299	296	298	297	291	234	299	293	306
No. of contigs [1-25] x min. coverage	{<1000}	0	0	0	0	0	0	0	0	0	0
Average coverage	{>50}	54	80	77	85	61	96	86	74	80	70
No. of reads (x 1000)		2018	3077	2945	3281	2300	3550	3185	2865	3035	2713
Average read length		151	151	151	151	151	151	151	151	151	151
Average insert size		307	310	325	313	300	308	296	308	306	307
N50 (kbp)		104	95	92	94	104	102	104	89	104	92

		Laboratory 222									
Parameters	**Ranges***	**9012**	**9094**	**9369**	**9681**	**9706**	**9719**	**9729**	**9818**	**9914**	**9990**
Detected species	{Ec}	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec	Ec
% Species 1		97.3	96.6	96.8	97.0	96.8	96.5	96.5	96.3	96.7	96.5
% Species 2		1.2	1.4	1.2	0.9	0.8	1.1	1.0	1.0	1.1	0.9
Unclassified reads (%)	{<100}	1.1	0.9	1.0	1.1	1.3	1.2	1.2	1.2	1.2	1.2
Length at >25 x min. coverage (Mbp)	{>45 ∧ <58}	5.5	5.3	5.6	5.4	5.6	5.4	5.6	5.5	5.5	5.5
Length [1-25] x min. coverage (kbp)	{<250}	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
No. of contigs at 25 x min. coverage	{>0}	#838	#651	#693	#531	#627	#647	#494	#515	#1322	#1340
No. of contigs [1-25] x min. coverage	{<1000}	#2	#0	#0	#0	#0	#0	#0	#0	#1	#0
Average coverage	{>50}	178	272	206	263	250	232	260	300	174	189
No. of reads (x 1000)		3133	4551	3501	4512	4564	3970	4709	5454	2925	3084
Average read length		318	323	336	319	312	325	315	309	341	349
Average insert size		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
N50 (kbp)		#15	#18	#20	#31	#24	#22	#33	#34	#8	#8

*Quality assessment made by the EQA-provider in-house quality control pipeline. *: indicative QC ranges; Ec: E. coli, Ss: Shigella sonnei, Pt: Pseudomonas tolaasii (listed if >5%).*
NA: not analysed.
#: QC values unreliable due to assembly issues for Ion Torrent data.

Annex 13. Results of the participants' assessment of the EQA provided genomes

	Characteristics identified by participants		EQA provider	
Lab ID	**Cluster**	**Genome 1**	**Contamination detected?**	**Cluster**
EQA provider	No	A non-cluster isolate (REF11) mixed with a different *Escherichia coli* (approx. 10%)	Yes	No
19	No	We would not include genome 1 in the cluster analysis because of a possible contamination. Genome 1 has many contigs, high multiple consensus loci, many unidentified bases, too large genome and failed in the species testing with Kraken - all indicating a possible contaminated sample. Despite that the genome would not be used for analysis before pure culture is available, when looking at allele differences (AD) to the cluster representative genome, genome 1 differs with 101 alleles and is therefore not considered closely related to the outbreak cluster.	Yes	No
34	No	Assembling by SKESA cgMLST by RIDOM SeqSphere+QC: excellent 97.2% good targets (only 70 missing) 929 contigs included in cluster analysis.	No	No
80	No	Our cluster threshold is <=10 allelic differences and genome number 1 is 91 allelic differences.	No	No
100	No	Genome 1 has 67 AD from the representative isolate.	No	No
108	No	Genome 1 is more than 771 SNP from 9 471 and is thereby not included in the cluster.	No	No
123	No	Genome 1 shows 98.5 % cgMLST targets (sufficient quality for routine analysis), it is ST 21, but another Complex Type (CT) (CT8917 and not CT3023 as the cluster isolates). It has 94 allelic differences to the representative isolate in the cluster 9 120 (threshold is 10). Because of the last two reasons, it does not belong to the cluster.	No	No
124	No	Genome 1 has a genome size of 5.9 Mb, a high number of N bases, and a high number of multiple alleles, suggesting a mix of closely related organisms. After running KmerFinder, a query coverage of 84.06% and a template coverage of 34.56% was obtained, with E.coli O26:H11 as template. 2nd: a query coverage of 34.56% and a template coverage of 54.80% was obtained, with E.coli 118UI as template. After running the cgMLST (Core Enterobase) analysis we observe that genome 1 has 100 AD with the cluster representative. With all this information we can presume that genome 1 is not part of the cluster.	Yes	No
127	No	Genome 1 is not a member of the outbreak cluster. Based on the cgMLST analysis, the difference greater than 10 alleles between genome 1 and representative isolate of the outbreak cluster (9 128) exists.	No	No
133	No	103 allele difference from representative strain 9 368.	No	No
134	No	The sequence quality of Genome 1 is quite good, with 97.5% of the targets found and a number of contigs slightly high but still correct. Allele difference (AD) with the cluster = 92. The isolate is excluded from the cluster.	No	No
135	No	Genome 1 does not meet quality criteria – the genome size is too high. It is therefore not possible to state reliably whether it is part of the cluster. Isolate should be checked for contamination and WGS repeated from the start. When included in comparison in spite of quality issues, 104 alleles different from closest cluster isolate.	Yes	No
136	No	We define a cluster threshold of <=10 allelic differences and genome 1 does not match the criteria.	No	No
137	No	839 SNPs distance indicating it is not part of the cluster as it is too distantly related.	No	No
139	No	Quality is OK using Enterobase passing filters. Using cgMLST as a typing method, we conclude that this isolate does not belong to the cluster (same HC50 only). We detect two different alleles for three loci (fumC, gyrB and icd) of MLST scheme suggesting a possible contamination with another E. coli/shigella isolate. However, after a rapid check, all combinations of alleles for these seven MLST genes lead to the same clonal complex STc29.A subculture of the isolate would allow to check the purity of the congelation stock.	Yes	No
222	No	2307/2360 loci of cgMLST scheme correctly mapped, satisfying our quality threshold set at 80% for reliability of cluster analysis. Moreover, the seven genes of conventional MLST (Warwick scheme) were all found 100% in length and with >=30 average depth of coverage. All quality criteria were satisfied for this sequence, which showed >=87 allelic differences from all the isolates part of the detected cluster. We set the threshold for considering a cluster at <=15 allelic differences, so this genome is not part of the cluster.	No	No

	Characteristics identified by participants		EQA provider	
Lab ID	**Cluster**	**Genome 2**	**QC Accepted**	**Cluster**
EQA provider	No	A non-cluster isolate (REF12) assembled with SKESA to a FASTA file. (76 AD to cluster isolate REF1/REF5).	Yes	No
19	No	Genome 2 has 74 AD to the cluster representative genome and is therefore not considered a part of the outbreak cluster.	Yes	No
34	No	Fasta cgMLST by RIDOM SeqSphere+QC: excellent 98.5% good targets (only 38 missing) included in cluster analysis.	Yes	No
80	No	Our cluster threshold is <=10 allelic differences and genome number 2 is 73 allelic differences.	Yes	No
100	No	Genome 2 has a too low percentage of good targets for cgMLST cluster analysis (0%). Not enough quality to perform cluster analysis. Isolate should be sequenced again in order to access if it is part of the cluster.	No	No
108	No	Genome 2 is more than 776 SNP from 9471 and is thereby not included in the cluster. The genome has a low average coverage (<20 times) and should be rerun before any certain conclusions can be made. Also, this was a fasta-file that usually gives a lower QC value.	Yes	No
123	No	Genome 2 shows 98,5 % cgMLST targets (sufficient quality for routine analysis), it is ST 21 (no CT can be calculated), and it has 74 allelic differences to the representative isolate in the cluster 9 120. Too many allelic differences (threshold is 10) for being part of the cluster.	Yes	No
124	No	Genome 2 has a genome size of 5.2 Mb, GC% of 53.03 and a low N50. The O-type could not be determined by the E.coli plugin tool in BioNumerics. After running KmerFinder, a query coverage of 93.27% and a template coverage of 95.20% was obtained, with E.coli O26:H11 as template. After running the cgMLST (Core Enterobase) analysis we observe that genome 2 has 70 AD with the cluster representative. With all this information we can presume that genome 2 is not part of the cluster.	Yes	No
127	No	An additional analysis was performed. Based on the reference-based SNP analysis (genome 2 = reference) in Bionumerics v7.6, the difference greater than 300 SNPs between genome 2 and representative isolate of the outbreak cluster (9128) exists.	Yes	No
133	No	74 allele differences from representative strain 9 368.	Yes	No
134	No	The quality of the assembly is good allowing 98.5% of the targets found. The number of contigs is correct with N= 549. AD = 74, so this isolate is not part of the cluster.	Yes	No
135	No	Genome 2 differs 74 alleles from closest cluster isolate.	Yes	No
136	No	We define a cluster threshold of <=10 allelic differences and genome 1 does not match the criteria.	Yes	No
137	No	614 SNPs distance, indicating it is not part of the cluster as it is too distantly related.	Yes	No
139	No	As fastQ files are not available for this isolate, we cannot check coverage quality nor determine the cgMLST for this isolate. However, using a SNIP-based phylogeny analysis, this isolate does not seem to be related to the cluster.	Yes	No
222	No	2336/2360 loci of cgMLST scheme correctly mapped, satisfying our quality threshold set at 80% for reliability of cluster analysis. Due to lack of availability of the original .fastq file, it was not possible to perform the additional quality check based on the depth of coverage of the seven genes of conventional MLST (Warwick scheme). For this reason, MLST was performed on the assembly, allowing correct identification of all the seven genes and call of ST21. Total length of assembled contigs was 5 290 121 bp and N50 was 32 079. The results were considered reliable (N50>30 000 and assembly length of about 5 Mb). The sequence showed >=71 allelic differences from all the isolates part of the detected cluster. We set the threshold for considering a cluster at <=15 allelic differences, so this genome is not part of the cluster.	Yes	No

	Characteristics identified by participants		EQA provider	
Lab ID	**Cluster**	**Genome 3**	**Contamination detected?**	**Cluster**
EQA provider	No	A non-cluster isolate (REF13) mixed with a *Klebsiella pneumonia (*approx. 10%).	Yes	No
19	No	We would not include genome 3 in the cluster analysis because of a possible contamination. Genome 3 had many contigs, high multiple consensus, many unidentified bases, double the size of expected genome size and failed in the species testing with Kraken – all indicating a possible contaminated sample. Despite that the genome would not be used for analysis before pure culture is available, when looking at AD to the cluster representative genome, genome 3 differ with 69 alleles and is therefore not considered closely related to the outbreak cluster.	Yes	No
34	No	Assembling by SKESA cgMLST by RIDOM SeqSphere+QC: bad 34.5% good targets (1 646 missing) no use for cluster analysis possible not included sequence data were contaminated (probably *E.coli* and *Klebsiella*) isolation of pure culture before rerunning.	Yes	No
80	No	Our cluster threshold is <=10 allelic differences and genome number 3 is 67 allelic differences	No	No
100	No	Genome 3 has 134 AD from the representative isolate.	No	No
108	No	This sample was contaminated (genome size 9,8Mbp, O26/O8:H11). SNP-analyses based on this contaminated sample shows that it´s not included in the cluster (more than 633 SNP from 9 471), but the sample must be recultivated before conclusions can be made.	Yes	No
123	No	Allthough Genome 3 shows only 41,7 %cgMLST targets (which is a not sufficient Quality for Routine Analysis, our treshold is 98 %cgMLST Targets normally we would rerun, or suggest to rerun this sequence), we already see in a pairwise comparison to the representative Isolat in the cluster 9 120 24 allelic differences. If more alleles could be compared, even more differences, and not fewer, would occur; therefore, it does not belong to the cluster.	Yes	No
124	No	Genome 3 has a genome size of 10.5 Mb, high number of N bases and a high number of multiple alleles, suggesting a mix of 2 distinct organisms (which was confirmed after running Kmer Finder: E.coli + Klebsiella pneumoniae) and probably contamination with other E.coli. The E.coli plugin tool in BioNumerics could determine the O-type (O26 or O8). After running the cgMLST (Core Enterobase) analysis we observe that genome 3 has 70 AD with the cluster representative. With all this information we can presume that genome 3 is not part of the cluster.	Yes	No
127	No	Genome 3 should be excluded from the analysis, because of sequence length (> 10 Mb) and high number of loci with multiple alleles. It seems that contamination might be present.	Yes	No
133	No	73 allele difference from representative strain 9368.	No	No
134	Yes	The quality of the raw reads is really bad, only 31.7% of the targets are found after the assembly. The number of contigs is too high as well. However, the vtx_1a variant is well identified for this isolate and AD = 26. In an outbreak situation, I would not exclude this isolate and rerun the sequencing (and re-do the DNA extraction as well).	Yes	Yes
135	No	genome 3 does not meet quality criteria: genome size too high, GC% too high, contig count too high, % assigned alleles too low, contamination too high (9% of total reads are Klebsiella pneumoniae). therefore it is not possible to reliably state if it is part of the cluster or not.isolate should be checked for contamination and WGS repeated from the start.	Yes	No
136	No	We define a cluster threshold of <=10 allelic differences and genome 1 does not match the criteria	No	No
137	No	3 989 SNPs distance, indicating it is not part of the cluster as it is too distantly related; it is also a different serotype, O177:H11.	No	No
139	No	Quality is OK using Enterobase passing filters. This isolate does not belong to the cluster as cgMLST is different (only the same HC100). We detect two different alleles for one loci (gyrB) of MLST scheme suggesting a possible contamination with another E. coli/shigella isolate. However, after a rapid check, the other possibility of combinations of alleles for these 7 MLST genes lead to the same clonal complex STc29. A subculture of the isolate would allow to check the purity of the congelation stock.	Yes	No
222	No	2344/2360 loci of cgMLST scheme correctly mapped, satisfying our quality threshold set at 80% for reliability of cluster analysis. Moreover, the 7 genes of conventional MLST (Warwick scheme) were all found 100% in length and with >=30 average depth of coverage. All quality criteria were satisfied for this sequence, which showed >=64 allelic differences from all the isolates part of the detected cluster. We set the threshold for considering a cluster at <=15 allelic differences, so this genome is not part of the cluster.	No	No

	Characteristics identified by participants		EQA provider	
Lab ID	**Cluster**	**Genome 4**	**QC Accepted**	**Cluster**
EQA provider	Yes	A Cluster isolate (REF14) without *stx* genes (0 AD to cluster isolate REF1/REF5/REF10).	Yes	Yes
19	Yes	Genome 4 has zero AD to the cluster representative genome and is therefore considered a part of the outbreak cluster.	Yes	Yes
34	Yes	assembling by SKESA cgMLST by RIDOM SeqSphere+QC: excellent98.8% good targets (only 29 missing)included in cluster analysis	Yes	Yes
80	Yes	Our cluster threshold is <=10 allelic differences and genome number 4 is identical.	Yes	Yes
100	Yes	Genome 4 has 0 AD from the representative isolate.	Yes	Yes
108	Yes	This sample differs by 4 SNP against 9471, and is thereby part of the cluster.	Yes	Yes
123	Yes	Genome 4 shows 99,5 %cgMLST Targets (good Quality), has the same ST and CT as the cluster isolates (21 and 3023, respectively), and only one allelic difference to the representative Isolat in the cluster 9120. This strain belongs to the cluster.	Yes	Yes
124	Yes	Genome 4 has a genome size of 5.6 Mb and a high number of N bases, suggesting impurity or contamination of the DNA extract. After running KmerFinder, a query coverage of 77.28% and a template coverage of 93.85% was obtained, with EHEC O26:H11 as template. 2nd: a query coverage of 2.27% and a template coverage of 52.26% was obtained, with Shigella as template. The E.coli plugin tool in BioNumerics could not detect stx. Normally, this would have been performed via PCR before performing NGS. After running the cgMLST (Core Enterobase) analysis we observe that genome 4 has 0 AD with the cluster representative. With all this information we can presume that genome 4 is part of the cluster.	Yes	Yes
127	Yes	Genome 4 is genetically linked to the outbreak cluster. Based on the cgMLST analysis, the two allele difference between genome 4 and representative isolate of the outbreak cluster (9128) exists.	Yes	Yes
133	Yes	only 1 allele difference from representative strain 9 368.	Yes	Yes
134	Yes	The sequencing data are really good, 99% targets are found and the number of contigs correct (N = 784). The AD = 1 from the cluster but the isolate does not harbor verotoxin target (no vtx1a). In outbreak situation, this isolate would have been considered as part of the cluster as stx genes may be lost during infection or in vitro during culturing steps. In addition the eae and ehxA genes are detected in this strain.	Yes	Yes
135	Yes	Genome 4 meets all quality criteria.it is identical (0 alleles difference) to the representative isolate.further isolate information needed to determine if there is an epidemiological link to confirm this genetic link.	Yes	Yes
136	Yes	We define a cluster threshold of <=10 allelic differences and genome 4 is two allelic difference	Yes	Yes
137	No	594 SNPs distance indicating it is not part of the cluster as it is too distantly related.	Yes	No
139	Yes	Sequencing quality seems OK using Enterobase passing filters. This isolate has the same Hierarchical clustering 2 (HC2) as the 4 isolates of the cluster. That's why we could consider it is related to the cluster. This isolate has the eae, ehxA, katP, espP genes suggesting that it belongs to the EHEC family but it lacks STX genes. This could be explained either by an insufficient sequencing quality or by the fact that STX genes have been lost by the isolate.To check that, a culture of the isolate is necessary and a rapid check by qPCR could be performed to check the presence or absence of the stx genes. Depending on this result, a second run of NGS could be performed and will allow to confirm the belonging of this isolate to the cluster.	Yes	Yes
222	Yes	2353/2360 loci of cgMLST scheme correctly mapped, satisfying our quality threshold set at 80% for reliability of cluster analysis. Moreover, the seven genes of conventional MLST (Warwick scheme) were all found 100% in length and with >=30 average depth of coverage. All quality criteria were satisfied for this sequence, which showed minimum 1 (from isolate 9729) and maximum 11 (from isolate 9990) allelic differences from all the isolates part of the detected cluster (four from 9 706 isolate used as cluster reference above and two from 9 914 isolate). We set the threshold for considering a cluster at <=15 allelic differences, so this genome is part of the cluster and could help solving the outbreak investigated.	Yes	Yes

	Characteristics identified by participants		EQA provider	
Lab ID	**Cluster**	**Genome 5**	**Quality issue**	**Cluster**
EQA provider	Yes	A Cluster isolate (REF9) with altered coverage (reduced to 12x)	Yes	Yes
19	Yes	It is likely that genome 5 is part of the outbreak cluster, but the genome has bad quality and needs to be re-sequenced to confirm the outbreak suspicion. Genome 5 has low coverage, many unidentified bases, many contigs, low N50 value and low cgMLST core%. Based on the only 51 core%, genome 5 cluster with the outbreak cluster, here indicating that genome 5 is likely part of the cluster, however not confirmed.	Yes	Yes
34	No	assembling by SKESA cgMLST by RIDOM SeqSphere+QC: bad45.5% good targets (1.370 missing)no use for cluster analysis possible not included recultivation of pure culture and new DNA- and library preparation before rerunning (probably small amount of DNA)	Yes	No
80	No	Genome 5 a cluster could not be performed for poor sequence quality	Yes	No
100	No	Genome 5 has a too low percentage of good targets for STEC cgMSLT cluster analysis (47,5 %). KmerFinder analysis showed coverage of approximately 80 with the best hit template coverage, which can indicate that contamination is possible.	Yes	No
108	No	We could not analyse this sequence, regarding SNP-analysis because the sample was too low (average coverage <15) to pass the QC values in our pipeline. This sample should be rerun.	Yes	No
123	No	Allthough Genome 5 shows only 91,6 %cgMLST targets (which is a not sufficient Quality for Routine Analysis, our threshold is 98 %cgMLST Targets normally we would rerun, or suggest rerunning this sequence), we see in a pairwise comparison to the representative Isolat in the cluster 9120 already 49 allelic differences. If more alleles could be compared even more differences, and not fewer would occur. Additionally, it shows a different CT (CT 8 918 and not CT 3 023 as the cluster strains). Because of these two reasons this strain does not belong to the cluster.	Yes	No
124	Yes	Genome 5 has a genome size of 5.4 Mb, a low coverage (average read coverage 14), a high number of N bases and a low number of confirmed alleles. After running KmerFinder, a query coverage of 77.28% and a template coverage of 95.22% was obtained, with EHEC O26:H11 as template. The E.coli plugin tool in BioNumerics could determine the O-type not the ST (seven genes). After running the cgMLST (Core Enterobase) analysis we observe that genome 5 has 0 AD with the cluster representative. With all this information, we could presume that genome 5 is part of the cluster but there are a lot of alleles missing.	Yes	Yes
127	No	Genome 5 did not pass quality control because of low coverage (< 30) and low N50. The sample should be made again.	Yes	No
133	No	Genome 5 failed out QC parameters. Therefore, it could not be determined if it is part of the cluster. In an outbreak situation, this case would be investigated as part of the cluster until repeat WGS confirmed whether or not it is part of the cluster.	Yes	No
134	Yes	Only 45.6 % of the targets are found after the assembly. The quality of the sequencing is not good enough to exclude this isolate from the cluster as it the vtx1a was also detected and AD = 2. In an outbreak situation, I would have rerun the sample (and redo DNA extraction as well).	Yes	Yes
135	No	Genome 5 does not meet quality criteria: N50 too low, contig count too high. It is therefore not possible to reliably state if it is part of the cluster or not. WGS can be repeated from isolated DNA if the quality of this DNA meets criteria. When included in comparison in spite of quality issues, 77 alleles different from closest cluster isolate.	Yes	No
136	No	We define a cluster threshold of <=10 allelic differences and genome 1 does not match the criteria.	No	No
137	Yes	Genome 5 was used as the reference strain for the cluster, therefore has a SNP distance of 0 and is part of the cluster.	No	Yes
139	-	We cannot conclude whether or not this isolate belongs to the cluster. What can be said is that the strain belongs to the same ST and has the same acquired resistance genes as isolates in the cluster. Due to the very insufficient sequencing quality, it is not possible to determine whether or not it belongs to the cluster. The insufficient sequencing coverage does not make it possible to decide on the presence of certain virulence genes or to determine the cgMLST. The only possibility is to re-sequence the isolate or to perform another typing method as PFGE.	Yes	-
222	No	2070/2360 loci of cgMLST scheme correctly mapped, satisfying our quality threshold set at 80% for reliability of cluster analysis. Nevertheless, the seven genes of conventional MLST (Warwick scheme) were not all found 100% in length or had with <30 average depth of coverage. Moreover, the N50 for the assembly was only 6 538 (lower than threshold set at 30 000). In an outbreak situation, we would consider cgMLST anyway to guide the analysis, but we would still repeat the sequencing for getting better and more reliable results. The cgMLST results >=54 allelic differences from all the isolates part of the detected cluster. We set the threshold for considering a cluster at <=15 allelic differences, so this genome is not part of the cluster, results to be confirmed with a better sequence.	Yes	No

-: no reported data/analysis performed.

Annex 14. Word format of the online form

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 (indicated by the 'Go to').

1. STEC EQA-10 2020

Dear Participant
Welcome to the tenth External Quality Assessment (EQA-10) scheme for typing of STEC in 2019-2020.
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 ecoli.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
Note: After pressing 'Submit results', you will not be able to review your results.

2. Country

- ❑ Austria
- ❑ Belgium
- ❑ Czechia
- ❑ Denmark
- ❑ Estonia
- ❑ Finland
- ❑ France
- ❑ Germany
- ❑ Greece
- ❑ Iceland
- ❑ Ireland
- ❑ Italy
- ❑ Latvia
- ❑ Lithuania
- ❑ Luxembourg
- ❑ Norway
- ❑ Poland
- ❑ Portugal
- ❑ North Macedonia
- ❑ Romania
- ❑ Scotland
- ❑ 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. Serotyping and virulence gene determination of STEC

8. Submitting results

(State one answer only)

- ❑ Submit serotyping/virulence gene determination results (please fill in the strain IDs in the next section) - Go to 9
- ❑ Did not participate in the serotyping nor virulence determination part(s) - Go to 21

9. Serotyping/virulence isolate IDs

Please enter the strain ID (4 digits)
We recommend to print this page out! To have the overview of isolate IDs and isolate No. 1-10, it will make the work easier.

STEC

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

10. Submitting results - Serotyping

(State one answer only)

- ❑ Both O group and H type - Go to 11
- ❑ Only O Group - Go to 11
- ❑ Only H type - Go to 13
- ❑ Did not participate in serotyping - Go to 15

11. Results for serotyping (O Group)

Please type the number of O Group by using (1-188)
Non Typable: 7777, Rough: 8888, Not done: ND

O Group

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

12. Please specify the method used:

Phenotypic or molecular (PCR based, WGS based)
(State only one answer per question)
Method

- ❑ Phenotypic
- ❑ PCR based
- ❑ WGS based

13. Results for serotyping (H Type)

Please type the number of H Type by using (1-56)
H-: 6666, Non Typable: 7777, Not done: ND
H type

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

14. Please specify the method used:

Phenotypic or molecular (PCR based, WGS based)
(State only one answer per question)
Method

- ❑ Phenotypic
- ❑ PCR based
- ❑ WGS based

15. Submitting results - Virulence gene determination

(State only one answer per question)

- ❑ Submit virulence gene determination data (*eae, aaiC, aggR, stx1a, stx2* or subtyping
- ❑ Did not participate in the virulence gene determination (*eae, aaiC, aggR, stx1a, stx2* or subtyping) - Go to 21

16. Please specify the method used for the virulence gene determination (incl. subtyping):

(State only one answer per question)

- ❑ WGS
- ❑ Other

17. Results for virulence gene determination

Please use 1 for detected and 0 for not detected, Not done: ND

	eae	*aaiC*	*aagR*	*stx1*	*stx2*
Isolate 1	___	___	___	___	___
Isolate 2	___	___	___	___	___
Isolate 3	___	___	___	___	___
Isolate 4	___	___	___	___	___
Isolate 5	___	___	___	___	___
Isolate 6	___	___	___	___	___
Isolate 7	___	___	___	___	___
Isolate 8	___	___	___	___	___
Isolate 9	___	___	___	___	___

Isolate 10 ___ ___ ___ ___ ___

18. Submitting results – subtyping results

(State one answer only)

❑ Submit subtyping data

❑ Did not participate in subtyping - Go to 21

19. Results for subtyping

Subtyping of *stx1,* select variant (*stx1a, stx1c, stx1d*)
All isolates have to be subtyped regardless of the results of the initial screening. 'Not done/ND' will by default be evaluated as an incorrect result.
(State one answer only)

	stx1a	*stx1c*	*stx1d*	*stx1a; stx1c*	*stx1a; stx1d*	*stx1c; stx1d*	Negative	ND
Isolate 1	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 2	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 3	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 4	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 5	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 6	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 7	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 8	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 9	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 10	❑	❑	❑	❑	❑	❑	❑	❑

20. Subtyping of *stx2* select variant (stx2a, stxb, stx2c, stx2d, stx2e, stx2f, stx2g)

All isolates have to be subtyped regardless of the results of the initial screening. 'ND' will by default be evaluated as an incorrect result.
(State one answer only)

	stx2a	*stx2b*	*stx2c*	*stx2d*	*stx2e*	*stx2f*	*stx2g*	*stx2a stx2b*	*stx2a stx2c*	*stx2a stx2d*	*stx2b; stx2c*	*stx2b stx2d*	*stx2c stx2d*	*stx2a stx2b stx2c*	*stx2a stx2c stx2d*	*stx2b stx2c stx2d*	*stx2a stx2b stx2c stx2d*	Negative	ND
Isolate 1	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 2	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 3	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 4	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 5	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 6	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 7	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 8	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 9	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑
Isolate 10	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑	❑

21. Submitting Cluster results

(State one answer only)

- ❑ Cluster analyses based on PFGE and/or WGS - Go to 22
- ❑ Did not participate in the Cluster part - Go to 132

22. Cluster isolate IDs

Please enter the cluster isolate ID (4 digits)
We recommend to print this page out!
To have the overview of isolate ID's and isolate No. 1-10, it will make the work easier.
Cluster strain ID
Isolate 1 ___
Isolate 2 ___
Isolate 3 ___
Isolate 4 ___
Isolate 5 ___
Isolate 6 ___
Isolate 7 ___
Isolate 8 ___
Isolate 9 ___
Isolate 10 ___

23. Submitting Cluster analysis results

(State one answer only)

- ❑ Cluster analysis based on PFGE - Go to 24
- ❑ Do not wish to submit any cluster results based on PFGE analysis - Go to 29

24. Cluster analysis based on PFGE data

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

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

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

Indicate the isolate ID

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

28. Results for cluster analysis - PFGE (xbaI)

Please use ND for not analysed

	xbaI - Total number of bands (>33kb)	xbaI - 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		

29. Submitting Cluster results

(State one answer only)

- ❑ Cluster analysis based on WGS data - Go to 30
- ❑ Do not wish to submit any cluster results based on WGS data - Go to 132

30. Cluster analysis based on WGS data

31. 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 33
- ❑ Allele based - Go to 40
- ❑ Other - Go to 32

32. If another analysis is used please describe your approach:

- Go to 47

33. Please report the used SNP-pipeline

Reference if publicly available or in-house pipeline

34. Please select the approach used for the SNP analysis

(State one answer only)

- ❑ Reference based - Go to 35
- ❑ Assembly based - Go to 38

35. Reference genome used

Please indicate Multi-locus Sequence Type (e.g. ST11) and isolate ID (e.g. one of the isolate from the current cluster, ID of a public reference isolate or an in-house isolate)

36. Please indicate the read mapper used (e.g. BWA, Bowtie2)

37. Please indicate the variant caller used (e.g. SAMtools, GATK)

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

39. Please specify the variant caller used (e.g. NUCMER)

40. Please select tools used for the allele analysis

(State one answer only)

- ❑ BioNumerics - Go to 42
- ❑ SeqPhere - Go to 42
- ❑ Enterobase - Go to 42
- ❑ Other - Go to 41

41. If another tool is used please enter here:

42. Please indicate allele calling method:

(State one answer only)

- ❑ Assembly-based and mapping-based - Go to 43
- ❑ Only assembly based - Go to 43
- ❑ Only mapping based - Go to 44

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

44. Please select scheme used for the allele analysis

(State one answer only)

- ❑ Applied Math (wgMLST) - Go to 46
- ❑ Applied Math (cgMLST/Enterobase) - Go to 46
- ❑ Enterobase (cgMLST) - Go to 46
- ❑ Other - Go to 45

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

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

47. 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 IDs for isolates in the cluster detected with the additional analysis.

48. Please list the IDs for the isolates included in the cluster

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

49. Select a representative isolate in the cluster

Indicate the isolate ID

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

Please use ND for not analysed

	7-Multi-locus Sequence Type (ST)	Distance/Difference (e.g. SNP or AD) 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	_____	_____

51. Analysis of the EQA provided genomes

The five genomes uploaded by the EQA provider should be included in the analysis and evaluated.

Please evaluate this part as a simulation, mimicking a large outbreak situation in your country.
These genomes (1-5) are very important because they might solve the outbreak.
Each of the provided genomes should be assessed whether it could be a part of the cluster defined in first part.

Explain your assessment of each genome in details, please not just suggesting rerunning the sequence, but explain what you observe and what you would suggest as the conclusion.

This part is not evaluated with a final score in the evaluation report, however the EQA provider list the characteristics of the isolates.

52. In an outbreak situation, would you consider the EQA provided genome 1 a part of the cluster of closely related isolates?

(State one answer only)

- ❑ Yes, genome 1 is a part of the cluster
- ❑ No, genome 1 is NOT a part of the cluster

53. Explain your assessment of genome 1 in details

Please not just suggest rerunning, but explain what you observe and what you would propose as the conclusion.

54. In an outbreak situation, would you consider the EQA provided genome 2 a part of the cluster of closely related isolates?

(State one answer only)

- ❑ Yes, genome 2 is a part of the cluster
- ❑ No, genome 2 is NOT a part of the cluster

55. Explain your assessment of genome 2 in details

Please not just suggest rerunning, but explain what you observe and what you would propose as the conclusion.

56. In an outbreak situation, would you consider the EQA provided genome 3 a part of the cluster of closely related isolates?

(State one answer only)

- ❑ Yes, genome 3 is a part of the cluster
- ❑ No, genome 3 is NOT a part of the cluster

57. Explain your assessment of genome 3 in details

Please not just suggest rerunning, but explain what you observe and what you would propose as the conclusion.

58. In an outbreak situation, would you consider the EQA provided genome 4 a part of the cluster of closely related isolates?

(State one answer only)

- ❑ Yes, genome 4 is a part of the cluster
- ❑ No, genome 4 is NOT a part of the cluster

59. Explain your assessment of genome 4 in details

Please not just suggest rerunning, but explain what you observe and what you would propose as the conclusion.

60. In an outbreak situation, would you consider the EQA provided genome 5 a part of the cluster of closely related isolates?

(State one answer only)

- ❑ Yes, genome 5 is a part of the cluster
- ❑ No, genome 5 is NOT a part of the cluster

61. Explain your assessment of genome 5 in details

Please not just suggest rerunning, but explain what you observe and what you would propose as the conclusion.

62. 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 an additional SNP analysis
(State one answer only)

- ❑ Yes - Go to 63
- ❑ No - Go to 102

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

(State one answer only)

- ❑ SNP-based - Go to 65
- ❑ Allele-based - Go to 72
- ❑ Other - Go to 64

64. If another analysis is used please describe your approach

- Go to 79

65. Please report the used SNP-pipeline

Reference if publicly available or in-house pipeline

66. Please select the approach used for the SNP analysis

(State one answer only)

- ❑ Reference-based - Go to 67
- ❑ Assembly-based - Go to 70

67. Reference genome used

Please indicate Multi-locus Sequence Type (e.g. ST8) and isolate ID (e.g. one of the isolate from the current cluster, ID of a public reference isolate or an in-house isolate)

68. Please indicate the read mapper used (e.g. BWA, Bowtie2)

69. Please indicate the variant caller used (e.g. SAMtools, GATK)

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

71. Please specify the variant caller used (e.g. NUCMER)

72. Please select tool used for the allele analysis

(State one answer only)

- ❑ BioNumerics - Go to 74
- ❑ SeqPhere - Go to 74
- ❑ Enterobase - Go to 74

❑ Other - Go to 73

73. If another tool is used please list here:

74. Please indicate allele calling method

(State one answer only)

❑ Assembly-based and mapping-based - Go to 75
❑ Only assembly-based - Go to 75
❑ Only mapping-based - Go to 76

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

76. Please select scheme used for the allele analysis

(State one answer only)

❑ Applied Math (wgMLST) - Go to 78
❑ Applied Math (cgMLST/Enterobase) - Go to 78
❑ Enterobase (cgMLST) - Go to 78
❑ Other - Go to 77

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

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

79. Additional analysis on data derived from WGS

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

Indicate the isolate ID

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

Please use ND for not analysed

	7-Multi-locus Sequence Type (ST)	Distance (e.g. SNP) to selected cluster isolate
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		

82. 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 an additional SNP analysis

(State one answer only)

- ❑ Yes - Go to 83
- ❑ No - Go to 102

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

(State one answer only)

- ❑ SNP-based - Go to 85
- ❑ Allele-based - Go to 92
- ❑ Other - Go to 84

84. If another analysis is used please describe your approach:

- Go to 99

85. Please report the used SNP-pipeline

Reference if publicly available or in-house pipeline

86. Please select the approach used for the SNP analysis

(State one answer only)

- ❑ Reference-based - Go to 87
- ❑ Assembly-based - Go to 90

87. Reference genome used

Please indicate Multi-locus Sequence Type (e.g. ST8) and isolate ID (e.g. one of the isolate from the current cluster, ID of a public reference isolate or an in-house isolate)

88. Please indicate the read mapper used (e.g. BWA, Bowtie2)

89. Please indicate the variant caller used (e.g. SAMtools, GATK)

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

91. Please specify the variant caller used (e.g. NUCMER)

92. Please select tool used for the allele analysis

(State one answer only)

- ❑ BioNumerics - Go to 94
- ❑ SeqPhere - Go to 94
- ❑ Enterobase - Go to 94
- ❑ Other - Go to 93

93. If another tool is used please enter here:

94. Please indicate allele calling method:

(State one answer only)

- ❑ Assembly-based and mapping-based - Go to 95
- ❑ Only assembly-based - Go to 95
- ❑ Only mapping-based - Go to 96

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

96. Please select scheme used for the allele analysis

(State one answer only)

- ❑ Applied Math (wgMLST) - Go to 98
- ❑ Applied Math (cgMLST/Enterobase) - Go to 98
- ❑ Enterobase (cgMLST) - Go to 98
- ❑ Other - Go to 97

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

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

99. Third analysis on data derived from WGS

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

Indicate the isolate ID

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

Please use ND for not analysed

	7-Multi-locus Sequence Type (ST)	Distance (e.g. SNP) to selected cluster isolate
Isolate 1		
Isolate 2		
Isolate 3		
Isolate 4		
Isolate 5		
Isolate 6		
Isolate 7		
Isolate 8		
Isolate 9		
Isolate 10		

102. Additional questions to the WGS part

103 Where was the sequencing performed

(State one answer only)

- ❑ In own laboratory
- ❑ Externally

104. Protocol used to prepare the library for sequencing:

(State one answer only)

- ❑ Commercial kits - Go to 105
- ❑ Non-commercial kits - Go to 107

105. Please indicate name of commercial kit:

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

- Go to 108

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

108. The sequencing platform used

(State one answer only)

- ❑ Ion Torrent PGM - Go to 110
- ❑ Ion Torrent Proton - Go to 110
- ❑ Genome Sequencer Junior System (454) - Go to 110
- ❑ Genome Sequencer FLX System (454) - Go to 110
- ❑ Genome Sequencer FLX+ System (454) - Go to 110
- ❑ PacBio RS - Go to 110
- ❑ PacBio RS II - Go to 110
- ❑ HiScanSQ - Go to 110
- ❑ HiSeq 1000 - Go to 110
- ❑ HiSeq 1500 - Go to 110
- ❑ HiSeq 2000 - Go to 110
- ❑ HiSeq 2500 - Go to 110
- ❑ HiSeq 4000 - Go to 110
- ❑ Genome Analyzer Iix - Go to 110
- ❑ MiSeq - Go to 110
- ❑ MiSeq Dx - Go to 110
- ❑ MiSeq FGx - Go to 110
- ❑ ABI SOLiD - Go to 110
- ❑ NextSeq - Go to 110
- ❑ MinION (ONT) - Go to 110
- ❑ Other - Go to 109

109. If another platform is used please list here:

110. 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 five EQA- provider selected criteria.
Next you will be asked to report 1-5 additional criteria of your own choice.
For each criteria please also report the threshold or procedure used to evaluate the current criteria.

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

- ❑ Yes
- ❑ No - Go to 115

112. Procedure used to evaluate confirmation of organism:

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

(State one answer only)

- ❑ Yes
- ❑ No - Go to 115

114. Procedure or threshold used for coverage:

115. Did you evaluate assembly quality?

(State one answer only)

- ❑ Yes
- ❑ No - Go to 117

116. Procedure used to evaluate assembly quality:

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

(State one answer only)

- ❑ Yes
- ❑ No - Go to 119

118. Procedure or threshold used for assembly length:

119. Did you evaluate allele calling result?

(State one answer only)

- ❑ Yes
- ❑ No - Go to 121

120. Procedure used to evaluate allele calling:

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

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

122. Other criteria used to evaluate the quality of sequence data – additional criteria 1

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

124. Other criteria used to evaluate the quality of sequence data – additional criteria 2:

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

126. Other criteria used to evaluate the quality of sequence data – additional criteria 3:

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

128. Other criteria used to evaluate the quality of sequence data – additional criteria 4:

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

130. Other criteria used to evaluate the quality of sequence data – additional criteria 5:

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

132. Comment(s):

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

133. Thank you for your participation

Thank you for your submission of STEC EQA-10 results.
For questions, please contact ecoli.eqa@ssi.dk or phone +45 3268 8341.
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.
For final submission, remember to press 'Submit results' after printing.
REMEMBER to upload your raw reads to the ftp-site.

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