TECHNICAL REPORT

Seventh external quality assessment scheme for typing of verocytotoxin-producing *Escherichia coli*

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Seventh external quality assessment scheme for verocytotoxin-producing *E. coli* (VTEC) typing

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Abbreviations

AEEC	Attaching and Effacing *E. coli*
aaiC	Chromosomal gene marker for Enteroaggregative *E. coli*
aggR	Gene encoding the master regulator in Enteroaggregative *E. coli*
BN	BioNumerics
bp	base pairs
eae	CVD434. *E. coli* attaching and effacing gene probe *ehx*A
EAEC	Enteroaggregative *E. coli*
ECDC	European Centre for Disease Prevention and Control
EIA	Enzyme Immunoassay
eltA	G119. Heat labile enterotoxin (LT) (almost identical to cholera toxin)
ESBL	Extended Spectrum Beta Lactamase
estA	DAS101. Heat stable enterotoxin (*estAp*; porcine variant: STp. *estAh*; human variant: STh) (STIa)
EQA	External Quality Assessment
EU/EEA	European Union/European Economic Area
FWD	Food- and Waterborne Diseases and Zoonoses
FWD-Net	Food- and Waterborne Diseases and Zoonoses Network
GFN	Global Foodborne Infections Network, Food Safety, WHO
HUS	Haemolytic Uraemic Syndrome
ipaH	WR390. Invasion plasmid antigen (found in several copies chromosomally as well as on plasmids)
MIC	Minimum Inhibitory Concentration
ND	Not Done
NSF	Non-Sorbitol Fermenter
NT	Non-Typeable
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
RFLP	Restriction Fragment Length Polymorphism
RT PCR	Real Time PCR
SOP	Standard Operating Procedure
SSI	Statens Serum Institut
STEC	Shiga toxin-producing *E. coli*, synonymous with VTEC
TESSy	The European Surveillance System
TESSy-MSS	TESSy Molecular Surveillance System
VCA	Vero Cell Assay
VT	Verocytotoxin
VT1	Verocytotoxin 1
VT2	Verocytotoxin 2
VTEC	Verocytotoxin-producing *E. coli*, synonymous with STEC
vtx1	Gene encoding VT1
vtx2	Gene encoding VT2
WGS	Whole Genome Sequencing

Executive summary

This report presents the results of the seventh round of the external quality assessment (EQA-7) scheme for typing of Shiga toxin/verocytotoxin-producing *Escherichia coli* (STEC/VTEC), organised for laboratories in the Food- and Waterborne Diseases and Zoonoses network (FWD-Net) and for EU candidate countries. Since 2012, the EQA scheme has covered the molecular typing method used for EU-wide surveillance of STEC/VTEC, namely Pulsed Field Gel Electrophoresis (PFGE). In addition, the surveillance of VTEC relies on conventional typing methods – i.e. O:H serotyping. The EQA-7 scheme was arranged by the Unit of Foodborne Infections at Statens Serum Institut (SSI), Denmark. The current EQA represents the final deliverable under the framework contract with ECDC and was executed from October 2015 to May 2016.

The objectives of the EQA scheme are to assess the quality and comparability of the typing data produced by the national public health reference laboratories in the FWD-Net. Test strains for the EQA were selected to cover currently relevant strains for public health in Europe and to challenge the methods included. A set of ten strains was selected for PFGE and another ten strains for O:H serotyping, virulence gene detection by PCR, and phenotyping. Thirty laboratories participated in the EQA exercise and submitted results; the highest number of participants throughout all the EQAs since 2012.

PFGE was included for the fourth time, and 19 (63%) of the laboratories produced a gel. A substantial improvement through the EQAs has been seen for both the quality of the gels and the gel analysis in BioNumerics (BN). The ability to produce comparable gels has increased from 45% (9/20) in EQA-4 to 74% (14/19) of the participants in the current EQA. For the gel analysis, improvement from 50% (6/12) to 92% (12/13) of the participants performing in accordance to the guidelines has been seen from EQA-4 to EQA-7. Although it has improved, the gel parameter 'Bands' has obtained a low average score in the EQAs (EQA-4, 2.2; EQA-5, 2.4; EQA-6, 2.6; EQA-7, 2.8), and further improvement of this parameter specifically would be beneficial for more comparable profiles. In addition, the performance of the band assignment could be increased, generating value to the profiles uploaded to TESSy.

O:H serotyping was performed by 57% (17/30) of the participants only, with a 90% average score. In general, the more common European serotypes generated the highest scores; 94% for O157:H7, while the less frequent O156:H4 obtained an average score of 12% only. Notably, not all laboratories demonstrated the capacity to determine all O groups and H types, and the participation in H typing was low (17/30). Capacity building, including a wider range of antisera, would be of advantage. The majority of incorrect results were reported as Not Done (ND) or Non-Typeable (NT) due to incomplete panel of anti-sera for both O grouping (66%; 59/89) and H typing (88%; 28/32).

The quality of the genotyping results was generally good with high average scores, *eae* (98%), *ehxA* (97%), *vtx1* (100%) and *vtx2* (98%), similar to previous EQAs. All participants identified the Enteroaggregative *E. coli* (EAEC) strain by correctly reporting the presence of the *aaiC* and *aggR* genes. Subtyping of *vtx1* and *vtx2* obtained a combined average score of 90%, identical to the previous EQAs (EQA-4, 90%; EQA-5, 92%, EQA-6, 91%), with the *vtx1* average score (99%) being higher than the *vtx2* score (90%). Incorrect *vtx2* results were mainly due to reporting two *vtx2* subtypes for strains harbouring only one type.

In comparison to the genotypic methods, the phenotypic methods were in general performed less frequently. The average scores varied from 84% for enterohaemolysin to 99% for sorbitol (93% for detection of verocytotoxin production (VT), 95% for β-glucuronidase and 98% for Extended Spectrum Beta Lactamase (ESBL) production.

The molecular surveillance system TESSy-MSS, relies on the capacity of the FWD-Net laboratories to produce comparable typing results, which should be reported to TESSy in real-time. The current EQA demonstrates that the majority of participating laboratories were able to produce good and comparable typing results. The issues identified could easily be improved by optimising laboratory procedures, training and capacity building.

In the longer term, whole genome sequencing (WGS)-based methods will gradually replace the current methods in the EQA. Thus, the EQA schemes should constantly adapt to and evaluate the typing techniques used in the FWD-Net laboratories to ensure harmonisation of surveillance and capacity for international comparisons, while taking into account the differences across EU. It is of utmost importance that laboratories submit their typing data to TESSy as close to real-time as possible. Only good-quality data submitted on time provide added value for EU-level surveillance.

1. Introduction

1.1 Background

The European Centre for Disease Prevention and Control (ECDC) is an independent European Union (EU) agency with a mandate to operate the dedicated surveillance networks. The mission of the Centre is to identify, assess and communicate current and emerging threats to human health from communicable diseases. The Centre shall foster the development of sufficient capacity within the Community network for diagnosis, detection, identification and characterisation of infectious agents, which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes [1].

External quality assessment (EQA) is an essential part of quality management ,using an external evaluator to assess the performance of laboratories on test samples supplied specifically for the purpose.

ECDC's disease networks organise a series of EQAs for EU/European Economic Area (EEA) countries. The EQAs aim to identify areas for improvement in the laboratory diagnostic and typing capacities relevant to epidemiological surveillance of communicable diseases as in the Decision No 1082/2013/EU [2], and to ensure reliability and comparability of the results generated by the laboratories across all EU/EEA countries.

The main objectives of the EQA schemes are:

- assessment of the general standard of performance ('state of the art')
- assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration)
- evaluation of individual laboratory performance
- identification and justification of common problem areas
- providing continuing education
- identification of needs for training activities.

Since 2012, the Unit of Foodborne Infections at Statens Serum Institut (SSI), Denmark has been the EQA provider for the typing of *Salmonella enterica* ssp. *enterica*, Shiga toxin/verocytotoxin-producing *Escherichia coli* (STEC/VTEC) and *Listeria monocytogenes*. The contract for the EQA scheme for VTEC covers Pulsed Field Gel Electrophoresis (PFGE), O:H serotyping, virulence gene detection, subtyping of *vtx* genes and common phenotypic traits of VTEC, including Extended Spectrum Beta Lactamase (ESBL) production. This report presents the results of the seventh VTEC EQA exercise (VTEC EQA-7).

1.2 Surveillance of VTEC infections

Verocytotoxin-producing *E. coli* is a group of *E. coli* characterised by the ability to produce verocytotoxins (VTs). Human pathogenic VTEC often harbour additional virulence factors important to the development of the disease. A large number of serotypes of *E. coli* have been recognised as VT producers. Notably, the majority of reported human VTEC infections are sporadic cases. The symptoms associated with VTEC infection in humans vary from mild diarrhoea, to life-threatening haemolytic uraemic syndrome (HUS) which is defined clinically by the triad of haemolytic anaemia, thrombocytopenia, and acute renal failure.

In 2014, the EU notification rate of VTEC infections was 1.56 cases per 100 000 population. The total number of confirmed cases of VTEC infections was 5 955, a slight decrease from 2013 (N=6 043). Seven deaths due to VTEC infection were reported, resulting in an EU case-fatality of 0.2%. As in previous years, the most commonly reported VTEC O group was O157 (46.3% of cases with known serogroup). O group O157 was followed by O26, O103, O145, O91, O146 and O111 [3].

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

The overall aims of integrating molecular typing data into EU level surveillance are to:

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

The molecular typing surveillance (TESSy-MSS) gives Member State users access to the EU-wide molecular typing data for the pathogens included. Furthermore, it provides users with the opportunity to perform cluster searches and cross-sector comparison of the EU level data, to determine whether isolates characterised by molecular typing at the national level(s) are part of a multinational cluster requiring cross-border response collaboration.

1.3 VTEC characterisation methods

The state-of-the-art characterisation of VTEC includes O:H serotyping in combination with a few selected virulence genes – i.e. the two genes for production of verocytotoxin VT1 (*vtx1*) and VT2 (*vtx2*), and the intimin (*eae*) gene associated with the attaching and effacing lesion of enterocytes, also seen in Attaching and Effacing non-VTEC *E. coli* (AEEC), including Enteropathogenic *E. coli* (EPEC). For some subtypes of VT2 the combination of the toxin genes is clinically relevant. The *vtx2a* in *eae* positive VTEC and the activatable *vtx2d* subtype in *eae* negative VTEC seem to be highly associated with the serious sequela HUS [4-6]. Gene *vtx2c* positive VTEC has also been associated with HUS [5,6]. Other specific subtypes or variants of VT1 and VT2 are primarily associated with milder course of disease without HUS [4-6], and *vtx2e* positive VTEC strains are probably non-pathogenic to humans [7]. Our understanding of the epidemiology of the *vtx* subtypes is therefore important for reducing the risk of VTEC infection and for the surveillance of VTEC.

Some of the existing methods for *vtx* subtyping using a combination of specific Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) are inadequate and may result in misleading conclusions. For example, subtyping of *vtx2* has been based on the absence of the PstI site as an indicator of the presence of the mucus-activatable *vtx2d* subtype [8-11]. However, the PstI site is absent in six variants of *vtx2a*, in two variants of *vtx2c*, in *vtx2f* and in all four variants of *vtx2g* [12]. Furthermore, the most commonly detected VTEC serotype, O157:H7, may be divided into two groups: one with the unusual property of failing to ferment sorbitol within the first 20 hours of incubation (the Non-Sorbitol Fermenters, NSF) and a highly virulent variant of O157 fermenting sorbitol. Non-sorbitol fermenting O157 is often characterised by failure to produce β-glucuronidase. Furthermore, approximately 75% of all VTEC strains produce enterohaemolysin, a toxin that can cause lysis of erythrocytes. Enterohaemolysin can either be detected phenotypically on sheep blood agar plates, or by detection of the *ehxA* gene.

The VTEC EQA-7 included O:H serotyping, genotyping of virulence genes (*eae*, *ehx*A, *vtx*1 and *vtx*2), genes for Enteroaggregative *E. coli* (EAEC), subtyping of *vtx* genes, and phenotypic detection of β-glucuronidase, enterohaemolysin, ESBL production, sorbitol fermentation and VT production.

1.4 Objectives of the EQA

1.4.1 Pulsed Field Gel Electrophoresis typing

The objectives of the VTEC EQA-7 were to assess a) the quality of standard PFGE typing and b) comparability of the collected test results among the participating laboratories. The exercise focused on the production of raw PFGE gels of high quality, normalisation of PFGE images and interpretation of the resulting PFGE profiles in BioNumerics (BN).

1.4.2 Serotyping

The EQA scheme assessed the determinations of somatic 'O' and flagellar 'H' antigens by using either serological or molecular typing methods (PCR and WGS).

1.4.3 Geno- and phenotyping (virulence determination)

The EQA scheme covered both genotypic and phenotypic methods for virulence determination, according to the virulence data currently collected at the EU level (with the possibility to report optional genes). The EQA included the following:

Genotyping

- Detection of EAEC genes (*aaiC* and *aggR*)
- Detection of the virulence genes *eae*, *ehxA*, *vtx1* and *vtx2*
- Subtyping of *vtx1* and *vtx2* genes

Phenotyping

- Production of β-glucuronidase
  Absence/presence of production
- Production of enterohaemolysin
  Defined as negative within 4-6 hours on washed sheep blood agar plates, and positive after 20 hours
- Production of ESBL
  Defined as at least a 3-fold decrease in Minimum Inhibitory Concentration (MIC) for either cefotaxime or ceftazidime when combined with clavulanic acid, versus MIC for one of these drugs alone
- Sorbitol fermentation
  Defined as positive within 20 hours
- Production of VT
  Absence/presence of production.

2. Study design

2.1 Organisation

The VTEC EQA-7 was funded by ECDC and arranged by SSI in accordance with the International Standard ISO/IEC 17043:2010 [13]. The EQA scheme included four parts; PFGE, serotyping, genotyping and phenotyping.

Invitations were e-mailed to the ECDC contact points in the FWD-Net (30 countries) by 10 October 2015 with a deadline to respond by 2 November 2015. In addition, invitations were sent to five EU candidate countries: Albania, Montenegro, the Former Yugoslav Republic of Macedonia, Serbia and Turkey.

Twenty-seven national public health reference laboratories in the EU/EEA and three laboratories in the EU candidate countries accepted the invitation to participate (Annex 1). The EQA test strains were sent to the laboratories on 20 January 2016. The participants were asked to submit their results to online sites by 18 March 2016.

The EQA protocol, submission of results instructions, preconfigured BN databases (including XML export) were distributed by e-mail and made available from two online sites (Annex 15-17).

2.2 Selection of strains

Twenty VTEC test strains (10 for PFGE and 10 for the sero-, geno- and phenotyping part) were selected to fulfil the following criteria:

- representing commonly reported strains in Europe
- remaining stable during the preliminary test period in the organising laboratory
- includeing repeat strains from EQA-4 through -7
- representing different subtypes of *vtx1*
- covering a variety of the seven different subtypes of *vtx2.*

Thirty-four candidate strains were analysed using the methods set out in the EQA before and after having been passaged ten times. A final selection of twenty test strains was made from among the candidate strains that remained stable using these methods. The 10 test strains for the PFGE part were selected to include both 'easy' and more 'difficult' profiles with double bands. The PFGE test strains were selected to represent the diversity of epidemiologically relevant profiles in Europe. In total, two repeat strains from EQA-4–EQA-7 were included, one in the PFGE part and one in the other parts (Table A). In the previous EQAs an additional two repeat strains had been included, however due to updated control restrictions they were excluded from the present EQA. The characteristics of the test strains used are listed as 'Original' in Annexes 4 and 7–13. In addition to the test strains, the participants could request the PFGE reference size marker *S. Branderup* H9812 and reference strains for the vtx subtyping (Annex 14).

Table A. Characteristics of the ten test strains for sero-, geno- and phenotyping

Method	Characterisation
O:H serotyping	O26:H11, O78:H2, O80:H2, O91:H14, O103:H2, O145:H34, O146:H21, O156:H4, O157:H-, O166:H15*
Genotyping	*aaiC*, *aggR*, *eae*, *ehxA*, *vtx1*, *vtx1a*, *vtx1c*, *vtx2*, *vtx2a*, *vtx2b*, *vtx2c*, *vtx2d*, *vtx2f*
Phenotyping	β-glucuronidase, enterohaemolysin, ESBL¤, sorbitol, VT

¤ Repeat strain included in EQA-4 through -7

2.3 Carriage of strains

In January 2016, all test strains were blind-coded and shipped on 20 January 2016, labelled as UN 3373 Biological Substance, Category B. As an extra precaution, an individual letter stating the unique strain IDs was included in the packages and distributed individually to the participants by e-mail on 19 January 2016 together with the protocol for the EQA. Seventeen participants received their dispatched strains within one day; 12 within five days and one participant received the strains 13 days after shipment. No participants reported damage to the shipment or errors in the specific strain IDs. The variation in the duration of shipment had no influence on the results.

On 21 January 2016, instructions on the procedure for submitting results were e-mailed to the participants. This included links to the online uploading site and submission forms, preconfigured BN databases with correct experiment settings (PFGE part) and the XML export file.

2.4 Testing

In the PFGE part, the participants could choose to perform the laboratory part only (submit TIFF image of the PFGE gel) or to furthermore complete an analysis of the gel (submit normalised profiles with assigned bands). For the laboratory procedures, the participants were instructed to use the laboratory protocol 'Standard PulseNet *E. coli* O157:H7 PFGE -One-Day (24-26 h) Standardised Laboratory Protocol for Molecular Subtyping of *Escherichia coli* O157:H7, Salmonella serotypes, *Shigella sonnei*, and *Shigella flexneri* by Pulsed Field Gel Electrophoresis (PFGE)' [14]. For the gel analysis, the participants were instructed to use the distributed preconfigured BN database and analyse the PFGE gel, including normalisation and band assignment. Submission of results included online uploading of PFGE images, as either TIFF file or XML export file including the BN analysis. Guidelines to correct image acquisition, setting up the BN database and export of XML files from BN were included in the EQA protocol (Annex 15-17).

In the other parts of the EQA, ten additional *E. coli* strains were included. These results were to be submitted online to Google Docs. The participants' ability to obtain the correct serotype, by either serological methods (suggested protocol [15]) or molecular typing (no international standard but the applied methods to be submitted together with the results) was tested.

In the genotyping part, the participants' ability to detect the virulence genes *eae*, *ehxA, vtx1* and *vtx2* genes and subtype *vtx1* (*vtx1a, vtx1c* or *vtx1d)* and *vtx2* (*vtx2a, vtx2b, vyx2c, vtx2d or vtx2f*) were assessed, as per the suggested protocol [16]. Additionally, two genes related to EAEC, the chromosomally encoded protein gene (*aaiC*) and enteroaggregative adherence transcription regulator gene (*aggR*), were included in the genotyping part of the scheme.

The phenotyping part of the EQA included detection of β-glucuronidase, enterohaemolysin, ESBL production, sorbitol fermentation and VT production by either Vero Cell Assay (VCA) or Enzyme Immunoassay (EIA).

2.5 Data analysis

As the participating laboratories submitted their results, the PFGE results were imported to a dedicated VTEC EQA-7 BN database. If errors were identified in the submission process, the EQA provider reported these to participants in order to ensure that results could be analysed. Re-submission of results was necessary for two participants (due to errors in the XML export or TIFF files.)

The PFGE gel quality was evaluated according to a modified version of the 'ECDC FWD MolSurv Pilot - SOPs 1.0, PulseNet US protocol PFGE Image Quality Assessment' ('TIFF Quality Grading Guidelines EQA-7', Annex 2) by scoring the gel according to seven parameters (scores in the range 1–4). The BN analysis was evaluated according to the 'BioNumerics Gel Analysis Quality Guidelines EQA-7' developed at SSI (Annex 3), grading the BN analysis according to five parameters (scores in the range 1–3). A score of 1 [Poor] in any of the parameters in the two guidelines corresponds to a gel/analysis which cannot be used for inter-laboratory comparison. Both guidelines were slightly modified from the EQA-6 versions in accordance with the performance of the participants by adding a sentence (Annex 2 and 3).

Results for the other parts of the EQA, submitted online, were exported by the EQA provider to an Excel spreadsheet where they were evaluated according to the percentage of correct results, generating a score from 0-100% for each method. If there were discrepancies between the submitted results and the prescribed methods, the EQA provider reported these to participants, thereby obtaining all results generated by the participants.

Individual evaluation reports and certificates of attendance were distributed to the participants during May 2016.

3. Results

3.1 Participation

The laboratories could choose to participate either in the full EQA scheme or only in selected parts. Approximately half (57%; 17/30) of the laboratories participated in the four parts of the scheme (PFGE, sero-, geno- and phenotyping) and three (10%) laboratories completed all methods in all parts of the scheme. In total, 19 (63%) laboratories participated in the PFGE part and almost all (97%; 29/30) participated in a selection of the other three parts. Most (68%; 13/19) of the participants in the PFGE part completed both the laboratory (gel) and the analysing (BN) part of the EQA (Table 2).

All 30 participants submitted results, however, deviation between the methods registered and the methods performed were identified for half (50%; 15/30) of the participants.

In the serotyping part, all of the 27 participants performed O grouping but only 63% (17/27) performed H typing (Table 3).

In the genotyping part, all of the 28 participants submitted results for *eae* and *vtx* genes, while 18 (64%) laboratories submitted results for the *ehxA* gene. Twenty-five laboratories (89%) submitted subtyping results, and 21 (75%) reported results for EAEC; *aaiC* (61%; 17/28) and *aggR* (75%; 21/28) (Table 3).

In the phenotyping part, the participation rate was highest for sorbitol fermentation (93%; 26/28) and lowest for VT detection (29%; 8/28). No more than 18 (64%) participants performed detection of β-glucuronidase, enterohaemolysin or ESBL production (Table 3).

Table 1. Number and percentage of laboratories submitting results for each part

	PFGE			Serotyping[1]	Genotyping[2]	Phenotyping[3]
	Gel + BN	Gel only	Total			
Number of participants	13	6	19	27	28	28
% of participants	68	32	63*	90*	93*	93*

Seventeen participants (57%) completed all four parts of the EQA scheme and three (10%) completed all methods in all parts.

[1] O grouping and/or H typing
[2] Detection of at least one gene (aaiC, aggR, eae, ehxA, vtx1 and vtx2) and/or subtyping of vtx1 and vtx2
[3] At least one phenotypic method (β-glucuronidase, enterohaemolysin, ESBL, sorbitol and VT)
**Percentage of the total number (30) of participating laboratories. BN, BioNumerics analysis.*

Table 2. Detailed participation information for the sero-, geno- and phenotyping parts

	Serotyping n=27		Genotyping n=28						Phenotyping n=28				
	O group	H type	*aaiC*	*aggR*	*eae*	*ehxA*	*vtx1* and *vtx2*	*vtx* subtyping	β-glucuronidase	Enterohaemolysin	ESBL	Sorbitol	VT
No. participants	27	17	17	21	28	18	28	25	15	14	18	26	8
% of participants^	100	63	61	75	100	64	100	89	54	50	64	93	29
% of participant*	90	57	57	70	93	60	93	83	50	47	60	87	27

^Percentage of participants in the respective part of the EQA
**Percentage of the total number (30) of participating laboratories*

3.2 Pulsed Field Gel Electrophoresis

Nineteen laboratories (63%) produced a PFGE gel image and thirteen (68%) of these also analysed the profiles and submitted the analysed data in XML export format.

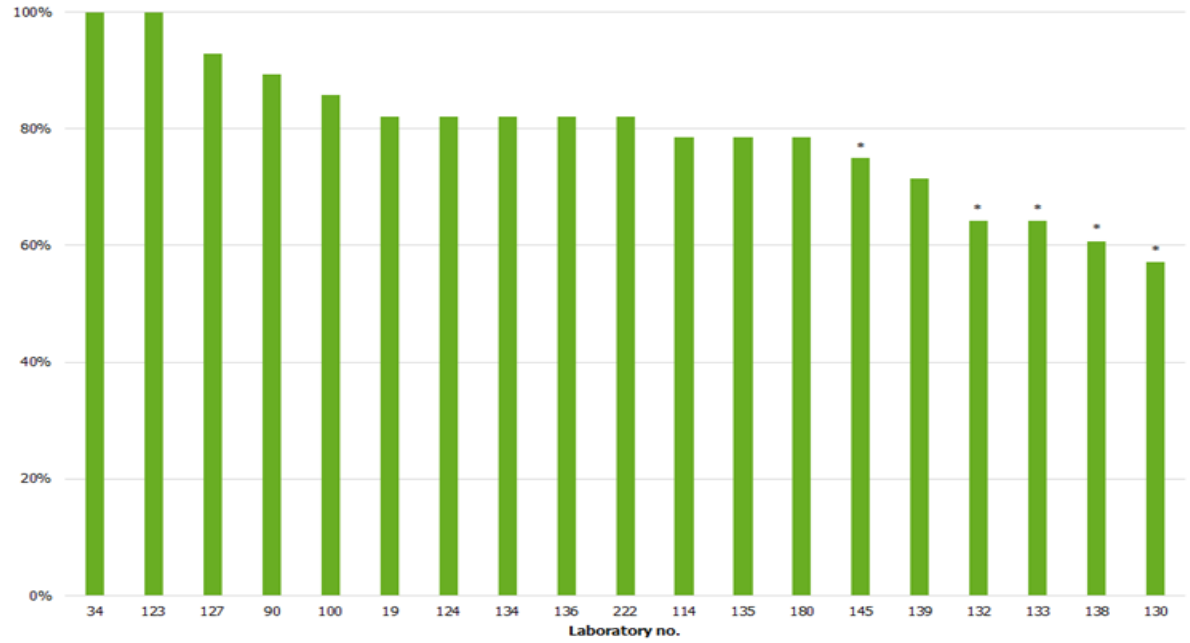
Annex 4 shows the profiles generated by the participants for test strains VTEC-5 and VTEC-7, including the profile produced by the EQA provider.

3.2.1 Gel quality

The gel quality varied considerably among the participants (Figure 1), resulting in a highly variable quality of the profiles for the individual test strains (Annex 4). Gels were graded according to the 'TIFF Quality Grading Guidelines EQA-7', evaluating seven gel parameters using four scores 1-4 (Annex 2). An acceptable gel quality (score of 2 'Fair' or better) should be achieved in each parameter since a low quality score of 1 'Poor' in just one parameter impacts on the ability to further analyse the image and compare profiles across laboratories. It is important to note that since a score of 1 in any parameter reflects an inter-laboratory incomparable gel, the total gel quality score alone cannot be used as a measure for quality.

Fourteen (74%) of the participating laboratories were able to produce a gel of sufficient quality to enable profile detection and inter-laboratory comparison (Figure 1, Annex 5). Two participants (Labs 34 and 123) produced a gel of excellent quality with respect to all parameters.

Figure 1. Participant percentage scores for PFGE gel quality



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

Table 3 shows the seven gel parameters, evaluated by the 'TIFF Quality Grading Guidelines EQA-7', the percentage of laboratories scoring 1–4 and the average score for all laboratories. In general, the average score was above 3, (i.e. between 'Good' and 'Excellent'). However, two parameters ('Bands' and 'DNA Degradation') obtained an average score below 3 (i.e. between 'Fair' and 'Good'). The majority (80%; 4/5) of the five inter-laboratory incomparable gels obtained a score of 1 'Poor' in one of these two parameters. For 'Bands' in particular, only a small percentage (21%) of the participants were able to obtain an 'Excellent' [4] score. In EQA-6, the parameter 'Bands' also obtained an average score below 3, with a low proportion (32%) of the participants producing 'Excellent' bands, and the majority (83%; 5/6) of incomparable gels obtaining a score of 1 'Poor', stressing the particular difficulties with this parameter. In the present EQA, four parameters generated an unsatisfactory score of 1 'Poor' (Table 3).

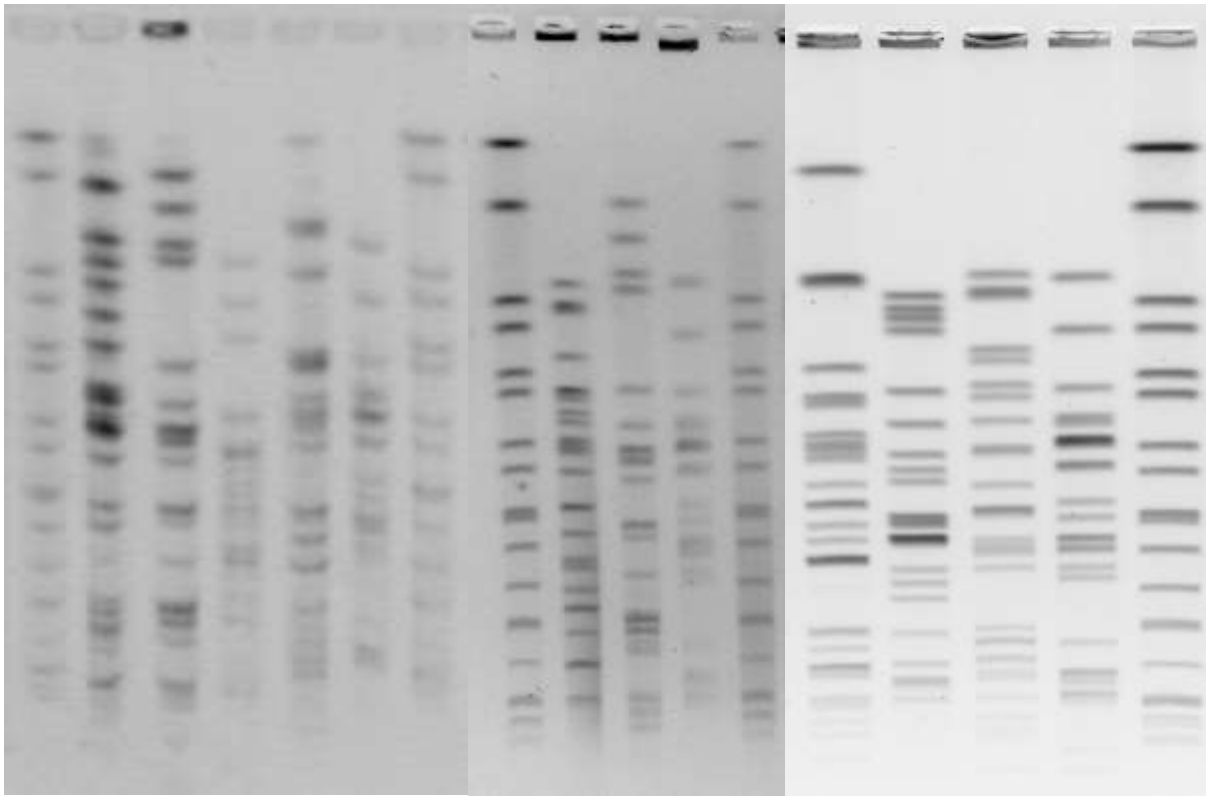
Table 3. Results of PFGE gel quality grading

	Grade [score in points]				
Parameter	**Poor [1]**	**Fair [2]**	**Good [3]**	**Excellent [4]**	**Average**
Image Acquisition and Running Conditions	16%	11%	26%	47%	3.1
Cell Suspension	0%	11%	26%	63%	3.5
Bands	16%	11%	53%	21%	2.8
Lanes	0%	11%	42%	47%	3.4
Restriction	5%	11%	21%	63%	3.4
Gel Background	0%	16%	53%	32%	3.2
DNA Degradation	5%	37%	21%	37%	2.9

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

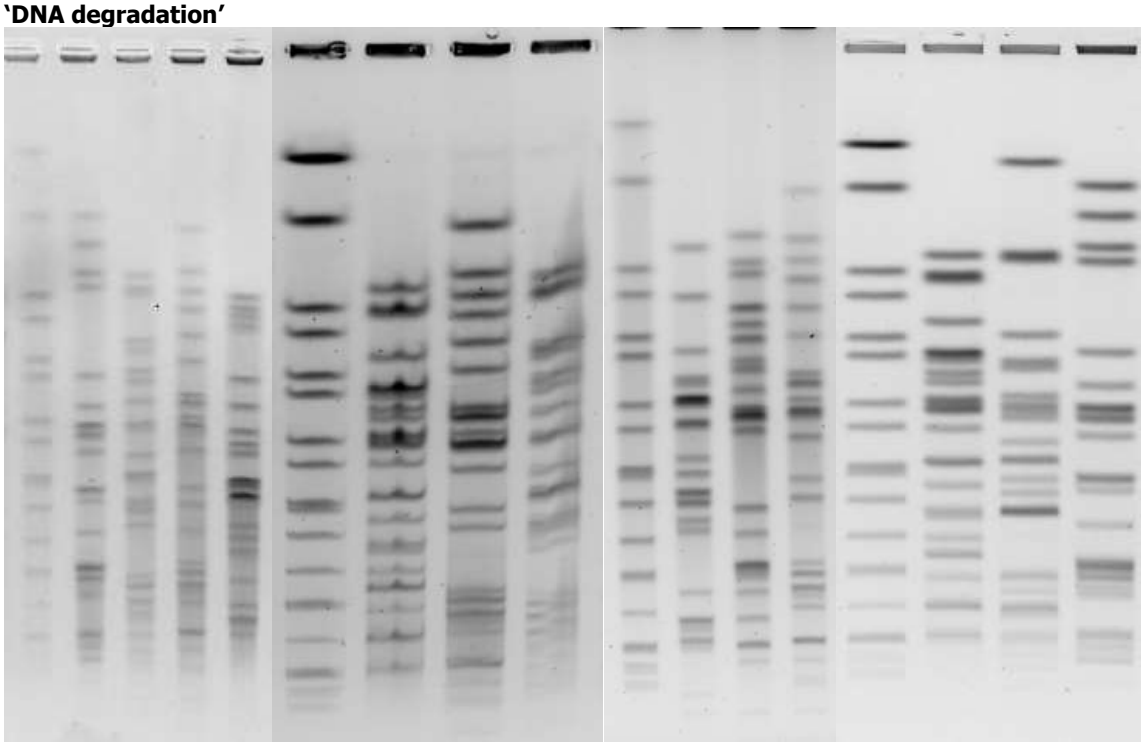
Figures 2 and 3 show gels of varying quality in the two parameters 'Bands' and 'DNA Degradation' scoring low in the present EQA. The scoring of 'DNA degradation' was lower this year than in the previous EQA. In particular, the problem with minor background smearing in many lanes, which resulted in the grading 'Fair' [2], was higher.

Figure 2. TIFF file examples of gel sections with fuzzy bands and a gel which scored Excellent [4] in the parameter 'Bands'



Left: Gel scored 1 [Poor]. Middle: Gel scored 2 [Fair]. Right: Gel scored 4 [Excellent] in the parameter 'Bands'

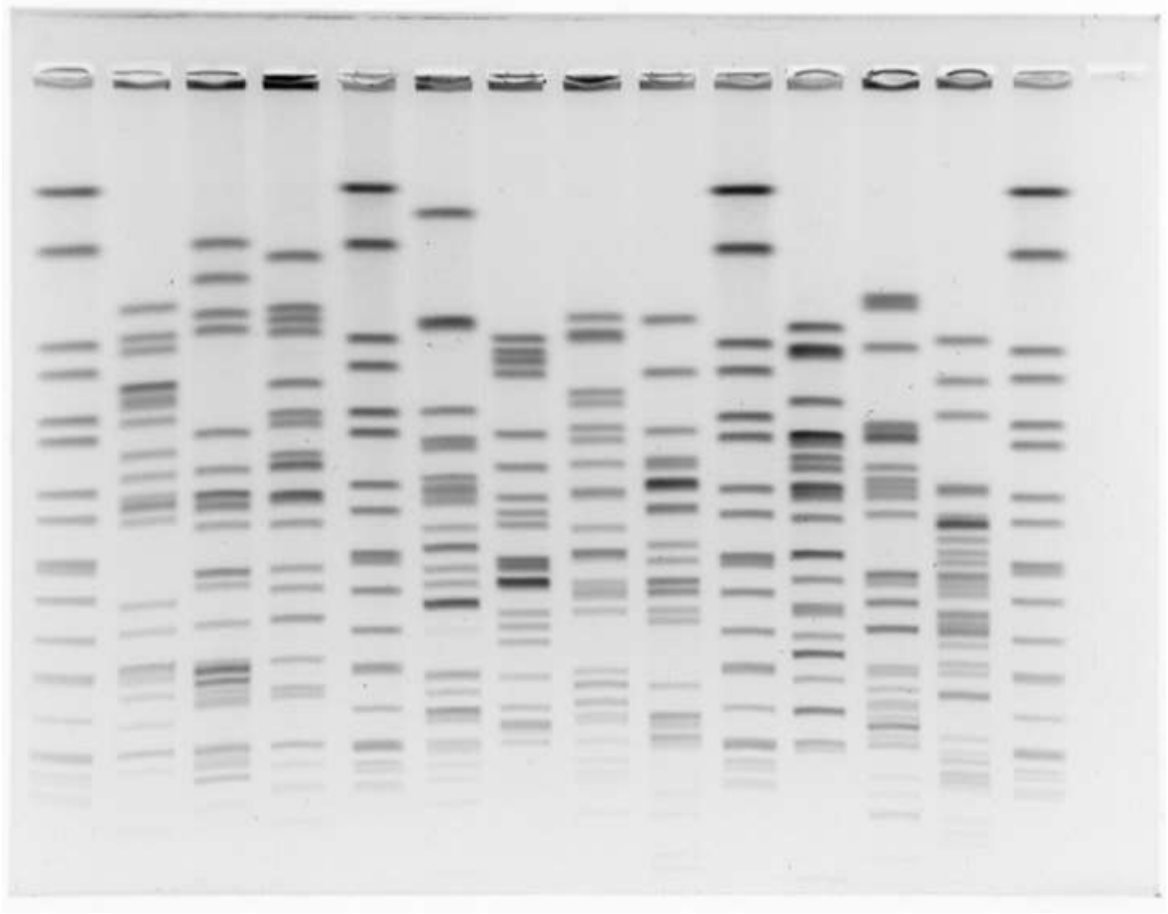
Figure 3. TIFF file examples of gels with problems and a gel which scored Excellent [4] in parameter 'DNA degradation'



Left: Gel scored 1 [Poor]. Middle: Two gels scored 2 [Fair]. Rightmost: Gel scored 4 [Excellent] in the parameter 'DNA Degradation'.

Figure 4 shows a gel with 'Excellent' [4] scores in all of the seven gel quality parameters. The image has been captured correctly, intensity is even, bands are sharp, and there are no background or shadow bands.

Figure 4. Gel with high scores in all seven PFGE gel quality parameters



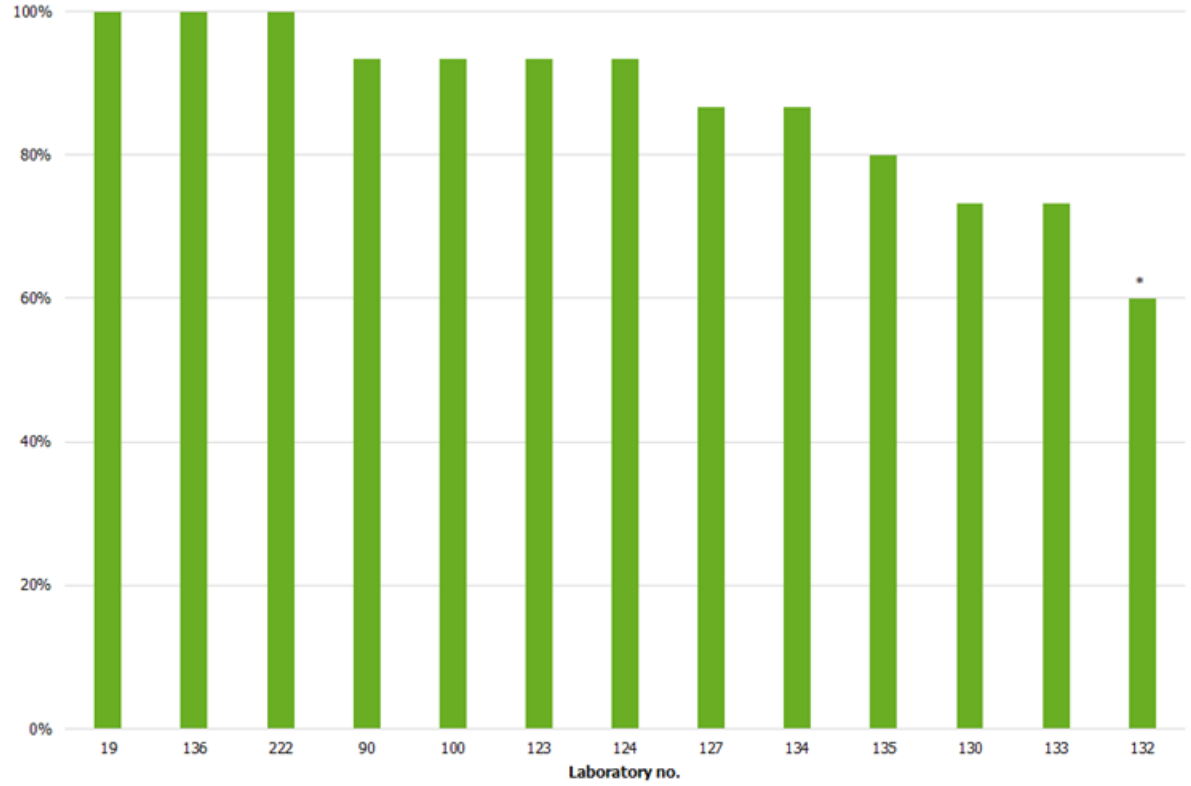
3.2.2 Gel analysis using the BioNumerics software

Thirteen laboratories (68%) analysed the PFGE gels in BN and were able to produce XML-export files according to the protocol. Re-submission of results was necessary for three participants. The participants' ability to perform gel analysis was graded according to the 'BioNumerics Gel Quality Grading Guidelines EQA-7'. The grading was made for five parameters with scores ranging 1—3 (Annex 3).

BioNumerics (BN) is a software initially developed for PFGE gel analysis. One of the critical steps in the analysis is the normalisation of the gel, but all steps in the analysis impact on the final profiles, and the possibility to perform an inter-laboratory comparison. To ensure identical experiment settings in BN, the EQA provider distributed pre-configured BN databases to the participants.

In comparison with the variation in gel quality among the participants, the gel analysis was more equal and participants demonstrated a high performance standard (Figure 5). Three laboratories (19, 36 and 222) produced a gel analysis of 'Excellent' [4] quality in all parameters.

Figure 5. Participant percentage scores for PFGE gel BN analysis



Arbitrary numbers represent the participating laboratories. Bars represent the total score in percentage of the maximal score of 15 points given according to evaluation of the gel analysis by five parameters graded 1-3.

**BN analysis not performed according to guidelines*

Table 4 shows the five gel analysis parameters evaluated by the *BioNumerics Gel Quality Grading Guidelines EQA-7*, the percentage of laboratories scoring 1–3 and the average score for all laboratories.
Twelve (92%) of the 13 participants performed a gel analysis of a 'Fair' [2] to 'Excellent' [3] quality (Table 4). The parameter 'Band Assignment' obtained the lowest average score (2.5), and only 54% (7/13) of the participants obtained an 'Excellent' band assignment score. However, no laboratory scored 'Poor' [1] in this parameter although producing fuzzy bands (Table 3, Annex 5). It is important to note that the quality of the band assignment is graded according to the quality of the gel, i.e. a laboratory producing a gel impossible to use for inter-laboratory comparison in regards to the gel quality can still achieve an 'Excellent' score in the BN analysis.

Table 4. Results of PFGE gel BN analysis

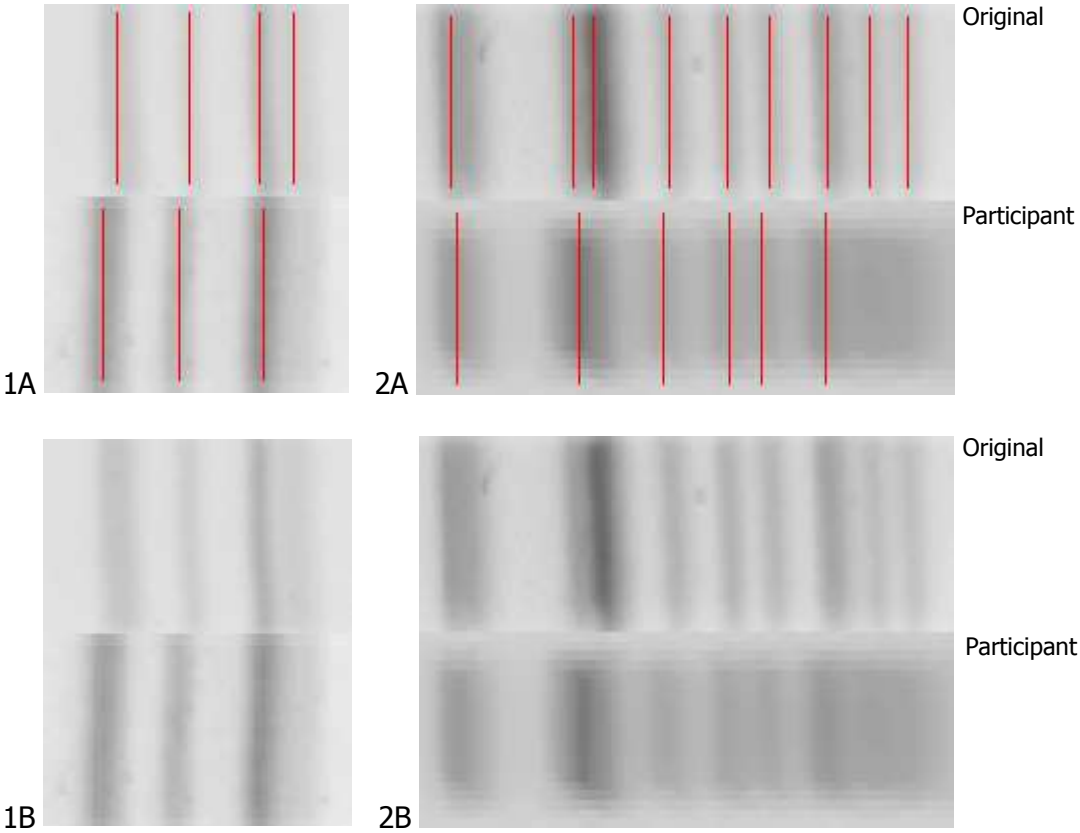
	Grade [score in points]			
Parameter	**Poor [1]**	**Fair [2]**	**Excellent [3]**	**Average**
Position of gel frame	0%	31%	69%	2.7
Strips	0%	38%	62%	2.6
Curves	0%	38%	62%	2.6
Normalisation	8%	23%	69%	2.6
Band assignment	0%	46%	54%	2.5

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

Correct assignment of bands is crucial, and highly dependent on the overall quality of the gel. Very fuzzy and/or thick bands make correct band assignment impossible. In the current EQA, half (3/6) of the 'Fair' band assignment scores were due to assignment of double bands as a single band, or vice versa. The other half were due to not all bands having been assigned a band being assigned where there were none. The sole laboratory producing an inter-laboratory incomparable gel analysis did not assign all bands in the reference lanes, thereby obtaining a 'Poor' [1] score in the 'Normalisation' parameter.

Double bands should be assigned when whitespace separates the bands, otherwise the band should be regarded as a single band. The examples in Figure 6 are shown in pairs with the band assignment at the top and the raw gel at the bottom.

Figure 6. TIFF file examples of gel analysis with band assignment problems



Analysis of two gels (1 and 2) with (A) and without (B) bands. The top part of every subfigure shows the original profiles and the bottom parts show participants with a score of 2 [Fair] in the parameter 'Band Assignment'

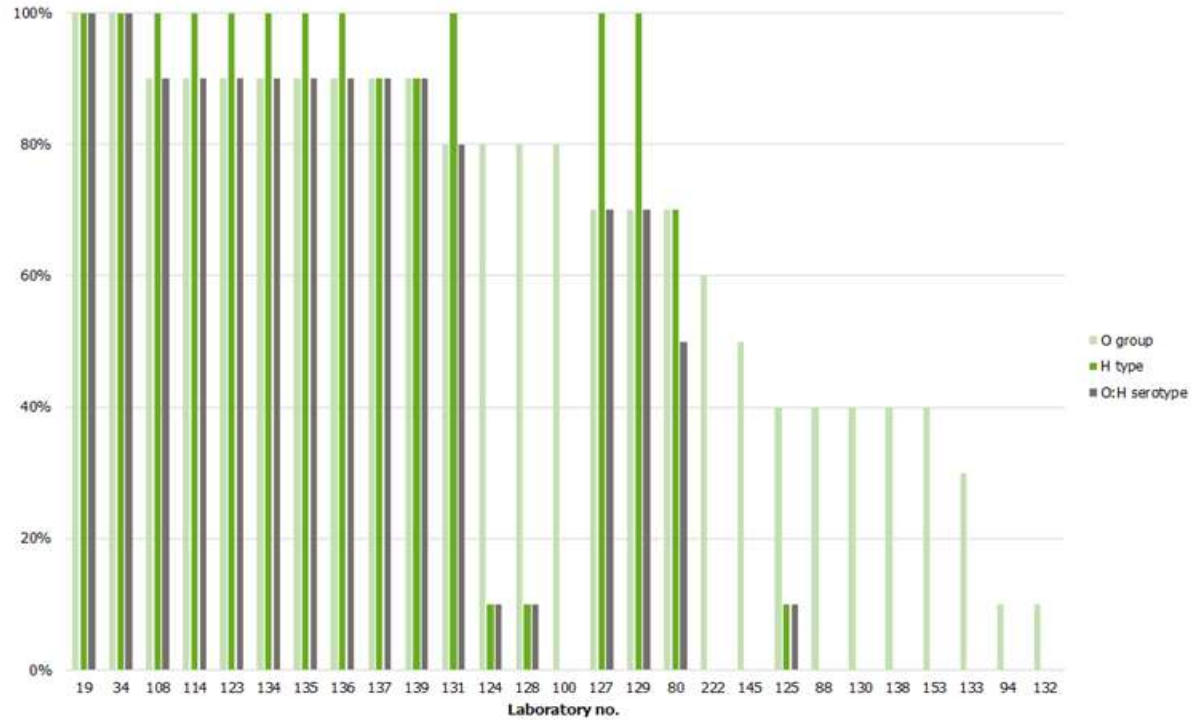
In Figure 6 Gel 1, the participant has produced an excellent gel, but failed to assign the band furthest to the right (1A) even though it is easy to discern whitespace between the bands (1B). In Gel 2, another participant has not assigned the two bands furthest to the right. The quality of Gel 2 is acceptable but not high and the participant should have been able to assign the two bands furthest to the right. In addition, a band is missing in the tight doublet, but since the band assignment is scored according to the quality of the gel, the notation of a single band is correct as no whitespace can be seen (2B).

3.3 Serotyping

Twenty-seven (90%) laboratories performed O grouping, and only two (7%) were able to type all ten test strains correctly, given an average score of 67% (Figure 7). The two laboratories with a 100% O-group score were the sole participants to report correct O-group for strain FF16 (O156), a rare O group (Figure 8). In addition, eight laboratories (30%) correctly O-grouped the nine other test stains, and additionally four (15%) correctly O-grouped eight test strains (incorrect O-group for AA11, O80, Annex 7). Thus, participants correctly O-grouping the eight or nine test strains had difficulties determining the O group of the same one (O156) or two (O156 and O80) test strains (Annex 6). The best performances were displayed for O157 (96%), O26 (93%), O103 (89%) and O145 (81%, Figure 6), all included in ECDC's minimum requirements [17]. Two laboratories (94 and 132) detected O157 only, generating incorrect (non-O157) results for the nine other strains (Figure 7, Annex 6). The majority (66%; 59/89) of incorrect O-grouping results was reported as Non-Typeable (NT) or Not Done (ND, Annex 7). Twelve (48%) laboratories reported an incorrect O-group for one or more strains (Annex 7).

Seventeen (57%) laboratories performed H typing, which was 63% (17/27) of the laboratories performing O-grouping. The general performance for H-typing was better than for O-grouping, with the majority (65%; 11/17) of participants correctly H-typing all ten test strains; average score of 81% (Figure 7). Four of the six (67%) participants obtaining incorrect H-type results, reported NT or ND results; only two reported an incorrect H-type (Annex 7). Therefore, as for O-grouping the majority (88%; 28/32) of incorrect H-types were due to a strain being reported as NT or ND.

Figure 7. Participant percentage scores for O grouping and H typing

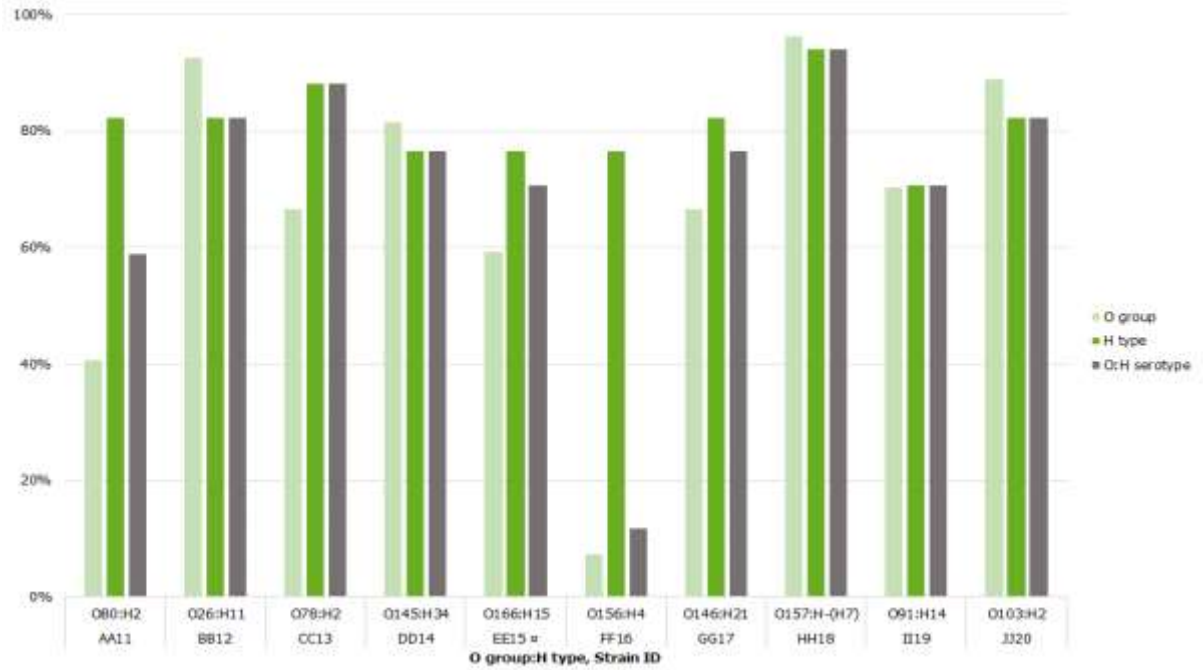


Arbitrary numbers represent the participating laboratories. Bars represent the number of correctly assigned O-groups (light green), n=27, H types (dark green), n=17, and combined O:H serotype (grey), n=17.

**O157 detection only*

Complete O:H serotyping was performed by 17 participants (57%) with an average score of 71%, ranging from 12% (2/17) for O156:H4 to 94% (16/17) for strain O157:H-(H7) of the participants reporting correct serotype (Figure 8). Two laboratories (19 and 34) identified the 100% correct O:H serotype for all 10 test strains (Annex 7).

Figure 8. Average percentage test strain score for serotyping of O and H



Bars represent the percentage of laboratories correctly assigning O groups (light green) (n=27), H types (dark green) (n=17), combined O:H serotypes (grey)), n=17. Average scores: O group, 67%, H type, 81% and combined O:H serotype 71%. ¤ Repeat strain in EQA-4, -5, -6 and -7.

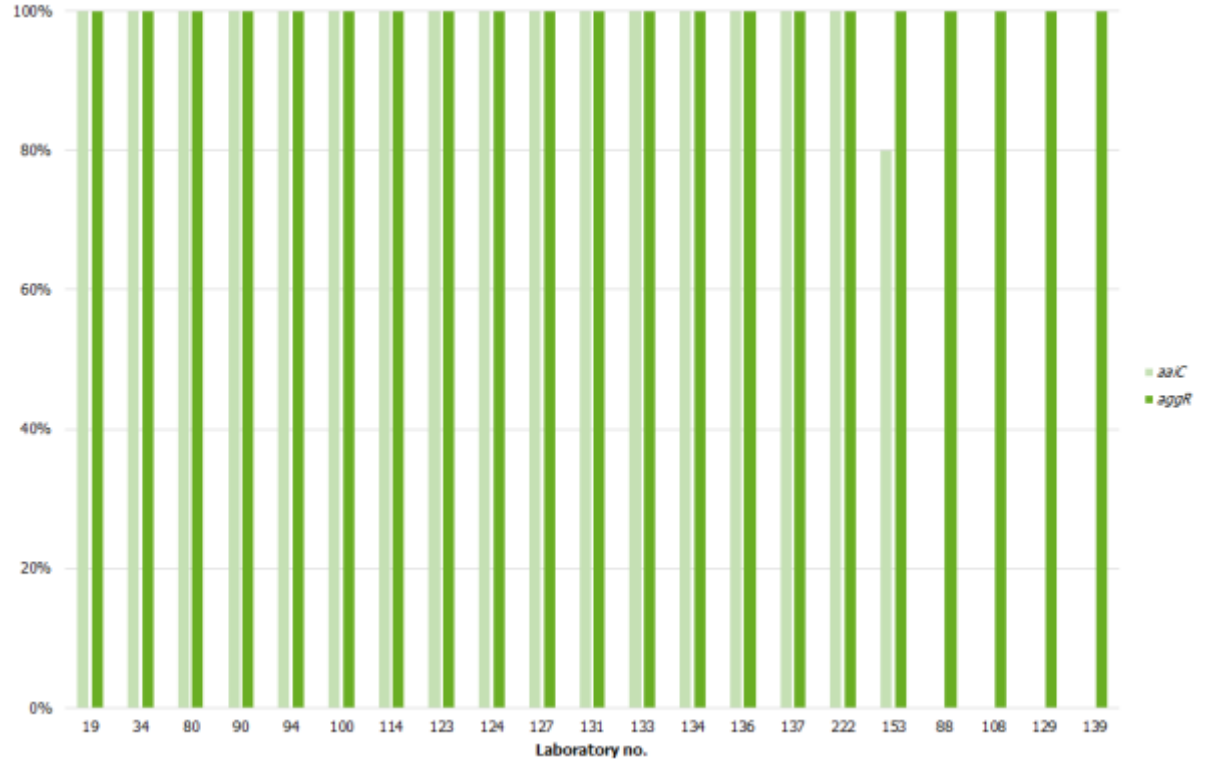
3.4 Genotyping

Twenty-eight laboratories participated in the genotyping part of the EQA scheme to detect EAEC genes (*aaiC* and *aggR*), virulence genes (*eae*, *ehxA*, *vtx1* and *vtx2*) and subtype *vtx1* and*vtx2* genes. All of the 28 participants submitted results for *eae* and *vtx* genes, while 18 (64%) laboratories submitted results for the *ehxA* gene. Twenty-five laboratories (89%) submitted subtyping results for *vtx1* and*vtx2* genes, and 21 (75%) reported results for EAEC; *aaiC* (61%; 17/28) and *aggR* (75%; 21/28).

3.4.1 Detection of EAEC genes (aaiC and aggR)

The performance of the 21 laboratories reporting genotyping results for EAEC, *aaiC* (61%; 17/28) and *aggR* (75%; 21/27), was good (Figure 9). All laboratories detecting *aaiC* and/or *aggR* reported correct presence of the genes in strain CC13. However, one laboratory (153) reported false positive results for *aaiC* in strain HH18 and II20, giving an average score of 99% (Annex 8).

Figure 9. Participant percentage scores for genotyping of aaiC and aggR



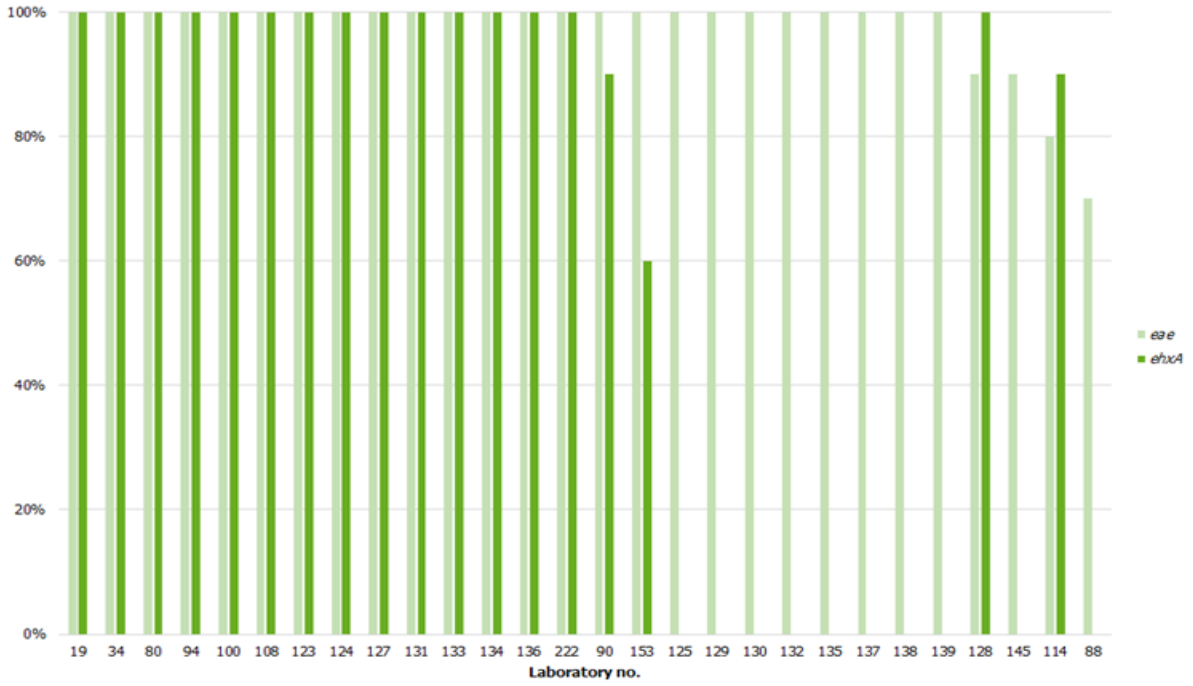
Arbitrary numbers represent the participating laboratories. Bars represent the number of correct genotyping of aaiC (light green), n=17 and aggR (dark green) n=21.

3.4.2 Detection of virulence genes *eae, ehxA, vtx1* and *vtx2*

The virulence genes *eae*, *ehxA, vtx1* and *vtx2* were genotyped by 28, 18 and 28 laboratories respectively, with generally good performances (Figure 10 and 11). For *eae* detection, 24 laboratories(86%) obtained a 100% score, and 15 laboratories (83%) obtained a 100% score for *ehxA* (Figure 8). Three of the four laboratories (88, 114, 128) reporting incorrect *eae* results, giving a false negative result for strain DD14 (Annex 8), whereas the other incorrect *eae* results were reported for different strains (Annex 9). In all, *eae* was misidentified seven times; five false negatives and three false positives.

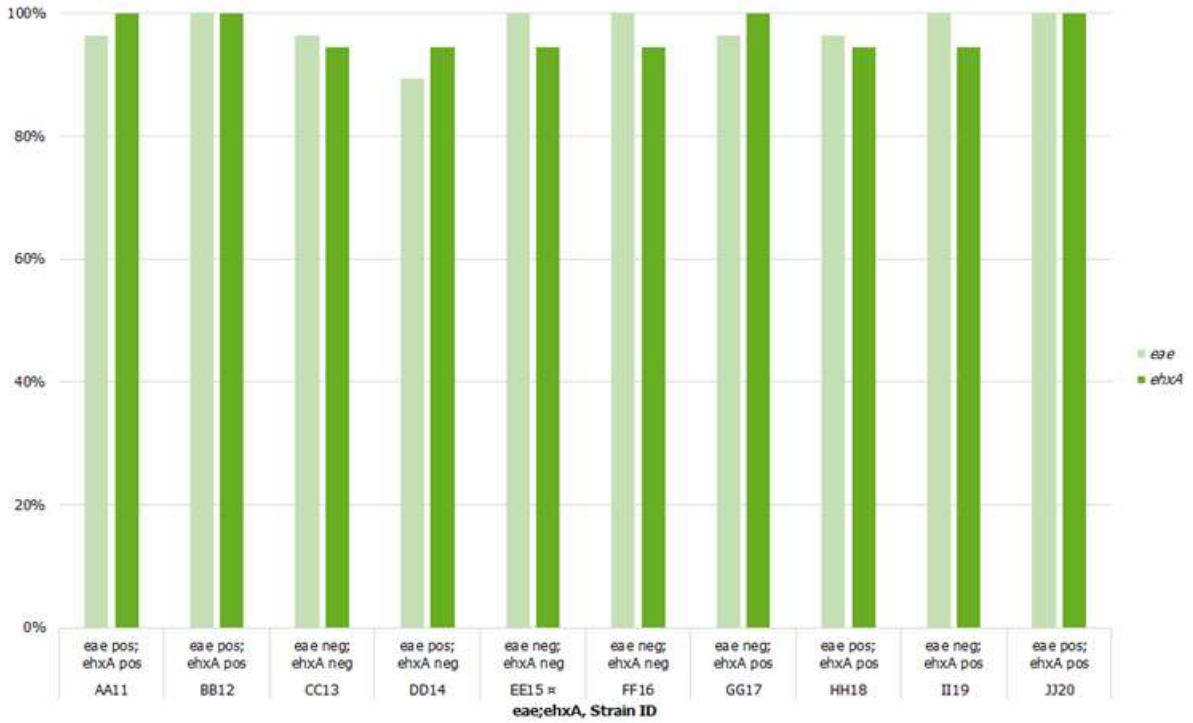
For *ehxA*, no strain generated more than one incorrect result (Annex 8). Thus, the three laboratories misidentifying *ehxA* had no incorrect results in common (Annex 8). In all, *ehxA* was misidentified six times, two false negatives and four false positives, the latter reported by one laboratory (153). One laboratory (114) missed the presence of both *eae* and *ehxA* in strain HH18 and also reported incorrect haemolysin and *vtx1* subtype for this strain.

Figure 10. Participant percentage scores for genotyping of *eae* and *ehxA*



Arbitrary numbers represent the participating laboratories. Bars represent the number of correct genotyping of eae (light green), n=28 and ehxA (dark green) (n=18).

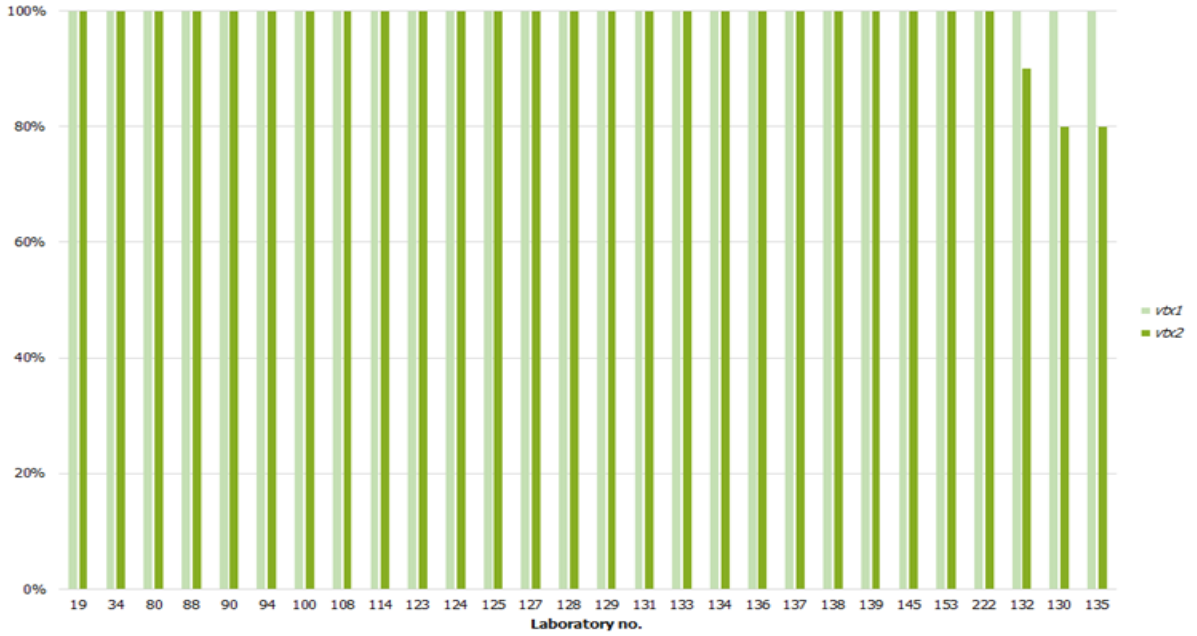
Figure 11. Average percentage test strain score for genotyping of *eae* and *ehxA*



Bars represent the percentage of laboratories correctly genotyping eae (light green), n=28 and ehxA (dark green) (n=18). Average scores: eae, 98% and ehxA, 97%. ¤ Repeat strain in EQA-4, -5, -6 and -7.

The genotyping of vtx1 and vtx2 genes was performed well; all laboratories reported 100% correct vtx1 results and 25 laboratories (89%) reported 100% correct vtx2 results (Figure 12). All of the five incorrect vtx2 results, obtained from three laboratories, were false negatives, and two laboratories missed the presence of vtx2 in DD14 (vtx2f) and HH18 (vtx2c) (Annex 10).

Figure 12. Participant percentage scores for genotyping of *vtx1* and *vtx2*



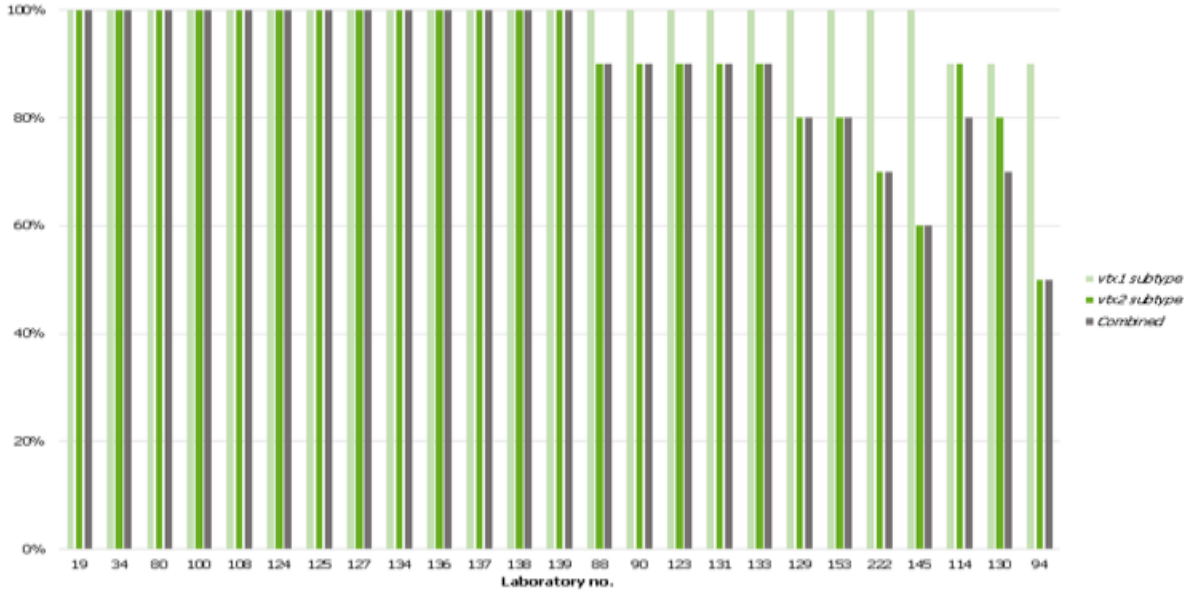
Arbitrary numbers represent the participating laboratories. Bars represent the number of correct genotyping of vtx1 (light green) and vtx2 (dark green). Number of laboratories =28. Average scores; vtx1, 100% and vtx2, 98%.

3.4.3 Subtyping of *vtx1* and *vtx2*

A total of 25 laboratories subtyped *vtx1* and *vtx2*, the majority of them (88%) (22/25) subtyping *vtx1* correctly but only half (52%) (13/25) reporting the correct *vtx2* subtype for all ten test strains (Figure 13). All laboratories correctly reported the absence of *vtx1* in the six *vtx1* negative test strains (AA11 through FF16), whereas three of the four test strains positive for *vtx1* were mis-subtyped by one laboratory each (Figure 14). Laboratories 94, 114 and 130 incorrectly reported *vtx1a* as *vtx1c* or vice versa in test strains GG17, HH18 or JJ20 (Annex 10).

In addition to the 25 laboratories performing complete subtyping of all *vtx1* and *vtx2* subtypes, one additional participant (128) correctly detected *vtxf* only (Annex 11). These results were not included in the analysis.

Figure 13. Participant percentage scores for subtyping of *vtx1* and *vtx2*

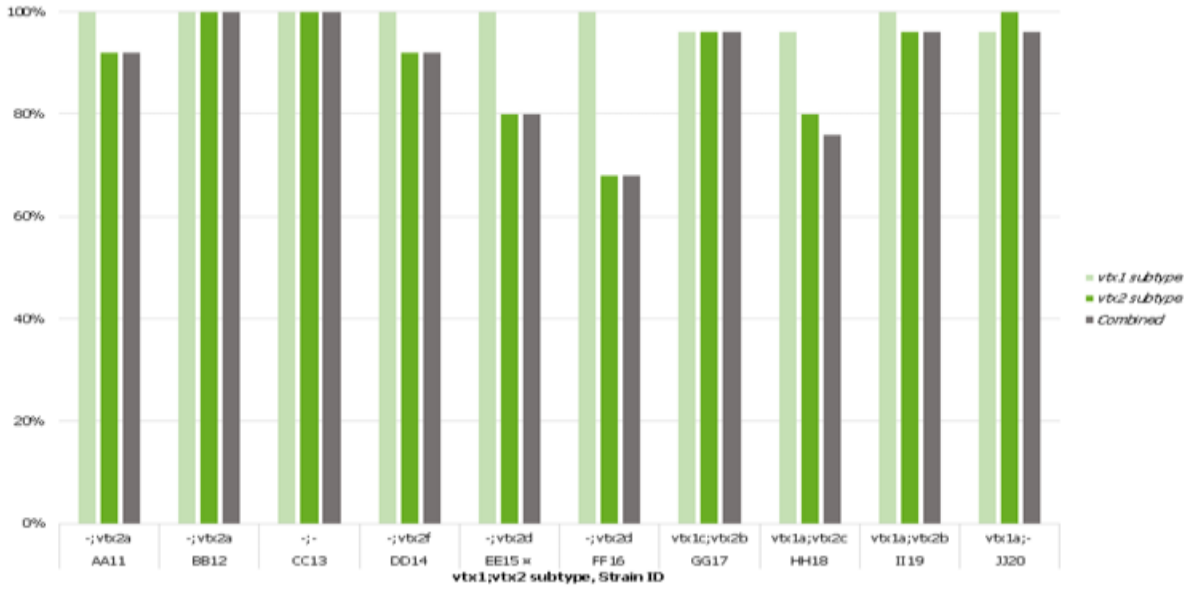


Arbitrary numbers represent the participating laboratories. Bars represent the number of correct subtyping of vtx1 *(light green),* vtx2 *(dark green), combined* vtx1 *and* vtx2 *(grey). Number of laboratories =25.*

Only half (13/25) of the participants were able to correctly *vtx2* subtype all ten test strains (Figure 13). The subtypes *vtx2a*, *vtx2b* and *vtx2f* generated the highest scores, and *vtx2c* and *vtx2d* generated the lowest scores (Figure 14).

Incorrect *vtx2* subtype results were reported 24 times, the majority (14/24) of which were due to reporting two different subtypes for a strain positive for only one *vtx2* subtype (Table 5). The ten test strains were positive for one *vtx2* subtype only. The 24 incorrect results could be divided into four categories: false negatives (2/24), incorrect subtype (8/24), one correct and one incorrect subtype (10/24) and two incorrect subtypes (4/24). The two false negative results were reported by one laboratory which also missed the presence of the *vtx2* gene in the genotyping part of the EQA. The laboratory may therefore not have performed the *vtx2* subtyping on all strains, although the protocol recommends subtyping all strains irrespective of the *vtx1* and *vtx2* genotyping results.

Figure 14. Average percentage test strain score for subtyping of *vtx1* and *vtx2*



Bars represent the percentage of laboratories correctly subtyping vtx1 (light green), vtx2 (dark green), combined vtx1 and vtx2 (grey). Number of laboratories =25. Average scores: vtx1, 99%, vtx2, 90% and combined vtx1 and vtx2, 90%. ¤ Repeat strain in EQA-4, -5, -6 and -7.

As for the *vtx1* negative test strains, the absence of *vtx2* was correctly reported by all participants for both *vtx2* negative test strains. One additional test strain (BB12, *vtx2a*) obtained correct subtyping results from all participants (Figure 14). The *vtx1a* positive test strain (AA11) was incorrectly given an additional subtype (*vtx2b* or *vtx2c*) by two participants (Table 5). The two *vtx2b* test strains were incorrectly subtyped as *vtx2g* by one laboratory (94), and the *vtx2f* strain was incorrectly subtyped as *vtx2e* or *vtx2e* + *vtx2f* by two laboratories (145 and 153). The *vtx2d* (EE15 and FF16) and *vtx2c* (HH18) strains generated the highest number of incorrect results (Figure 14). All participants identified the *vtx2d* gene in the repeat test strain EE15 (O166:H15), however, five laboratories reported an additional subtype (*vtx2a* or *vtx2c*). The other *vtx2d* positive test strain (FF16) was also incorrectly given a *vtx2a* and/or *vtx2c* subtype, however, the *vtx2d* gene was not reported by the eight participants generating incorrect results for this strain. The *vtx2c* positive test strain (HH18) was incorrectly subtyped as either *vtx2c* + *vtx2d* or *vtx2a* + *vtx2d*, meaning that four participants reported an additional *vtx2d* gene for this strain. The incorrect *vtx2* subtyping results are summarised in Table 5.

Table 5. Incorrect *vtx2* subtype results

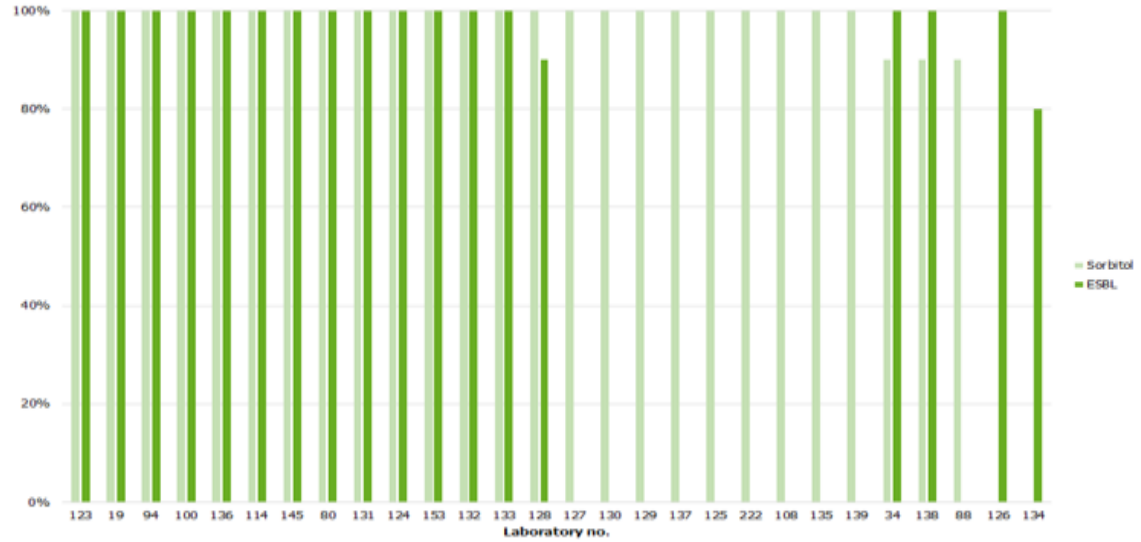
Strain ID	Original	Incorrect *vtx2* subtype results				
		False negative	One incorrect	Correct + incorrect	Two incorrects	Total no.
AA11	*vtx2a*			*vtx2a* + *vtx2b* (1), *vtx2a* + *vtx2c* (1)		2
BB12	*vtx2a*					0
CC13	-					0
DD14	*vtx2f*		*vtx2e* (1)	*vtx2f* + *vtx2e* (1)		2
EE15	*vtx2d*			*vtx2d* + *vtx2a* (1), *vtx2d* + *vtx2c* (4)		5
FF16	*vtx2d*	1	*vtx2a* (3), *vtx2c* (2)		*vtx2a* + *vtx2c* (2)	8
GG17	*vtx2b*		*vtx2g* (1)			1
HH18	*vtx2c*	1		*vtx2c* + *vtc2d* (2)	*vtx2a* + *vtx2d* (2)	5
II19	*vtx2b*		*vtx2g* (1)			1
JJ20	-					0
Total		2	8	10	4	24

3.5 Phenotyping

Twenty-eight laboratories participated in the phenotyping part of the exercise, and the participation rate ranged from 29% (VT detection) to 93% (sorbitol fermentation). Fourteen to 18 (64%) participants detected production of β-glucuronidase, enterohaemolysin or ESBL (Annex 12-13).

Twenty-three (88%) of the participants detecting sorbitol fermentation obtained a 100% correct score (Figure 15). Three laboratories reported an incorrect result; two false positives (strain DD14 and HH18) and one false negative (strain CC13) results (Annex 12). For ESBL, a similar high number (89%; 16/18) of participants reported correct results (Figure 15). All laboratories correctly detected ESBL production for strain EE15, however, two laboratories (128 and 134) reported one or two false positive results for three different strains (AA11, CC13 and DD14, Annex 12).

Figure 15. Participant percentage scores for detection of sorbitol and ESBL production



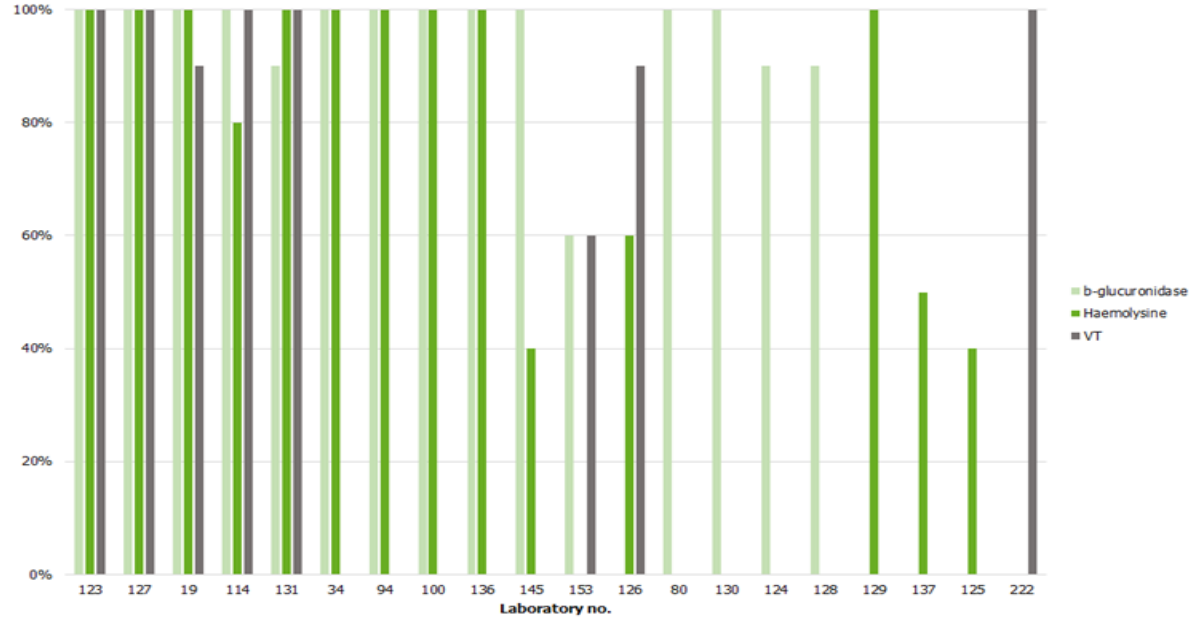
Arbitrary numbers represent the participating laboratories. Bars represent the number of correct detections of sorbitol production (light green) n=26 and ESBL (dark green) n=18.

Fifteen participants detected β-glucuronidase production; eleven (73%) obtained a 100% score (Figure 16). Four participants (124, 128, 131 and 153) reported false negative results for strain JJ20, and Laboratory 153 reported additional false negative results for three strains (AA11, BB12, II19, Annex 13).

The performance for detection of enterohaemolysin production was fairly poor, with nine of the 14 (64%) participants reporting 100% correct results (Figure 16). Most (83%; 19/23) of the incorrect results were false negatives reported by four participants (114, 125, 137 and 145) for two to six strains (AA11, BB12, GG17, HH18, II19, JJ20). One laboratory (126) reported false positive results (alfa) for four strains (CC13, DD14, EE15, FF16) (see Annex 13).

Similarly, the performance for detection of VT production was fairly poor. Five (63%) of the eight participants obtained a 100% score (Figure 14). False negative results were reported for strain BB12 by two laboratories (19 and 153), and Laboratory 153 reported an additional three false negative results (DD14, EE15, FF16). One laboratory (126) reported a false positive result for strain CC13 (Annex 13).

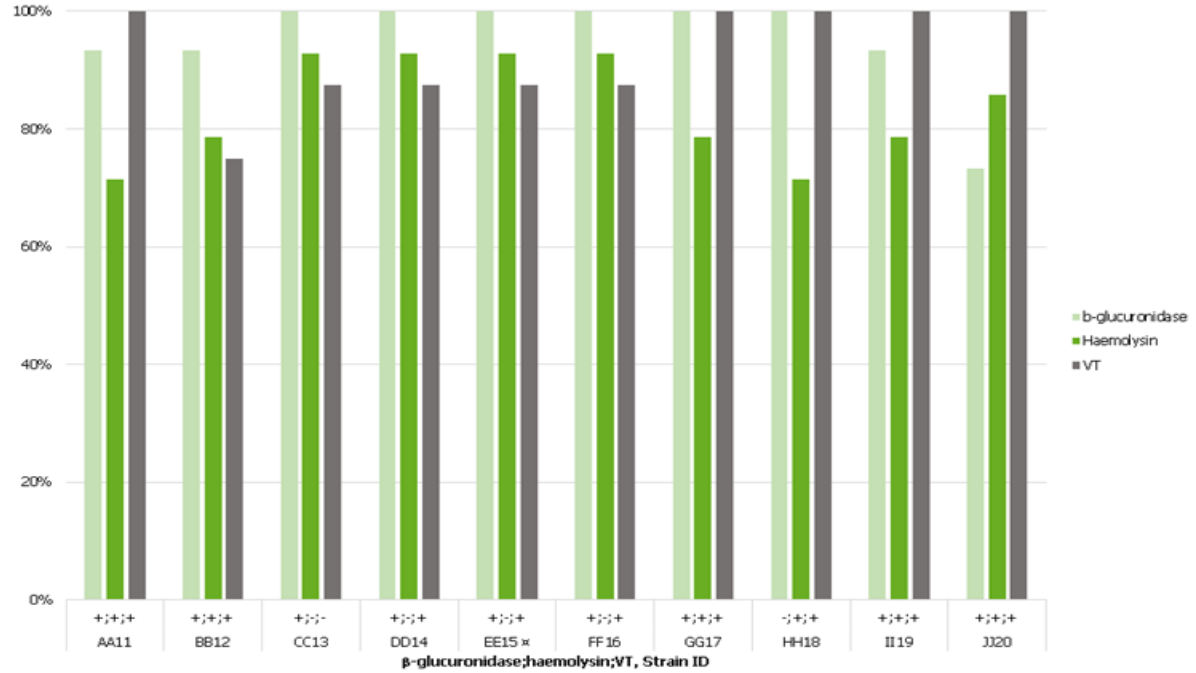
Figure 16. Participant percentage scores for detection of β-glucuronidase, haemolysin and VT production



Arbitrary numbers represents the participating laboratories. Bars represent the number of correct detections of β-glucuronidase production (light green) n=15, enterohaemolysin (dark green) (n=15) and VT (grey) (n=8).

Half (14) of the 28 laboratories participating in the phenotypic part of the EQA were able to report 100% correct results for all methods they performed (Annexes 12–13). For each test, two to five participants (11 to 37%) reported incorrect results. Specific strains generated common difficulties (incorrect result from two or more laboratories); strain JJ20 for β-glucuronidase; strain AA11, BB12, GG17, HH18, II19 and JJ20 for enterohaemolysin; BB12 for VT (Figure 17, Annex 13).

Figure 17. Average percentage test strain score for detection of β-glucuronidase, haemolysin and VT production



Bars represent the percentage of laboratories correctly detecting β-glucuronidase production (light green) n=15, enterohaemolysin (dark green) n=15 and VT (grey) n=8. Average scores: β-glucuronidase, 95%, enterohaemolysin, 84% and VT 93%. ¤ Repeat strain in EQA-4, -5, -6 and -7.

4. Conclusion

Thirty laboratories participated in the EQA-7 scheme, the highest number of participants of all the EQAs. The overall participation was high for serotyping (90%), genotyping (93%) and phenotyping (93%) methods and lower for PFGE (63%; 19/30). Half (57%) of the laboratories participated in all four parts of the scheme, and three (10%) completed all methods in all parts of the scheme.

PFGE was included for the fourth time, and a substantial improvement has been seen through the EQAs for both the gel and the gel analysis performances. The gel performance has increased from 45% (9/20) in EQA-4 to 74% (14/19) of the participants producing comparable gels. The gel analysis has increased from 50% (6/12) to 92% (1/13) performing in accordance with the guidelines. The gel parameter 'Bands' obtained a low average score, as in previous EQAs, and improvement of this parameter specifically would be beneficial. In addition, the performance of the band assignment could be increased, generating value to the profiles uploaded to TESSy.

The state-of-the art typing method for VTEC, O:H serotyping, was performed by 57% (17/30) of the participants only, with a 90% average score. As in previous EQAs, the participation in O-grouping was higher than in H-typing. Notably, not all laboratories demonstrated the capacity to determine all O-groups and H-types, capacity building to include a wider range of serotypes would be advantageous. The majority of incorrect results were reported as ND or NT. In general, the more common European serotypes generated the highest scores. Serotype O156:H4 generated the lowest scores, correctly reported by only two out of 27 laboratories.

In both the genotyping and phenotyping part of the exercise the participation and detection rate varied considerably, as in previous EQAs.

The performances for detection of *aaiC* /*aggR* have been high throughout the three EQAs, including an EAEC strain (EQA-4, -5 and -7). The presence of the two genes has been detected by all participants, and false positive results have only been reported by a few laboratories. The present EQA has yielded the best performance for *aggR* of all the EQAs, with a 100% average score.

There have been high participation rates and high average scores for the genotyping of *eae* in all the EQAs, with a tendency towards improvement (EQA-4, 96%; EQA-5, 98%; EQA-6, 97%). On the other hand, there have been low participation rates and high average scores for the genotyping of *ehxA* in the EQAs, with a tendency towards decreasing scores (EQA-4, 99%; EQA-5, 98%; EQA-6, 98%; EQA-7, 97%).

Similar to the previous EQAs, the participation and average score for *vtx1* and *vtx2* gene detection were high, 100% average score for *vtx1* and 98% for *vtx2*. Subtyping of *vtx1* and *vtx2* is highly valuable since specific subtypes (*vtx1*a and *vtx2*f) have been associated with HUS irrespective of other characteristics. The fairly high participation and good average score of 90% is therefore encouraging. The average score for subtyping of both *vtx1* and *vtx2* has been to a large extent unchanged in all the EQAs (EQA-4, 90%; EQA-5, 92%, EQA-6, 91%). In the current EQA, the incorrect *vtx2* results were mainly due to reporting two *vtx2* subtypes for strains harbouring one type only.

The phenotypic characterisation was performed with varying average scores; 84% for enterohaemolysin, 93% for VT, 95% for β-glucuronidase, 98% for ESBL, and 99% for sorbitol. In general, phenotypic characterisation was performed less frequently than genotypic characterisation, except for sorbitol fermentation (87%). Participation rates for the other phenotypic methods were: enterohaemolysin production (47%), detection of VT production (27%), β glucuronidase production (50%) and ESBL production (60%). The participation rate in each phenotypic test has decreased during the EQAs.

The large number of participating laboratories and their good performance overall is reassuring. The molecular surveillance system implemented as part of TESSy (TESSy-MSS) relies on the capacity of the FWD-Net laboratories to produce comparable typing results. The current EQA demonstrates that the majority of participating laboratories were able to produce good and comparable typing results. For the majority of the issues identified, an acceptable quality could be achieved by optimising procedures in laboratories, troubleshooting assistance, training and capacity building. Furthermore, completion of the methods for all strains could enhance performance.

5. Discussion

5.1 Pulsed Field Gel Electrophoresis

Nineteen laboratories participated in the PFGE gel part, and their gels were graded according to the *TIFF Quality Grading Guidelines EQA-7*, where seven parameters are used for grading given scores between 1 and 4 (Poor, Fair, Good and Excellent).

Fourteen (74%) of the participating laboratories produced an acceptable TIFF quality gel. Four parameters generated an unsatisfactory score of 1 [Poor], two ('Bands' and 'DNA Degradation') of which obtained an average score below 3. Four of the five inter-laboratory incomparable gels scored Poor [1] in either of these two parameters. In EQA-6, the parameter 'Bands' also obtained an average score below 3, with a low proportion (23%) of the participants producing 'Excellent' bands, and the majority (83%; 5/6) of incomparable gels obtained a score of 1 [Poor] in this parameter, stressing the particular difficulties with this parameter.

Similarly, in the present EQA, only four (21%) participants were able to obtain an 'Excellent' [4] score in the parameter 'Bands' and three (16%) gels scored 1 [Poor] in this parameter. Most of the low 'Band' scores were due to band distortion and fuzzy bands. Band distortion often comes from either physical damage to gel slices or an electrophoresis machine that does not work to specification. Regarding fuzzy bands, the easiest, and often best way to improve the sharpness of the bands is to use wider wells, but fuzzy bands can have several causes. Some of the most common are:

- Bad image capture due to improper focussing or use of an improper aperture size
- Use of an excessively small image
- Use of a gel comb with narrow wells. When using these the margin of error is greatly reduced. The recommended comb sizes are 10 wells in a 14 cm wide gel and 15 wells in 21 cm wide gel
- Cutting very thick gel slices. The recommended thickness is ~2 mm
- The staining procedure. The acceptable alternatives to EtBr are GelRedTM, SYBR® Safe, SYBR® Gold. Laboratories are strongly encouraged to follow the manufacturer's instructions. If one of the EtBr alternative stains is used, the de-staining steps should be omitted.

'Image Acquisition and Running Conditions' generated three Poor [1] scores due to incorrect running conditions (laboratories 130, 132 and 145). The use of correct running conditions, as described for the relevant organism, is very important. Failure to follow the protocol strongly affects the subsequent normalisation and band assignment in BN. Furthermore, it is vital that the equipment is properly maintained and works within specifications and that the buffer temperature is in accordance to the protocol. The electrophoresis time should also be adjusted in each laboratory, as failure to do this results in a bottom band that is not 1–1.5 cm from the bottom of the gel.

Following the gel electrophoresis, proper image capture of the gel is a critical step in obtaining a good quality TIFF image. Another common deviation from the protocol involved not allowing the gel to fill the whole TIFF. This is less critical than using incorrect running conditions, but can still strongly affect the ability to assign bands correctly.

In EQA-6, six participants (27%; 6/22) produced an inter-laboratory incomparable gel, two of these participants did not participate in the PFGE part of the current EQA, one improved the performance and obtained an acceptable gel (139). Two laboratories (132 and 138) still obtained a score of 1 [Poor] in the parameter 'Bands' and in one additional parameter. One laboratory (133) still obtained a single 1 [Poor] score, however this was in a different parameter. In EQA-6, only two parameters ('Bands' and 'Restriction') generated incomparable gels; this year additionally two parameters were graded 1 [Poor] (Image Acquisition and Running Conditions and DNA Degradation). In particular, the problem with use of incorrect running conditions increased in the present EQA. Incorrect running conditions generated no 1 [Poor] scores in the previous EQA. Consequently, two laboratories (130 and 145) saw their performance reduced to 'Poor' in the current EQA (from 'Good' in the previous round) due to incorrect running conditions. In general, gel performance has increased through the EQAs, with a higher percentage of the participants producing comparable gels in each round (EQA-4, 45%; EQA-5, 60%; EQA-6 73%; EQA-7, 74%).

The performance of the gel analysis was very good and all but one laboratory produced a BN analysis in accordance with the guidelines. The general performance of the BN analysis has increased in each round since PFGE was introduced to the EQA scheme. It has improved from 50% (6/12) of the participants performing gel analysis in accordance with the guidelines in EQA-4 to 92% (12/13) in EQA-7. Two laboratories (124 and 153) have obtained a 'Poor' BN analysis score twice, and Laboratory 132 has scored 1 [Poor] three times.

Even though the band assignments were well performed it is important to note that the evaluation of the band assignment is based on the quality of the gel. Therefore, it would still be useful if participants could improve their band assignment performance.

In general, caution should be exercised when comparing EQA results between the years. The results of the EQA are influenced by the laboratories which participate in the respective EQA round and by the nature of the test strains.

5.2 Serotyping

The participation in O-group typing has been almost unchanged from EQA-4 to EQA-7, with 90% of the participants performing O grouping (26/28; 26/29; 26/29; 27/30). However, there was a slight reduction in the H-typing participation rate from 64% (18/28) to 57% (17/30) between EQA-4 and EQA-7.

The performance of O-grouping was poor this year, mainly due to strain FF16 (O156), and only two (7%) participants reported the correct O group for all ten test strains. O group O156 is uncommon in Europe. Furthermore, participants using WGS-based serotyping would potentially be unable to O-group this variant of O156, since it is different from the common variants. The more common O groups, also included in the minimum requirements of ECDC, generated the highest performances (O157 (96%), O26 (93%)). The average score was lower (67%) in the current EQA than in the previous EQA (78%).

The general performance for H-typing was higher than O-grouping, with the majority (65%, 11/17) of participants correctly H-typing all ten test strains (Figure 5). Compared to the previous EQA, the average score of 81% correct results was unchanged (82% in EQA-6).

The O:H serotyping results ranged from 94% (16/17) for strain O157:H-(H7) to only 12% (2/17) of the participants reporting correct serotype for O156:H4. The average percentage of correct O:H serotyping scores in this EQA was lower (71%) than in EQA-6 (78%), and unchanged compared to EQA-5 (69%). The O:H serotyping score was highly influenced by the O group score, therefore the inclusion of O156 in the current EQA affected the results.

In general, the less common European serotypes generated the lowest scores, and vice versa. The performance of serotyping (O group/H type) is highly affected by the range of available antisera. Laboratories using a limited panel of antisera were encouraged to report serotype results as NT for strains they were unable to type. The majority of incorrect serotype results (both O group and H type results) was reported as not NT or ND, and no systematic typing error was observed.

In addition to O grouping, H typing is crucial for outbreak detection, epidemiological surveillance, taxonomic differentiation of *E. coli*, and detection of pathogenic serotypes. One of the main challenges is therefore to enable more of the national public health reference laboratories to perform complete and reliable O:H serotyping, particular H typing.

5.3 Genotyping

Twenty-eight laboratories participated in the genotyping part of the EQA scheme, and the participation rate and performance varied significantly between the different tests. As in previous EQAs the participation rate was highest for the genotypic detection of the *vtx* genes (93%) and lowest for the detection of *ehxA* (66%).

The performances for detection of the two EAEC genes were good; all participants detected *aaiC* and/or *aggR* correctly in the one EAEC strain included in the EQA. However, two false positive results for *aaiC* were reported by one laboratory. The performances for detection of *aaiC* /*aggR* in EAEC strains have been good in all the three EQAs including an EAEC strain (EQA-4, -5 and -7). The presence of the two genes has been detected correctly by all participants, and false positive results were only reported by two (EQA-4), three (EQA-5) or one laboratory (EQA-7). The present EQA demonstrates the best performance (100% average score) for *aggR* of all the EQAs.

Genotyping of *eae* had a high participation rate (28/30) and performance; 24 (86%) laboratories obtained a 100% score, giving an average score of 98%. The average correct score has been fairly unchanged through the EQAs, although it has tended towards improvement (EQA-4, 96%; EQA-5, 98%; EQA-6, 97%).

The participation rate for *ehxA* detection was low (60%; 18/30) but the performance was good; 15 (83%) laboratories obtained a 100% score. Compared to EQA-4, the average score for *ehxA* has slightly decreased (EQA-4, 99%; EQA-5, 98%; EQA-6, 98%; EQA-7, 97%).

Both the participation (28/30) and detection rates were high for genotyping of *vtx1* (100%) and *vtx2* genes (98%), which is similar to the previous EQAs. It is worth noting that the majority of false negative results originated from test strain DD14 (*vtx2f*) and HH18 (*vtx2c*). Recent cases of HUS caused by strains harbouring *vtx2f* have been described. The importance of *vtx2f* awareness has been described by Friesema et al. 2014 [18] and routine detection of *vtx2f* should be included in the expected repertoire of VTEC in Europe. Thus, the one additional participant performing detection of *vtx2f* was encouraging.

Correct subtyping of both *vtx1* and *vtx2* obtained an average score of 90%, a similar performance to previous EQAs (EQA-4, 90%; EQA-5, 92%, EQA-6, 91%). Similarly, the performance for *vtx1* subtyping has been higher than for *vtx2* through the EQAs (EQA-4, 94% vs. 93%; EQA-5 98% vs. 92%; EQA-6 100% vs. 91%; EQA-7, 99%

vs. 90%). Since the number of *vtx1* subtypes is lower (3) than the number of *vtx2* subtypes (7), the performance of the former could be expected to be higher. In the current EQA, the incorrect *vtx2* results were mainly due to reporting two *vtx2* subtypes, especially *vtx2c* and *vtx2d*, for strains harbouring one type only. The simultaneous presence of *vtx2a* and *vtx2c* in *eae* positive O157 strains is quite common. However, *vtx2c* and *vtx2d* genes occur very rarely, and then most often in *eae* negative strains.

5.4 Phenotyping

Participation in the phenotyping part of the exercise ranged from eight (VT detection) to 26 (sorbitol fermentation) laboratories. The participation rate for the various phenotypic tests has decreased during the EQAs, especially for VT (EQA-4, 10/28; EQA-5 8/29; EQA-6 7/29; EQA-7, 8/30) and β-gluruconidase (EQA-4, 19/28; EQA-5 15/29; EQA-6 16/29; EQA-7, 15/30). Detection of VT production is a classic phenotypic test for VTEC, however, the low participation rate suggests that these appliances are not readily available in national public health reference laboratories.

Eleven (73%) of the 15 participants detecting β-glucuronidase production obtained a 100% score. Four participants reported false negative results for the same strain (JJ20).

Fermentation of sorbitol can be considered the most important phenotypic test since detection of the highly virulent sorbitol fermenting O157:H7 clone is possible. The high participation (26/30) and detection rate (99% average score) was therefore encouraging. Participation in the sorbitol part has been high and fairly unchanged throughout the EQAs (EQA-4, 26/28; EQA-5, 26/29; EQA-6, 24/29; EQA-7, 26/30).

A similar strong performance was displayed for ESBL, with an average score of 98%. All the 18 laboratories detected the ESBL producing strain (EE15), however, two laboratories reported one or two false positive results for different strains.

Detection of enterohaemolysin production generated the lowest average score (84%) of the phenotypic tests, with 64% (9/14) of the participants obtaining 100% correct results. Three laboratories reported negative results for nine or all ten test strains, suggesting that the assay was performed for one or a few strains, and the negative results were reported as ND was not an option. An alternative to the detection of enterohaemolysin production is detection of the *ehxA* gene, for which performance was good in the current EQA.

Half (14/28) of those who participated in the phenotypic part of the EQA were able to report 100% correct results for all methods they performed. For each test, two to five participants (11 to 37%) reported incorrect results. For some tests (β-glucuronidase; enterohaemolysin; VT) specific strains generated common difficulties.

In summary, the performance level for phenotypic characterisation was high for sorbitol and ESBL and lower for VT and enterohaemolysin. Overall, the participation rate in each test was lower than in EQA-4 and has decreased during the EQAs, possibly in part due to replacement of phenotypic assays with WGS-based methods. Phenotypic assays, especially detection of ESBL and VT production, are important since the gene product and not the presence of the gene provides the phenotype.

5.5 General remarks

The inconsistency in the number of tests performed per strain and per laboratory have been a recurrent problem throughout the EQAs so far. The participants have not explained why a specific test was not performed on all 10-test strains. This was particularly evident for O grouping and H typing where laboratories submitted multiple entries for ND. These inconsistencies reduce comparability between the tests and the laboratories and complicate the analyses. Furthermore, in all EQAs there have been deviations between the methods registered and the methods performed.

6. Recommendations

6.1 Laboratories

By evaluating the results from the FWD-Net laboratories participating in this EQA it has been possible to identify a number of technical issues reducing the quality of the typing results. For each method, performance could be improved by introducing a number of initiatives.

The quality of PFGE profiles is directly dependent on application of controlled laboratory procedures. Therefore, laboratories can optimise their performance by strictly adhering to the protocol. A high quality gel is dependent on a variety of details such as temperatures, running times, number of repeated washing steps, etc. All these should be performed strictly according to the protocol for the relevant organism. The gel parameter 'Bands' caused most problems in this and the previous EQA. 'Bands' is one of the most complex parameters in the TIFF guidelines and there are many reasons for not obtaining crisp bands. Individual laboratories should therefore evaluate their particular situation carefully and assess their own problems, following advice provided in the evaluation report. Overexposure of the gels was less of a problem than in previous EQAs. A number of other errors could easily have been avoided by carefully reading the instructions on how to create and send TIFF and XML files of the PFGE results. We therefore encourage participants to use the troubleshooting team.

O:H serotyping is essential for characterisation of VTEC, however, participation has been fairly low throughout the EQAs for complete O:H serotyping, mainly due to limited participation in H typing. Furthermore, performance has been strongly affected by laboratories using a limited panel of antisera, reporting ND or NT results. Capacity building to include a wider range of antisera would be useful.

The major problem in subtyping the *vtx2* genes was the simultaneous detection of *vtx2c* and *vtx2d*. This has been shown to relate to the sensitivity of different PCR cycler equipment and use of different DNA polymerases. Laboratories are advised to test and calibrate their PCR cycler (increasing the annealing temperature up to 67°C or higher) in order to improve the distinction of *vtx2c* and *vtx2d.*

6.2 ECDC and FWD-Net

The participation rate was high (86%, 30 accepted out of 35 laboratories invited from the FWD-Net and candidate countries). Future EQAs should aim to include and provide improved assistance for new participants, possibly through technical training, or by including laboratories repeatedly displaying identical difficulties, even after receiving guidance from individual evaluation reports and the EQA report.

Pulsed Field Gel Electrophoresis was included for the fourth time, and 19 (63%) of the laboratories produced a gel. A considerable proportion (6/19) of the participants in the PFGE part did not perform the gel analysis, and the participation rate for the gel analysis part has remained unchanged. There is still a need for capacity building in laboratory procedures, gel analysis and interpretation using BN, although the improved, and in some cases strong performances during the course of the EQAs are reassuring.

The reasons behind the relatively low rates of participation in complete O:H typing should be explored.

Few laboratories performed the phenotypic tests for VT production and β-glucuronidase production and these may be excluded in future EQAs.

6.3 The EQA provider

The guidelines used for grading the PFGE gel quality is part of the ECDC SOP for molecular typing data in TESSy, adapted from PulseNet USA. The scheme was slightly modified to ensure correspondence between the score and the suitability of the gel for inter-laboratory comparability. Similarly, the guidelines used for grading the BN gel analysis were adapted to the performances of the participants in the current EQA. For future reference, we propose that a short guide on when to separate doublets would be the best way to significantly increase the performance of the band assignment. The other four BN analysis parameters are easier to improve without actual training and simply involve strict observation of a detailed protocol.

It may be useful to consider a request for specification of the O:H serotype methods applied since some participants performed WGS-based serotyping instead of conventional serotyping, which could have affected their performance.

Once again, this year, the EQA provider improved the instructions to the participants, with additional details and online submission forms similar to those used in previous years. Nevertheless, some participants still submitted unacceptable XML-exports; failed to use the specific strain ID as the key in BN; did not include the Lab ID in the TIFF file, did not use correct running conditions or did not test all ten strains for each of the methods performed.

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Annex 1. List of participants

Country	Laboratory	National institute
Austria	NRC *Escherichia coli* including Verotoxin producing *E. coli*	Austrian Agency for Health and Food Safety (IMED Graz / AGES)
Belgium	Department of microbiology and infection control	UZ Brussel
Bulgaria	NRL for Enteric Pathogens	National Center of Infectious and Parasitic Diseases
Cyprus	NRL for Salmonella and other Enteric Pathogens	Medical and Public Health Services
Czech Republic	NRL for *E.coli* and *Shigella*	National Institute of Public Health
Denmark	Foodborne Infections	Statens Serum Institut
Estonia	Laboratory of Communicable Diseases	Health Board
Finland	Bacteriology Unit	National Institute for Health and Welfare (THL)
France	Centre National de Référence des *E. coli*, *Shigella* et *Salmonella*	Institut Pasteur
Germany	NRC for *Salmonella* and other Bacterial Enteric Pathogens	Robert Koch Institute
Greece	NRC for *Salmonella*, *Shigella*, VTEC	National School of Public Health
Hungary	National Public Health Reference Laboratory for enteric bacteria	National Center for Epidemiology
Iceland	Department of Clinical Microbiology	Landspítali University Hospital
Ireland	VTEC-RL	Public Health Laboratory
Italy	Dipartimento di Sanità Pubblica Veterinaria e Sicurezza Alimentare, Reparto di Zoonosi trasmesse da alimenti	Istituto Superiore di Sanità
Latvia	National Reference Laboratory	Infectology Centre of Latvia
Lithuania	National Public Health Surveillance Laboratory	Budget Organization
Luxembourg	Surveillance Epidémiologique	Laboratoire National de Santé
Norway	National Reference Laboratory of Enteropathogenic Bacteria	Norwegian Institute of Public Health
Poland	Department of Bacteriology	National Institute of Public Health - National Institute of Hygiene
Portugal	Lab. de Salmonella, *E.coli* e outras bactérias entéricas	Instituto Nacional de Saúde Doutor Ricardo Jorge
Republic of Macedonia	Food Institute	Faculty of veterinary medicine-Skopje
Romania	Molecular Epidemiology Laboratory	Cantacuzino National Institute of Research
Serbia	Molecular Genetics	Institute of Epidemiology
Slovenia	Department for Public Health Microbiology	National Laboratory of Health, Environment and Food
Spain	Unidad de Enterobacterias	National Centre for Microbiology, Institute of Health Carlos III
Sweden	Mikrobiologi	Folkhälsomyndigheten
The Netherlands	Centrum Infectieziekte-onderzoek Diagnostiek en Sceening	RIVM
Turkey	National Reference Laboratory for Enteric Pathogens	Public Health Institution of Turkey
United Kingdom	Gastrointestinal Bacteria Reference Unit	Public Health England

Annex 2. TIFF Quality Grading Guidelines EQA-7

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

In extension of the EQA-6 Guidelines the sentence 'Too few reference lanes included' was added to the Poor [1] score in the parameter Image Acquisition and Running Conditions.

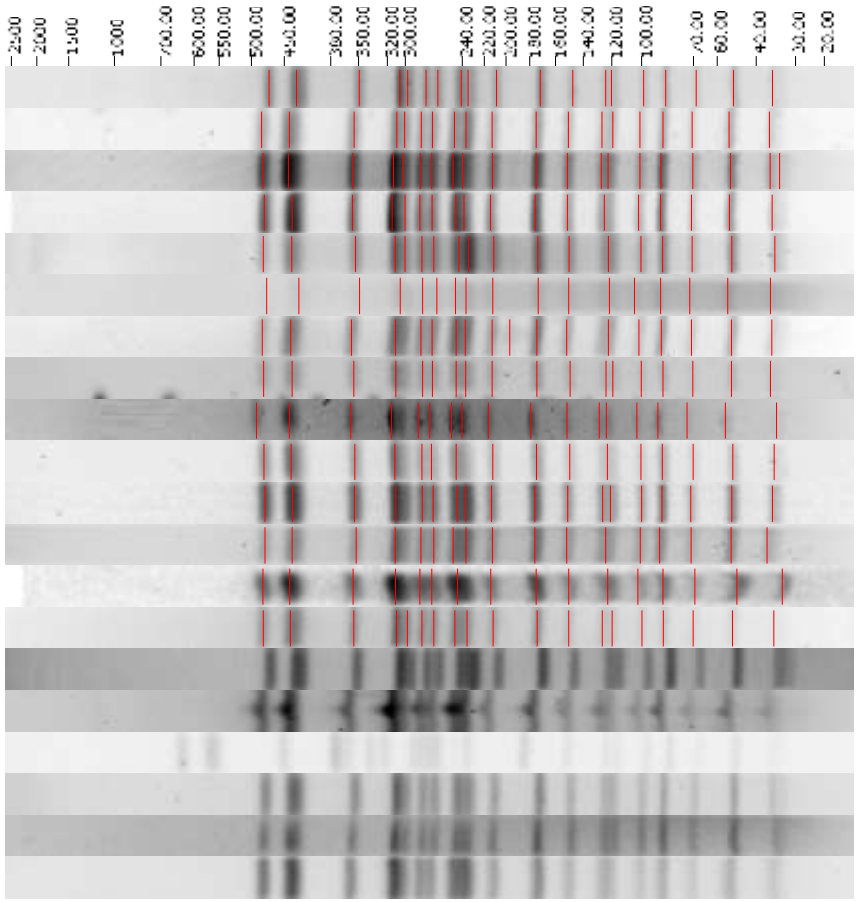
Annex 3. BioNumerics Gel Analysis Quality Guidelines EQA-7

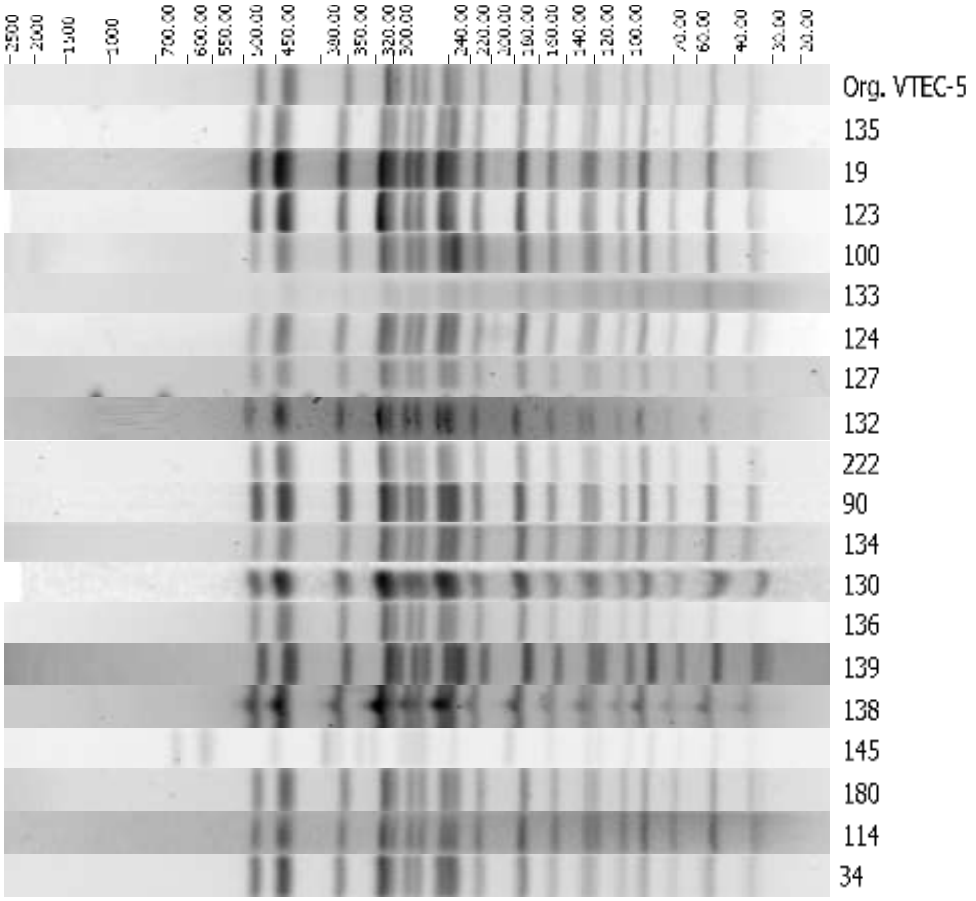
Parameter	Grade [score in points]		
	Poor [1]	Fair [2]	Excellent [3]
Position of Gel Frame	- Wells wrongly included when placing the frame - Gel is not inverted	- The frame is positioned too low - Too much space framed at the bottom of the gel - Too much space framed on the sides of the gel.	Excellent placement of frame and gel is inverted.
Strips	Lanes incorrectly defined	- Lanes are defined too narrow (or wide) - Lanes are defined outside profile - A single lane is not correctly defined.	All lanes correctly defined.
Curves	Curve set so that artefacts will cause wrong band assignment	Curve extraction is defined as either too narrow or includes almost the whole Lane.	1/3 or more of the lane is used for averaging curve extraction
Normalisation	- Many bands not assigned in the reference lanes - The references were not included when submitting the data - Assignment of band(s) in reference lane(s) to incorrect size(s).	- Bottom bands <33kb are not assigned in some or all of the reference lanes - Some bands wrongly assigned in reference lane(s).	All bands correctly assigned in all reference lanes
Band Assignment	Incorrect band assignment making inter-laboratory comparison impossible.	- Few double bands assigned as single bands or single bands assigned as double bands. - Few shadow bands are assigned. - Few bands are not assigned. - Few bands assigned where there are none.	Excellent band assignment with regard to the quality of the gel.

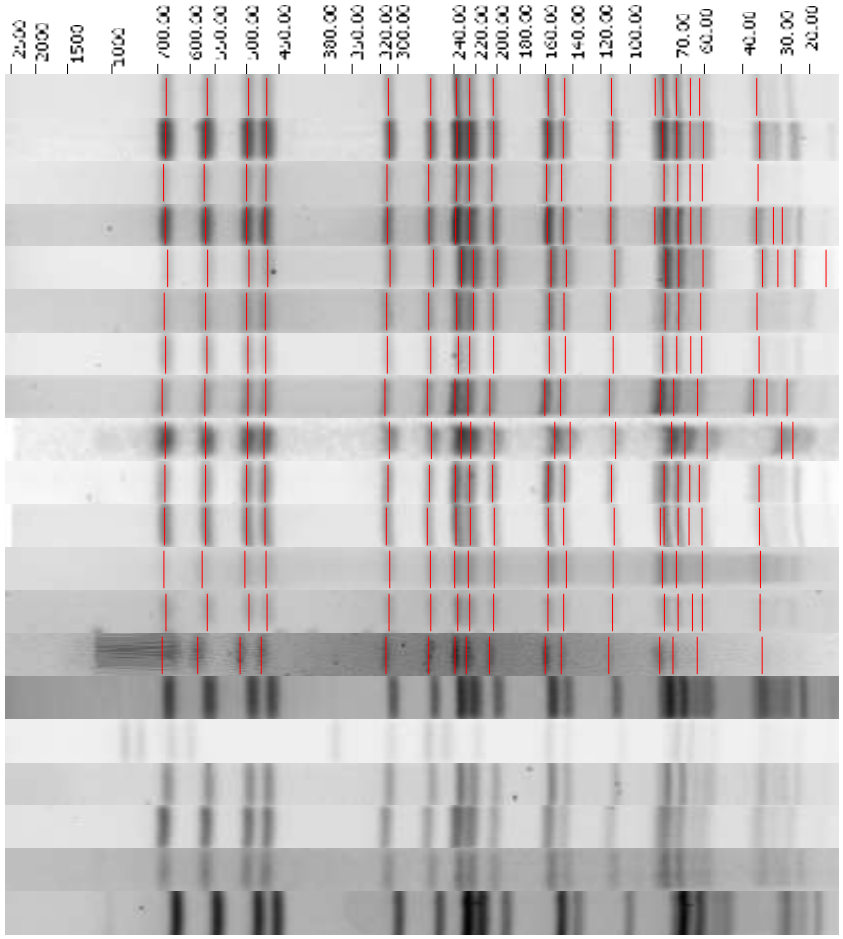
In extension of the EQA-6 Guidelines, two sentences were added to the Normalisation parameter: 'Assignment of band(s) in reference lane(s) to incorrect size(s)' in the Poor [1] score and 'Some bands wrongly assigned in reference lane(s)' was added in the Fair [2] score; and one sentence to the Band Assignment parameter in the Fair [2] score: 'Few bands assigned where there are none'.

Annex 4. PFGE profiles of two test strains

Nineteen PFGE profiles (13 with band assignment, BN analysis) produced by the participants and the original profiles of test strain VTEC-5 (top) and VTEC-7 (bottom) cut with *XbaI*.



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600.00
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500.00
450.00
380.00
340.00
320.00
300.00
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70.00
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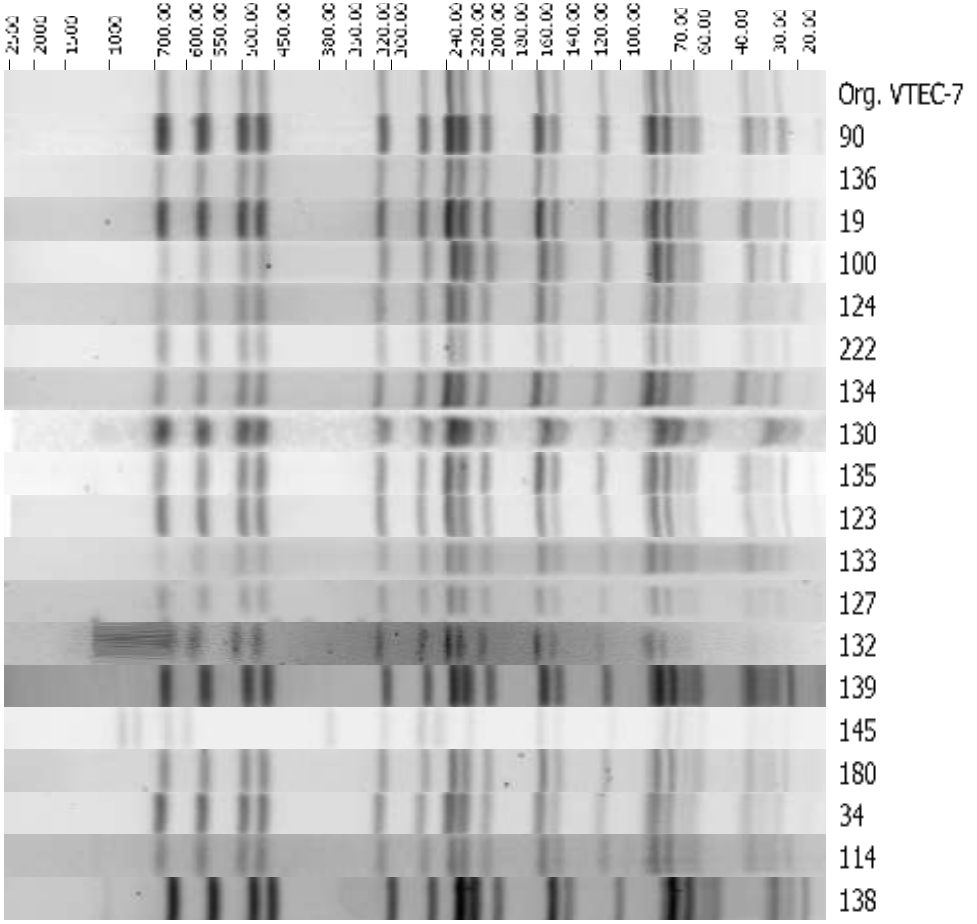
20.00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100.00
70.00
60.00
40.00
30.00
20.00
Org. VTEC-7
90
136
19
100
124
222
134
130
135
123
133
127
132
139
145
180
34
114
138

Annex 5. Scores of the PFGE results

Gel quality

	Laboratory no.																		
Parameter	**19**	**34**	**90**	**100**	**114**	**123**	**124**	**127**	**130**	**132**	**133**	**134**	**135**	**136**	**138**	**139**	**145**	**180**	**222**
Image Acquisition and Running Conditions	4	4	4	3	4	4	4	4	1	1	2	3	3	3	3	2	1	4	4
Cell Suspension	3	4	4	4	3	4	4	4	2	3	2	4	4	3	4	4	4	3	4
Bands	3	4	3	3	2	4	3	3	1	1	3	4	3	4	1	3	3	3	2
Lanes	4	4	3	4	3	4	3	4	4	3	4	3	4	3	3	2	2	4	3
Restriction	4	4	3	4	4	4	4	4	3	4	3	4	2	4	1	4	4	2	3
Gel Background	3	4	4	4	4	4	2	3	3	2	3	3	3	3	3	2	3	4	3
DNA Degradation	2	4	4	2	2	4	3	4	2	4	1	2	3	3	2	3	4	2	4
Total Quality	23	28	25	24	22	28	23	26	16	18	18	23	22	23	17	20	21	22	23

Participant scores 1–4 (Poor, Fair, Good, Excellent) obtained for each of the seven TIFF Quality Grading Guidelines parameters and the total score

Poor score

BN analysis

	Laboratory no.																		
Parameter	**19**	**34**	**90**	**100**	**114**	**123**	**124**	**127**	**130**	**132**	**133**	**134**	**135**	**136**	**138**	**139**	**145**	**180**	**222**
Position of the Gel	3	-	2	3	-	3	3	3	3	2	3	2	2	3	-	-	-	-	3
Strips	3	-	3	3	-	3	3	2	2	2	2	3	2	3	-	-	-	-	3
Curves	3	-	3	2	-	3	3	3	2	2	2	2	3	3	-	-	-	-	3
Normalisation	3	-	3	3	-	3	2	3	2	1	2	3	3	3	-	-	-	-	3
Band assignment	3	-	3	3	-	2	3	2	2	2	2	3	2	3	-	-	-	-	3
Total Quality	15	-	14	14	-	14	14	13	11	9	11	13	12	15	-	-	-	-	15

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

Poor score

Annex 6. Original data

Strain ID	Serotyping		Genotyping								Phenotyping					Additional virulence genes	Pathotype
	O group	H type	*aaiC* gene	*aggR* gene	*eae* gene	*ehxA* gene	*vtx1* gene	*vtx2* gene	*vtx1* subtypes	*vtx2* subtypes	β-glucuronidase production	ESBL production	Haemolysin production	Sorbitol fermentation	Verocytotoxin production		
AA11	O80	H2	-	-	+	+	-	+	-	*vtx2a*	+	-	+	+	+		VTEC
BB12	O26	H11	-	-	+	+	-	+	-	*vtx2a*	+	-	+	+	+		VTEC
CC13	O78	H2	+	+	-	-	-	-	-	-	+	-	-	+	-	*aatA*	EAEC
DD14	O145	H34	-	-	+	-	-	+	-	*vtx2f*	+	-	-	-	+		VTEC
EE15	O166	H15	-	-	-	-	-	+	-	*vtx2d*	+	+	-	+	+	*eltA*	VTEC-ETEC
FF16	O156	H4	-	-	-	-	-	+	-	*vtx2d*	+	-	-	+	+		VTEC
GG17	O146	H21	-	-	-	+	+	+	*vtx1c*	*vtx2b*	+	-	+	+	+		VTEC
HH18	O157	H-(H7)	-	-	+	+	+	+	*vtx1a*	*vtx2c*	-	-	+	-	+		VTEC
II19	O91	H14	-	-	-	+	+	+	*vtx1a*	*vtx2b*	+	-	+	+	+	*saa*	VTEC
JJ20	O103	H2	-	-	+	+	+	-	*vtx1a*	-	+	-	+	+	+		VTEC

- negative; + positive; EAEC enteroaggregative *E. coli*; ESBL extended spectrum beta-lactamase; ETEC enterotoxigenic *E. coli*; VTEC verocytotoxin-producing *E. coli*
Verocytotoxin production, intermediate result evaluated as a positive result
Enterohaemolysin, alfa result evaluated as a positive result.

Annex 7. O-group and H-type results

O-group

	Laboratory no.																											
Strain ID	Org.	19	34	80	88	94	100	108	114	123	124	125	127	128	129	130	131	132	133	134	135	136	137	138	139	145	153	222
AA11	O80	O80	O80	NT	O80	NT	O41	O80	O80	O80	NON-O157	O113	NT	ND	NT	NT	NT	NON-O157	ND	O80	O80	O80	O80	O126	O80	O91	ND	O119
BB12	O26	O26	O26	O26	O26	NT	O26	O26	O26	O26	O26	O26	O26	O26	O26	O26	O26	NON-O157	O26	O26	O26	O26	O26	O26	O26	O26	O26	O26
CC13	O78	O78	O78	O78	NT	NT	O78	O78	O78	O78	O78	O78	O78	O78	O78	NT	O78	NON-O157	ND	O78	O78	O78	O78	NT	O78	O142	ND	NT
DD14	O145	O145	O145	O145	NT	NT	O145	O145	O145	O145	O145	O103	O145	O145	O145	O145	O145	NON-O157	ND	O145	O145	O145	O145	O145	O145	O145	O145	O145
EE15 ¤	O166	O166	O166	NT	O166	NT	O166	O166	O166	O166	O166	O142	O166	O166	NT	NT	O166	NON-O157	ND	O166	O166	O166	O166	NT	O166	O109	ND	NT
FF16	O156	O156	O156	NT	NT	NT	O36	NT	NT	Rough	NON-O157	NT	NT	O41	NT	NT	Rough	NON-O157	ND	NT	O108	NT	NT	NT	NT	O157	ND	NT
GG17	O146	O146	O146	O146	NT	NT	O146	O146	O146	O146	O146	O146	NT	O146	O146	NT	O146	NON-O157	ND	O146	O146	O146	O146	NT	O146	O76	ND	O146
HH18	O157	O157	O157	O157	NT	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157	O157
II19	O91	O91	O91	O91	NT	NT	O91	O91	O91	O91	O91	O63	O91	O91	O91	O104	O91	NON-O157	ND	O91	O91	O91	O91	NT	O91	O91	ND	O91
JJ20	O103	O103	O103	O103	O103	NT	O103	O103	O103	O103	O103	O145	O103	O103	O103	O103	O103	NON-O157	O103	O103	O103	O103	O103	O103	O103	O103	O103	O103

n= 27 participants

H-type

	Laboratory no.																											
Strain ID	Org.	19	34	80	88	94	100	108	114	123	124	125	127	128	129	130	131	132	133	134	135	136	137	138	139	145	153	222
AA11	H2	H2	H2	H2	-	-	-	H2	H2	H2	H-	ND	H2	ND	H2	-	H2	-	-	H2	H2	H2	H2	-	H2	-	-	-
BB12	H11	H11	H11	H11	-	-	-	H11	H11	H11	NT	ND	H11	ND	H11	-	H11	-	-	H11	H11	H11	H11	-	H11	-	-	-
CC13	H2	H2	H2	H2	-	-	-	H2	H2	H2	NT	ND	H2	H2	H2	-	H2	-	-	H2	H2	H2	H2	-	H2	-	-	-
DD14	H34	H34	H34	NT	-	-	-	H34	H34	H34	NT	ND	H34	ND	H34	-	H34	-	-	H34	H34	H34	H34	-	H34	-	-	-
EE15 ¤	H15	H15	H15	NT	-	-	-	H15	H15	H15	NT	ND	H15	ND	H15	-	H15	-	-	H15	H15	H15	H15	-	H15	-	-	-
FF16	H4	H4	H4	H4	-	-	-	H4	H4	H4	NT	ND	H4	ND	H4	-	H4	-	-	H4	H4	H4	H17	-	H4	-	-	-
GG17	H21	H21	H21	H21	-	-	-	H21	H21	H21	NT	ND	H21	ND	H21	-	H21	-	-	H21	H21	H21	H21	-	H21	-	-	-
HH18	H-(H7)	H-	H7	H7	-	-	-	H7	H-	H7	H7	H-	H7	ND	H7	-	H-	-	-	H7	H-	H7	H7	-	H7	-	-	-
II19	H14	H14	H14	NT	-	-	-	H14	H14	H14	H-	ND	H14	ND	H14	-	H14	-	-	H14	H14	H14	H14	-	H10	-	-	-
JJ20	H2	H2	H2	H2	-	-	-	H2	H2	H2	NT	ND	H2	ND	H2	-	H2	-	-	H2	H2	H2	H2	-	H2	-	-	-

n= 17 participants

Incorrect result - negative; + positive; ND Not Done; NT Non-Typeable

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 8. *aaiC* and *aggR* genotyping results

aaiC

		Laboratory no.																				
Strain ID	Org.	19	34	80	88	90	94	100	108	114	123	124	127	129	131	133	134	136	137	139	153	222
AA11	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
BB12	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
CC13	+	+	+	+		+	+	+		+	+	+	+		+	+	+	+	+		+	+
DD14	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
EE15 ¤	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
FF16	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
GG17	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
HH18	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		+	-
II19	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		-	-
JJ20	-	-	-	-		-	-	-		-	-	-	-		-	-	-	-	-		+	-

n= 17 participants

aggR

		Laboratory no.																				
Strain ID	Org.	19	34	80	88	90	94	100	108	114	123	124	127	129	131	133	134	136	137	139	153	222
AA11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BB12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CC13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DD14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EE15 ¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FF16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GG17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HH18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JJ20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n= 21 participants

Incorrect result - negative; + positive

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 9. *eae* and *ehxA* genotyping results

eae

		Laboratory no.																											
Strain ID	***eae***	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
BB12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CC13	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DD14	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
EE15 ¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FF16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GG17	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HH18	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JJ20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

n= 28 participants

ehxA

		Laboratory no.																											
Strain ID	***ehx A***	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	+	+	+	+		+	+	+	+	+	+	+		+	+			+		+	+		+					+	+
BB12	+	+	+	+		+	+	+	+	+	+	+		+	+			+		+	+		+					+	+
CC13	-	-	-	-		-	-	-	-	-	-	-		-	-			-		-	-		-					+	-
DD14	-	-	-	-		-	-	-	-	-	-	-		-	-			-		-	-		-					+	-
EE15 ¤	-	-	-	-		-	-	-	-	-	-	-		-	-			-		-	-		-					+	-
FF16	-	-	-	-		-	-	-	-	-	-	-		-	-			-		-	-		-					+	-
GG17	+	+	+	+		+	+	+	+	+	+	+		+	+			+		+	+		+					+	+
HH18	+	+	+	+		+	+	+	+	-	+	+		+	+			+		+	+		+					+	+
II19	+	+	+	+		-	+	+	+	+	+	+		+	+			+		+	+		+					+	+
JJ20	+	+	+	+		+	+	+	+	+	+	+		+	+			+		+	+		+					+	+

n= 18 participants

Incorrect result - negative; + positive

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 10. *vtx1* and *vtx2* genotyping results

vtx1

		Laboratory no.																											
Strain ID	**Org.**	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BB12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CC13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DD14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EE15 ¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FF16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GG17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HH18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
II19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JJ20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

n= 28 participants

vtx2

		Laboratory no.																											
Strain ID	**Org.**	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128**	**129**	**130**	**131**	**132**	**133**	**134**	**135**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BB12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CC13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DD14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+
EE15 ¤	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FF16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
GG17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HH18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+
II19	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
JJ20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

n= 28 participants

Incorrect result - negative; + positive

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 11. *vtx1* and *vtx2* subtyping results

vtx1

	Laboratory no.																									
Strain ID	**Org.**	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128**	**129**	**130**	**131**	**133**	**134**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
BB12	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
CC13	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
DD14	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
EE15 ¤	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
FF16	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-
GG17	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1a*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*		*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*	*vtx1c*
HH18	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1c*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*		*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*
II19	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*		*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*
JJ20	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*		*vtx1a*	*vtx1c*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*	*vtx1a*

n= 25 participants

vtx2

	Laboratory no.																									
Strain ID	**Org.**	**19**	**34**	**80**	**88**	**90**	**94**	**100**	**108**	**114**	**123**	**124**	**125**	**127**	**128***	**129**	**130**	**131**	**133**	**134**	**136**	**137**	**138**	**139**	**145**	**153**	**222**
AA11	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	ND	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a+ vtx2c*	*vtx2a*	*vtx2a+ vtx2b*
BB12	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	ND	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*	*vtx2a*
CC13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-
DD14	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2f*	*vtx2e+ vtx2f*	*vtx2e*	*vtx2f*
EE15 ¤	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2c+ vtx2d*	*vtx2a+ vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	ND	*vtx2c+ vtx2d*	*vtx2d*	*vtx2c+ vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2c+ vtx2d*
FF16	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2c*	*vtx2d*	*vtx2a*	*vtx2d*	*vtx2d*	*vtx2a*	*vtx2a*	*vtx2d*	*vtx2d*	*vtx2d*	ND	*vtx2d*	-	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2d*	*vtx2c*	*vtx2a+ vtx2c*	*vtx2a+ vtx2c*
GG17	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2g*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	ND	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*
HH18	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2a+ vtx2d*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	ND	*vtx2c+ vtx2d*	-	*vtx2c*	*vtx2c+ vtx2d*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2c*	*vtx2a+ vtx2d*	*vtx2c*	*vtx2c*
II19	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2g*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	ND	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*	*vtx2b*
JJ20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	-	-	-	-	-	-	-	-	-	-	-

n= 25 participants, * participant performing detection of *vtx2f* only, not included in the analysis of the subtyping results

Incorrect result - negative; ND Not Done

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 12. ESBL and sorbitol results

ESBL

		Laboratory no.																										
Strain ID	Org.	19	34	80	88	94	100	108	114	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	145	153	222
AA11	-	-	-	-		-	-		-	-	-		-		-			-	-	-	+		-		-		-	-	
BB12	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	
CC13	-	-	-	-		-	-		-	-	-		-		-			-	-	-	+		-		-		-	-	
DD14	-	-	-	-		-	-		-	-	-		-		+			-	-	-	-		-		-		-	-	
EE15 ¤	+	+	+	+		+	+		+	+	+		+		+			+	+	+	+		+		+		+	+	
FF16	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	
GG17	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	
HH18	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	
II19	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	
JJ20	-	-	-	-		-	-		-	-	-		-		-			-	-	-	-		-		-		-	-	

n= 18 participants

Sorbitol

		Laboratory no.																										
Strain ID	Org.	19	34	80	88	94	100	108	114	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	145	153	222
AA11	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
BB12	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
CC13	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	-	+	+	+	+
DD14	-	-	+	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
EE15 ¤	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
FF16	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
GG17	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
HH18	-	-	-	-	+	-	-	-	-	-	-	-		-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
II19	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
JJ20	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+

n= 26 participants

Incorrect result - negative; + positive

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 13. β-gluruconidase, enterohaemolysin and VT results

β-gluruconidase

		Laboratory no.																		
Strain ID	Original	19	34	80	94	100	114	123	124	125	126	127	128	129	130	131	136	137	145	153	222
AA11	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	-	
BB12	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	-	
CC13	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	+	
DD14	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	+	
EE15 ¤	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	+	
FF16	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	+	
GG17	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	+	
HH18	-	-	-	-	-	-	-	-	-			-	-		-	-	-		-	-	
II19	+	+	+	+	+	+	+	+	+			+	+		+	+	+		+	-	
JJ20	+	+	+	+	+	+	+	+	-			+	-		+	-	+		+	-	

n= 15 participants

Enterohaemolysin

		Laboratory no.																			
Strain ID	Original	19	34	80	94	100	114	123	124	125	126	127	128	129	130	131	136	137	145	153	222
AA11	+	+	+		+	+	-	+		-	+	+		+		+	+	-	-		
BB12	+	+	+		+	+	+	+		-	+	+		+		+	+	-	-		
CC13	-	-	-		-	-	-	-		-	+	-		-		-	-	-	-		
DD14	-	-	-		-	-	-	-		-	+	-		-		-	-	-	-		
EE15 ¤	-	-	-		-	-	-	-		-	+	-		-		-	-	-	-		
FF16	-	-	-		-	-	-	-		-	+	-		-		-	-	-	-		
GG17	+	+	+		+	+	+	+		-	+	+		+		+	+	-	-		
HH18	+	+	+		+	+	-	+		-	+	+		+		+	+	-	-		
II19	+	+	+		+	+	+	+		-	+	+		+		+	+	-	-		
JJ20	+	+	+		+	+	+	+		-	+	+		+		+	+	+	-		

n= 14 participants

VT

		Laboratory no.																			
Strain ID	Original	19	34	80	94	100	114	123	124	125	126	127	128	129	130	131	136	137	145	153	222
AA11	+	+					+	+			+	+				+				+	+
BB12	+	-					+	+			+	+				+				-	+
CC13	-	-					-	-			+	-				-				-	-
DD14	+	+					+	+			+	+				+				-	+
EE15 ¤	+	+					+	+			+	+				+				-	+
FF16	+	+					+	+			+	+				+				-	+
GG17	+	+					+	+			+	+				+				+	+
HH18	+	+					+	+			+	+				+				+	+
II19	+	+					+	+			+	+				+				+	+
JJ20	+	+					+	+			+	+				+				+	+

n= 8 participants

Incorrect result - negative; + positive

¤ Repeat strain in EQA-4, -5, -6 and -7

Annex 14. *vtx* subtypes reference strains

SSI collection D number	Strain	Toxin subtype	Toxin variant designation	GenBank accession no.	Results	Serotype	Additional virulence genes
D2653	EDL933	VT1a	VT1a-O157-EDL933	M19473	*vtx1a + vtx2a*	O157:H7	*eae, ehxA, astA*
D3602	DG131/3	VT1c	VT1c-O174-DG131-3	Z36901	*vtx1c + vtx2b*	O174:H8	
D3522	MHI813	VT1d	VT1d-O8-MHI813	AY170851	*vtx1d*	O8:K85ab:Hrough	*eae*
D3428	EH250	VT2b	VT2b-O118-EH250	AF043627	*vtx2b*	O118:H12	*astA*
D3431	F35790	VT2c	VT2c-O157-310/ VT2c-O157-Y350-1	ND	*vtx2c*	O157:H7	*eae, ehxA, astA*
D4134	1112R15035	VT2d	ND	ND	*vtx2d*	O166:H15	
D3648	S1191	VT2e	VT2e-O139-S1191	M21534	*vtx2e*	O139:K12:H1	
D3546	T4/97	VT2f	VT2f-O128-T4-97	AJ010730	*vtx2f*	O128ac:H2	*eae, bfpA, astA*
D3509	7v	VT2g	VT2g-O2-7v	AY286000	*vtx2g*	O2:H25	*ehxA, astA, estAp*

ND = Not Done.

Annex 15. Guide to setup a BN database

An EQA database can be set up in two ways. If you have BioNumerics version 6 or 7 you can simply use the ready-made database(s) that was sent out together with this instruction.

Two important things:

YOU NEED TO SET UP A NEW database; do not use any of your existing databases, not even those for the previous EQAs. This is important in order to be able to submit correctly formatted results – use guide **(A).**

If (and only if) you have a BioNumerics version **prior to 6.0**, use the instruction on setting up a database from scratch **(B).**

A) Setting up a database if you have BioNumerics 6.0 – 7.x

1. The database is packaged in the zip archive called "Listeria EQA-4 BN<6/7>.zip", "Salmonella EQA-7 BN<6/7>.zip" or "E coli EQA-7 BN<6/7>.zip". Note that there are two versions of each, one for version 6 and one for version 7 of BioNumerics.
2. Please choose the correct file and download the files from links found in the e-mail containing the submission details to your own PC.
3. Unzip the files into the folder "XX" where you would like to have your database.
4. The archive contains the complete ready-made database (one file and one folder).
5. Open the BioNumerics program and change the home directory to where you placed your database.

6. Press the third button from the left (look at the picture above) and choose the first option "Change home directory".
7. Browse to find the pre-configured database (desktop or the "XX" folder where you saved the files).
8. In the open pre-configured database - the only option visible is the STD_H9812Ec.
9. Then import your TIFF, and use the four-digit strain no. as KEY (USE the guide to change the TIFF from a 16-bit to an 8-bit file correctly).
10. Fill in Lab ID, e.g. "DK_SSI".
11. Make the BioNumerics analysis.
12. Afterwards follow the XML export guide below - it is important that you select your strains, before making the export.

B) Set up a database from scratch

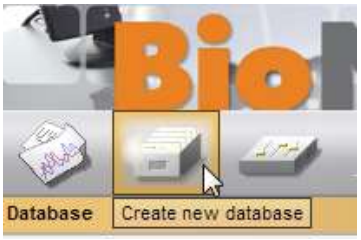
All the images in this instruction refer to *E. coli* so just exchange "E coli" for either "Salmonella" or "Listeria" when setting up these databases.

The screen shots are from version 6 of BioNumerics so it may look slightly different in your version.

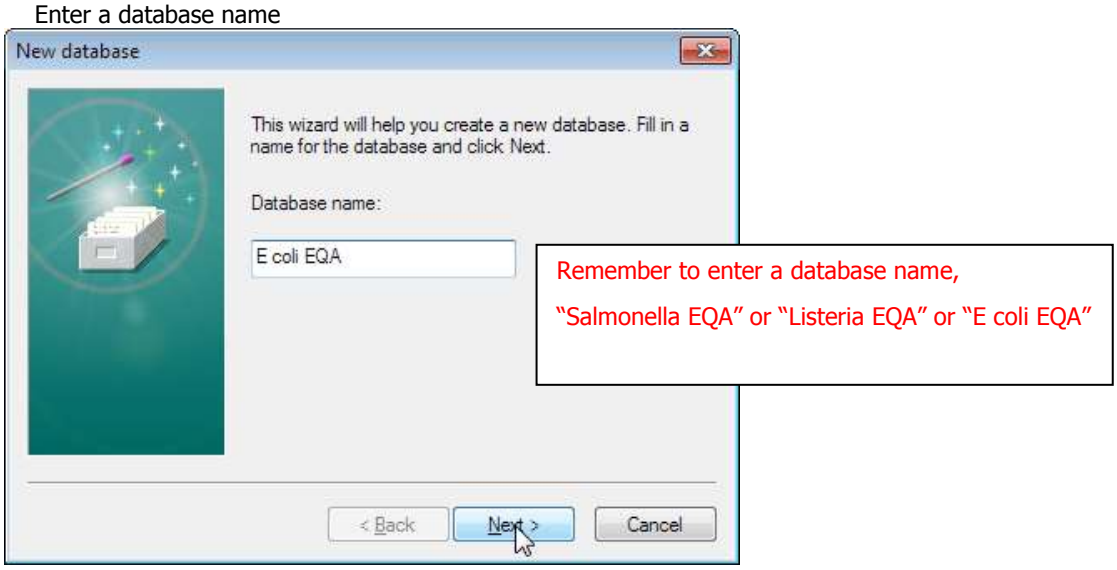
Set up the database by first creating an empty database. Then make an import of an XML file containing experiment settings and field definitions.

Set up the empty database

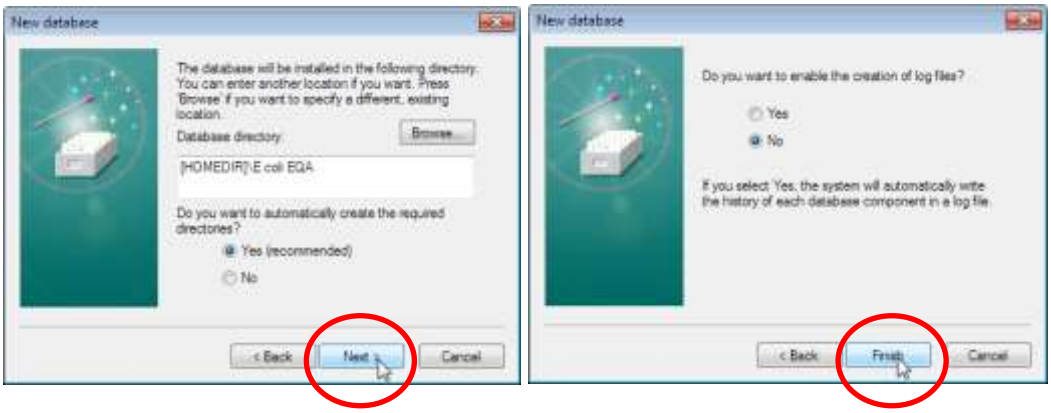
1. Choose to "Create a new database"



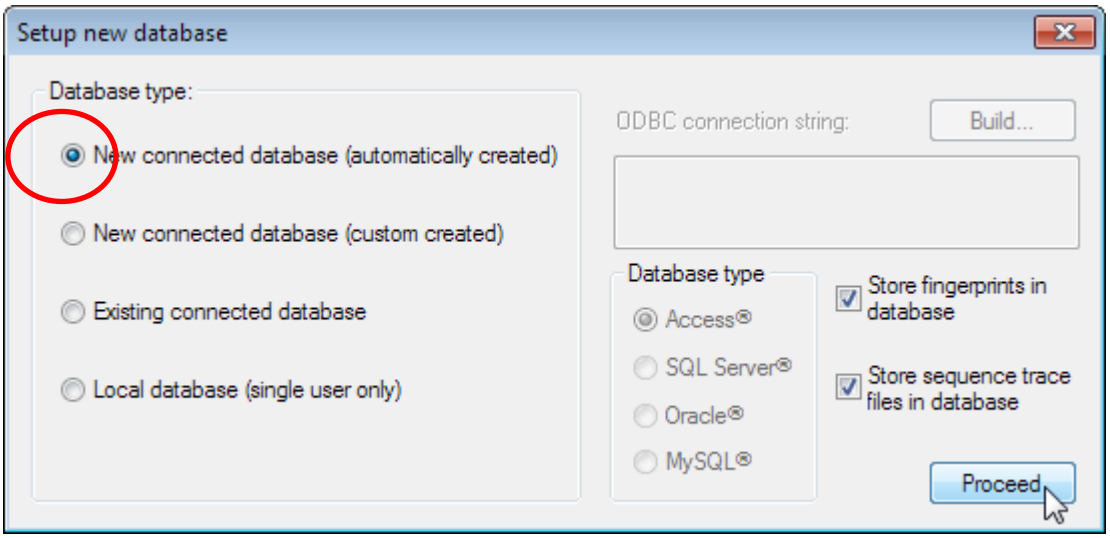
Enter a database name



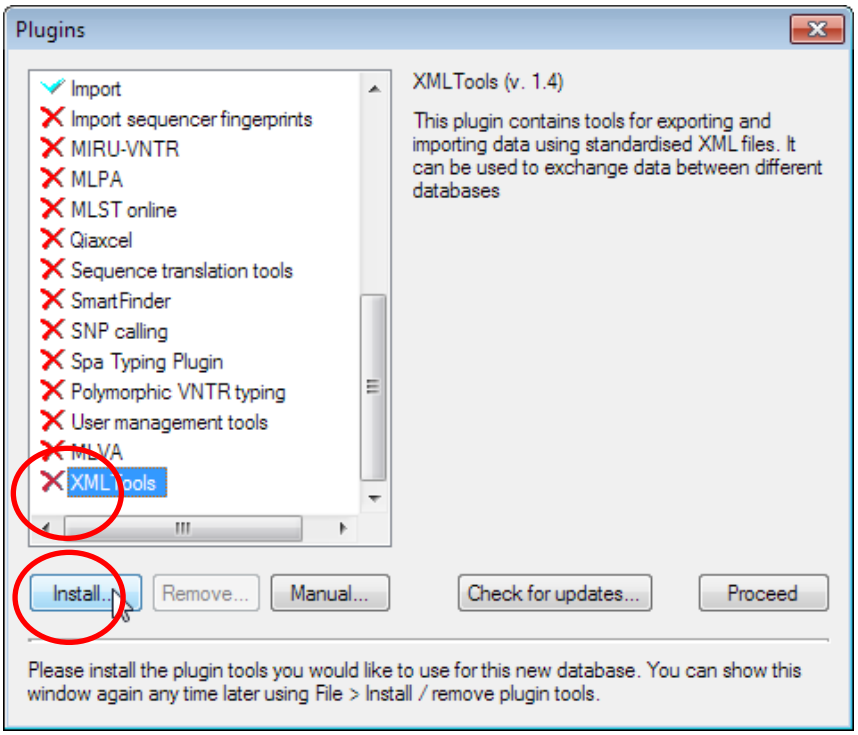
2. Use default values



3. Choose a new connected "Access" type database



4. When choosing plugins, add the "XML Tools" plugin by selecting the plugin in the list and press "Install…"

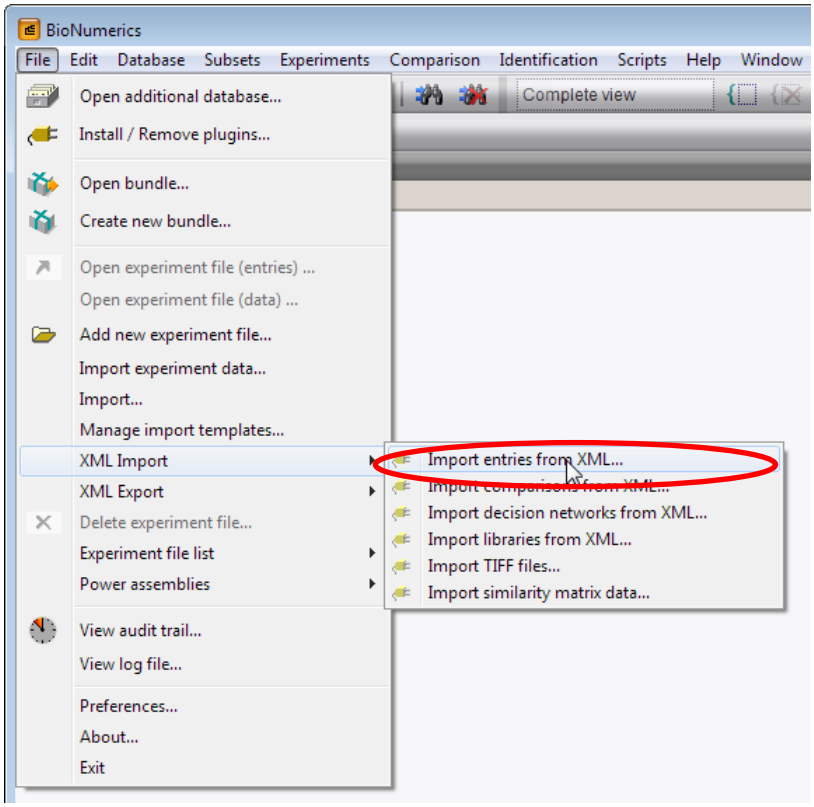


5. Proceed to the next window. The database is now set up and ready to import the database definitions.

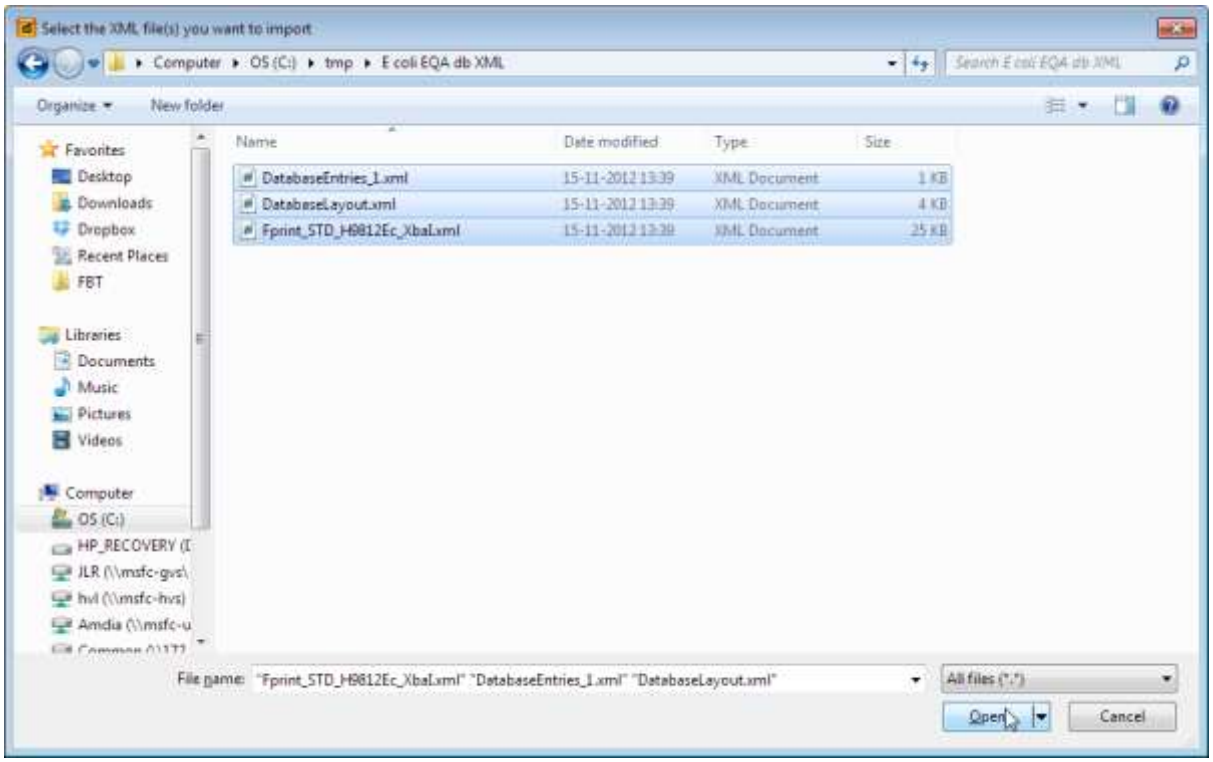
Importing the XML structure

6. Unzip the contents of the supplied file "Listeria EQA db XML.zip", "Salmonella EQA db XML.zip" or "Ecoli EQA db XML.zip" into the folder where you would like to place the files.

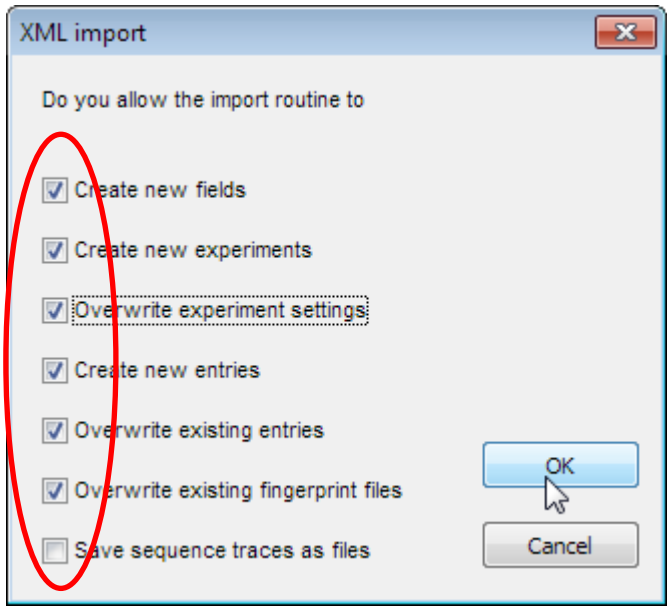
7. Select the menu item "Import entries from XML".



8. Locate your newly unzipped files. Select all of them and click "Open".



9. Mark the box "Overwrite experiment settings" and click "OK".

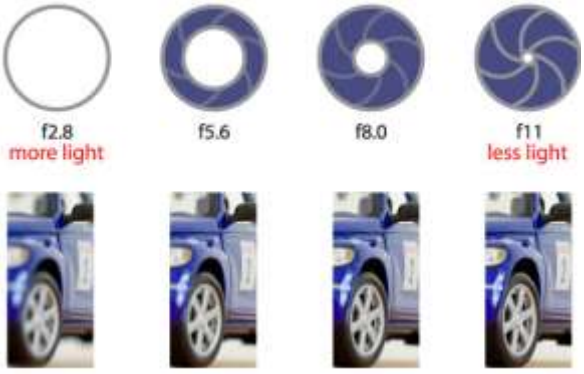


10. Restart the database.

Annex 16. Guide to Image Acquisition

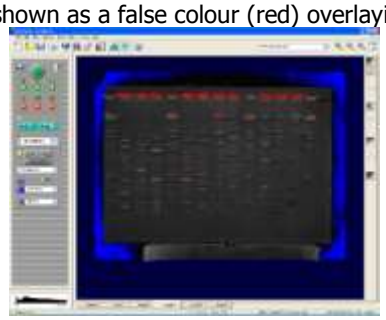
The following SOP is written in general terms since various laboratories use different equipment. Use your image acquisition software as per the manufacturers' instruction.

1. Ensure the agarose gel is adequately stained and destained.
2. Carefully remove the gel from the appropriate container with gloved hands or gel scoop; drain excess liquid from gel and place it in your imaging equipment.
3. Turn on the white light and using the computer monitor to visualise the gel, centre the gel on screen with the wells parallel to the top of the screen so that they are still visible.
4. To obtain high-quality gel profiles it is vital to minimise the possibility of blur/fuzziness:
    a. Adjust the aperture (f-stop) of your camera (either directly on the camera or through the software) so that you never use a wide open aperture (very low f-stop).
    b. A wide open aperture gives you soft/blurry images with focusing problems in the corners of the image.



    c. If your instrument's wide-open aperture (minimum f-stop) is: f:1.8, close the aperture by increasing the value (stopping down) to at least f:4.

5. Zoom in or out until the image completely fills the imaging window,making sure that the wells are included on the top of the screen.
6. Using a flat ruler or grid, focus the image until it is sharp.
    – If necessary, once the image is in focus make minor adjustments by zooming in or out to ensure that the image size is appropriate. Minor adjustments to the image size should not change the focus.
7. Turn off the white light, and turn on the UV light. If you have the option use a weaker UV intensity. This might be called 'Analytical' (weak) or 'Preparative'.
8. Adjust the exposure time until a satisfactory image is obtained.
    a. This might mean integration of several images or a single exposure, consult your machine's manuals.
    b. Bands on every lane should be visible without excessive brightness.
    c. NOTE: Optimise the exposure time by using the 'saturation view' of the image, this is usually shown as a false colour (red) overlaying the image.



    d. Adjusting the exposure time of the camera so that the strongest sample band (DNA) is just below the point of saturation (no red showing).
    e. Saturation in the gel wells may be present and is acceptable. If the image is not visible, increase the exposure times or check the aperture on the camera (top ring).

9. Adjust the aperture to the appropriate level of brightness by opening it up to the maximum setting. If the image is still not visible, the gel may have to be restained.

10. Once the desired image has been captured, turn off the UV light to avoid quenching the DNA in the gel.

11. Save the captured image, **as a TIFF file in its original size**. **Do not resize or change dpi of the image.**

12. If you have images in 12 bit (NB these might appear as 16-bit images) format you can find some guidelines in the next appendix.

- Let the gel fill the whole image.
- Capture images at your instrument's highest resolution.
- Be careful to focus your camera properly.
- 'Stop down' your aperture slightly.
- Expose so that the strongest sample band is just below saturation.
- Do not resize or change the dpi of the image.
- Do not perform any post processing of the image, either in the image capture software, or with any external image editing tools, such as Photoshop, etc.

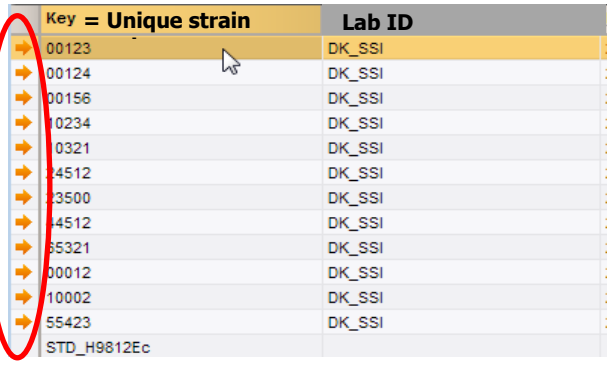
Annex 17. Guide to exporting XML data from BN

After analysing your data, export all your results in XML format. The procedure looks slightly different in BioNumerics version 6 (A) and 7 (B).

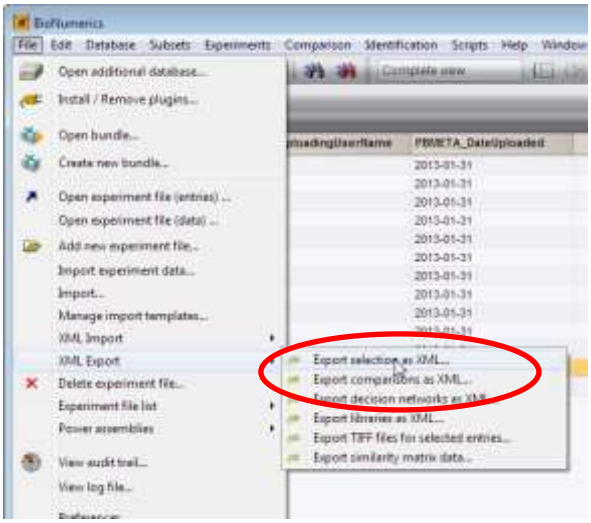
A) BioNumerics version 6

In BioNumerics version 6 and earlier, you need to export TIFF files separately from the analysed data. Follow all the steps in the guide below.

1. Select all isolates that you would like to export.

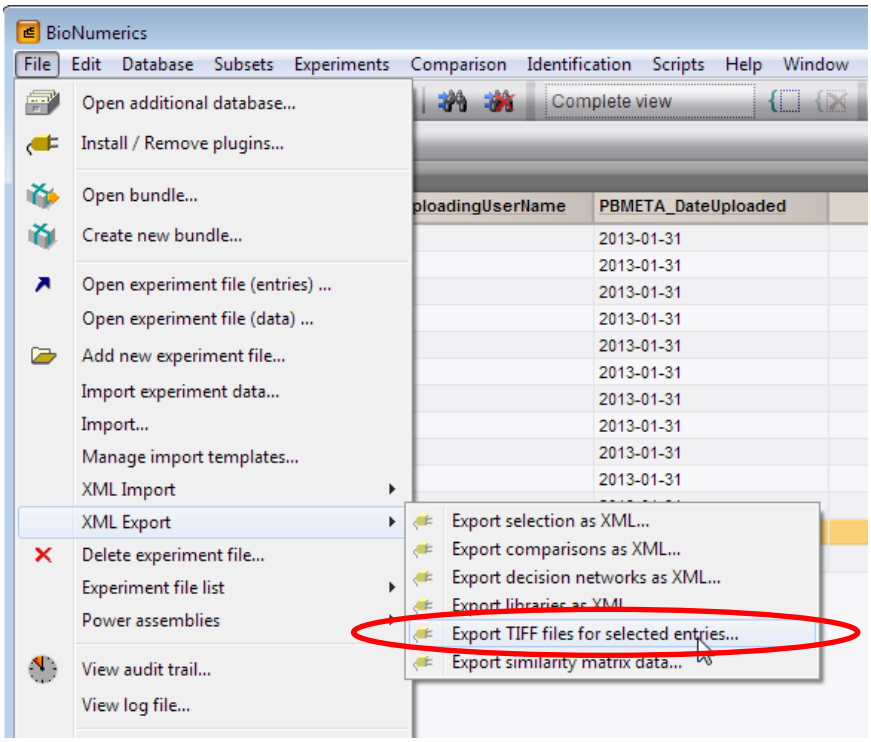


2. Export selection as "XML".

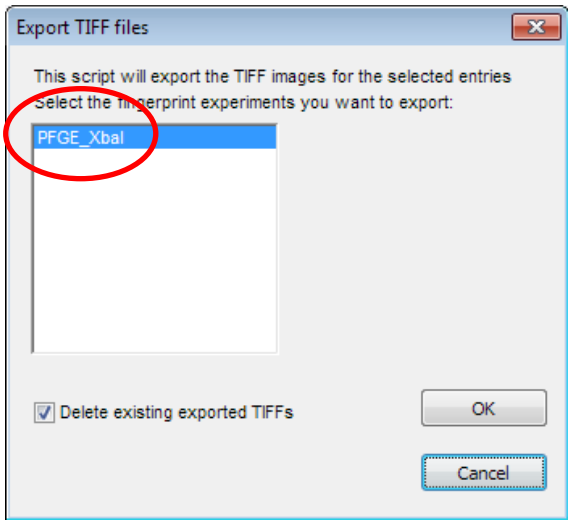


3. De-select the check box "Only export selected fingerprint lanes" and make sure all experiments and all fields are marked.

4. Now export the TIFF file(s).

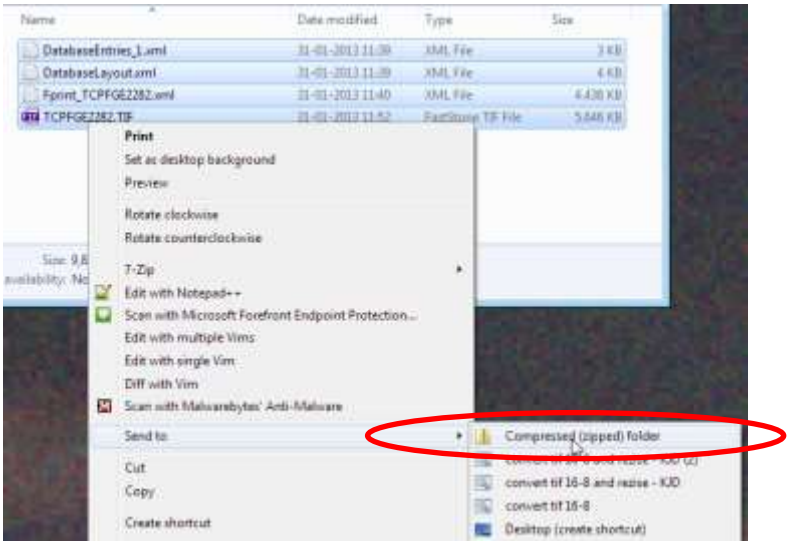


5. Select which experiments to export; in the case of *Listeria* you can export both enzymes at the same time.



6. Now locate the EXPORT directory in your database directory. Remember to check that the TIFF file is included.

7. Please compress the files into a zip archive. One way of creating the zip archive is to mark all the XML and TIFF files, right click on them and choose "Send to → Compressed (zipped) folder".



8. Submit all XML and TIFF files to the EQA provider at https://sikkerftp.ssi.dk

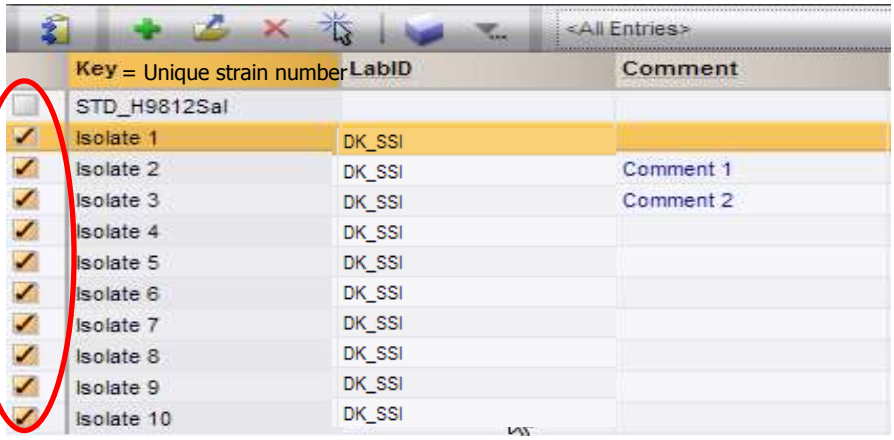
- Username: EQAParticipant
- Password: Kun4Upload
- Open the folder 2015-16
- Open the folder VTECEQA
- Choose "Add files"
- Locate your file
- Click "Start"

Remember to entitle the files with your Lab ID and "EQA-7" for easy recognition e.g. "DK_SSI_EQA-7".

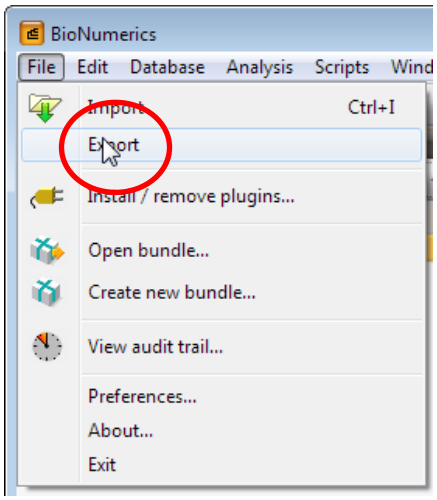
B) BioNumerics version 7

In BioNumerics 7 all data is exported in a single step.

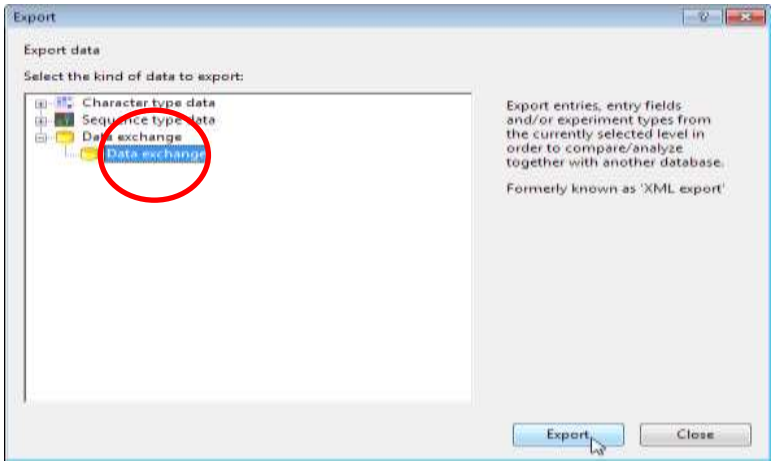
1. Select all isolates that you would like to export.



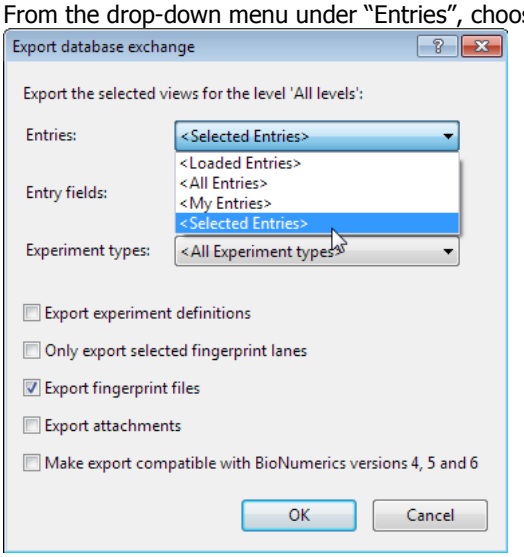
2. Click "File" → "Export", choose "Data exchange"



3. and click "Export".

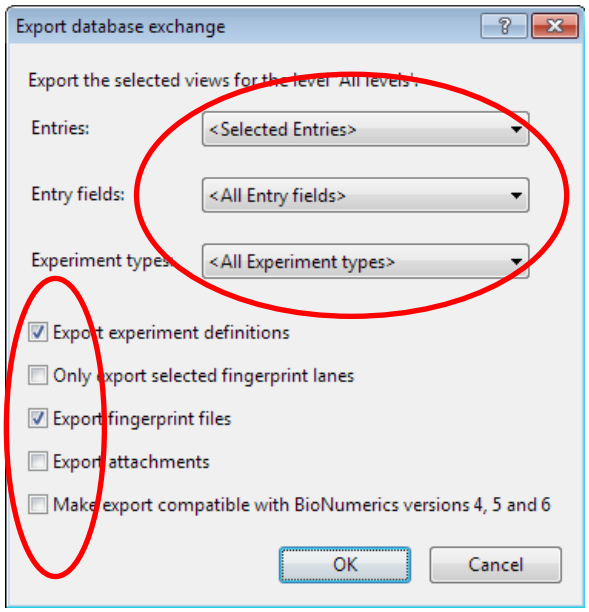


4. From the drop-down menu under "Entries", choose "<Selected Entries>".



5. From the drop-down menu under "Entry fields", select "<All Entry Fields>".
6. From the drop-down menu under "Experiment types", select "<All experiment types>".

7. In the checkboxes tick, both "Export experiment definitions" and "Export fingerprint files".



8. Now locate the EXPORT directory in your database directory.
9. The export described will yield a file called "export.zip" that contains all data.
10. Rename the file with your Lab_ID and "EQA-7" for easy recognition (e.g. DK_SSI_EQA-7).
11. Submit the file to the EQA provider at https://sikkerftp.ssi.dk

- Username: EQAParticipant
- Password: Kun4Upload
- Open the folder 2015-16
- Open the folder VTECEQA
- Choose "Add files"
- Locate your file
  Click "Start

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