TECHNICAL REPORT

External quality assurance scheme for typing of verocytotoxin-producing *E.coli* (VTEC)

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External quality assurance scheme for typing of verocytotoxin-producing *E. coli* (VTEC)

as part of the European Food- and Waterborne Diseases and Zoonoses Programme

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Abbreviations

DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EIA	Enzyme immunoassay
EPEC	Enteropathogenic *Escherichia coli*
EQA	External quality assurance
EU	European Union
FWD-Net	Food- and Waterborne Diseases Network
GFN	WHO Global Foodborne Infections Network
HUS	Haemolytic uremic syndrome
NSF	Non-sorbitol-fermenters
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
QMS	Quality management systems
SF	Self-funded
SF	Sorbitol-fermenters
SSI	Statens Serum Institut, Denmark
VCA	Vero cell assay
VTEC	Verocytotoxin-producing *Escherichia coli*
WHO	World Health Organization

Executive summary

Main findings

- Forty-five laboratories from 38 countries participated in the second international external quality assurance scheme by ECDC on typing of VTEC. Twenty-six of the laboratories were from 23 EU/EEA countries and nineteen laboratories were from 15 non-EU countries.
- An average of 78% of the EU/EEA countries were able to correctly O:H serotype the ten VTEC strains that were included in the EQA. Correct phenotypic characterisation was generally very high – over 92% – in the EU/EEA laboratories.
- About 86% of the non-EU/self-funded (SF) laboratories were able to correctly O:H serotype the ten VTEC strains. Correct phenotypic characterisation was higher than 85% in the non-EU/SF laboratories.
- Accurate gene detection was very high except for subtypes *vtx1d* and *vtx2f*. Accurate detection of *vtx2c* was at 100% for EU/EEA laboratories. In non-EU/SF laboratories, correct detection of *vtx2c* was 95%. Improvements need to be made in some laboratories regarding the methodology used for specific subtypes, preparation of template DNA to avoid cross-contamination and choice of primers for the detection of the *eae* gene. The fact that only 9/17 (53%) laboratories detected verocytotoxin production in the O157:H7 strain underlines the importance of genotypic detection.

This report presents the results of the second round of the external quality assurance (EQA) scheme for typing of verocytotoxin-producing *Escherichia coli* (VTEC) funded by the European Centre for Disease Prevention and Control (ECDC). The EQA was conducted between December 2009 and June 2010 and included O:H serotyping, detection and typing of *eae*, *vtx1*, *vtx2* and *ehxA* genes, phenotypic detection of verocytotoxin/shiga toxin-production, fermentation of sorbitol and production of β-glucuronidase and enterohaemolysin for VTEC.

In February 2010, the laboratories of the Food- and Waterborne Diseases Network (FWD-Net) were contacted and asked which parts of the scheme they wanted to participate in (serotyping, virulence typing or both). In March 2010, the WHO Global Foodborne Infections Network (GFN) kindly offered to translate the invitation to participate in the EQA into Chinese, French, Spanish, Portuguese and Russian, and circulated the invitation through the GFN network in April and May of 2010.

Forty-five laboratories from 38 countries participated, of which 23 were from the EU/EEA countries: Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Denmark, England, Finland, France (two laboratories), Germany (three laboratories), Greece, Hungary, Ireland, Italy, Lithuania, Luxembourg, the Netherlands, Norway, Poland, Romania, Slovenia, Spain and Sweden. Only one EU/EEA laboratory per country was funded by the ECDC EQA programme. In Denmark, nine regional hospitals also participated in this round of EQA and in addition to the ten VTEC strains the Danish EQA programme also included typing of five non-VTEC strains. Malta, Portugal and Scotland accepted the invitation to participate but did not meet the extended deadline of 31 August 2010 for submission of results.

Eighteen self-funded (SF) laboratories from 14 non-EU countries participated: Argentina, Australia, Bangladesh, Brazil, Canada (three laboratories), India, Japan, Mexico, New Zealand, Philippines, South Africa, Switzerland, USA (three laboratories) and Vietnam. In addition, one EU candidate country (Turkey) was funded by ECDC.

The participating laboratories could choose whether to participate in full typing or only a selection of the methods and/or strains. Participation for full O:H serotyping among the EU/EEA countries was 57–70%, which was slightly lower than participation by the non-EU/SF laboratories. Of these, an average of 78% of EU/EEA laboratories could correctly O:H serotype all the strains. The more common a serotype is, the higher the quality of typing was, ranging from 100% correct typing of O157:H7 to 38% of O41:H26 among the EU/EEA countries and 71% of O128ac:[H2] and O177:[H25] among the non-EU/SF laboratories.

Participation for EU/EEA countries in phenotypic detection was 57% for β-glucuronidase, 39% for haemolysin, 35% for verocytotoxin while fermentation of sorbitol was 83%. The percentages of results that were correct in EU/EEA countries were 92% for β-glucuronidase, 98% for haemolysin and 96% for verocytotoxin while fermentation of sorbitol was 97%. Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by 21/23 (91%) of the EU/EEA participants with 95% correct detection, followed by the detection of the *ehxA* gene which was detected 99% correctly by 87% of the EU/EEA participants.

Surprisingly, only 5/8 (63%) of the EU/EEA laboratories were able to phenotypically detect verocytotoxin in the O157:H7 strain (JJ10) which encodes subtype *vtx2c* – a subtype known to be weakly expressed due to a weak

upstream promoter. None of the EU/EEA laboratories, however, failed to detect the gene for *vtx2*. Detection of the gene of *vtx2f* in strain CC3 was correctly made by 15/21 (71%) of the EU/EEA laboratories. Detection of *vtx1d* was correct in 20/21 (95%) EU/EEA laboratories. False positive *vtx1* genes were reported once by one EU/EEA laboratory. One EU/EEA laboratory detected three false positive *vtx2* strains. The *eae* gene was detected correctly by all EU/EEA laboratories except for one negative.

Participation in full O:H serotyping among the non-EU/SF laboratories was 64–82%. An average of 86% non-EU/SF laboratories could correctly O:H serotype all the strains. Participation of non-EU/SF countries in phenotypic detection was 55% for β-glucuronidase, 50% for haemolysin, and 41% for verocytotoxin while fermentation of sorbitol was 77%. The percentages of results that were correct in non-EU/SF countries were 87% for β-glucuronidase, 85% for haemolysin, and 87% for verocytotoxin while fermentation of sorbitol was 96%. Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by 20/22 (91%) of the non-EU/SF participants with results of 92% correct detection or above, followed by the detection of the *ehxA* gene which was detected 98% correctly by 73% of the non-EU/SF participants.

Only 4/9 (44%) of the non-EU/SF laboratories were able to phenotypically detect verocytotoxin in the O157:H7 strain (JJ10) which encodes subtype *vtx2c* . Only one of the non-EU/SF laboratories, however, failed to detect the gene for *vtx2*. Detection of the gene of *vtx2f* in strain CC3 was correctly made by 7/20 (35%) of non-EU/SF laboratories. Detection of *vtx1d* was correct in 13/20 (65%) non-EU/SF laboratories. False positive *vtx1* genes were reported twice by one non-EU/SF laboratory. One non-EU/SF laboratory in two strains and another laboratory in one strain detected three false positive *vtx2* strains. The non-EU/SF laboratories reported four false positive *eae* genes, one failed to detect one *eae* gene and one failed to detect four *eae* genes.

1 Background

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to operate the dedicated surveillance networks and to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Within its mission, ECDC shall 'foster the development of sufficient capacity within the Community for the 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.' (Article 5.3, EC 851/2004[1]).

External quality assurance (EQA) is part of quality management systems (QMS) and evaluates performance of laboratories, by an outside agency on material that is supplied especially for the purpose. ECDC's disease specific networks organise a series of EQA schemes for EU/EEA countries. In some specific networks non-EU/EEA countries are also involved in the EQA activities organised by ECDC. The aim of EQA is to identify needs for improvement in laboratory diagnostic capacities relevant to surveillance of the diseases listed in Decision No 2119/98/EC[2] and to ensure comparability of results in laboratories from all EU/EEA countries. The main purposes of EQA schemes include:

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

For many years the European Commission funded Enter-net, an international surveillance network for national reference laboratories and surveillance centres on selected human gastrointestinal infections. Custodianship of Enter-net migrated to ECDC in October of 2007. In 2008, a framework contract for external quality assurance for *Salmonella* and verocytotoxin-producing *E. coli* (VTEC) was put in place for the years 2008–2011.

The contract for the VTEC EQA was awarded to the laboratory of the International *Escherichia* and *Klebsiella* Centre (WHO) – hereafter referred to as 'The Centre' – at Statens Serum Institut (SSI) in Denmark. This laboratory now arranges annual EQA rounds for the national reference laboratories in the EU and EEA countries on serotyping and virulence typing for VTEC.

[1] Regulation (EC) no 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control. OJ L 142, 30.4.2004, p. 1–11.

[2] Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. OJ L 268, 3.10.1998, p. 1–7.

2 Introduction

The International *Escherichia* and *Klebsiella* Centre at the Statens Serum Institute (SSI) in Denmark has played a leading role in establishing a worldwide international network of quality evaluation and assurance for the typing of *E. coli* since 2002. The first two ring trials with serotyping were organised in 2002 and 2003. In 2005, the third ring trial was launched, including serotyping, virulence typing and typing by pulse field gel electrophoresis (PFGE) of *E. coli*. The PFGE part of the ring trial was co-ordinated with PulseNet Europe[3] as part of the 'PulseNet Europe Feasibility Study' of VTEC. The fourth international ring trial in 2006 was, apart from the regular O:H serotyping, centred on the capacity to detect the *vtx* genes. In 2007, the fifth ring trial EQA scheme for sero- and virulence-typing of VTEC was arranged and in 2008, the Centre was awarded the tender for EQA for VTEC organised by ECDC.

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 verocytotoxin VT1 (*vtx1*) and VT2 (*vtx2*), and the *eae* gene associated with the attaching and effacing lesion of enterocytes also seen in enteropathogenic *E. coli* (EPEC). The combination of some of these toxin determinants is clinically relevant in that some subtypes of VT2 (VT2a in *eae*-positive VTEC and two activatable VT2d variants in *eae*-negative VTEC) seem to be highly associated with the serious sequelae haemolytic uremic syndrome (HUS) [1,2], but VT2c-positive VTEC can also cause HUS [2,3]. Other specific subtypes or variants of VT1 and VT2 are primarily associated with milder course of disease [2,3], and VT2e-positive VTEC strains are probably not pathogenic to humans [4]. Our understanding of the epidemiology of the VT subtypes is therefore important for reducing the risk of VTEC infection and for the surveillance of VTEC. Finally, some of the existing subtyping methods 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, typing of *vtx2* has been based on the absence of the *Pst*I site as an indicator of the presence of the mucus-activatable *stx2d* subtype [1,5–7]. However, the *Pst*I site is also absent in six variants of *vtx2a*, in two variants of *vtx2c*, in *stx2f* and in all four variants of subtype *stx2g*.

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 one highly virulent variant of O157 which will ferment sorbitol (SF). NSF O157 is most often also characterised by failure to produce β-glucuronidase. Approximately 75% of all VTEC also produce enterohaemolysin, a toxin which can cause lysis of erythrocytes. Enterohaemolysin may either be detected phenotypically on sheep blood agar plates or by detection of the gene *ehxA* encoding enterohaemolysin.

Proficiency testing therefore included O:H serotyping, detection and typing of *eae*, *vtx1*, *vtx2* and *ehxA* genes, phenotypic detection of Verocytotoxin/Shiga toxin-production through vero cell assay (VCA) or enzyme immunoassay (EIA), fermentation of sorbitol, and production of β-glucuronidase and enterohaemolysin.

[3] PulseNet Europe is the molecular surveillance network for food-borne infections in Europe. http://www.pulsenetinternational.org/networks/Pages/europe.aspx

3 Materials and methods

3.1 Organisation

The second round of the VTEC EQA funded by ECDC was arranged in December 2009 and included O:H serotyping, detection and typing of *eae*, *vtx1*, *vtx2* and *ehxA* genes, phenotypic detection of verocytotoxin/shiga toxin-production, fermentation of sorbitol and production of β-glucuronidase and enterohaemolysin for VTEC. Invitations were sent to the VTEC laboratory experts in the FWD network in February 2010. In March and April 2010, the invitation to participate was further circulated through the WHO Global Foodborne Infections Network (GFN), translated into Chinese, French, Spanish, Portuguese and Russian. Nine Danish regional hospitals also participated in this round. In total, 45 laboratories participated, of which 23 were in EU/EEA countries and one was a candidate country whose participation was also funded by ECDC (Annex 2). By the end of May 2010, the strains had been sent to the laboratories, which were required to return their results by 18 June. Each laboratory received their individual results in September 2010. Due to a delay in shipping the strains, the overall results could only be presented in brief at the second annual meeting of the European Food- and Waterborne Diseases and Zoonoses Network in Dublin, 22–23 June 2010.

3.2 Selection of strains

Strains were selected for the EQA programme based on three criteria: a) they should represent the most commonly reported strains, b) they should remain stable during the preliminary testing period at the organising laboratory and c) they should be easy to type. Nine of the ten strains included in the programme were carefully chosen from the top thirty sero-and virulence types of 5 160 strains reported to the dedicated surveillance network Enter-net in the years 2000–2006 (see Annex 3). One of these strains, O157:H7 only encoding *vtx2c,* was chosen because this subtype of verocytotoxin is often poorly expressed leading to failure in phenotypic detection. Another strain, O128:[H2] encoding *vtx2f,* was chosen to represent this particular virulence profile in order to test the participants' capacity to detect *vtx2f*. The tenth strain encoding *vtx1d* was added to the panel because *vtx1d* is rarely isolated from humans and inclusion of this strain could give an indication of whether this is due to a technical detection problem.

The characteristics of the 10 VTEC strains included are listed in Table 1.

3.3 Carriage of strains

By the end of May 2010, all strains were packed and mailed as 'dangerous goods' to all but one EU (Malta) and one non-EU/SF (USA) participant.

Some of the parcels were delayed in delivery due to holding at customs and courier-related problems, and therefore not all the involved laboratories were able to meet the deadline (18 June 2010) for returning their results. Later, strains were mailed as 'diagnostic specimens' (UN3373) with a door-to-door courier to the participants. All parcels were received in good time to meet the extended dead line of 31 August.

3.4 Testing

The EQA tests included O:H serotyping, detection and typing of *eae*, *vtx1*, *vtx2* and *ehxA* genes, phenotypic detection of verocytotoxin/shiga toxin-production, fermentation of sorbitol and production of β-glucuronidase and enterohaemolysin for VTEC. Subtyping of *vtx* genes was also encouraged on a voluntary basis but suffers from the lack of universal typing procedures and commonly accepted nomenclature.

Participants were requested to test for additional virulence genes at their own convenience and capacities. This voluntary and additional testing is not a core part of the EQA programme but is meant as a source for sharing information on the capacities found within the network of participating laboratories. It provides additional information on the test strains, which may be valuable if and when laboratories wish to set up new tests.

3.5 Data analysis

When the results from each individual laboratory were received by the Centre, they were immediately entered into the EQA database and scores provided to the relevant laboratory.

Table 1. Characteristics of the 10 VTEC strains used in the second VTEC EQA 2009–2010 [†]

Ranking[a]	Strain No.	Serotype	Sorbitol fermentation	β-glucuronidase activity	Haemolysin production	VCA	*eae*	*vtx1*	*vtx2*	*ehxA*	Subtyping of *vtx* genes [b]	Other virulence genes	Comments
19	AA1	O174:H 8	pos.	pos.	neg.	pos.	neg.	pos.	pos.	neg.	*vtx1c + vtx2b*		
23	BB2	O55:H 7	neg.	pos.	neg.	pos.	pos.	pos.	neg.	neg.	*vtx1a*	*astA*	
9[c]	CC3	O128ac:[H2][d]	pos.	pos.	neg.	pos.	pos.	neg.	neg.	pos.	*vtx2f*	*bfpA, astA*	
16	DD4	O177:[H25][d]	neg.	pos.	Ent.	pos.	pos.	neg.	pos.	neg.	*vtx2c + vtx2d*		Lactose neg.
7	EE5	O111:[H8][d]	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	*vtx1a + vtx2a*		
12	FF6	O113:H4	pos.	pos.	Ent.	pos.	neg.	pos.	pos.	neg.	*vtx1c + vtx2b*	*astA*	
2	GG7	O103:H2	pos.	pos.	Ent.	pos.	pos.	pos.	neg.	neg.	*vtx1a*		
3	HH8	O26:H11	pos.	pos.	neg.	pos.	pos.	pos.	neg.	neg.	*vtx1a*		
NR	ii9	O41:H26	pos.	pos.	neg.	pos.	neg.	pos.	neg.	neg.	*vtx1d*		
1 [e]	JJ10	O157:H 7	neg.	neg.	Ent.	(pos.)	pos.	neg.	pos.	neg.	*vtx2c*	*astA*	

[†] All results from WHO Collaborating Centre for Reference and Research on Escherichia *and* Klebsiella *according to standard of accreditation DS/EN ISO/IEC 17025 described in DANAK accreditation No. 397. ALL strains were negative for the following genes: EAF, saa, ipaH, aatA (CVD432), LT, STh and STp.*

[a] Sero-and virulence types reported to Enter-net in 2000–2006 are ranked by prevalence (highest to lowest) (see Annex 3).

[b] Results were obtained using a new subtyping PCR protocol developed by the WHO Collaborating Centre for Reference and Research on Escherichia *and* Klebsiella *for the subtyping of the vtx1 and vtx2 genes not included in the accredited analyses (In prep.).*

[c] A strain of O128:[H2] encoding vtx2f was chosen to represent this virulence profile in order to test participants' capacity to detect vtx2f.

[d] Non-motile strains where H types were confirmed by fliC PCR/RFLP by five participating laboratories and not by the organising laboratory at the Centre. Submission of NM as a result was also considered correct.

[e] A strain of O157:H7 only encoding vtx2c was chosen because this subtype of verocytotoxin is often poorly expressed leading to failure in phenotypic detection.

Genes detected

astA: pSS126. Enteroaggregative heat stable toxin (EAST1).

bfpA: MSD207. Bundle forming pilus gene probe.

eae: CVD434. *E. coli* attaching and effacing gene probe.

ehxA: CVD419. Plasmid encoded O157-enterohaemolysin.

vtx1: NTP705. Verotoxin 1; Almost identical with the Shiga toxin.

vtx2 : DEP28. Verotoxin 2; Variants exist. Approx. 60% homology to VT1.

Genes not detected[†]

[†] all strains were negative for the following genes:

aatA: Plasmid marker (formerly the CVD432) encoding a dispersin translocator in Enteroaggregative *E. coli.*

EAF: JPN16. EPEC Adherence Factor gene probe.

ipaH: WR390. Invasion plasmid antigen. These genes are found in several copies chromosomally as well as on plasmids.

LT (*eltB*): G119. Heat labile enterotoxin. Almost identical to cholera toxin.

saa: STEC Auto agglutinating adhesion; PCR.

ST_h (STIb; *estAh*): DAS100. Heat stable enterotoxin (human variant).

ST_p (STIa; *estA*): DAS101. Heat stable enterotoxin (porcine variant).

Table 2. Summary of participation rates* for each test– EU/EEA laboratories funded by ECDC and non-EU/SF laboratories

	Serotyping			Phenotypic detection				Genotypic detection				
	O:H Serotype	O group	H type	Sorbitol fermentation	β-glucuronidase activity	Haemolysin production	VCA/EIA	*eae*	*vtx1*	*vtx2*	*ehxA*	Subtyping of *vtx*
EU/EAA participants	13–16/23 57–70%	15–19/23 (19 for O26, O103 & O157) 65-83%	13-16/23 (16 for H7 and H11) 57-70%	19/23 83%	13/23 57%	9/23 39%	8/23 35%	21/23 91%	21/23 91%	21/23 91%	21/23 91%	3–11/23 13–48%
Non-EU/self-funded (SF) laboratories	14–18/22 64–82%	19–21/22 (21 for O26, O103, O111 and 22 for O157) 86–95%	14-18/22 (17 for H7) 64-82%	17/22 77%	12/22 55%	11/22 50%	9/22 41%	20/22 91%	20/22 91%	20/22 91%	16/22 73%	4–10/22 18–45%

* *Number of laboratories participating per method.*

4 Results

The overall results for both the EU/EEA and the non-EU/SF laboratories are listed in Table 2. The specific results for the EU/EEA laboratories are presented in Table 4 (Annex 1) and for the non-EU/SF laboratories in Table 5 (Annex 1). Subtyping results for *vtx* are listed in Table 6 (Annex 1) for both EU/EEA and non-EU/SF laboratories and results for additional virulence genes presented in Table 7 (Annex 1).

4.1 Serotyping

Participation of EU/EEA laboratories in O:H serotyping was slightly lower than the non-EU/SF laboratories (13–16 laboratories (57–70%) and 14–18 laboratories (64–82%), respectively).

An average of 78% (average 11.4; median 11) of EU/EEA laboratories could correctly O:H serotype the ten strains, compared with 86% (average 12.9; median 12.5) of non-EU/SF laboratories.

Correct O:H serotyping ranged from 100% correct typing of O157:H7 for all participating laboratories to 38% of O41:H26 among the EU/EEA countries and 71% of O128ac:[H2] and O177:[H25] among the non-EU/SF laboratories. Among the EU/EEA participants, O157:H7 was correctly serotyped by all laboratories, followed by serotypes O55:H7 (94%), O103:H2 (93%), O26:H11 (88%), O128:H2 (79%), O111:[H8] (79%) and O113:H4 (79%) (Annex 1, Table 4). Lower scores were obtained for O174:H8 (71%) followed by O177:[H25] (57%) and O41:H26 (38%). The latter (strain ii9) was included because of the presence of *vtx1d*, see Annex 1, Table 6. Thus, the less common a serotype is, the more difficult it was for the participants to serotype the strain correctly.

4.2 Phenotypic detection

Participation of EU/EEA and non-EU/SF countries, respectively, in phenotypic detection was 57% and 55% for β-glucuronidase, 39% and 50% for haemolysin, and 35% and 41% for verocytotoxin while fermentation of sorbitol was 83% and 77%. Of the results for EU/EEA and non-EU/SF countries, respectively, the percentage correct were 92% and 87% for β-glucuronidase, 98% and 85% for haemolysin, and 96% and 87% for verocytotoxin, while fermentation of sorbitol was 97% and 96%. Five out of eight (63%) EU/EEA and three out of nine (33%) non-EU/SF laboratories were able to phenotypically detect verocytotoxin in the O157:H7 strain (JJ10) which encodes subtype *vtx2c*. However, only one laboratory (non-EU/SF) failed to detect the gene for *vtx2*.

4.3 Genotypic detection

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

There was a similar level of participation in gene detection of virulence genes *eae*, *vtx1* and *vtx2* for EU/EEA laboratories as non-EU/SF laboratories, being performed by 21/23 (91%) of the EU/EEA and 20/22 (91%) of the non-EU/SF participants, while genotypic detection of *ehxA* was performed by a higher percentage (87% or 20/23) of the EU/EEA laboratories than the non-EU/SF laboratories (73% or 16/22). All four genes were detected at a very high score of 95% to 99.5% for EU/EEA laboratories and 92% to 98% of the non-EU/SF participants (Annex 1, Tables 4 and 5). Failure to detect the gene of *vtx2f* in strain CC3 occurred in 6/21 (29%) of EU/EEA and in 13/20 (65%) of non-EU/SF laboratories. Detection of *vtx1d* only failed in 1/21 (5%) EU/EEA laboratories compared to 7/20 (35%) non-EU/SF laboratories.

The *eae* gene was detected correctly by all EU/EEA laboratories except for one that failed to detect *eae* in strain number EE5 (serotype O111:[H8]). Among the non-EU/SF laboratories, two false positive *eae* genes were reported by one laboratory in strains AA1 (serotype O174:H8) and FF6 (serotype O113:H4) and two laboratories reported a single false positive *eae* detection: one in strain AA1 (serotype O174:H8) and one in ii9 (serotype O41:H26). One laboratory failed to detect the *eae* gene in DD4 (serotype O177:[H25]) and one laboratory failed detection of the *eae* gene in four strains: CC3 (serotype O128ac:[H2]), DD4 (serotype O177:[H25]), GG7 (serotype O103:H2) and HH8 (serotype O26:H11).

One EU/EEA laboratory detected one false positive *vtx1* gene in strain CC3 (serotype O128) and one laboratory detected three false positive *vtx2* strains: BB2 (serotype O55:H7), GG7 (serotype O103:H2) and HH8 (serotype O26:H11). One non-EU/SF laboratory reported two false positive *vtx1* genes in strains CC3 (serotype O128) and DD4 (serotype O177:[H25]) and another laboratory in strain in ii9 (serotype O41:H26).

4.3.2 Subtyping of *vtx1* and *vtx2*

The number of laboratories participating in subtyping of *vtx* genes was generally low, ranging from 3 to 11 (13–48%) among EU/EEA laboratories and from 4 to 10 (32–45%) among non-EU/SF laboratories. Correct

nomenclature and subtyping of both *vtx1* and *vtx2* genes ranged from 2/6 to 3/3 corresponding to 30–100% in EU/EEA laboratories and from 3/9 to 7/7 corresponding to 33–100% for the non-EU/SF laboratories. Ten out of 11 EU/EEA laboratories correctly typed *vtx2f* and 4/5 typed *vtx1d* correctly. The numbers of non-EU/SF laboratories correctly typing *vtx2f* were 7/7 and for *vtx1d*, 3/4. Subtyping results for *vtx* genes are presented in Annex 1, Table 6.

4.3.3 Detection of other virulence genes

Results for additional virulence genes are presented in Annex 1, Table 7. Subtyping of the *eae* gene was obtained for the seven *eae*-positive strains by 2–4 laboratories with similar results. Two to five LEE (Locus for enterocytes effacement) encoded genes were detected in these *eae*-positive strains, and the *tccP* gene was found by one laboratory in all these except for strain HH8 (O26:H11). One to three non-LEE encoded genes (*nle*) were detected in six strains. Long polar fimbrial genes (*lpf*) using a non-comparable nomenclature were detected in nine of the strains by four laboratories. Of the four laboratories examining for *iha*, only three strains were found positive by all four laboratories, while three strains were found positive three times, one strain twice and one strain once.

One laboratory found all ten strains negative for *bfpA* and another all ten strains negative for *bfpB*. One laboratory found six strains positive for *bfpA* including strain CC3 (serotype O128ac:[H2]), which was found positive by the Centre in two independent tests. Three to seven laboratories found strains BB2 (O55:H7), CC3 (O128ac:[H2]), FF6 (O113:H4) and JJ10 (O157:H7) positive for *astA*, *efa1* (or the similar *toxB*) was found by 3–4 laboratories in strains BB2 (O55:H7), EE5 (O111:[H8]), GG7 (O103:H2), HH8 and JJ10 (O157:H7).

5 Discussion

The participation rate varied substantially between the different tests included in the EQA, being highest for the genotypic detection of *eae* and the *vtx* genes and lowest for the subtyping of *vtx*.

The serotyping results ranged from 100% correct typing of O157:H7 for all laboratories to 38% correct for O41:H26 among the EU/EEA countries, and 71% for O128ac:[H2] and O177:[H25] among the non-EU/SF laboratories. The general trend corresponded to how commonly the serotypes have been detected in humans in recent years, as the more common a serotype is, the higher the performance was (Table 3). Among the EU/EEA participants, O157:H7 was correctly serotyped by all laboratories followed by serotypes O55:H7 (94%), O103:H2 (93%), O26:H11 (88%), O128:H2 (79%), O111:[H8] (79%) and O113:H4 (79%). Except for O103:H2 and O113:H4, these serotypes may also be EPEC serotypes, which have traditionally been looked for by many laboratories. Furthermore, commercial kits focus on these particular serotypes. Lower scores were obtained for the two strains with more recent O groups that lie outside the traditional panel with O174:H8 (71% correctly serotyped) followed by O177:[H25] (57%) and O41:H26 (38%), the latter included because of the presence of *vtx1d*.

Participation in phenotypic detection was relatively low. The highest participation was for sorbitol fermentation with high average scores for both EU/EEA and non-EU/SF laboratories. The sorbitol fermentation test is important for identifying the highly virulent sorbitol-fermenting O157:H7 clone. Surprisingly, only 5/8 (63%) EU/EEA and 3/9 (33%) of the non-EU/SF laboratories were able to phenotypically detect verocytotoxin in the O157:H7 strain (JJ10) which encodes subtype *vtx2c* – a subtype which has been shown to be weakly expressed due to a weak upstream promoter. However, only one laboratory (non-EU/SF) failed to detect the gene for *vtx2*..

Based on the results of *vtx* subtyping, it is recommended that six EU/EEA and 13 non-EU/SF laboratories review their genetic detection methodology for *vtx2f* and that one EU/EEA and seven non-EU/SF laboratories review their detection for *vtx1d*.

Two EU/EEA and four non-EU/SF laboratories should review their sample preparation procedures of template DNA for PCR, bearing in mind that false positive contamination is best minimised when DNA template preparation is done separately from running the PCR reaction. One non-EU/SF laboratory should review their choice of primers for detection of the *eae* gene.

Table 3. Serotyping scores by the EU/EEA and non-EU/SF participants in relation to the ranking of the serotype in Europe*. Table sorted in descending order of scores for EU/EEA laboratories.

Ranking	Strain No.	Serotype	Percentage of results correct for O:H serotyping by the EU/EEA laboratories n = 14–17	Percentage of results correct for O:H serotyping by the self-funded laboratories n = 14–18	Prevalence of reported O:H serotype in the EU/EEA
1	JJ10	O157:H7	100%	100%	28%
23	BB2	O55:H7	94%	100%	0.3%
2	GG7	O103:H2	93%	93%	11%
3	HH8	O26:H11	88%	89%	9%
9	CC3	O128ac:[H2]	79%	71%	2%
7	EE5	O111:[H8]	79%	79%	2%
12	FF6	O113:H4	79%	86%	1%
19	AA1	O174:H8	71%	93%	0.4%
16	DD4	O177:[H25]	57%	71%	1%
NR	ii9	O41:H26	38%	79%	<1%
	Average score		78%	86%	Cumulated prevalence 57%

** Ranking based on the number of O:H serotyped cases reported to Enter-net in 2000–2006 (see also Annex 3, Table 8).*
n: Number of laboratories participating per method.
NR: not ranked in the Enter-net database.

Comments on discrepancies and validity of *vtx* subtyping and other virulence genes

The nomenclature of the subtypes and variants of the *vtx* genes varies – see footnote b in Table 6 (Annex 1) for how interpretation was implemented for this EQA. The previously proposed nomenclature is currently being finalised. An update on the revised proposal for the revision of the *vtx* nomenclature was presented on 30 October 2009 at the fourth annual workshop of the National Reference Laboratories for *E. coli* in the EU, organised by the

Community Reference Laboratory for *E. coli*, Department of Veterinary Public Health and Food Safety, Unit of Food-borne Zoonoses and Veterinary Epidemiology, Istituto Superiore di Sanità in Rome. In relation to this work, the WHOCC has developed a subtyping PCR protocol for subtyping *vtx1* and *vtx2* genes. The protocol has now been blind tested on a reference collection of 63 VTEC strains in seven (two German, one Belgian, one Italian, two US and one Danish) independent laboratories with similar results, and will be included in the next VTEC EQA round for 2010–11.

The results for detection of *bfpA* seem to indicate that both positive and negative reporting from two laboratories may not be trusted. One laboratory had 5/6 false positives and one laboratory failed to detect the one *bfpA*-positive strain. Reporting on *astA* also varied from three to seven laboratories for the four *astA*-positive strains indicating that this gene may be underreported.

Standardisation of both the nomenclature and methodology of *bfpA*, *lpf*, *efa1*/*lifA* and *toxB* genes needs further validation in order to be properly included in the EQA programme.

6 Conclusions

In conclusion, an average of 78% of the EU/EEA laboratories correctly O:H serotyped the ten VTEC strains representing 57% of the total number of reported serotypes to Enter-net in the period 2000–2006. Phenotypic performance was generally very high – higher than 92% – in the EU/EEA laboratories. Performance in gene detection was very high except for subtypes *vtx1d* and *vtx2f*.

Six EU/EEA laboratories should review their genetic detection methodology for *vtx2f* and one laboratory should review their detection for *vtx1d*. Two EU/EEA laboratories should reduce their false-positive PCR results by looking into the possible reasons for this, including procedures for sample preparation of template DNA for PCR in order to minimise the risk of cross-contamination

An average of 86% of the non-EU/SF laboratories correctly O:H serotyped the ten VTEC strains. Phenotypic performance was higher than 85% in the non-EU/SF laboratories. Performance in gene detection was very high except for subtypes *vtx1d* and *vtx2f*.

Thirteen non-EU/SF laboratories should review their genetic detection methodology for *vtx2f* and seven laboratories should review their detection for *vtx1d*. Four non-EU/SF should reduce their false-positive PCR results by looking into the possible reasons for this including procedures for sample preparation of template DNA for PCR in order to minimise the risk of cross-contamination. Finally, one non-EU/SF laboratory should review their choice of primers for detection of the *eae* gene.

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Annex 1. Results of the participants by strain

Table 4. Participants' scores – EU/EEA participants (percentage). The percentages represent the number of results in accordance with the intended result

Strain No.	O:H Serotype n = 13-16	O group n=15-19 (n=19 for O26, O103 & O157)	H type n=13-16 (n=16 for H7 & H11)	Sorbitol fermentation n=19	β-glucuronidase activity n=13	Haemolysin production n=9	VCA/ EIA n=8	*eae* n=21	*vtx1* n=21	*vtx2* n=21	*ehxA* n=20	Subtyping of *vtx*; Correct/tests
AA1	71	73	75	100	100	100	100	100	95	100	100	4/7 (57%)
BB2	94	94	88	84	100	100	100	100	100	95	100	3/3 (100%)
CC3	79	88	80	100	100	100	100	100	95	71	100	10/11 (91%)
DD4	57	63	87	95	69	89	100	100	100	100	95	3/8 (38%)
EE5	79	100	73	100	69	100	100	95	100	95	100	2/6 (33%)
FF6	79	88	80	100	100	100	100	100	95	100	90	4/8 (50%)
GG7	93	100	87	100	92	89	100	100	100	95	100	3/3 (100%)
HH8	88	100	82	100	92	100	100	100	100	95	100	3/3 (100%)
ii9	38	44	57	95	100	100	100	100	91	100	100	4/5 (80%)
JJ10	100	100	94	100	100	100	63	100	100	100	100	5/7 (71%)
Average score	78	85	80	97	92	98	96	99.5	98	95	99	41/61 (67%)

n: Number of laboratories participating per method

Table 5. Participants' scores – non-EU/SF laboratories (percentage). The percentages represent the number of results in accordance with the intended result

Strain No.	Serotype n = 14-18	O group n=19-22 (n=21 for O26, O103, O111 and 22 for O157	H type n=14-18 (n=17 for H7)	Sorbitol fermentation n=17	β-glucuronidase activity n=12	Haemolysin production n=11	VCA/ EIA n=9	*eae* n=20	*vtx1* n=20	*vtx2* n=20	*ehxA* n=16	Subtyping of *vtx*; correct/tests
AA1	93	74	93	100	100	100	89	90	95	95	100	3/10 (30%)
BB2	100	95	100	88	100	91	100	100	100	100	94	5/5 (100%)
CC3	71	75	86	94	100	91	56	90	90	35	94	7/7 (100%)
DD4	71	63	86	100	75	36	89	90	95	100	100	3/9 (33%)
EE5	79	95	79	94	58	91	100	100	100	100	100	4/7 (57%)
FF6	86	80	86	94	83	91	100	95	90	95	100	3/9 33%)
GG7	93	90	93	94	75	73	100	95	100	100	100	4/4 (100%)
HH8	89	100	89	100	83	91	100	95	100	100	100	4/4 (100%)
ii9	79	63	86	94	100	91	89	95	65	95	94	3/4 (75%)
JJ10	100	100	100	100	92	91	33	95	95	95	94	4/8 (50%)
Average score	86	84	90	96	87	85	86	95	93	92	98	40/67 (60%)

n: Number of laboratories participating per method

Table 6. Results of *vtx1* and *vtx2* subtyping – EU/EEA and non-EU/SF laboratories. Numbers in brackets correspond to the number of laboratories reporting this result

Strain No.	Subtype by the WHOCC [a]	EU/EEA participants' results [b]		Non-EU/SF participants' results	Number of laboratories getting the correct *vtx* subtypes/ number of laboratories testing	
		Subtype	Variant	Subtype	EU/EEA	Non-EU/SF
AA1	*vtx1c* + *vtx2b*	*vtx1c* + *vtx2b* (4) *vtx2b* (2) *vtx2d* (1)		*vtx1c* + *vtx2b* (3) *vtx2d* (3) *vtx2cd*-neg; *vtx2e*-neg (1) *vtx2e*-absent (1) *vtx1* + *vtx2* (1) *vtx1* (1)	4/7	3/10
BB2	*vtx1a*	*vtx1a* (3)		*vtx1a* (5)	3/3	5/5
CC3	*vtx2f*	*vtx2f* (10) *vtx2g* (1)		*vtx2f*	10/11	7/7
DD4	*vtx2c* + *vtx2d*	*vtx2c* + *vtx2d* (2) *vtx2a* (2) *vtx2d* (1) *vtx2*-NV206 (1) *vtx2c* (1)	*vtx2c* + *vtx2d*-O174-EC1720a (1)	*vtx2c* + *vtx2d* (3) *vtx2a* + *vtx2c* (2) *vtx2A* + *vtx2B* (1) *vtx2c* (1) *vtx1* + *vtx2* (1) *vtx2a* (1)	3/8	3/9
EE5	*vtx1a* + *vtx2a*	*vtx1a* + *vtx2a* (2) *vtx1a* (1) *vtx2a* (2)	*vtx2a*-O157-SF-258-98 (1)	*vtx1a* + *vtx2a* (4) *vtx2cd*-pos; *vtx2e*-neg (1) *vtx2A* + *vtx2B* (1) *vtx2* untypable (1)	3/6	4/7
FF6	*vtx1c* + *vtx2b*	*vtx1c* + *vtx2b* (4) *vtx2a* (1) *vtx2b* (2) *vtx2d* (1)		*vtx1c* + *vtx2b* (3) *vtx2d* (2) *vtx1* + *vtx2* (1) *vtx2cd*-neg; *vtx2e*-neg (1) *vtx1A* + B subunit (1) *vtx2e*-absent (1)	4/8	3/9
GG7	*vtx1a*	*vtx1a*		*vtx1a*	3/3	4/4
HH8	*vtx1a*	*vtx1a*		*vtx1a*	3/3	4/4
ii9	*vtx1d*	*vtx1d* (4) *vtx1a* (1)		*vtx1d* (3) *vtx1a* (1)	4/5	¾
JJ10	*vtx2c*	*vtx2c* (5) *vtx2a* (1) *vtx2b* (1)		*vtx2c* (5) *vtx2a* (1) *vtx2a* + *vtx2c* (1) *vtx2A* + *vtx2B* O157 (1)	5/7	5/8

[a] *Results for* vtx1 *were by a triplex PCR (unpublished) with specific primers for* vtx1a, vtx1c *and* vtx1d, *and for* vtx2 *by a prototype PCR (unpublished) with specific primers for the seven subtypes:* vtx2a, vtx2b, vtx2c, vtx2d, vtx2e, vtx2f *and* vtx2g.

[b] *The submitted results for subtyping of* vtx *genes were interpreted as follows:* stx *as* vtx*;* vtx1 *as* vtx1a*;* vtx2 *as* vtx2a*;* vtx2d-*Ount and* vtx2-*O118 as* vtx2b, vtx2v-*ha as* vtx2c *and* vtx2-*NV206 as* vtx2d.

Table 7. Results of additional virulence genes by all participating laboratories

Strain No	Antigen related genes	Virulence genes (n)
A1	*fliC*H8	*eaaA* *espI* *lpfA*, *lpfA2-1* *iss* *iha* (3)
BB2	*fliC*H7	*astA* (3) *bfpA* [a] *eae* γ (3), *eae* γ1 *efa1* (4) *espA*_O157H11 *espF* *espJ* *etpD* (4) *iha* *iss* *lpf*O157/OI-154, *lpf*O157/OI-141, *lpf*O141, *lpfA*1-3, l*pfA*2-2 *nleA* *nleB* *nleC* *pagC* *tccP* *tir*_O157H7
CC3		*astA* (7) *bfpA* (2) [b] *cba* *cdt* *cif* *cma* *eae* β (3), *eae* β1 *espB* *espA*_O103H2, *espF*_O103H2 *iss* *lpfA1-2*, *lpfA*O26nleA *nleC* *tccP* *tir*_O103H2
DD4		*cif* *eae* β (3), *eae* β1 *espA*_O103H2, *espF*_O103H2 *espB* *espJ* *espI* *espP* (3) *iha* (4) *katP* (5) *lpfA2-1*, *lpfA* *nleA* *nleB* *nleC* *terE* *tccP* *tir*_O103H2
EE5	*rfb*O111 *fliC*H8	*bfpA* [a] *cba* *celb* *cif* *eae* θ (2) *efa1* (4) *espA* *espF* *espJ* *iha* (3) *lpfA*, *lpfA1-2*, *lpfA2-1*, *lpfA*O26 *nleA* *nleB* *nleC* *pagC* *tccP* *tir* *terE* *wbdL*
FF6	*rfb*O113	*astA* (6) *cba* *celb* *cma* *eaaA* *espI* *iha* (4) *saa* *senB*

Strain No	Antigen related genes	Virulence genes (n)
GG7	*wzx*O103	*bfpA* [a] *cba* *cma* *cif* *eae* ε (3) *efa1* (4) *espA*_O49H12 *espB* *espF*_O103H2 *espJ* *etpD* (4) *katP* (5) *lpfA1*-2, *lpfA*O26 *nleA* *nleB* *pagC* *tccP* *tir*_O103H2
HH8	*wbuA*O26	*bfpA* [a] *eae* β (3) *eae* β1 *efa1* (3) *iha* (2) *lpfA1-2*, *lpfA2-1*, *lpfA*O26 *terE*
ii9		*cba* *cma* *mchB* *mchC* *mchF* *lpfA1-5*, *lpf*O141, *lpf*O157/OI-154, *lpf*O157/OI-141
JJ10	*rfb*O157 (2) *fliC*H7	*astA* (2) *bfpA* *eae* γ (3), *eae* γ1 *efa1* (2), *efa1-1* *espA*_O157H11 *espF* *espJ* *espP* (3) *etpD* (5) *iha* (4) *katP* (5) *lpf*O157/OI-154, *lpf*O157/OI-141, *lpfA1-3*, *lpfA2-2*, *lpf*O141 *nleA* *nleB* *nleC* *pagC* *rfbE* *tccP* *terE* *tir*_O157H7 *toxB* (2) *uidA*

[a] *One laboratory has these six strains positive for* bfpA. *These strains were found negative by one participant and the Centre except for strain CC3.*

[b] *Found negative by two participants for* bfpA *and by one participant for* bfpB.

Genes found by participating laboratories

astA, bfpA, cba, cdt, celb, cif, cma, eae α, *eae* β, *eae* β1, *eae* γ, *eae* γ1, *eae* ε, *eae* θ, *efa1, efa1*-1, *espA, espA*_O103H2, *espF*_O103H2, *espA*_O157H11, *espA*_O49H12, *espB, espF, espF*_O103H2, *espI, espJ, espP, etpD, eaaA, iha, iss, katP, lpfA, lpfA1-2, lpfA2-1, lpfA*O26, *lpfA1-5*, *lpf*O141, *lpf*O157/OI-154, *lpf*O157/OI-141, *lpfA1-3*, *lpfA2-2, mchB, mchC, mchF, nleA, nleB, nleC, pagC, rfbE, senB, saa, tccP, terE, tir, tir*_O103H2, *tir*_O157H7, *toxB, uidA and wbdL .*

Annex 2. List of the participants

ECDC-funded EU/EEA participants

Laboratory	Institution	Country
Bereich Humanmedizin, Institut für Medizinische Mikrobiologie und Hygiene Bereich Humanmedizin	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	Austria
Dept. Microbiology	Universitair Ziekenhuis	Belgium
Nat Ref Lab for Enteric Pathogens	National Centre of Infectious and Parasitic Diseases	Bulgaria
Microbiology Department	Nicosia General Hospital	Cyprus
NRL for *E. coli* and Shigella	National Institute of Public Health	Czech Republic
Foodborne bacteria and typing, Dept. of Microbiological Surveillance and Research	Statens Serum Institut	Denmark
Head ESYV Reference Unit, Laboratory of Enteric Pathogens	Specialist and Reference Microbiology Division, Health Protection agency	England
Unit of Bacteriology	National Institute for Health and Welfare (THL)	Finland
Centre National de Référence des *Escherichia coli* et Shigella	Unité Biodiversité des Bactéries Pathogènes Emergentes, Institut Pasteur	France
NRC Salmonella and other Enterics	Robert Koch Institute, Bereich Wernigerode	Germany
Department of Microbiology	National School of Public Health	Greece
'B. Johan'	National Center for Epidemiology	Hungary
SWAHB, Public Health Laboratory	Cherry Orchard Hospital	Ireland
Dipartimento di Sanità Alimentare e Animale	Istituto Superiore di Sanità	Italy
Microbiology department	National Public Health Surveillance Laboratory	Lithuania
Laboratoire National de Santé	Division Microbiologie	Luxembourg
Diagn. Lab. for Infectious Diseases and Perinatal screening	National Institute of Public Health and the Environment, Centre for Infectious	Netherlands
Dept. of Foodborne Infections, Division of Infectious Disease Control Division of Infectious Disease Control	Norwegian Institute of Public Health	Norway
Department of Bacteriology	National Institute of Hygiene; Narodowy Instytut Zdrowia Publicznego	Poland
Reference Laboratory for Molecular Epidemiology	National Institute of Research-Development for Microbiology and immunology "Cantacuzino"	Romania
Laboratory of Enteropathogenic bacteria, Department of Medical Microbiology	National Institute of Public Health	Slovenia
Sección de Enterobacterias, Servicio de Bacteriología	Carretera de Majadahonda a Pozuelo, Centro Nacional de Microbiologia	Spain
Dept. of Bacteriology	Swedish Institute of Infectious Disease Control	Sweden

ECDC-funded non-EU/EEA country

Laboratory	Institution	Country
National Reference Laboratory for Enteric Pathogens, Communicable Diseases Research Department	Refik Saydam Public Health Agency	Turkey

Self-funded participants

Laboratory	Institution	Country
Servicio Fisiopatogenia	Inei-Anlis 'Dr. Carlos G. Malbrán'	Argentina
Microbiological Diagnostic Unit Public Health Laboratory	Department of Microbiology and Immunology, University of Melbourne	Australia
Enteric and Food Microbiology Laboratory	ICDDR,B	Bangladesh
Secao de Bacteriologia, Laboratorio Central De Saude Publica	Instituto Adolfo Lutz	Brazil
Head Identification and Serotyping, Enteric Disease Program	National Microbiology Laboratory	Canada
E. coli Laboratory, Laboratory for Foodborne Zoonoses	Public Health Agency of Canada	Canada
Reference Laboratory for Escherichia coli, GREMIP, Faculté de médecine vétérinaire	Université de Montréal	Canada
Service de microbiologie Laboratoire associé au CNR *E. coli*-Shigella	Hôpital Robert Debré	France
Abteilung Mikrobiologischer Verbraucherschutz	Institut für Hygiene und Umwelt	Germany
Nationales Referenzlabor für Escherichia coli (NRL-*E. coli*)	Bundesinstitut für Risikobewertung (BfR)	Germany
Department of Bacteriology	National Institute of Cholera and Enteric Diseases (Indian Council of Medical Research)	India
Department of Bacteriology	National Institute of Infectious Diseases	Japan

Public Health Laboratory	Department of Public Health, Faculty of Medicine, National Autonomous University Mexico	Mexico
Enteric Reference Laboratory; ESR NCBID – Wallaceville	Institute for Environmental Science and Research Ltd	New Zealand
Microbiology Department	Research Institute for Tropical Medicine, Department of Health	Philippines
Enteric Diseases Reference Unit (EDRU)	National Institute for Communicable Diseases, NHLS (previously SAIMR)	South Africa
Nationales Zentrum für enteropathogene Bakterien (NENT)	Institut für Lebenmittelsicherheit und -hygiene	Switzerland
E. coli Reference Center	The Pennsylvania State University	USA
National Reference Laboratory for *Escherichia coli* and Shigella, DASH, Unir 7	Centers for Disease Control and Prevention	USA
	U.S. FDA, HFS-711, Washington	USA
	The National Institute For Food Control	Vietnam

Annex 3. Prevalence of sero- and virulence types (Enter-net)

Table 8. Prevalence of sero-and virulence types reported to Enter-net 2000–2006, ranked according to the reported number of human cases and the verocytotoxin combination

Ranking	O group	H type	Number of cases with given virulence profile				Number of cases	Prevalence of virulence profile within the given O:H serotype				Prevalence of O:H serotype in Enter-net database
			vtx1 + vtx2	*vtx1*	*vtx2*	neg. [a]		*vtx1 + vtx2*	*vtx1*	*vtx2*	neg. [a]	
1	157	7	303	11	1144	11	1469	21%	1%	78%	1%	28%
2	103	2	1	584	1	7	593	0%	98%	0%	1%	11%
3	26	11	43	364	56	17	480	9%	76%	12%	4%	9%
4	157	NM	278	14	160	18	470	59%	3%	34%	4%	9%
5	91	NM	46	250	1	0	297	15%	84%	0%	0%	6%
6	145	NM	9	19	124	6	158	6%	12%	78%	4%	3%
7	111	[H8]	48	77	1	1	127	38%	61%	1%	1%	2%
8	91	14	1	120	0	0	121	1%	99%	0%	0%	2%
9	128	[2]	84	11	18	3	116	72%	9%	16%	3%	2%
10	26	NM	17	69	13	8	107	16%	64%	12%	7%	2%
11	146	21	48	21	10	0	79	61%	27%	13%	0%	2%
12	113	4	59	0	13	0	72	82%	0%	18%	0%	1%
13	146	28	8	1	46	0	55	15%	2%	84%	0%	1%
14	76	19	19	33	0	0	52	37%	63%	0%	0%	1%
15	117	7	0	48	0	0	48	0%	100%	0%	0%	1%
16	177	[25]	1	27	11	1	40	3%	68%	28%	3%	1%
17	5	NM	6	30	0	0	36	17%	83%	0%	0%	1%
18	121	19	1	1	21	1	24	4%	4%	88%	4%	0%
19	174	8	16	5	2	0	23	70%	22%	9%	0%	0.4%
20	146	NM	6	5	10	0	21	29%	24%	48%	0%	0%
21	8	19	1	1	15	0	17	6%	6%	88%	0%	0%
22	91	21	5	2	9	0	16	31%	13%	56%	0%	0%
23	55	7	0	6	4	5	15	0%	40%	27%	33%	0.3%
24	156	25	0	12	2	1	15	0%	80%	13%	7%	0%
25	166	28	7	8	0	0	15	47%	53%	0%	0%	0%
26	112	2	3	10	1	0	14	21%	71%	7%	0%	0%
27	115	10	0	14	0	0	14	0%	100%	0%	0%	0%
28	22	8	10	1	2	0	13	77%	8%	15%	0%	0%
29	103	25	0	2	2	9	13	0%	15%	15%	69%	0%
30	103	NM	3	9	0	1	13	23%	69%	0%	8%	0%

Shading denotes the types chosen for this EQA.

[a] *These strains were probably reported on the basis of phenotypic detection and not genotyped, or the method used for genotypic confirmation was inadequate in detecting certain subtypes of the* vtx *genes.*

Annex 4. Individual results for all laboratories

Table 9. Individual results for O:H serotyping, all participants

Lab No.	Strain No.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score (%) (O/H)
Result	**O174:H8**	**O55:H7**	**O128ac:H-**	**O177:H-**	**O111:H-**	**O113:H4**	**O103:H2**	**O26:H11**	**O41:H26**	**O157:H7**	
1	O174:H8	O55:H7	O128ac:H-	O177:H-	O111:H-	O113:H4	O103:H2	O26:H11	O41:NM	O157:H7	100 / 90
2	?:?	?:NM	?:NM	?:NM	O111:NM	?:?	O103:?	O26:?	?:?	O157:H7	40 / 40
3	N.D.:	O55:H7	O127:	O177:ND	O111:ND	O113: ND	O103: ND	O26: ND	N.D.:	O157:H7	70 / 20
4	O103:H6	O55:H7	O128ab:H10	O118:ND	O111:ND	ND:H4	O103:H2	O26:H11	ND:H41	O157:H7	60 / 50
5	O174:H8	O55:H7	O128:NM	O15:NM	O111:H8	O113:H4	O103:H2	O26:H11	O15:H26	O157:H7	80 / 100
6	O174:*fliC*H8a	O55:*fliC*H7	O128:*fliC*H8a	O177:*fliC*H25	O111:*fliC*H2 or H8a	O113:*fliC*H4	O103:*fliC*H2	O26:*fliC*H11	ONT:*fliC*H26	O157:*fliC*H7	90 / 80
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0 / 0
9	ND	O55:ND	ND	ND	O111:ND	ND	ND	O26:ND	ND	O157:ND	40 / 0
10	O174:H8	O55:H7	O128abc:HNM	O177:HNM	O111:HNM	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
11	ND	O55:ND	O128:ND	ND	O111:ND	ND	O103:ND	O26:ND	O115:ND	O157:ND	60 / 0
12	O174:H8	O55:H7	O128:H-	O177:H-	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
13					O111:ND		O103: ND	O26: ND		O157: ND	40 / 0
14	O174:H8	O55:H7	O128:NM	O177:NM	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
15	NT:H8	O55:H7	O128:NM	OR:NM	O111:NM	O113:H4	O103:H2	O26:H11	O41:NT	O157:H7	80 / 90
16	O174:H8	O55:H7	O128:HNM	O177:HNM	O111:HNM	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
17	O174:	O55:H7	O128:	O177:	O111:	O113:	O103:	O26:H11	ONTa:	O157:H7	90 / 30
18	O174:H8	O55:H7	O128:H2 (NM)	NT:H25 (NM)	O111:H8 (NM)	O113:H4	O103:H2	O26:H11	NT:H26	O157:H7	80 / 100
19	NT:ND	O55:ND	NT:ND	NT:ND	O111:ND	NT:ND	O103:ND	O26:ND	NT:ND	O157:ND	50 / 0
20	?:H8	O55:H7	O128:NM 2	O177:NM	O111:NM 8	O113:?	O103:H2	O26:H11	?:?	O157:H7	80 / 80
21											0 / 0
22											0 / 0
23	O174:H8 (*fliC*H8)	O55:H7 (*fliC*H7)	O128:NM (*fliC*H2)	O145:NM (*fliC*H25)	O111:NM (*fliC*H8)	O113:H4 (*fliC*H4)	O103:H2 (*fliC*H2)	O26:H11 (*fliC*H11)		O157:H7 (*fliC*H7)	80 / 90
24											0 / 0
25	O174:H8	O55:H7	O128abc:NM	O177:NM	O111:NM	O113:H4	O103:H2	O26:H11	NT:H26	O157:H7	90 / 100
26	ND	ND	O 128:ND	ND	ND	ND	ND	O26:ND	ND	O157:ND	30 / 0
28	O174:H8	O55:H7	O128:NM	O177:NM	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
29	O174:H8	O55:H7	O128ad:H-	O177:H-	O111ac:H49	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 90
50	O174:H8	O55:H7	O128ac:H-	O177:H-	O111ac:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
51	O174:H8	O55:H7	O128ac:H[2]	O177:H[25]	O111:H[8]	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
52	O174:H8	O55:H7	O128ab:NM	O177:NM	O111:NM	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
53	O174:H8	O55:H7	O128:NM	O177:NM	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
54	O174:H8	O55:H7	O128:NT	O177:NT	NT:H24	O113:H17	O103:H2	O26:H11	O41:H26	O157:H7	90 / 60
55	NT:	O55:H7	O128:ND	O145:ND	O111:ND	O113:ND	O103:ND	O26:H11	O45:ND	O157:H7	70 / 30
56	O174:H8	O55:H7	O128:H-	O177:H-	O111:H-	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
57	O174:H8	O55:H7	Orough:NM	NT:NM	O111:H8	O113:H4	O103:H2	O26:H11	NT:NT	O157:H7	70 / 90
58			No growth:								0 / 0
59	O174:H8	O55:H7	O128:HNM	O177:HNM	O111:HNM	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
60	O174:H8	O55:H7	OX38:H2	Neg:H25	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	80 / 100
61	O174:H8	O55:H7	O128:NM	O177:NM	O111:NM	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
62	O174:H8	O55:H7	O128:NM	O177:NM	O111:H8	O113:H4	O103:H2	O26:H11	O41:H26	O157:H7	100 / 100
63	O174:ND	O55:ND	O128:ND	O177:ND	O111:ND	O113:ND	O103:ND	O26:ND	O41:ND	O157:ND	100 / 0
64	not TOP5:ND	not TOP5:ND	not TOP5:ND	not TOP5:ND	O111:ND	not TOP5:ND	O103:ND	O26:ND	not TOP5:ND	O157:ND	40 / 0
403		O55:H7			O111:ND	O113:ND	O103:ND	O26:H11		O157:H7	60 / 30
404			O145:		O111:ND		O103:ND	O26:ND		O157:H7	40 / 10
406	NT:NT	O55:H7	O128:NT	NT:NT	O111:NT	NT:NT	O8:NT	O26:H11	NT:NT	O157:H7	50 / 30
409	NT:ND	O55:ND	O128:ND	NT:ND	O111:ND	NT:ND	NT:ND	O26:ND	NT:ND	O157:ND	50 / 0

Shading denotes incorrect results.
NM = non motile
ND = not done
NT = Non typeable

Table 10. Individual results for fermentation of sorbitol, all participants

Lab No.	Strain No										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score (%)
Result	**pos.**	**neg.**	**pos.**	**neg.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**neg.**	
1	pos.	neg.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
2	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
4	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
5	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
6	pos.	neg.	neg.	pos.	neg.	neg.	neg.	neg.	neg.	pos.	100
8	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
9	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
10	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
12	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
13	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
14	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
15	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
16	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	90
17	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
18	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
19	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
20	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
22	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
23	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
25	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
26	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.ND	100
28	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
29	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
50	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
51	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
52	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
54	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	90
55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
56	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
57	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
58	pos.	neg.	ND	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
60	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
61	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
62	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
63	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
64	pos.	neg.	pos.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	90
403	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
404	pos.	neg.	pos.	neg.	pos.	neg.	neg.	pos.	pos.	neg.	80
406	pos.	neg.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	100
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Shading denotes incorrect results.

Table 11. Individual results for β-glucuronidase production, all participants

Lab No.	Strain No										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score (%)
Result	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**neg.**	
1	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
2	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
4	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
5	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
8	pos.	pos.	pos.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	80
9	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
10	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
14	pos.	pos.	pos.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	80
15	pos.	pos.	pos.	pos.	neg.	pos.	neg.	pos.	pos.	neg.	80
16	pos.	pos.	pos.	pos.	neg.	pos.	pos.	neg.	pos.	neg.	80
17	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
20	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
23	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
25	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
26	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
28	pos.	pos.	pos.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	80
29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
51	pos.	pos.	pos.	pos.	neg.	pos.	neg.	pos.	pos.	neg.	80
52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
54	pos.	pos.	pos.	neg.	neg.	neg.	neg.	neg.	pos.	neg.	50
55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
56	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	90
57	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
60	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
61	pos.	pos.	pos.	pos.	neg.	neg.	neg.	neg.	pos.	neg.	60
62	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
403	pos.	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	neg.	90
404	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	100
406	pos.	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	neg.	90
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Shading denotes incorrect results.
ND = not done

Table 12. Individual results for verocytotoxin production, all participants

Lab No.	Strain No										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score (%)
Result	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	**pos.**	
1	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
3	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
9	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
10	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
15	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
17	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
21	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
23	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
25	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	80
26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
51	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
52	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	Inter-mediate	100
53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
54	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
57	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	100
58	neg.	pos.	ND	pos.	pos.	pos.	pos.	pos.	pos.	pos.	80
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
62	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	90
63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
64	pos.	pos.	neg.	Inter-mediate	pos.	pos.	pos.	pos.	Inter-mediate	neg.	80
403	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
404	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
406	pos.	pos.	neg.	pos.	pos.	pos.	pos.	pos.	pos.	neg.	80
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Shading denotes incorrect results.

Table 13. Individual results for haemolysin production, all participants

Lab No.	Strain No										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score (%)
Result	**neg.**	**neg.**	**neg.**	**pos.**	**pos.**	**pos.**	**pos.**	**neg.**	**neg.**	**pos.**	
1	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
5	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
9	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
10	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
14	neg.	neg.	neg.	α hly	pos.	pos.	α hly	neg.	neg.	pos.	80
15	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
17	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
18	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
21	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
23	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
24	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
25	neg.	neg.	neg.	α hly	pos.	pos.	pos.	neg.	neg.	pos.	90
26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
28	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
50	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
51	neg.	neg.	neg.	pos.	pos.	pos.	pos.	neg.	neg.	pos.	100
52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
54	neg.	neg.	neg.	α hly	pos.	pos.	α hly	neg.	neg.	pos.	80
55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
56	neg.	neg.	neg.	α hly	pos.	pos.	α hly	neg.	neg.	pos.	80
57	neg.	neg.	neg.	α hly	pos.	pos.	pos.	neg.	neg.	pos.	90
58	neg.	neg.	ND	pos.	pos.	pos.	pos.	neg.	neg.	pos.	90
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
60	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	50
61	neg.	neg.	neg.	α hly	pos.	pos.	pos.	neg.	neg.	pos.	90
62	neg.	pos.	neg.	α hly	pos.	pos.	pos.	pos.	pos.	pos.	60
63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
403	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
404	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
406	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Shading denotes incorrect results.

Table 14. Individual results for gene detection of *eae*, *vtx1* and *vtx2*, all participants

Lab No.	Strain No																																
	AA1			BB2			CC3			DD4			EE5			FF6			GG7			HH8			ii9			JJ10			Score (%)		
	eae	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*
Result	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**-**	**+**	**+**	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**+**	**+**	**+**	**-**	**+**	**+**	**-**	**-**	**+**	**-**	**+**	**-**	**+**			
1	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
2	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
3	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
4	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
5	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
6	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
8	-	+	+	+	+	-	+	-	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**90**	**100**	**100**
9	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
10	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0**	**0**	**0**
12	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
13	-	+	+	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	**100**	**100**	**60**
14	-	+	+	+	+	-	+	-	+	+	-	+	+	-	-	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
15	-	+	+	+	+	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
16	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
17	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
18	-	+	+	+	+	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**90**	**100**
19	-	+	+	+	+	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	+	-	+	ND	ND	ND	**80**	**80**	**70**
20	-	+	+	+	+	-	+	-	-	+	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0**	**0**	**0**
22	-	-	+	+	+	-	+	-	-	+	-	+	+	+	+	-	-	+	+	+	-	+	+	-	-	-	-	+	-	+	**100**	**70**	**90**
23	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
24	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
25	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**80**	**90**	**90**
26	ND	+	-	ND	+	-	ND	-	-	ND	-	+	ND	+	+	ND	+	-	ND	+	-	ND	+	-	ND	+	-	ND	-	+	**0**	**100**	**70**
28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0**	**0**	**0**
29	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
50	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**90**	**90**	**90**
51	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
52	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
53	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
54	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
55	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
56	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
57	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
58	-	-	+	+	+	-	ND	-	+	+	-	+	+	+	+	-	-	+	+	+	-	+	+	-	-	+	-	+	-	+	**90**	**70**	**90**
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	**0**	**0**	**0**
60	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**

Lab No.	Strain No																														
	AA1			BB2			CC3			DD4			EE5			FF6			GG7			HH8			ii9			JJ10			Score (%)		
	eae	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*
61	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
62	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**100**
63	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**100**	**90**	**90**
64	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	-	+	-	+	**100**	**100**	**90**
403	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**100**	**90**	**90**
404	-	+	-	+	+	-	-	+	+	-	+	+	+	+	+	-	-	-	-	+	-	-	+	-	-	+	-	+	-	+	**60**	**70**	**80**
406	-	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**100**	**90**	**90**
409	-	+	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	-	+	-	+	**90**	**90**	**90**

Shading denotes incorrect results.

Table 15. Individual results for gene detection of *ehxA* all participants

Lab No.	Strain No										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score
Result	**-**	**-**	**-**	**+**	**+**	**+**	**+**	**-**	**-**	**+**	
1	-	-	-	+	+	+	+	-	-	+	100
2	-	-	-	+	+	+	+	-	-	+	100
3	-	-	-	+	+	+	+	-	-	+	100
4	-	-	-	+	+	+	+	-	-	+	100
5	-	-	-	+	+	+	+	-	-	+	100
6	-	-	-	+	+	+	+	-	-	+	100
8	-	-	-	+	+	+	+	-	-	+	100
9	-	-	-	+	+	+	+	-	-	+	100
10	-	-	-	+	+	+	+	-	-	+	100
11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
12	-	-	-	+	+	+	+	-	-	+	100
13	-	-	-	+	+	-	+	-	-	+	90
14	-	-	-	+	+	+	+	-	-	+	100
15	-	-	-	+	+	+	+	-	-	+	100
16	-	-	-	-	+	+	+	-	-	+	90
17	-	-	-	+	+	+	+	-	-	+	100
18	-	-	-	+	+	+	+	-	-	+	100
19	-	-	-	+	+	+	+	-	+	ND	80
20	-	-	-	+	+	+	+	-	-	+	100
21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
23	-	-	-	+	+	+	+	-	-	+	100
24	-	-	-	+	+	+	+	-	-	+	100
25	-	-	-	+	+	+	+	-	-	+	100
26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
29	-	-	-	+	+	-	+	-	-	+	90
50	-	+	-	+	+	+	+	-	-	+	90
51	-	-	-	+	+	+	+	-	-	+	100
52	-	-	-	+	+	+	+	-	-	+	100
53	-	-	-	+	+	+	+	-	-	+	100
54	-	-	-	+	+	+	+	-	-	+	100
55	-	-	-	+	+	+	+	-	-	+	100
56	-	-	-	+	+	+	+	-	-	+	100
57	-	-	-	+	+	+	+	-	-	+	100
58	-	-	ND	+	+	+	+	-	-	+	90
59	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
60	-	-	-	+	+	+	+	-	-	+	100
61	-	-	-	+	+	+	+	-	-	+	100
62	-	-	-	+	+	+	+	-	-	+	100
63	-	-	-	+	+	+	+	-	-	+	100
64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
403	-	-	-	+	+	+	+	-	-	+	100
404	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
406	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
409	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Shading denotes incorrect results.