TECHNICAL REPORT

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

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Taina Niskanen (Food- and Waterborne Diseases Programme, ECDC) and produced by Statens Serum Institut, Denmark.

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Contents

Abbreviations v
Executive summary 1

1 Background 3

2 Introduction 4

3 Materials and methods 5
3.1 Organisation 5
3.2 Selection of strains 5
3.3 Carriage of strains 5
3.4 Testing 5
3.5 Data analysis 6

4 Results 8
4.1 Serotyping 12
4.2 Phenotypic detection 12
4.3 Genotypic detection 13
4.3.1 Detection of virulence genes *eae*, *vtx1*, *vtx2* and *ehxA* 13
4.3.2 Subtyping of *vtx1* and *vtx2* 13
4.3.3 Detection of other virulence genes 14

5 Discussion 15

6 Conclusions 17

7 References 18

Annex 1. List of participants 19
Annex 2. Prevalence of sero-and virulence types 21

Tables

Table 1. Number of laboratories according to network affiliation and the part of the joint EQA/PT programme they had accepted to participate in 2
Table 2. Characteristics of the 10 VTEC strains used in the third VTEC EQA 2010-2011† 6
Table 3. Summary of participation rates for each test; laboratories with funding from ECDC, EU NRL and non-EU/SF laboratories 6
Table 4. Serotyping scores by ECDC, EU NRL and non-EU/SF participants in relation to the ranking of the serotype in Europe, based on the number of O:H serotyped cases reported to Enter-net in 2000–2006 and TESSy 2007–2010 and ranked according to the reported number of human cases (Appendix, Table A1) 9
Table 5. Participants' scores. Third international external quality programme for VTEC 2010–11; ECDC contact points, including four EU NRL (veterinary) laboratories (Group 1) 10
Table 6. Participants' scores. Third international external quality programme for VTEC 2010–11; EU NRL (veterinary) laboratories (Group 2) 10
Table 7. Participants' scores. Third international external quality programme for VTEC 2010–11; non-EU/self-funded laboratories (Group 3) 10
Table 8. Results of *vtx1* & *vtx2* subtyping VTEC EQA 2009–2010; ECDC contact points, including four EU NRL (veterinary) laboratories and non-EU/SF laboratories 11
Table 9. Specificity and sensitivity of the subtyping of *vtx1* and *vtx2* genes, VTEC EQA 2009–2010; all laboratories: ECDC contact points including 4 EU NRL (veterinary) laboratories and non-EU/SF laboratories combined 12
Table 10. Results of additional virulence genes as submitted by all participating laboratories 14
Table 11. ECDC-funded participants 19
Table 12. EU NRL participants 19
Table 13. Self-funded participants 20

Table A1. Prevalence of sero-and virulence types reported to Enter-net in 2000–2006 and to TESSy 2007–2010, ranked according to reported number of human cases and combination of the *eae* and verocytotoxin genes 21
Table A2. Individual results for O:H serotyping; all participants 27
Table A3. Individual results for fermentation of sorbitol; all participants 28
Table A4. Individual results for β-glucuronidase production all participants 30
Table A5. Individual results for vero cytotoxin production all participants 31
Results that are not in accordance with the results table are shaded grey. 32
Table A6. Individual results for haemolysin production all participants 32
Table A7. Individual results for gene detection of *vtx1*, *vtx2* and *eae* all participants 34
Table A8. individual results for gene detection of *ehxA* all participants 35
Table A9. Individual results for *vtx* suptyping all participants 37

Abbreviations

EIA	Enzyme immunoassay
FWD-Net	European Food- and Waterborne Diseases and Zoonoses network
HUS	Haemolytic uremic syndrome
ISS	Istituto Superiore di Sanità; EU reference laboratory for VTEC
GFN	Global Foodborne Infections Network, Food Safety, WHO
Non-EU/SF	Non-EU member state funded by ECDC and self-funded participants
NRL	National reference laboratory (veterinary)
PCR	Polymerase chain reaction
PT	Proficiency testing
RFLP	Restriction fragment length polymorphism
SSI	Statens Serum Institut
STEC	Shiga toxin-producing *Escherichia coli*. STEC is synonymous with VTEC
VCA	Vero cell assay
VT1	Verocytotoxin 1
VT2	Verocytotoxin 2
VTEC	Verocytotoxin-producing *Escherichia coli*. VTEC is synonymous with STEC
vtx1	The gene encoding VT1
vtx2	The gene encoding VT2
WHOCC	WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*

Executive summary

- Eighty-one laboratories from 41 countries signed up for ECDC's third international external quality assessment (EQA) scheme for the typing of verocytotoxin-producing *Escherichia coli* (VTEC). Two laboratories failed to submit results. The sixty-two laboratories are located in 30 EU/EEA countries.
- An average of 68% of the ECDC-funded laboratories were able to correctly determine the O:H serotype of the ten VTEC strains that were included in the third VTEC EQA. Ninety-three per cent of the EU NRLs and 79% of the non-EU/self-funded (SF) laboratories correctly serotyped the ten VTEC strains.
- Phenotypic characterisation was generally very high (above 89%) for ECDC-funded laboratories. EU NRLs and non-EU/SF laboratories scored above 78%.
- Gene detection was 96–100% correct, except for the *eae* gene in one strain (i9), which was a false negative (three laboratories), and *vtx2f*, which was not detected by seven laboratories. Improvements need to be made by non-EU/SF laboratories regarding the methodology used for *vtx2f*. Three ECDC-funded laboratories need to reconsider their choice of primers for the detection of the *eae* gene.
- The prototype *vtx* subtyping protocol was tested by 50–95% of the participants but the results indicated significant sensitivity to different PCR cycler equipment probably due to incorrect or non-comparable temperature calibration.

This report presents the results of the 3rd round of the external quality assessment (EQA) scheme for the typing of verocytotoxin-producing *E. coli* (VTEC). The EQA was funded by ECDC and conducted in collaboration with the 6th EU RL proficiency testing (PT) by the Directorate-General for Health and Consumers (DG SANCO). The third VTEC EQA was conducted between November 2010 and February 2011 and included O:H serotyping, detection and typing of *eae*, *vtx1*, *vtx2* and *ehxA* genes, subtyping of the *vtx* genes, phenotypic detection of verocytotoxin/Shiga toxin-production, fermentation of sorbitol, and production of β-glucuronidase and enterohaemolysin for VTEC.

The prototype PCR protocol for *vtx* subtyping is the preliminary result of an international collaboration between seven research and reference laboratories coordinated by the EU reference laboratory for VTEC (EU RL VTEC) at Istituto Superiore di Sanità (ISS), Rome, Italy, and the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* at Statens Serum Institut (SSI), Copenhagen, Denmark.

In November 2011, the laboratories of the European Food- and Waterborne Diseases and Zoonoses network (FWD-Net) were contacted and asked to inform SSI regarding the parts of the scheme they wanted to participate in (serotyping, virulence typing, *vtx* subtyping, or any combination of the three parts). In addition, invitations were circulated to 30 EU-national reference laboratories (EU NRLs), representing 25 EU Member States, Croatia, Norway, Switzerland, and previous EQA participants, including members of the WHO Global Foodborne Infections Network (GFN).

Eighty-one laboratories from 41 countries signed up for the third VTEC EQA, 62 of which were from (applicant) EU or EEA countries: Austria, Belgium (3), Bulgaria, Croatia, Cyprus (2), Czech Republic (2), Denmark (2), England (2), Estonia (2), Finland (2), France (5), Germany (3), Hungary (2), Ireland (2), Italy (5), Latvia (2), Lithuania (2), Luxembourg, Netherlands (2), Norway (2), Poland (2), Portugal (2), Romania, Slovakia (2), Slovenia (2), Spain (4), Sweden (3), Switzerland and Turkey. Twenty-two laboratories were funded by ECDC, 26 were funded by the EU NRL network, and four by both networks. Two of the laboratories (one ECDC-funded laboratory from Latvia and one EU NRL lab from Denmark) accepted the invitation to participate but communicated that they were not able to perform the tests and are not included in the result tables.

Nineteen self-funded (SF) laboratories from 11 non-EU countries participated: Argentina, Australia (3), Bangladesh, Brazil, Canada (6), Japan, Mexico, New Zealand, Philippines, South Africa, and the USA (2).

In order compare the results of the second EQA scheme (2010) with those of the third VTEC EQA, all ECDC-funded laboratories from 23 EU countries, including four EU NRL (veterinary) laboratories (which are both public health laboratories and veterinary national reference laboratories), one applicant and one EEA country are presented together in this report as Group 1. Twenty-two EU NRLs and 32 non-EU/SF laboratories are presented as two additional groups: Group 2 and Group 3 – see Table 1 for a summary.

The combined results for all 29 participating EU NRLs, including four ECDC-funded public health/EU NRL (vet) laboratories and a total of three laboratories in Switzerland, Norway and Croatia, are presented in the 'Report of the 6th interlaboratory study on verocytotoxin-producing *E. coli* (VTEC) identification and typing – 2010–2011'.

The participating laboratories could choose whether to participate in the full scheme or only a selection of the methods and/or strains (Table 1). The participation in full O:H serotyping was highest for the laboratories whose participation in the third VTEC EQA was funded by ECDC (54–82%); participation was lower for EU NRLs (9%) and non-EU/SF laboratories (44–53%). An average of 68% of ECDC-funded laboratories could correctly determine the

O:H serotype of all strains (Table 5). Better results were delivered by EU NRLs (93%) (Table 6) and non-EU/SF laboratories (79%) (Table 7).

Table 1. Number of laboratories according to network affiliation and the part of the joint EQA/PT programme they had accepted to participate in

Network affiliation	Full scheme	O:H and virulence	Virulence and subtyping	*vtx* subtyping	Total	Group	For this report
ECDC and EU NRL (veterinary)*	4				4	**Group 1** ECDC-funded laboratories	25
ECDC contact points in FWD-Net	17[a]	4	1		22		
EU NRL (veterinary)	23[a]				23	**Group 2** funded by DG SANCO	22
Non-EU NRL (veterinary), funded by DG SANCO	3				3	**Group 3** non-EU/self-funded (SF) laboratories	32
Self-funded (human and veterinary; EU and non-EU)	16	3		4	23		
Self-funded, get strains through contact point, EU	3	2	1		6		
Total	**66**	**9**	**2**	**4**	**81**		**79**

[*] *Includes national public health reference laboratories and veterinary NRLs in the EU.*

[a] *After receiving the samples, two laboratories (one ECDC-funded from Latvia, one EU NRL from Denmark) communicated that they were not able to perform the tests. Both laboratories are not included in the result tables.*

The overall results for ECDC-funded laboratories including 4 EU NRLs (veterinary), the EU NRLs (veterinary) and the non-EU/self-funded (SF) laboratories are listed in Table 3. The specific results for the ECDC-funded laboratories, including four EU NRL (veterinary) laboratories, are presented in Table 5; results for the EU NRL (veterinary) are in Table 6, and the non-EU/SF laboratories are covered in Table 7. Subtyping results for *vtx* are listed in Table 8 for both EU/EEA and non-EU/SF laboratories, and specificity and sensitivity of the subtyping of *vtx1* and *vtx2* genes are given in Table 9. The results for additional virulence genes are presented in Table 10.

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 assessment schemes." [1]

External quality assessment (EQA) is an element of quality management systems and involves an external agency evaluating the performance of laboratories, by an outside agency on material supplied specially for the purpose.

ECDC's disease-specific networks organise a series of EQA for EU/EEA countries, with the aim to identify needs for improvement in laboratory diagnostic capacity relevant to the surveillance of diseases listed in Decision No 2119/98/EC[2], and to ensure the reliability and comparability of results from laboratories in all EU/EEA countries. The main objectives of EQA schemes include:

- 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 problem areas;
- provision of continuing education; and
- identification of training needs.

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

The EQA framework contract was awarded to the laboratory of the International *Escherichia* and *Klebsiella* Centre (WHO) at Statens Serum Institut (SSI), Denmark. This laboratory now conducts annual EQA rounds for the national reference laboratories in the EU/EEA on serotyping and virulence typing for VTEC.

The laboratories at ISS and SSI jointly conducted the third ECDC VTEC EQA and combined it with the 6th EU RL proficiency testing (PT) programme. Funds were provided by ECDC and DG SANCO. The joint EQA and PT programme included O:H serotyping and virulence typing of ten VTEC strains, as well as the testing of a jointly developed prototype protocol for subtyping the ten *vtx* subtype genes through conventional gel-based polymerase chain reaction (PCR).

[1] Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control, Article 5.3.

[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.

2 Introduction

The WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* (WHO-CC) at Statens Serum Institut (SSI) in Denmark has played a leading role in establishing an international network for quality evaluation and assessment for the typing of *E. coli* since 2002. The first two ring trials for serotyping were carried out in 2002 and 2003. In 2005, the third ring trial was launched, which included serotyping, virulence typing and typing by pulse field gel electrophoresis (PFGE) of *E. coli*. The PFGE part of the ring trial was coordinated with Pulse Net Europe as part of the 'PulseNet Europe feasibility study' for VTEC (6). 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 verocytotoxin-producing *E. coli* (VTEC) was arranged, and in 2008, the International *Escherichia* and *Klebsiella* Centre won the tender for the ECDC-funded VTEC EQAs.

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 verocytotoxin VT1 (*vtx1*) and VT2 (*vtx2*), and the *eae* gene associated with the attaching and effacing lesion in 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 the activatable VT2d subtype in *eae*-negative VTEC) seem to be highly associated with a serious sequela, namely haemolytic-uremic syndrome (HUS) [2,5,9], but VT2c-positive VTEC can also cause HUS [5,9].

Other specific subtypes or variants of VT1 and VT2 are primarily associated with a milder course of the disease [2,5,9], and VT2e-positive VTEC strains are probably not pathogenic to humans [11]. A better understanding of the epidemiology of the VT subtypes is therefore important for reducing the risk of VTEC infection and the surveillance of VTEC. Finally, some of the existing subtyping methods using a combination of 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 *PstI* site as an indicator of the presence of the mucus-activatable *stx2d* subtype [1,3,7,8]. However, the *PstI* 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 (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, production of β-glucuronidase and enterohaemolysin, and the testing of a jointly developed prototype protocol for subtyping the ten *vtx* subtype genes by conventional gel-based PCR.

The study was conducted jointly with the network of EU reference laboratory for VTEC (EU RL VTEC) at ISS, Rome, Italy. The aim of such a liaison was the harmonisation of typing methods, which would make it possible to compare human and nonhuman data across all current monitoring programmes and databases.

This document features a complete set of results from the laboratories that participated in the study.

The results produced by the network of VTEC NRLs in the veterinary and food safety fields (Regulation (EC) No 882/2004) have been published by ISS under the title 'Report of the 6th inter-laboratory study on verocytotoxin-producing *E. coli* (VTEC) identification and typing – 2010–2011' . The report is available online: http://www.iss.it/binary/vtec/cont/PT6_Report.pdf).

3 Materials and methods

3.1 Organisation

The third ECDC-funded VTEC EQA was arranged in collaboration with the network of EU reference laboratory for VTEC (EU RL VTEC) at ISS, Rome, Italy, in October 2010. It 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, and the testing of a jointly developed prototype protocol for subtyping the ten *vtx* subtype genes by conventional-gel based PCR.

In October–November 2010, invitations were sent to the VTEC contact points in the network for Food- and Waterborne Diseases and Zoonoses (FWD-Net) and EU RL for VTEC (EU National Reference Laboratories – EU NRLs (veterinary)); also invited were previous EQA participants, including members of the WHO Global Foodborne Infections Network (GFN). In total, 79 laboratories participated, 25 of which were ECDC-funded (four of which were also EU NRLs). Twenty-two laboratories from the EU NRL network were funded through DG SANCO, and 32 funded their own participation and took part as 'non-EU/self-funded' (SF) laboratories (Tables 1, 11–13). In the beginning of December 2010, the strains were sent to all laboratories, which later submitted their results online at the EU RL VTEC website; deadline was 25 February 2011. Each laboratory received their individual results upon submission.

3.2 Selection of strains

Strains were selected for the third VTEC EQA programme based on four criteria:

- strains should be commonly reported;
- they should remain stable during the preliminary testing period at the organising laboratory;
- they should easily serotyped; and
- they should represent the three different subtypes of *vtx1* and the seven different subtypes of *vtx2* in positive and stable strains.

Five of the ten strains included in the third EQA were chosen from the top-thirty sero- and virulence types of the 5 462 strains reported to the dedicated surveillance network Enter-net from 2000–2006 and to TESSy from 2007–2010 (Appendix: Table A1). Only strains with complete information on O:H serotype and the three genes *eae*, *vtx1* and *vtx2* were analysed. Five additional strains were chosen because of the relevance of their genotype: four of those were included in the Enter-net/TESSy databases but ranked below the top-thirty sero- and virulence types; one was neither included in the Enter-net nor the TESSy database.

The characteristics of the 10 VTEC strains used in the third EQA are listed in Table 2.

3.3 Carriage of strains

Shipping of the strains took place between 26 November and 10 December 2010. The parcels were shipped from SSI in Copenhagen, labelled as 'UN 3373 Biological Substance, Category B'.

By 19 December, all participating laboratories had received the dispatched strains. One late participant received the strains on 5 January 2011.

On 14 January, user ID, password, laboratory code, and instructions on how to log on to the restricted area of the EU RL VTEC website (http://www.iss.it/vtec) were e-mailed to all participants.

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 encouraged (prototype PCR protocol) [12].

Participants were requested to test for additional virulence genes at their own convenience. Voluntary and additional testing was not a core part of the EQA programme but was seen as indicative of the capacities of the laboratories in the network. It provided additional information on the test strains, which may be valuable if laboratories wish to set up new tests.

3.5 Data analysis

After data submission on the restricted website, results were e-mailed to SSI in an Excel spreadsheet. Results were immediately analysed and scores submitted to all participating laboratories.

Table 2. Characteristics of the 10 VTEC strains used in the third VTEC EQA 2010-2011[†]

Ranking[a]	Strain no.	Serotype	Sorbitol fermentation	β-glucuronidase activity	Haemolysin production	VCA	*eae*	*vtx1*	*vtx2*	Subtyping of *vtx* genes[b]	Other virulence genes
2	1=B2	O103:H2	pos.	pos.	pos.	pos.	pos.	pos.	neg.	*vtx1a*	*ehxA*
11	2=C3	O146:H21	neg.	pos.	pos.	pos.	neg.	pos.	pos.	*vtx1c + vtx2a*	*ehxA, astA*
36	3=D4	O154:H31	pos.	pos.	neg.	pos.	neg.	pos.	neg.	*vtx1d*	
1	4=E5	O157:H7	neg.	neg.	pos.	pos.	pos.	neg.	pos.	*vtx2a + vtx2c*	*ehxA, astA*
24	5=A1	O91:H21	pos.	pos.	pos.	pos.	neg.	neg.	pos.	*vtx2d*	*ehxA, saa*
NA	6=F6	O2:H25	pos.	pos.	neg.	pos.	neg.	neg.	pos.	*vtx2g*	*astA*
28	7=G7	O22:H 8	pos.	pos.	pos.	pos.	neg.	pos.	pos.	*vtx1c + vtx2b*	*ehxA*
40	8=H8	O139:H 1	pos.	pos.	alpha	pos.	neg.	neg.	pos.	*vtx2e*	
31	9=i9	O145:H34	neg.	pos.	neg.	pos.	pos.	neg.	pos.	*vtx2f*	*bfpA, astA*
40	10=J10	O171:H 2	pos.	pos.	neg.	pos.	neg.	neg.	pos.	*vtx2b + vtx2c + vtx2d*	

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

[a] *Prevalence of sero- and virulence types reported to Enter-net from 2000–2006 and TESSy 2007–2010 are ranked in the left column (Appendix, Table A1).*

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

Haemolysis (HLY) (DIR 2271):
alpha: α haemolysin

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
saa	STEC Auto-agglutinating adhesion; PCR
vtx1	NTP705. Verotoxin 1; almost identical with the Shiga toxin.
vtx2	DEP28. Verotoxin 2; variants exist. Approximately 60% homology to VT1

Genes not detected:

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
ST_h (STIb; *estAh*)	DAS100. Heat-stable enterotoxin (human variant)
ST_p (STIa; *estA*)	DAS101. Heat-stable enterotoxin (porcine variant)

Table 3. Summary of participation rates for each test; laboratories with funding from ECDC, EU NRL 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*
Group 1 n=25 ECDC-funded laboratories, including four EU NRL (veterinary) laboratories	12–18 54–82%	20–22 (22 for O103 and O157 84–88%	13–18 for all 10 strains (18 for H7); 1/1 for H7 52–72%; 4%	19 for all 10 strains; 1 for only 5 strains 76%; 4%	14 for all 10 strains; 1 for only 6 strains 56%; 4%	10 for all 10 strains; 1 for only 1 strain; 1 for only 5 strains 40%; 4%; 4%	10 40%	23 for all strains; 1 for 5 strains 92%; 4%	23 for all strains; 1 for 5 strains 92%; 4%	23 for all strains; 1 for 5 strains 92%; 4%	17 68%	16–17; 64–68%; 18 for *vtx2f* 72%

	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***
Average participation rate	**53%**	**83%**	**56%**	**78%**	**58%**	**42%**	**40%**	**94%**	**94%**	**94%**	**68%**	**68%**
Group 2 n=22 EU NRL (veterinary)	1 for all strains; 3–4 for 5 strains 5%; 18%	2 for all strains; 20 for 5 strains 9%; 91%	1 for all strains; 4 for 5 strains 5%; 18%	2 for all strains; 4 for 5 strains 9%; 18%	2 for all strains; 3 for 5 strains 9%; 14%	1 for all strains; 3 for 5 strains 5%; 14%	1 for all strains 5%	2 for all strains; 20 for 5 strains 9%; 91%	2 for all strains; 20 for 5 strains 9%; 91%	2 for all strains; 20 for 5 strains 9%; 91%	1 for all strains; 2 for 5 strains 5%; 9%	2 for all strains; 17–19 for 5 strains 9%; 77–86%
Average rate	**9%**	**55%**	**10%**	**18%**	**16%**	**11%**	**5%**	**55%**	**55%**	**55%**	**9%**	**50%**
Group 3 n=32 non-EU/SF laboratories	14–17 44–53%	23 for all strains; 4 for 5 strains 72%; 13%	14–17 for all strains; 1 for H7 and H2; 2 for H7 44–53%; 3%; 6%	15–17 for all strains 47–53%	10–11 for all strains 31–34%	10 31%	10 31%	24 for all strains; 6 for 5 strains 75%; 19%	25 for all strains; 6 for 5 strains 75%; 19%	25 for all strains; 6 for 5 strains 75%; 19%	12 38%	16–24 50–75%
Average participation rate	**45%**	**78%**	**45%**	**49%**	**33%**	**31%**	**31%**	**84%**	**88%**	**88%**	**38%**	**63%**

Note: Number of participating laboratories per method

4 Results

4.1 Participation of laboratories

Group 1 (ECDC-funded laboratories)

An average of 53% (range: 54–82%) participated in full O:H serotyping. Participation in phenotypic detection was 78% for fermentation of sorbitol, 58% for β-glucuronidase, 42% for haemolysin, and 40% for verocytotoxin. Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by an average of 94% (23/25 for all the strains, and 1/25 for 5 strains), 68% for *ehxA*, and 68% participated in subtyping of *vtx* genes (Table 3).

Group 2 (funded by DG-SANCO)

An average of 9% participated in full O:H serotyping. The low participation rate may be explained by the fact that veterinary laboratories rarely need to perform H typing (only one laboratory performed full H typing of all 10 strains) and that Group 2 focussed on O grouping of the top-5 O groups by RT-PCR.

All 22 laboratories participated in O grouping of five strains. Participation in phenotypic detection was 18% for fermentation of sorbitol, 16% for β-glucuronidase, 11% for haemolysin, and 5% for verocytotoxin. Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by an average of 55% (2/22 for all strains, and 20/22 for five strains), 9% (1/22 for all the strains, and 2/22 for five strains) for *ehxA*, and 50% participated in subtyping of *vtx* genes (Table 3).

Group 3 (Non-EU/SF)

An average of 45% (44–53%) participated in full O:H serotyping. Participation in phenotypic detection was 45% for fermentation of sorbitol, 33% for β-glucuronidase, 31% (5–18%) for haemolysin, and 31% for verocytotoxin. Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by an average of 84–88% (24–25/32 for all strains, 5–6/32 for five strains, 38% for *ehxA*, and 63% participated in subtyping of *vtx* genes (Table 3).

4.2 Scores

Group 1 (ECDC-funded laboratories)

Of 12–18 participants, an average of 68% could correctly O:H serotype all strains. Correct results were 100% for fermentation of sorbitol, 97% for β-glucuronidase, 89% for haemolysin, and 94% for verocytotoxin. Genotypic detection of *eae*, *vtx1* and *vtx2* was very high, with scores of 96–98% and even higher for the detection of the *ehxA* gene, which was detected correctly by 99% of the Group 1 participants.

False positive *vtx1* genes were reported in two strains by two different laboratories; two laboratories did not detect *vtx1* genes in two different strains. One laboratory failed to detect *vtx2* in two strains, while two other laboratories failed to detect *vtx2* in one strain each. The *eae* gene was detected as false positive once in strain A1 and once in strain C3 by two laboratories. Two laboratories were not able to detect the *eae* gene in three strains (C3, E5 and i9), and one laboratory failed to detect *eae* in strain i9 (Table 5).

Group 2 (funded by DG-SANCO)

Of only 1–5 participants, an average of 93% could correctly determine the O:H serotype of all strains. However, of the five strains of which Group 2 had been requested to determine the O group, only 71% were correctly typed. The results were 100% correct for fermentation of sorbitol, 96% correct for β-glucuronidase, 82% for haemolysin, and 100% for verocytotoxin.

Genotypic detection of *eae*, *vtx1* and *vtx2* was performed by two of 22 laboratories for all strains, and 20 laboratories of 22 carried out genotypic detection for five strains with scores of 98–100%. One of 22 laboratories conducted genotypic detection for all strains, and two of 22 laboratories conducted genotypic detection of the *ehxA* gene for five strains (100% correct). Only one laboratory failed to detect *vtx1* in one strain, and all laboratories detected *vtx2* correctly.

The *eae* gene was detected as a false positive three times by one laboratory (Table 6).

Group 3 (Non-EU/SF)

Of 14–17 participants, an average of 79% correctly determined the O:H serotype of all strains. The fermentation of sorbitol was correctly determined by 94% of laboratories, and β-glucuronidase was correctly determined by 97% of the participants; 82% of the laboratories reported correct results for haemolysin, and 90% did so for verocytotoxin.

Twenty-four of 32 participants (75%) determined *eae* for all the strains; results were 99% correct. Equally good results were achieved when 25 of 32 laboratories genotyped all strains for *vtx1* and *vtx2* (97–98%).

Four laboratories failed to detect *vtx1* in two strains, and one of these laboratories also had a false positive *vtx1* report in one strain. Five laboratories were unable to detect *vtx2f* in strain i9, and one of these laboratories also failed to detect *vtx2* in two additional strains. The *eae* gene was detected as a false positive in three strains by one laboratory; another laboratory detected it as a false positive in one strain (Table 7).

4.3 All laboratories (Groups 1–3)

More common serotypes led to better typing results: O157:H7 was typed correctly by almost all laboratories (94–100%); correct results were lower for other serotypes, for example O171:H2 in Group 1 (17%), O154:H31 in Group 2 (33%), O2:H25 and O139:H1 in Group 3 (57%) (Tables 5–7).

Seven out of a total of 21 (33%) laboratories did not phenotypically detect VT2e (VT2e is only rarely found in humans and probably does not cause diarrhoea). Three laboratories did not detect VT2g, three laboratories did not detect VT2f, and three different laboratories failed to detect VT1d or combinations of VT1c + VT2a or VT1c + VT2b. No false positives were reported.

False positive *vtx1* genes were reported twice by two Group 1 laboratories and once by one Group 3 laboratory. One Group 1 laboratory detected one false positive *vtx2* strain.

The *eae* gene was detected as a false positive four times in Group 1, three times in Group 2, and four times in Group 3. False negative results for *vtx1* ranged between 2 and 9: Group 1 (2), Group 2 (1) and Group 3 (9). False negatives for *vtx2* ranged between 5 and 6: Group 1 (6) and Group 3 (5); Group 1 detected seven false positives for *eae*.

The gene of *vtx2f* in strain i9 was correctly determined by 21 of 23 laboratories (91%) in Group 1, two of two labotatories in Group 2, and 20 of 25 laboratories (80%) in Group 3.

Table 4. Serotyping scores by ECDC, EU NRL and non-EU/SF participants in relation to the ranking of the serotype in Europe, based on the number of O:H serotyped cases reported to Enter-net in 2000–2006 and TESSy 2007–2010 and ranked according to the reported number of human cases (Appendix, Table A1)

Strain no.	ECDC no.	Serotype	Scores for O:H serotyping			Prevalence of reported O:H serotype (rank)
			ECDC-funded laboratories, including four EU NRL (veterinary) laboratories (n=15–17)	**EU NRL (veterinary)** (n=1/22 for all strains; 4/22 for 5 strains)	**Non-EU/SF laboratories** (n=14–17)	
4	E5	O157:H7	94%	100%	100%	27% (1)
1	B2	O103:H2	79%	100%	93%	11% (2)
2	C3	O146:H21	92%	100%	71%	2% (11)
5	A1	O91:H21	92%	100%	93%	0.3% (24
7	G7	O 22:H 8	69%	100%	79%	0.2% (28)
9	i9	O145:H34	77%	100%	93%	0.2% (31)
3	D4	O154:H31	42%	33%	80%	0.1% (36)
8	H8	O139:H 1	58%	100%	57%	0.02% (40)
10	J10	O171:H 2	17%	100%	71%	0.02% (40)
6	F6	O 2:H25	62%	100%	57%	NR
		Average score	**68%**	**93%**	**79%**	**Cumulated prevalence 41%**

n: Number of laboratories participating per method

NR: Not ranked in the combined Enter-net/TESSy database

Note: Table sorted in descending order of scores for EU/EEA laboratories

Table 5. Participants' scores. Third international external quality programme for VTEC 2010–11; ECDC-funded laboratories, including four EU NRL (veterinary) laboratories (Group 1)

Strain no.	O:H Serotype n=12–18	O group n=20–22 (22 for O103 and O157	H type n=13–18 for all 10 strains (18 for H7)	Sorbitol fermentation n=19 for all 10 strains, 1 for only 5 strains	β-glucuronidase activity n=14 for all 10 strains, 1 for only 6 strains	Haemolysin production n=10–11 for all 10 strains, 1 for only 5 strains	VCA/EIA n=10	*eae* n=23 for all 10 strains, 1 for only 5 strains	*vtx1* n=23 for all 10 strains, 1 for only 5 strains	*vtx2* n=23 for all 10 strains, 1 for only 5 strains	*ehxA* n=17	Subtyping of *vtx*; *vtx1*/*vtx2* n=15–18
B2	79%	100%	79%	95%	93%	83%	100%	92%	100%	100%	100%	100%/NA
C3	92%	67%	92%	100%	100%	73%	100%	96%	96%	96%	94%	94%/88%
D4	42%	29%	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%/NA
E5	94%	100%	94%	100%	87%	73%	100%	92%	96%	100%	100%	NA/88%
A1	92%	76%	93%	100%	100%	91%	100%	96%	100%	100%	100%	NA/94%
F6	62%	60%	79%	100%	100%	100%	80%	100%	100%	100%	100%	NA/89%
G7	69%	45%	93%	100%	93%	90%	100%	100%	96%	100%	100%	88%/88%
H8	58%	45%	62%	100%	100%	80%	70%	100%	96%	96%	100%	NA/94%
i9	77%	95%	79%	100%	100%	100%	90%	87%	100%	91%	100%	NA/100%
J10	17%	25%	62%	100%	100%	100%	100%	100%	100%	100%	100%	NA/94%
Average score	**68%**	**64%**	**82%**	**100%**	**97%**	**89%**	**94%**	**96%**	**98%**	**98%**	**99%**	**96%/92%**

n: Number of laboratories participating per method

Note: Percentages represent results in accordance with the intended result for those laboratories that performed the test

Table 6. Participants' scores. Third international external quality programme for VTEC 2010–11; EU NRL (veterinary) laboratories (Group 2)

Strain no.	O:H Serotype n=1/22 for all strains; 3–4/22 for 5 strains	O group n=2 for all strains; 20 for 5 strains	H type n=1 for all strains; 4 for 5 strains	Sorbitol fermentation n=2 for all strains; 4 for 5 strains	β-glucuronidase activity n=2 for all strains; 3 for 5 strains	Haemolysin production n=1 for all strains; 3 for 5 strains	VCA/EIA n=1 for all strains	*eae* n=2 for all strains; 20 for 5 strains	*vtx1* n=2 for all strains; 20 for 5 strains	*vtx2* n=2 for all strains; 20 for 5 strains	*ehxA* n=1 for all strains; 2 for 5 strains	Subtyping of *vtx*; *vtx1vtx2* n=19–21
B2	100%	95%	100%	100%	100%	75%	100%	95%	100%	100%	100%	100%/NA
C3	100%	64%	100%	100%	80%	25%	100%	95%	100%	100%	100%	90%/90%
D4	33%	14%	100%	100%	100%	100%	100%	95%	95%	100%	100%	NA/100%
E5	100%	100%	100%	100%	80%	25%	100%	100%	100%	100%	100%	NA/95%
A1	100%	82%	100%	100%	100%	50%	100%	95%	100%	100%	100%	NA/86%
F6	100%	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	NA/100%
G7	100%	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%/50%
H8	100%	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	NA/100%
i9	100%	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	NA/100%
J10	100%	50%	100%	100%	100%	100%	100%	100%	100%	100%	100%	NA/100%
Average score	**93%**	**60%**	**100%**	**100%**	**96%**	**78%**	**100%**	**98%**	**100%**	**100%**	**100%**	**91%/93%**

n: Number of laboratories participating per method

Note: Percentages represent results in accordance with the intended result for those laboratories that performed the test

Table 7. Participants' scores. Third international external quality programme for VTEC 2010–11; non-EU/self-funded laboratories (Group 3)

Strain no.	Serotype n=14–17	O group n=27 (15 for O146, 16 for O121, 17 for O113 and O128, 19 for O55, O91, 22 for O103 and O154, 24 for O26 and O111)	H type 14/27 for all strains; 1/27 for H7 and H2; 2/27 for H7	Sorbitol fermentation n=15/27 for all strains; 2/27 for 5 strains	β-glucuronidase activity n=9–10/27 for all strains; 1/27 for 5 strains	Haemolysin production n=10	VCA/EIA n=10	*eae* n=24 for all strains; 6 for 5 strains	*vtx1* n=25 for all strains; 6 for 5 strains	*vtx2* n=25 for all strains; 6 for 5 strains	*ehxA* n=12	Subtyping of *vtx*; *vtx1*/*vtx2*
B2	93%	74%	100%	94%	90%	60%	100%	100%	100%	100%	100%	100%/NA
C3	71%	56%	93%	81%	100%	60%	90%	97%	94%	100%	100%	83%/78%
D4	80%	56%	93%	100%	100%	100%	90%	97%	94%	100%	100%	91%/NA
E5	100%	100%	100%	100%	82%	70%	100%	100%	100%	100%	100%	NA/88%
A1	93%	67%	93%	88%	100%	80%	100%	97%	100%	100%	92%	NA/92%
F6	57%	57%	71%	93%	100%	100%	90%	100%	96%	96%	100%	NA/100%

Strain no.	Serotype n=14–17	O group n=27 (15 for O146, 16 for O121, 17 for O113 and O128, 19 for O55, O91, 22 for O103 and O154, 24 for O26 and O111)	H type 14/27 for all strains; 1/27 for H7 and H2; 2/27 for H7	Sorbitol fermentation n=15/27 for all strains; 2/27 for 5 strains	β-glucuronidase activity n=9–10/27 for all strains; 1/27 for 5 strains	Haemolysin production n=10	VCA/EIA n=10	*eae* n=24 for all strains; 6 for 5 strains	*vtx1* n=25 for all strains; 6 for 5 strains	*vtx2* n=25 for all strains; 6 for 5 strains	*ehxA* n=12	Subtyping of *vtx*; *vtx1*/*vtx2*
G7	79%	52%	100%	93%	100%	80%	90%	100%	100%	100%	92%	94%/94%
H8	57%	52%	57%	100%	100%	70%	60%	96%	100%	96%	100%	NA/94%
i9	93%	70%	93%	93%	100%	100%	80%	100%	100%	80%	100%	NA/100%
J10	71%	43%	50%	100%	100%	100%	100%	100%	100%	100%	100%	NA/67%
Average score	**79%**	**63%**	**85%**	**94%**	**97%**	**82%**	**90%**	**99%**	**98%**	**97%**	**98%**	**92%/89%**

n: Number of laboratories participating per method

Note: Percentages represent results in accordance with the intended result for those laboratories that performed the test

Table 8. Results of *vtx1* & *vtx2* subtyping VTEC EQA 2009–2010; ECDC-funded laboratories, EU NRL (veterinary) laboratories and non-EU/SF laboratories

Strain No.	Subtype by the WHOCC[a]	ECDC-funded laboratories including 4 EU NRL (veterinary) laboratories **Group 1** (n=16 for all strains; 17 for strains 1–9; 18 for strain i9)		EU NRL (veterinary) **Group 2** (n=2 for all strains; 19-21 for 5 strains)		Non-EU/SF laboratories **Group 3** (n=16–23)	
		vtx1 Correct (%) false negative (%) **false positive (%)**	*vtx2* Correct (%) false negative (%) **false positive (%)**	*vtx1* Correct (%) false negative (%) **false positive (%)**	*vtx2* Correct (%) false negative (%) **false positive (%)**	*vtx1* Correct (%) false negative (%) **false positive (%)**	*vtx2* Correct (%) false negative (%) **false positive (%)**
1=B2	*vtx1a*	17/17 (100%)		20/20 (100%) 0 **2 *vtx1c* (10%)**		23/23 (100%)	
2=C3	*vtx1c + vtx2a*	16/17 (94%) 0	15/17 (88%) 1/17 (6%) **14/17 *vtx2b* (82%); 1/17 *vtx2e* (6%)**	19/21 (90%) 2 (10%) **2 *vtx1a* (10%)**	19/21 (90%) 2 (10%) **2/21 *vtx2b* (10%); 2/21 *vtx2d* (10%); 5 *vtx2c* (43%)**	20/24 (83%) 4/24 (17%) **2 *vtx1a* (8%)**	19/24 (79%) 4/17 (17%) **17/24 *vtx2b* (71%); 3/24 *vtx2c* (13%); 1/24 *vtx2c* & *vtx2d* (4%); 1/24 *vtx2g* (4%)**
3=D4	*vtx1d*	17/17 (100%)		19/19 (100%) 0 **2 *vtx1a* &*vtx1c* (11%); 2 *vtx1c* (11%)**		21/23 (91%) 2 (9%) **2 *vtx1c* (9%) 1 *vtx1a* (4%)**	
4=E5	*vtx2a + vtx2c*	**1/17 *vtx1c* (6%)**	15/17 (88%) 1/17 *vtx2a* (6%); 1/17 *vtx2c* (6%) **3/17 *vtx2d* (18%)**		20/21 (95%) 1 *vtx2a* (5%) **8 *vtx2d* (38%)**		21/24 (88%) 2 *vtx2a* (8%); 1 *vtx2c* (4%) **4 *vtx2d* (17%)**
5=A1	*vtx2d*		16/17 (94%) 1/17 (6%) **1/17 *vtx2a* & *vtx2b* (6%); 1/17 *vtx2a* & *vtx2c* (6%); 5/17 *vtx2c* (29%)**		18/21 (86%) 1 (5%) **12 *vtx2c* (57%)**		22/24 (92%) 2 (8%) **9 *vtx2c* (38%); 2 *vtx2a* (8%)**
6=F6	*vtx2g*		16/17 (89%) 0 **1/17 *vtx2b* (6%)**		2/2 (100%)	**1 *vtx1d* (6%)**	16/16 (100%)
7=G7	*vtx1c + vtx2b*	15/17 (88%) 2/17 *vtx1c* (12%)	15/17 (88%) 0 **1/17 *vtx2d* (6%); 1/17 *vtx2g* (6%)**	2/2 (100%)	1/2 (50%) 1/2 *vtx2b* (50%)	15/16 (94%) 1 *vtx1c* (6%) **1 *vtx1a* (6%)**	15/16 (94%) 1/16 *vtx2b* (6%) **1 *vtx2d* (6%); 1 *vtx2f* (6%)**
8=H8	*vtx2e*	**1/17 *vtx1c* (6%)**	16/17 (94%) 0 **1/17 *vtx2b* (6%)**		2/2 (100%)		16/17 (94%) 0 **1 *vtx2a* (6%)**
9=i9	*vtx2f*		18/18 (100%)		2/2 (100%)		18/18 (100%)
10=J10	*vtx2b + vtx2c + vtx2d*		15/16 (94%) 1/16 *vtx2b* and *vtx2d* (6%)		1/2 (100%) 1/2 *vtx2b* (100%)		12/18 (67%) 3 *vtx2b* (17%); 4 *vtx2d* (22%) **1 *vtx2a* (6%)**

[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*

Note: Percentages within brackets correspond to the proportion of laboratories reporting this result

Table 9. Specificity and sensitivity of the subtyping of *vtx1* and *vtx2* genes, VTEC EQA 2009–2010; all laboratories: ECDC-funded laboratories, EU NRL (veterinary) laboratories and non-EU/SF laboratories combined

	vtx1a	*vtx1c*	*vtx1d*	*vtx2a*	*vtx2b*	*vtx2c*	*vtx2d*	*vtx2e*	*vtx2f*	*vtx2g*
Sensitivity	1.00	0.96	0.95	0.93	0.94	0.99	0.92	0.94	1.00	0.97
Specificity	0.98	0.97	0.997	0.98	0.86	0.90	0.94	0.997	0.997	0.99

4.4 Serotyping

There was a significantly lower EU NRL participation (Group 2) in O:H serotyping (only one laboratory typed all strains, while four laboratories typed five strains; average: 9%) compared with the ECDC-funded laboratories (Group 1): 12–18 (54–82%) typed all strains. Fourteen of 17 non-EU/SF laboratories (Group 3) typed all strains (44–53%).

An average of 68% (average 9.2, median 9.5) of Group 1 laboratories could correctly determine the O:H serotype in the ten strains, compared with 93% of the EU NRLs in Group 2 (five strains: average 2, median 2.6; 10 strains: average 1, median 1), and 79% of the non-EU/SF laboratories in Group 3 (average 11.6; median 11.5).

Correct O:H serotyping ranged from 100% correct typing results of O157:H7 in Groups 2 and 3 to a much lower percentage of correct results in other groups: O171:H2 in Group 1: 17% of all results were correct; O154:H31 in Group 2: 33% correct; O139:H1 and O2:H25 in Group 3: 57% correct. See Tables 5–7.

In Group 1, O157:H7 was correctly serotyped by 94% of all participants, followed by serotypes O91:H21 (92%), O146:H21 (92%), O103:H2 (79%), O145:H34 (77%), O22:H8 (69%), O2:H25 (62%) and O139:H1 (58%) (see Table 5). Lower scores were obtained for O154:H31 (42%), followed by O171:H2 (17%) (see Table 2). This leads to the conclusion that it is more difficult for laboratories to serotype a strain correctly if the serotypes are less common.

Among the five Group 2 participants performing O:H serotyping, all strains were typed correctly, except for O154:H31, which was only typed correctly by 33% of the participants (Table 6).

Among the Group 3 participants, O157:H7 was correctly serotyped by all laboratories, followed by O91:H21 (93%), O145:H34 (93%), O103:H2 (93%), O154:H31 (80%), O22:H8 (79%), O171:H2 (71%) and O146:H21 (71%). Lower scores were obtained for O139:H34 (57%) and O2:H25 (57%) (Table 7).

One laboratory using molecular typing for H reported that they got the same identity for H1, H2 and H12 in strain H8 (O139:H1) and for H2, H8 and H35 in strain J10 (O171:H2).

4.5 Phenotypic detection

The participation of ECDC-funded laboratories with respect to phenotypic detection was 78%. Please refer to Table 3 for details. Correct results were 100% for the fermentation of sorbitol, 97% for β-glucuronidase, 89% for haemolysin, and 94% for verocytotoxin. See Table 5.

Only 18% of EU NRLs engaged in phenotypic detection, so only a few laboratories presented results for β-glucuronidase, haemolysin, verocytotoxin, and the fermentation of sorbitol. For details please refer to Table 3. The EU NRLs reported flawless results for the fermentation of sorbitol and for verocytotoxin; slightly lower results were achieved for β-glucuronidase and (96% correct) and haemolysin (82%). See Table 6.

Of the 32 non-EU/SF laboratories, 49% (15–17 laboratories) provided results on the fermentation of sorbitol, 33% (10–11 laboratories) on β-glucuronidase, 31% (10 laboratories) on haemolysin, and 31% (10 laboratories) on verocytotoxin (see Table 3). Correct results for the non-EU/SF laboratories were 94% for the fermentation of sorbitol, 97% for β-glucuronidase, 82% for haemolysin, and 90% for verocytotoxin (see Table 7).

Only two ECDC-funded laboratories did not detect verocytotoxin production in strains F6 (VT2g) and H8 (VT2e), and one laboratory was not able to detect verocytotoxin production in strains H8 (VT2e) and i9 (VT2f) (see Table 5). One EU NRL detected verocytotoxin production in all 10 strains. In Group 3, one laboratory did not detect verocytotoxin production in strains C3 (VT1c and VT2a), D4 (VT1d), G7 (VT1c and VT2b), i9 (VT2f) and H8 (VT2e); this was also not detected by three other non-EU/SF laboratories. Finally, one laboratory did not detect verocytotoxin production in F6 (VT2g).

In summary, seven laboratories did not detect VT2e (VT2e probably does not cause diarrhoea and is only rarely found in humans). Three laboratories did not detect VT2g, three laboratories did not detect VT2f, and three different laboratories failed to detect VT combinations of VT1c + VT2a, VT1c + VT2b or VT1d. No false positives were reported.

4.6 Genotypic detection

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

With regard to the detection of virulence genes, the three groups of laboratories (ECDC-funded, EU NRL and non-EU/SF) showed participation levels which were similarly high for genes *eae*, *vtx1* and *vtx2*. For details please refer to Table 5.

Two of 22 EU NRLs detected the genotype for all submitted strains; 20/22 did so for five strains. The submitted scores were very high (98–100%) (see Table 6). The genotype for all strains was detected by 24–25/32 of the non-EU/SF participants; six of 32 restricted themselves to five strains. Accuracy was very high (97–99%) (Table 7). With the exception of one laboratory, all ECDC-funded participants and all EU NRLs participated in the detection of *eae*, *vtx1* and *vtx2* genes, whereas 94% of the non-EU/SF participants reported on *eae* virulence genes and 97% reported on *vtx1* and *vtx2* virulence genes.

All laboratories were able to detect the three genes accurately (96–100%) (Tables 5, 6 and 7). The *eae* gene was detected correctly by 96% of the ECDC-funded participants, 98% of the EU NRLs, and 99% of the non-EU/SF laboratories. Three of the ECDC-funded laboratories reported false *eae* negatives for strain i9, serotype O145:H34; two laboratories also reported a false negative for this strain (strain B2, serotype O103:H2; strain E5, serotype O157:H7). Furthermore, two ECDC-funded laboratories reported *eae* as false positives for either strain A1, serotype O91:H21, or strain C3, serotype O146:H21.

Of the EU NRLs, one laboratory reported false *eae* positives for strain C3, serotype O146:H21; strain D4, serotype O154:H31; and strain A1, serotype O91:H21. One of the EU NRLs reported a false *eae* negative for strain B2, serotype O103:H2.

Of the non-EU/SF laboratories, one laboratory reported three false positive *eae* genes in strain C3, serotype O146:H21; strain D4, serotype O154:H31; and strain A1, serotype O91:H21. One non-EU/SF laboratory reported a false positive for strain H8, serotype O139:H1.

One ECDC laboratory detected a false positive *vtx1* gene and a false negative *vtx1* gene in strain C3, serotype O146:H21, and strain E5, serotype O157:H7. One laboratory reported a false *vtx1* positive for strain H8, serotype O139:H1, while another laboratory reported a false *vtx1* negative for strain G7, serotype O22:H8. In connection with the detection of the *vtx2* gene, four ECDC laboratories reported one false negative result each for strain C3, serotype O146:H21; strain H8, serotype O139:H1; and strain I9, serotype O145:H34.

Of the EU NRLs, one laboratory reported false negative *vtx1* genes for strain D4, serotype O154:H31. All participants reported correct results for the *vtx2* gene.

Two non-EU/SF laboratories reported false negative results for the *vtx1* gene for strain C3, serotype O146:H21, and a further two participants reported false negatives for strain D4, serotype O154:H31. One of the laboratories reported a false positive for strain F6, serotype O 2:H25. One laboratory reported false negative results for strain F6, serotype O 2:H25, and H8, serotype O139:H1. Moreover, five laboratories reported false negative results for strain I9, serotype O145:H34.

Gene detection of *ehxA* showed a significantly lower average participation rate: 68% (Group 1), 9% (Group 2), and 38% (Group 3). Of the ECDC-funded participants, genotypic detection of *ehxA* was performed by 17/25 laboratories for all the strains. Only one of the 22 EU NRLs genotyped all strains. Two of the 22 EU NRLs genotyped five strains, as did 12 of the 32 non-EU/SF participants. In both instances the scores reached 98–100%.

Results submitted as Alfa-hly were considered as incorrect because α-haemolysin is different from enterohaemolysin. Results submitted as hlyA and hly were considered as enterohaemolysin and hence correct but reflect the fact that there is no universal nomenclature.

4.6.2 Subtyping of *vtx1* and *vtx2*

The number of laboratories subtyping *vtx* genes was very high, ranging from 50–95%. The following participation rates were recorded: 64–72% (16–18 ECDC-funded laboratories), 86–95% (19–21 EU NRLs), and 50–75% (16–24 non-EU/SF laboratories).

Subtyping results of *vtx* genes are presented in Table 8; sensitivity and specificity for all laboratories (51–63) is summarised in Table 9.

Fifty-five of 62 laboratories correctly subtyped *vtx1c* in strain C3 and 32/35 in strain G7. Eight laboratories did not subtype *vtx1c* correctly; only one laboratory failed to subtype in both strains (C3 and G7).

Thirty-eight of 40 participants correctly typed *vtx1d*. Two laboratories did not subtype *vtx1d,* and three reported also *vtx1c* or *vtx1a*.

Fifty-three and 56/62 laboratories subtyped *vtx2a* correctly in strains C3 and E5. Many false positives were reported: 33 for *vtx2b*, 9 for *vtx2c*, three for *vtx2d*, one for *vtx2e* and one for *vtx2g* in C3, and 15 *vtx2d* in E5.

Thirty-one of 35 laboratories subtyped *vtx2b* correctly in strain G7. Two also reported *vtx2d*. Two laboratories each reported false positives for *vtx2f* and *vtx2g*. Twenty-eight of 36 laboratories subtyped *vtx2b* correctly in J10, and only one false positive *vtx2a* was reported.

Fifty-three of 62 and 28/36 laboratories subtyped *vtx2c* correctly in strains E5 and J10. False positives were 15 *vtx2d* in E5 and one *vtx2a* in J10.

Fifty-six out of 62 laboratories correctly subtyped *vtx2d* in strain A1, but false positives of combinations of *vtx2a* + *vtx2b* (1), *vtx2a* + *vtx2c* (1) or *vtx2a* (2), *vtx2c* (26) were also reported. In strain J10, *vtx2d* was correctly reported by 28/36 laboratories, and only one false positive *vtx2a* was reported.

Thirty-four of 36 laboratories subtyped *vtx2e* correctly; two false positives *vtx2a* and *vtx2b* (one each) and one false positive *vtx1c* were reported.

All 38 participants correctly subtyped *vtx2f*.

Thirty-four of 35 correctly subtyped *vtx2g*. One false positive *vtx2b* and one false positive *vtx1d* were reported.

The highest sensitivity was for *vtx1a* and *vtx2f* (1.0) followed by *vtx2c* (0.99), *vtx2g* (0.97), *vtx1c* (0.96), *vtx1d* (0.95), *vtx2b* and *vtx2e* (0.94), *vtx2a* (0.93). Sensitivity was lowest for *vtx2d* (0.92).

Specificity was highest for *vtx1d*, *vtx2e* and *vtx2f* (0.997) followed by *vtx2g* (0.99), *vtx1a* and *vtx2a* (0.98), *vtx1c* (0.97), *vtx2d* (0.94), *vtx2c* (0.9). Specificity was lowest for *vtx2b* (0.86).

4.6.3 Detection of other virulence genes

Results for additional virulence genes are presented in Table 9. Subtyping of the *eae* gene was obtained for the three *eae*-positive strains (B2, E5 and i9) by 2–3 laboratories with similar results for strains E5 (O157:H7) and i9 (O145:H34). Two laboratories subtyped the *eae* gene in strain B2 (O103:H2) as *eae* ε and one as *eae* ξ. ξB is identical to ε5, but ξR has also been used as synonymous with β2. The *eae* nomenclature is rather confusing and ξ may be interpreted as two quite different subtypes of *eae*. pO157 plasmid-encoded genes *espP*, *etpD* and *katP* were detected in *eae*-positive strain E5 (O157:H7), *etpD* and *katP* in *eae*-positive strain B2 (O103:H2) and finally *espP* and *katP* in *eae*-negative strain F6 (O2:H25). Long polar fimbrial genes (*lpf*) using a non-comparable nomenclature were detected in eight of the strains by four laboratories. In a database analysis of the genes encoding the two loci *lpfA1* and *lpfA2*, Torres et al. [13] identified several polymorphisms and classified the major fimbrial variants into distinct variants. The authors also presented a PCR scheme for the subtyping of *lpfA1-1* through *lpfA1*-5 and *lpfA2-1* through *lpfA2*-3. It is strongly recommended that laboratories use the PCR procedure and nomenclature from this important paper [13]. The *saa* gene was correctly identified by eight laboratories in strain A1 (O91:H21), which was also found positive for *subAB* by one laboratory.

The *iha* (chromosomal iron-regulated gene A homolog adhesin) was detected in four strains.

Two laboratories found one strain positive for *bfpA*. One to five laboratories found strains C3 (O146:H21), E5 (O157:H7), F6 (O2:H25), H8 (O139:H1) and i9 (O145:H34) positive for *astA*. OI122 pathogenicity island gene *efa1* (or the similar *toxB*) was found by 1–2 laboratories in strains B2 (O103:H2) and E5 (O157:H7), and *pagC* in E5 (O157:H7), A1 (O91:H21) and J10 (O171:H2).

Three laboratories detected ETEC-related gene st1a (*estA*) in strain F6 (O2:H25), and one of these laboratories also detected st1b (*estB*) in strain H8 (O139:H1) and lt (*elt*) in strain J10 (O171:H2). The cytolethal toxin gene *cdt* was detected by one laboratory in strains A1 (O91:H21) and i9 (O145:H34); it was not specified which variant of *cdt* was detected. One laboratory also reported the tellurite resistance gen *terE* in strains C3 (O146:H21) and J10 (O171:H2).

Table 10. Results of additional virulence genes as submitted by all participating laboratories

Strain no.	Antigen-related genes	Virulence genes (n)
1=B2		*eae* epsilon (2) *eae* subtype Xi *efa1* *etpD* (2) *katP* (3) *lpfA*1-2 *lpfA*O26

Strain no.	Antigen-related genes	Virulence genes (n)
2=C3		*astA* (5) *iha* *lpfA 2-1* *lpfA1-2,* *lpfA*O26 *terE*
3=D4		*lpfA1-5* *lpfA*O157/OI-141
4=E5	*rfbO157* (2)	*astA* *eae* gamma (3) *efa1* *espP* (2) *etpD* (2) *iha* *katP* (3) *lpfA1-3* *lpfA2-2* *lpfA*O157/OI-141 *pagC* *terE* *toxB* *uidA* (O157)
5=A1		*cdt* *iha* *lpfA1-2* *lpfA2-1* *lpfAO26* *pagC* *saa* (8) *subAB*
6=F6		*astA* (3) *espP* (2) *estA* (STIa-STp) *katP* (3) *st1a* (2) *st1b*
7=G7		*lpfA1-2* *lpfA2-1* *lpfAO26*
8=H8	For the molecular H typing we got same identity on both H1, H2 and H12	*astA* (2) l*pfA2-3* *st1b*
9=i9		*astA* (5) *bfpA* (2) *cdt* *eae i*ota *eae* iota-1
10=J10	For the molecular H typing we got same identity on H2, H8 and H35	*iha* *lpfA2-1* *lt* *pagC* *terE*

Genes found by participating laboratories:
astA, bfpA, cdt, eae γ, eae ι, eae ι-1, eae ε, eae ξ, efa1, espP, estA (STIa-STp), etpD, iha, katP, lpfA1-2, lpfA2-1, lpfA2-3, lpfAO26, lpfA1-5, lpfO157/OI-141, lpfA1-3, lpfA2-2, lt, pagC, saa, st1a, st1b, subAB, terE, toxB, and uidA (O157).

5 Discussion

This third VTEC EQA had a much higher number of participants than the second EQA [10], 81 laboratories from 41 countries, compared with 45 laboratories from 38 countries. The participation rate varied substantially between the different tests included in the EQA. It was highest for the genotypic detection of *eae* and the *vtx* genes and lowest for the detection of *ehxA*. In all three participant groups (ECDC-funded laboratories, EU NRLs and non-EU/SF laboratories), the fermentation of sorbitol was performed more often than any of the other phenotypic tests. The second highest participation was for β-glucuronidase activity, followed by haemolysin and toxin production. The prototype protocol for subtyping of *vtx* genes was tested by more than two thirds of the participants, depending on the number of strains tested.

Correct O:H serotyping ranged from 100% correct typing of O157:H7 by EU NRLs (Table 6) and non-EU/SF laboratories (Table 7) to 17% of O171:H2 by ECDC-funded laboratories (group 1) (Table 5).

Correct O:H serotyping in the third VTEC EQA was generally lower than in the second EQA, where the corresponding percentages were 78% for the ECDC-funded laboratories and 86% for the non-EU/SF laboratories.

This may in part be explained by the fact that the third VTEC EQA strains were chosen to test the prototype protocol for *vtx* gene subtyping and therefore included more rare O:H serotypes than in the second EQA. The general trend in both EQAs was the same: more common serotypes were identified more reliably.

Serotype O157:H7 was correctly typed by all laboratories except for one laboratory which typed the H antigen as H rough. It is not known whether this laboratory used supplementary incubation at 30 °C and/or room temperature in order to improve typeability, but this approach should be considered when the *H. flagella* antigen initially is typed as H rough. Serotypes O146:H21 and O91:H21 were correctly typed by 92–94% of ECDC-funded laboratories, serotypes O103:H2, O91:H21 and O145:H34 were correctly typed by 93% of non-EU/SF laboratories. All strains were correctly typed by the one EU NRL which typed all strains. Only one serotype (O154:H31) was not correctly determined by two EU NRLs. No systematic typing errors were observed.

Participation in phenotypic detection was relatively low. The highest participation rate was 78% (ECDC-funded laboratories), compared with 83% earlier (second EQA, fermentation of sorbitol). Eighteen per cent of the EU NRL participants reported data on the fermentation of sorbitol; accuracy was 100% in both ECDC-funded and EU NRL. The test for fermentation of sorbitol is important for the screening of non-sorbitol-fermenting O157 and for identifying the highly virulent sorbitol-fermenting O157:H7 clone. Surprisingly, only 14/21 (66%) were able to phenotypically detect VT2e in the O139:H1 strain (H8). Three participants failed to detect VT2f, and three failed to detect VT2g. It should be noted that VT2c was not explicitly tested in this third VTEC EQA. However, in the second EQA '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'* [10]. These results are in accordance with the recently published results from Feng et al. who found that 'most of the serological assays examined did not detect Stx2c, Stx2e, Stx2f and Stx2g' [4].

The *vtx1c* gene was missed by four participants and *vtx1d* by three. Only one ECDC-funded laboratory failed to detect the clinically relevant gene *vtx2a*; the less relevant *vtx2e*, *vtx2f* and *vtx2g* subtypes were missed by 2, 7 and 1 participants, respectively. Laboratories should review their genetic detection methodology for *vtx1d*, *vtx1c* and *vtx2f*.

Two ECDC-funded participants did not detect the *eae* gene in the three relevant strains and should revise their detection of *eae*. This is lower than in the second EQA where only one laboratory failed to detect the *eae* gene in one strain. Only one EU NRL participant did not detect the *eae* gene in one of the strains. These failures to detect the *eae* gene are of concern because *eae*-positive VTEC include strains that are primarily associated with HUS cases. The use of different PCR primers should also be examined and harmonised.

Comments on the prototype PCR protocol for *vtx* subtyping

The prototype PCR protocol for subtyping of *vtx* genes was tested by two thirds of ECDC-funded laboratories and non-EU/SF participants. The protocol was also tested by more than half of the EU NRLs, where only two laboratories subtyped all strains and 17–19 subtyped only five strains. This was included in the third VTEC EQA programme in order to see whether the protocol developed in the two organising laboratories (SSI and ISS) could also be used in other laboratories and thus form the basis of a universal subtyping scheme. Feedback and questions from some participants indicated from an early stage that this was not possible, and supplementary advice was given with respect to annealing temperatures which could be raised in order to distinguish between certain subtypes.

In general, good results were obtained for *vtx1a* in strain B2, which was correctly subtyped by all laboratories; two *vtx2c* false positives were also reported.

In general, the major problem was to distinguish between *vtx2a*, *vtx2b*, *vtx2c* and *vtx2d*. A few dedicated participants made extra efforts to collect data on the gradient calibration of PCR cyclers. Further testing in six research and reference laboratories resulted in a recommendation that annealing temperatures for the subtyping of both *vtx1* and *vtx2* to 64–66 °C should be annealed, depending on the individual PCR cyclers. A review of *vtx* nomenclature and the revised subtyping protocol was published in September 2012 [12]. The revision of the nomenclature using the prototype protocol is a major improvement in the ability to compare results from different laboratories. The differences in nomenclature make a comparison with the results from the second EQA difficult because different methods (sequencing, RFLP, etc.) were used in the second EQA, and only one prototype protocol was tested in the third VTEC EQA. However, there was a higher participation in the third VTEC EQA: 51–63 laboratories compared with 7–21 in the second EQA, and correct subtyping of *vtx* genes was generally higher: 88–100% compared with 33–100% for ECDC-funded laboratories and 67–100% compared with 30–100% for non-EU/SF laboratories.

6 Conclusions

Eighty-one laboratories from 41 countries signed up for the third VTEC EQA. Two laboratories failed to submit results. Sixty-two of the laboratories were located in one the 30 EU/EEA countries.

An average of 68% of the ECDC-funded laboratories (including four EU NRL (veterinary) laboratories), 93% of the EU NRLs, and 79% of the non-EU/self-funded laboratories were able to correctly identify the O:H serotype of the ten VTEC strains. These strains represent 41% of the total number of serotypes reported to the combined Enter-net/TESSy database. Sixty to 64% of the participants correctly identified the O group; H typing with correct result was performed by 82–100% of the participants.

Phenotypic characterisation was generally very high (above 89%) for ECDC-funded laboratories and higher than 78% for both EU NRLs and non-EU/self-funded laboratories. Phenotypic characterisation is not performed as often as genotypic characterisation. Only 40% of ECDC-funded laboratories participants perform detection of verocytotoxin production, and values were even lower for non-EU/SF laboratories (31%) and EU NRLs (5%).

Gene detection was 96–100%, except for the *eae* gene in one strain (i9), which was reported as a false negative by three laboratories, and *vtx2f* , which was not detected by seven laboratories. Improvements need to be made by non-EU/self-funded laboratories regarding the methodology used for *vtx2f*. Three ECDC-funded laboratories need to reconsider their choice of primers for the detection of the *eae* gene.

The prototype *vtx* subtyping protocol was tested by 50–95% of the participants. It showed significant sensitivity to different PCR cycler equipment, probably due to incorrect or non-comparable temperature calibration. Nevertheless, correct results ranged between 91 and 96 per cent for the subtyping of *vtx1* and between 89 and 93 per cent for *vtx2*.

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Annex 1. List of participants

Table 11. ECDC-funded participants

Institution	Laboratory	Country
Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	Bereich Humanmedizin, Institut für Medizinische Mikrobiologie und Hygiene Bereich Humanmedizin	Austria
Universitair Ziekenhuis	Dept. Microbiology	Belgium
Nicosia General Hospital	Microbiology Department (C/O Dr. Panayiota Maikanti-Charalampous)	Cyprus
National Institute of Public Health	National reference laboratory for *E.coli* and Shigella	Czech Republic
Statens Serum Institut	Foodborne bacteria and typing, Dept. of Microbiological Surveillance and Research	Denmark
HPA Centre for Infections	GI Reference Unit, Laboratory of Gastrointestinal Pathogens (GEZI)	England
Central Laboratory of Communicable Diseases for Health Protection Inspectorate	Laboratory of Communicable Diseases	Estonia
National Institute for Health and Welfare (THL)	Bacteriology Unit, SUBA, Department of Infectious Disease Surveillance and Control	Finland
Unité Biodiversité des Bactéries Pathogènes Emergentes, Institut Pasteur	Centre National de Référence des Escherichia coli et Shigella	France
Robert Koch Institute, Bereich Wernigerode	NRC *Salmonella* and other enterics	Germany
National Center for Epidemiology	'B. Johan'	Hungary
Cherry Orchard Hospital	SWAHB, Public Health Laboratory	Ireland
Istituto Superiore di Sanità	Dipartimento di Sanità Alimentare e Animale	Italy
Infectology Center of Latvia		Latvia
Surveillance et Epidémiologie des Maladies Infectieuses	Laboratoire National de Santé	Luxembourg
National Institute of Public Health and the Environment - Centre for Infectious Disease Control	Laboratory for Infectious Diseases and Perinatal Screening, Epidemiological Typing Reference Unit	Netherlands
Norwegian Institute of Public Health	Department of Foodborne Infections, Division of Infectious Disease Control Division of Infectious Disease Control	Norway
National Institute of Hygiene - National Institute of Public Health	Department of Bacteriology	Poland
Instituto Nacional de Saúde Dr. Ricardo Jorge	Laboratório Nacional de Referência de Salmonella, *E. coli* e outras Enterobacterias	Portugal
National Institute of Research-Development for Microbiology and immunology 'Cantacuzino'	Reference Laboratory for Molecular Epidemiology	Romania
National Reference Centre of Environmental Microbiology	Public Health Authority of Slovak Republic, UVZSR	Slovakia
National Institute of Public Health	Laboratory of Enteropathogenic bacteria, Department of Medical Microbiology	Slovenia
Instituto de Salud Carlos III, Centro Nacional de Microbiología	Unidad de Enterobacterias	Spain
Swedish Institute of Infectious Disease Control	Department of Bacteriology	Sweden
Refik Saydam Public Health Agency	National Reference Laboratory for Enteric Pathogens, Communicable Diseases Research Department	Turkey

Table 12. EU NRL participants

Institution	Laboratory	Country
Scientific Institute of Public Health	Direction Opérationnelle Maladies Transmissibles et Infectieuses	Belgium
Veterinary and Agrochemical Research Centre (CODA-CERVA)	Operational Directorate Bacterial Diseases	Belgium
National Diagnostic and Research Veterinary Institute		Bulgaria
Veterinary Services	Laboratory for the Control of Food of Animal Origin (LCFAO)	Cyprus
Veterinary Research Institute		Czech Republic
Veterinary and Food Laboratory		Estonia
Finnish Food Safety Authority Evira	Veterinary Bacteriology Research Unit	Finland
VetAgro Sup Campus Vétérinaire de Lyon	Unité de Microbiologie Alimentaire et Prévisionnelle (UMAP)	France
Federal Institute for Risk Assessment (BfR)	Division 'Microbial Toxins', NRL-*E. coli*	Germany
Central Agricultural Office, Food and Feed Safety Directorate	Feed Investigation National Reference Laboratory	Hungary
Department of Agriculture & Food Laboratories		Ireland
Institute of Food Safety, Animal Health and Environment 'BIOR'		Latvia
National Food and Veterinary Risk Assessment Institute	Bacteriology Unit	Lithuania
Food and Consumer Product Safety Authority		Netherlands
National Veterinary Research Institute	Department of Hygiene of food of animal origin	Poland
Laboratório Nacional de Investigação Veterinária (LNIV)	Unidade de Higiene Pública/ Microbiologia dos Alimentos	Portugal
tate Veterinary and Food Institute Dolný Kubín	Department of Molecular Biology	Slovakia
University of Ljubljana, National Veterinary Institute	Laboratory for Bacteriology and Mycology	Slovenia
Laboratorio Central de Sanidad Animal		Spain
The Spanish Food Safety and Nutrition Agency (AESAN)	Servicio de Microbiología Alimentaria	Spain

Institution	Laboratory	Country
National Veterinary Institute, SVA	Dept of Bacteriology	Sweden
National Food Administration, SLV	Microbiology Division	Sweden

Table 13. Self-funded participants

Institution	Laboratory	Country
Servicio Fisiopatogenia	Departamento de Bacteriología – Inei-Anlis 'Dr. Carlos G. Malbrán'	Argentina
Public Health Microbiology	Forensic and Scientific Services	Australia
MDU PHL – Department of Microbiology and Immunology	University of Melbourne	Australia
CSIRO Food Nutritional Sciences	Block 10 Loading Dock	Australia
Enteric and Food Microbiology Laboratory	ICDDR, B	Bangladesh
Laboratorio Central de Saude Public	Secao de Bacteriologia	Brazil
Public Health Agency of Canada	*E. coli* Laboratory, Laboratory for Foodborne Zoonoses	Canada
Head Identification and Serotyping, Enteric Disease Program	National Microbiology Laboratory	Canada
Université de Montréal	Reference Laboratory for *Escherichia coli*, GREMIP, Faculté de médecine vétérinaire	Canada
Provincial Laboratory for Public Health		Canada
Canadian Food Inspection Agency	Research and Development Section, Ottawa Laboratory (Carling)	Canada
Ontario Agency for Health Protection and Promotion	Enteric, Environmental, and Bacterial Sexually Transmitted Infections Laboratories; Public Health Laboratories	Canada
The Poultry Center of the Croatian Veterinary Institute	Feed Analysis Laboratory	Croatia
ANSES Laboratoire de Securite des alimentes	SRPI-QMA	France
Hôpital Robert Debré	Service de microbiologie Laboratoire associé au CNR *E. coli*-Shigella	France
Ecole Nationale Veterinaire de Toulouse		France
Institut für Hygiene und Umwelt	Abteilung mikrobiologischer Verbraucherschutz	Germany
Istituto Zooprofilattico Sperimentale Piemonte, Liguria e Valle d'Aosta	Laboratorio Controllo Alimenti	Italy
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche	Laboratorio Contaminanti Biologici PGCB	Italy
Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana	Dir. Op. Controllo degli Alimenti, Centro di Rif. Reg. Enterobatteri Patogeni	Italy
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale'	Laboratorio Regionale di Riferimento per Enterobatteri Patogeni (LRREP-A)	Italy
National Institute of Infectious Diseases	Department of Bacteriology	Japan
3er piso edificio de Investigación, Facultad de Medicina	Universidad Nacional Autónoma de México	Mexico
Institute for Environmental Science and Research Ltd	Enteric Reference Laboratory, ESR NCBID – Wallaceville	New Zealand
National Veterinary Institute	Section for food bacteriology and GMO	Norway
Research Institute for Tropical Medicine, Department of Health	Microbiology Department	Philippines
National Institute of Communicable Disease, a division of National Health Laboratory services	Enteric Diseases Reference Unit, Upper North Wing, SAVP Building	Republic of South Africa
Lotian University Hositals NHS Trust, Western General Hospital	Department of Clinical Microbiology	Scotland
University of Santiago de Compostela	Departament of Microbiology and Parasitology, Faculty of Veterinary	Spain
University of Zurich, Vetsuisse Faculty	Institute for food safety and hygiene	Switzerland
The Pennsylvania State University	*E. coli* Reference Center	USA
Centers for Disease Control and Prevention	National Reference Laboratory for *Escherichia coli* and Shigella, DASH, Unit 7	USA

Annex 2. Prevalence of sero-and virulence types

Table A1. Prevalence of sero-and virulence types reported to Enter-net in 2000–2006 and to TESSy 2007–2010, ranked according to reported number of human cases and combination of the *eae* and verocytotoxin genes

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
		eae	*vtx1 + vtx2*	*vtx1*	*vtx2*		*vtx1 + vtx2*	*vtx1*	*vtx2*		
1	O157:H7	+	307	11	1184	1502	20%	1%	79%	27%	*vtx2a + vtx2c*
2	O103:H2	+		609	1	610	0%	100%	0%	11%	*vtx1a*
3	O157:HNM	+	366	15	166	547	67%	3%	30%	10%	
4	O26:H11	+	42	403	57	502	8%	80%	11%	9%	
5	O91:HNM	-		254		254	0%	100%	0%	5%	
6	O145:HNM	+	9	19	126	154	6%	12%	82%	3%	
7	O111:HNM	+	47	78	3	128	37%	61%	2%	2%	
8	O91:H14	-	1	121		122	1%	99%	0%	2%	
9	O128:H2	-	84	11	19	114	74%	10%	17%	2%	
10	O26:HNM	+	15	68	13	96	16%	71%	14%	2%	
11	O146:H21	-	53	23	12	88	60%	26%	14%	2%	*vtx1c + vtx2a*
12	O113:H4	-	61	1	15	77	79%	1%	19%	1%	
13	O146:H28	-	10	1	52	63	16%	2%	83%	1%	
14	O117:H7	-		62		62	0%	100%	0%	1%	
15	O76:H19	-	20	36		56	36%	64%	0%	1%	
16	O91:HNM	-	55			55	100%	0%	0%	1%	
17	O177:HNM	+	1	25	12	38	3%	66%	32%	1%	
18	O121:H19	+	2	1	28	31	6%	3%	90%	1%	
19	O5:HNM	+	1	26		27	4%	96%	0%	0%	
20	O174:H8	-	17	5	2	24	71%	21%	8%	0%	
21	O146:HNM	-	6	5	12	23	26%	22%	52%	0%	
22	O166:H28	-	7	10	2	19	37%	53%	11%	0%	
23	O8:H19	-	1	1	15	17	6%	6%	88%	0%	
24	O91:H21	-	5	2	9	16	31%	13%	56%	0%	*vtx2d*
24	O156:H25	+		14	2	16	0%	88%	13%	0%	
25	O112:H2	-	3	11	1	15	20%	73%	7%	0%	
25	O128:HNM	-	11	2	2	15	73%	13%	13%	0%	
26	O157:H7	-	3		12	15	20%	0%	80%	0%	
27	O103:H2	-	1	13		14	7%	93%	0%	0%	
27	O115:H10	-		14		14	0%	100%	0%	0%	
28	O22:H8	-	10	1	2	13	77%	8%	15%	0%	*vtx1c + vtx2b*
29	O113:H21	-	2		10	12	17%	0%	83%	0%	
29	O174:H21	-		1	11	12	0%	8%	92%	0%	
29	O103:HNM	+	2	9	1	12	17%	75%	8%	0%	
30	O106:H18	-		10	1	11	0%	91%	9%	0%	
30	O5:HNM	-	7	4		11	64%	36%	0%	0%	
31	O181:H16	-	3	7		10	30%	70%	0%	0%	
31	O78:HNM	-	3	7		10	30%	70%	0%	0%	
31	O145:H34	+			10	10	0%	0%	100%	0%	*vtx2f*
31	O55:H7	+		6	4	10	0%	60%	40%	0%	
32	O157:HNM	-	4	1	4	9	44%	11%	44%	0%	
32	O163:H19	-	2		7	9	22%	0%	78%	0%	
32	O174:H2	-	8	1		9	89%	11%	0%	0%	
32	O178:H19	-	4		5	9	44%	0%	56%	0%	
32	O23:H15	-	3	6		9	33%	67%	0%	0%	
32	O118:H16	+		9		9	0%	100%	0%	0%	
33	O100:HNM	-			8	8	0%	0%	100%	0%	
33	O2:H6	-		1	7	8	0%	13%	88%	0%	
33	O75:H8	-	6		2	8	75%	0%	25%	0%	
33	O76:HNM	-	5	3		8	63%	38%	0%	0%	
33	O145:H28	+		6	2	8	0%	75%	25%	0%	
33	O98:HNM	+		8		8	0%	100%	0%	0%	
34	O40:H8	-		2	5	7	0%	29%	71%	0%	

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
34	O55:H12	-		7		7	0%	100%	0%	0%	
34	O8:HNM	-			7	7	0%	0%	100%	0%	
34	O87:H16	-			7	7	0%	0%	100%	0%	
35	O113:HNM	-	3		3	6	50%	0%	50%	0%	
35	O6:H10	-		6		6	0%	100%	0%	0%	
35	O77:H18	-	2	1	3	6	33%	17%	50%	0%	
35	O63:H6	+	1		5	6	17%	0%	83%	0%	
35	O80:HNM	+		3	3	6	0%	50%	50%	0%	
36	O115:HNM	-		5		5	0%	100%	0%	0%	
36	O148:H8	-		1	4	5	0%	20%	80%	0%	
36	O15:H8	-			5	5	0%	0%	100%	0%	
36	O15:HNM	-	5			5	100%	0%	0%	0%	
36	O154:H31	-		5		5	0%	100%	0%	0%	*vtx1d*
36	O156:H7	-		5		5	0%	100%	0%	0%	
36	O111:H8	+		5		5	0%	100%	0%	0%	
36	O118:HNM	+	1	4		5	20%	80%	0%	0%	
36	O177:H45	+	2	3		5	40%	60%	0%	0%	
37	O111:HNM	-	4			4	100%	0%	0%	0%	
37	O175:H16	-			4	4	0%	0%	100%	0%	
37	O178:H7	-		4		4	0%	100%	0%	0%	
37	O179:H8	-			4	4	0%	0%	100%	0%	
37	O181:H49	-	4			4	100%	0%	0%	0%	
37	O38:H26	-	4			4	100%	0%	0%	0%	
37	O7:H18	-		4		4	0%	100%	0%	0%	
37	O74:H6	-			4	4	0%	0%	100%	0%	
37	O8:H21	-			4	4	0%	0%	100%	0%	
37	O88:HNM	-	1	1	2	4	25%	25%	50%	0%	
37	O91:H10	-		1	3	4	0%	25%	75%	0%	
37	O103:H11	+	1	3		4	25%	75%	0%	0%	
37	O103:H25	+		2	2	4	0%	50%	50%	0%	
37	O156:HNM	+	1	3		4	25%	75%	0%	0%	
37	O165:H25	+			4	4	0%	0%	100%	0%	
37	O177:H11	+		4		4	0%	100%	0%	0%	
38	O101:HNM	-	1		2	3	33%	0%	67%	0%	
38	O102:H6	-			3	3	0%	0%	100%	0%	
38	O104:H4	-			3	3	0%	0%	100%	0%	
38	O110:H8	-		1	2	3	0%	33%	67%	0%	
38	O112:H18	-		3		3	0%	100%	0%	0%	
38	O112:H8	-		1	2	3	0%	33%	67%	0%	
38	O117:H-	-		3		3	0%	100%	0%	0%	
38	O14:H15	-		3		3	0%	100%	0%	0%	
38	O152:HNM	-		3		3	0%	100%	0%	0%	
38	O166:H15	-		1	2	3	0%	33%	67%	0%	
38	O174:H28	-			3	3	0%	0%	100%	0%	
38	O176:HNM	-	2		1	3	67%	0%	33%	0%	
38	O26:HNM	-	1	2		3	33%	67%	0%	0%	
38	O38:H26	-		3		3	0%	100%	0%	0%	
38	O43:H2	-	2		1	3	67%	0%	33%	0%	
38	O74:H28	-			3	3	0%	0%	100%	0%	
38	O75:HNM	-		1	2	3	0%	33%	67%	0%	
38	O78:H-	-		3		3	0%	100%	0%	0%	
38	O81:H21	-		3		3	0%	100%	0%	0%	
38	O82:H8	-	1		2	3	33%	0%	67%	0%	
38	O86:HNM	-		3		3	0%	100%	0%	0%	
38	O9:HNM	-			3	3	0%	0%	100%	0%	
38	O115:HNM	+			3	3	0%	0%	100%	0%	
38	O182:H25	+		3		3	0%	100%	0%	0%	
38	O26:H46	+		3		3	0%	100%	0%	0%	
38	O76:H19	+	1	2		3	33%	67%	0%	0%	
38	O80:H2	+			3	3	0%	0%	100%	0%	
38	O84:HNM	+		3		3	0%	100%	0%	0%	
38	O91:HNM	+		3		3	0%	100%	0%	0%	
39	O103:H18	-		2		2	0%	100%	0%	0%	
39	O103:HNM	-	2			2	100%	0%	0%	0%	
39	O104:H7	-		2		2	0%	100%	0%	0%	
39	O11:H4	-	1		1	2	50%	0%	50%	0%	

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
39	O112:H31	-		2		2	0%	100%	0%	0%	
39	O117:H8	-		2		2	0%	100%	0%	0%	
39	O118:H12	-			2	2	0%	0%	100%	0%	
39	O119:H4	-	1	1		2	50%	50%	0%	0%	
39	O138:H8	-			2	2	0%	0%	100%	0%	
39	O14:H21	-			2	2	0%	0%	100%	0%	
39	O14:H28	-	2			2	100%	0%	0%	0%	
39	O153:H21	-			2	2	0%	0%	100%	0%	
39	O156:HNM	-	1	1		2	50%	50%	0%	0%	
39	O17:H18	-			2	2	0%	0%	100%	0%	
39	O170:H7	-	2			2	100%	0%	0%	0%	
39	O174:H35	-			2	2	0%	0%	100%	0%	
39	O174:HNM	-	1	1		2	50%	50%	0%	0%	
39	O175:H21	-	1	1		2	50%	50%	0%	0%	
39	O175:H28	-			2	2	0%	0%	100%	0%	
39	O22:H1	-			2	2	0%	0%	100%	0%	
39	O22:HNM	-	2			2	100%	0%	0%	0%	
39	O26:H11	-		2		2	0%	100%	0%	0%	
39	O3:H21	-		2		2	0%	100%	0%	0%	
39	O3:HNM	-	1		1	2	50%	0%	50%	0%	
39	O55:HNM	-		2		2	0%	100%	0%	0%	
39	O6:H1	-			2	2	0%	0%	100%	0%	
39	O6:HNM	-	2			2	100%	0%	0%	0%	
39	O71:HNM	-	2			2	100%	0%	0%	0%	
39	O74:H27	-			2	2	0%	0%	100%	0%	
39	O79:H14	-		2		2	0%	100%	0%	0%	
39	O8:H14	-			2	2	0%	0%	100%	0%	
39	O8:H25	-		2		2	0%	100%	0%	0%	
39	O8:H8	-		1	1	2	0%	50%	50%	0%	
39	O8:H9	-			2	2	0%	0%	100%	0%	
39	O87:H2	-		2		2	0%	100%	0%	0%	
39	O9:H21	-			2	2	0%	0%	100%	0%	
39	O91:HNM	-			2	2	0%	0%	100%	0%	
39	O103:H7	+		2		2	0%	100%	0%	0%	
39	O111:H31	+	1	1		2	50%	50%	0%	0%	
39	O113:H6	+			2	2	0%	0%	100%	0%	
39	O119:HNM	+		1	1	2	0%	50%	50%	0%	
39	O125:H6	+			2	2	0%	0%	100%	0%	
39	O127:H7	+		2		2	0%	100%	0%	0%	
39	O15:H2	+			2	2	0%	0%	100%	0%	
39	O156:H1	+		2		2	0%	100%	0%	0%	
39	O26:H21	+		1	1	2	0%	50%	50%	0%	
39	O45:H2	+		2		2	0%	100%	0%	0%	
39	O49:HNM	+		2		2	0%	100%	0%	0%	
39	O51:H49	+			2	2	0%	0%	100%	0%	
39	O55:HNM	+		1	1	2	0%	50%	50%	0%	
39	O6:HNM	+	1	1		2	50%	50%	0%	0%	
39	O69:H11	+		2		2	0%	100%	0%	0%	
39	O70:H11	+		1	1	2	0%	50%	50%	0%	
39	O70:HNM	+	1	1		2	50%	50%	0%	0%	
39	O84:H2	+		2		2	0%	100%	0%	0%	
39	O84:H28	+		2		2	0%	100%	0%	0%	
40	O1:H12	-		1		1	0%	100%	0%	0%	
40	O1:H20	-		1		1	0%	100%	0%	0%	
40	O1:H7	-		1		1	0%	100%	0%	0%	
40	O104:H2	-			1	1	0%	0%	100%	0%	
40	O104:H21	-	1			1	100%	0%	0%	0%	
40	O105:H4	-	1			1	100%	0%	0%	0%	
40	O106:HNM	-		1		1	0%	100%	0%	0%	
40	O107:HNM	-			1	1	0%	0%	100%	0%	
40	O110:H31	-	1			1	100%	0%	0%	0%	
40	O113:H31	-	1			1	100%	0%	0%	0%	
40	O113:H40	-	1			1	100%	0%	0%	0%	
40	O114:H41	-		1		1	0%	100%	0%	0%	
40	O12:HNM	-		1		1	0%	100%	0%	0%	
40	O120:H10	-			1	1	0%	0%	100%	0%	

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
40	O123:HNM	-	1			1	100%	0%	0%	0%	
40	O126:H20	-			1	1	0%	0%	100%	0%	
40	O127:H21	-			1	1	0%	0%	100%	0%	
40	O128:H1	-			1	1	0%	0%	100%	0%	
40	O128:H31	-		1		1	0%	100%	0%	0%	
40	O132:H10	-			1	1	0%	0%	100%	0%	
40	O132:H21	-	1			1	100%	0%	0%	0%	
40	O136:H20	-		1		1	0%	100%	0%	0%	
40	O136:H6	-			1	1	0%	0%	100%	0%	
40	O136:H7	-			1	1	0%	0%	100%	0%	
40	O138:H48	-			1	1	0%	0%	100%	0%	
40	O139:H1	-			1	1	0%	0%	100%	0%	*vtx2e*
40	O145:HNM	-			1	1	0%	0%	100%	0%	
40	O146:H11	-	1			1	100%	0%	0%	0%	
40	O146:H2	-			1	1	0%	0%	100%	0%	
40	O146:H31	-	1			1	100%	0%	0%	0%	
40	O146:H32	-			1	1	0%	0%	100%	0%	
40	O146:H8	-		1		1	0%	100%	0%	0%	
40	O148:HNM	-			1	1	0%	0%	100%	0%	
40	O149:H1	-		1		1	0%	100%	0%	0%	
40	O15:H7	-		1		1	0%	100%	0%	0%	
40	O153:H16	-			1	1	0%	0%	100%	0%	
40	O153:H18	-		1		1	0%	100%	0%	0%	
40	O153:H2	-	1			1	100%	0%	0%	0%	
40	O153:H25	-	1			1	100%	0%	0%	0%	
40	O156:H21	-			1	1	0%	0%	100%	0%	
40	O156:H9	-		1		1	0%	100%	0%	0%	
40	O157:H21	-	1			1	100%	0%	0%	0%	
40	O159:HNM	-			1	1	0%	0%	100%	0%	
40	O16:H6	-		1		1	0%	100%	0%	0%	
40	O169:HNM	-		1		1	0%	100%	0%	0%	
40	O17:H41	-			1	1	0%	0%	100%	0%	
40	O17:H45	-			1	1	0%	0%	100%	0%	
40	O171:H2	-			1	1	0%	0%	100%	0%	*vtx2b + vtx2c + vtx2d*
40	O171:HNM	-			1	1	0%	0%	100%	0%	
40	O175:H7	-			1	1	0%	0%	100%	0%	
40	O176:H21	-		1		1	0%	100%	0%	0%	
40	O177:HNM	-		1		1	0%	100%	0%	0%	
40	O178:H2	-		1		1	0%	100%	0%	0%	
40	O18:H31	-			1	1	0%	0%	100%	0%	
40	O181:H40	-		1		1	0%	100%	0%	0%	
40	O181:HNM	-		1		1	0%	100%	0%	0%	
40	O2:H29	-			1	1	0%	0%	100%	0%	
40	O20:H16	-		1		1	0%	100%	0%	0%	
40	O20:HNM	-		1		1	0%	100%	0%	0%	
40	O21:H21	-			1	1	0%	0%	100%	0%	
40	O22:H11	-	1			1	100%	0%	0%	0%	
40	O23:H12	-			1	1	0%	0%	100%	0%	
40	O23:H21	-			1	1	0%	0%	100%	0%	
40	O23:H4	-		1		1	0%	100%	0%	0%	
40	O25:H7	-		1		1	0%	100%	0%	0%	
40	O28:H6	-			1	1	0%	0%	100%	0%	
40	O30:H24	-			1	1	0%	0%	100%	0%	
40	O36:HNM	-			1	1	0%	0%	100%	0%	
40	O38:H39	-			1	1	0%	0%	100%	0%	
40	O38:H49	-	1			1	100%	0%	0%	0%	
40	O4:H10	-	1			1	100%	0%	0%	0%	
40	O4:HNM	-		1		1	0%	100%	0%	0%	
40	O40:H7	-	1			1	100%	0%	0%	0%	
40	O40:HNM	-			1	1	0%	0%	100%	0%	
40	O45:H8	-		1		1	0%	100%	0%	0%	
40	O5:H19	-	1			1	100%	0%	0%	0%	
40	O51:H42	-			1	1	0%	0%	100%	0%	
40	O54:H21	-			1	1	0%	0%	100%	0%	
40	O55:H31	-		1		1	0%	100%	0%	0%	

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
40	O6:H33	-		1		1	0%	100%	0%	0%	
40	O6:H7	-			1	1	0%	0%	100%	0%	
40	O64:H31	-		1		1	0%	100%	0%	0%	
40	O65:H2	-		1		1	0%	100%	0%	0%	
40	O69:H17	-		1		1	0%	100%	0%	0%	
40	O69:HNM	-	1			1	100%	0%	0%	0%	
40	O7:H7	-			1	1	0%	0%	100%	0%	
40	O70:H7	-			1	1	0%	0%	100%	0%	
40	O70:HNM	-	1			1	100%	0%	0%	0%	
40	O73:H18	-			1	1	0%	0%	100%	0%	
40	O74:H42	-	1			1	100%	0%	0%	0%	
40	O77:H41	-			1	1	0%	0%	100%	0%	
40	O77:H45	-		1		1	0%	100%	0%	0%	
40	O79:H23	-		1		1	0%	100%	0%	0%	
40	O8:H10	-			1	1	0%	0%	100%	0%	
40	O8:H16	-			1	1	0%	0%	100%	0%	
40	O8:H31	-			1	1	0%	0%	100%	0%	
40	O87:HNM	-			1	1	0%	0%	100%	0%	
40	O88:H25	-	1			1	100%	0%	0%	0%	
40	O88:H8	-			1	1	0%	0%	100%	0%	
40	O89:H4	-			1	1	0%	0%	100%	0%	
40	O9:H9	-	1			1	100%	0%	0%	0%	
40	O90:H45	-			1	1	0%	0%	100%	0%	
40	O91:H42	-		1		1	0%	100%	0%	0%	
40	O91:H8	-	1			1	100%	0%	0%	0%	
40	O92:H18	-			1	1	0%	0%	100%	0%	
40	O92:H2	-	1			1	100%	0%	0%	0%	
40	O92:HNM	-	1			1	100%	0%	0%	0%	
40	O93:H46	-			1	1	0%	0%	100%	0%	
40	O96:H19	-	1			1	100%	0%	0%	0%	
40	O98:H14	-		1		1	0%	100%	0%	0%	
40	O102:H6	+		1		1	0%	100%	0%	0%	
40	O103:H18	+		1		1	0%	100%	0%	0%	
40	O103:H21	+		1		1	0%	100%	0%	0%	
40	O104:H7	+		1		1	0%	100%	0%	0%	
40	O111:H2	+	1			1	100%	0%	0%	0%	
40	O111:H4	+			1	1	0%	0%	100%	0%	
40	O115:H8	+			1	1	0%	0%	100%	0%	
40	O116:H2	+		1		1	0%	100%	0%	0%	
40	O117:H8	+		1		1	0%	100%	0%	0%	
40	O118:H11	+		1		1	0%	100%	0%	0%	
40	O119:H25	+		1		1	0%	100%	0%	0%	
40	O121:H21	+			1	1	0%	0%	100%	0%	
40	O121:H6	+			1	1	0%	0%	100%	0%	
40	O123:H2	+		1		1	0%	100%	0%	0%	
40	O126:H2	+		1		1	0%	100%	0%	0%	
40	O126:HNM	+			1	1	0%	0%	100%	0%	
40	O127:H40	+		1		1	0%	100%	0%	0%	
40	O128:H2	+			1	1	0%	0%	100%	0%	
40	O132:H34	+			1	1	0%	0%	100%	0%	
40	O134:H27	+		1		1	0%	100%	0%	0%	
40	O142:HNM	+			1	1	0%	0%	100%	0%	
40	O143:H11	+		1		1	0%	100%	0%	0%	
40	O145:H42	+			1	1	0%	0%	100%	0%	
40	O145:H46	+		1		1	0%	100%	0%	0%	
40	O146:HNM	+	1			1	100%	0%	0%	0%	
40	O150:HNM	+	1			1	100%	0%	0%	0%	
40	O153:H19	+		1		1	0%	100%	0%	0%	
40	O157:H2	+	1			1	100%	0%	0%	0%	
40	O158:H40	+		1		1	0%	100%	0%	0%	
40	O162:H10	+			1	1	0%	0%	100%	0%	
40	O167:H49	+	1			1	100%	0%	0%	0%	
40	O167:H9	+			1	1	0%	0%	100%	0%	
40	O17:H18	+			1	1	0%	0%	100%	0%	
40	O172:HNM	+			1	1	0%	0%	100%	0%	
40	O174:H2	+	1			1	100%	0%	0%	0%	

Ranking	O:H serotype	*eae*	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 the combined Enter-net and TESSy databases	Selected *vtx* genotype
40	O177:H25	+		1		1	0%	100%	0%	0%	
40	O177:H30	+		1		1	0%	100%	0%	0%	
40	O180:H2	+		1		1	0%	100%	0%	0%	
40	O184:H2	+		1		1	0%	100%	0%	0%	
40	O2:H6	+			1	1	0%	0%	100%	0%	
40	O20:H12	+	1			1	100%	0%	0%	0%	
40	O20:H25	+		1		1	0%	100%	0%	0%	
40	O21:H21	+		1		1	0%	100%	0%	0%	
40	O21:H8	+			1	1	0%	0%	100%	0%	
40	O25:H11	+		1		1	0%	100%	0%	0%	
40	O25:H4	+		1		1	0%	100%	0%	0%	
40	O38:H26	+		1		1	0%	100%	0%	0%	
40	O5:H11	+			1	1	0%	0%	100%	0%	
40	O6:H10	+		1		1	0%	100%	0%	0%	
40	O68:H18	+	1			1	100%	0%	0%	0%	
40	O71:H8	+	1			1	100%	0%	0%	0%	
40	O75:H55	+		1		1	0%	100%	0%	0%	
40	O80:H21	+			1	1	0%	0%	100%	0%	
40	O82:H10	+		1		1	0%	100%	0%	0%	
40	O84:H31	+		1		1	0%	100%	0%	0%	
40	O87:H2	+		1		1	0%	100%	0%	0%	
40	O87:HNM	+			1	1	0%	0%	100%	0%	
40	O91:H14	+		1		1	0%	100%	0%	0%	
40	O91:H21	+			1	1	0%	0%	100%	0%	
NA	O2:H25	-	NA	NA	NA	0	0%	0%	0%	0%	*vtx2g*

Note: Strains are only shown if complete information on O:H serotypes and eae, vtx1 *and* vtx2 *was available.*
Shaded green: types chosen for this VTEC EQA

Annex 3. Individual results

Table A2. 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	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	
L01	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L02	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L03	O91:H21	O103:H2	ONT:H21	ONT:H31	O157:H7	ONT:H25	ONT:H8	ONT:NT	O145:H34	ONT:NT	40/80
L07	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L08	O91:ND	O103:ND	O26:ND	O26:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L10	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L100	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H-	100/90
L101	ONT:ND	O103:H2	ONT:ND	ONT:ND	O157:H7	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	20/20
L11	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L12	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H-	100/90
L13	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L14	O91:ND	O103:ND	O146:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/0
L15	O91:ND	O103:ND	O146:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/0
L17	O91:H11	O103:H41	O146:H21	O154:H31	O157:H7	O2:H6	O22:H8	O139:NT	O145:H34	O134:H-	90/50
L19	O91:H21	O103:H2	O146:H21	ONT:ND	O157:H7	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:H2	40/50
L20	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L21	O121:ND	O103:ND	ONT:ND	O121:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L22	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L23	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L24	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L25	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L28	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:H7	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/20
L29	O91:ND	O103:ND	O146:H21	ONT:H31	O157:H7	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/60
L34	ONT:ND	O103:ND	O146:ND	O55:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L36	O91:ND	O103:ND	O146:ND	O154:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	100/0
L37	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L41	O91:ND	O103:ND	ONT:ND	O154:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/0
L42	O91:ND	O103:ND	O146:ND	ONT:H31	O157:H7	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/40
L45	O91:ND	O103:ND	O146:ND	ONT:ND	O157:H7	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/20
L46	O91:ND	O103:ND	O146:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	50/0
L49	O91:ND	O103:ND	O146:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	80/0
L50	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	60/0
L51	O91:ND	O111:ND	ONT:ND	O148:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	40/0
L52	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L53	ND:H21	O103:H16	ND:H-	ND:H31	O157:H7	ND:H25	ND:H8	ND:H12	ND:H34	ND:H2	20/70
L54	O91:H21	O103:H2	O146:H21	ONT:H31	O157:H7	O2:H2	ONT:H8	ONT:H1	O145:H34	ONT:H2	60/90
L55	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L56	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L57	O91:ND	O103:ND	O146:ND	O154:ND	O157:HR	O2:ND	O22:ND	ONT:ND	O145:ND	ONT:ND	80/0
L58	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L60	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H6	O22:H8	O139:H1	O145:H34	O171:H-	100/80
L61	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:H7	ONT:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	30/10
L62	O91:ND	O103:ND	O146:ND	O154:ND	O157:ND	O2:ND	O22:ND	O139:ND	O145:ND	O46:ND	90/0
L63	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L64	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H-	100/90
L65	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L66	ONT:ND	ONT:ND	ONT:ND	ONT:H7	O157:H7	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	10/10
L67	O91:H21	O103:H2	O146:H21	ONT:NT	O157:H7	O2:NT	ONT:H8	ONT:NT	O145:NT	ONT:H2	60/60
L68	O91:H21	O103:H2	O146:H21	ONT:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H-	90/90
L69	ONT:ND	ONT:ND	ONT:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	10/0
L70	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H5	O22:H8	O139:H1	O145:H34	O171:H2	100/90
L71	O91:H21	O103:H2	ONT:H21	O114:H41	O157:H7	ONT:H25	O44:H-	ONT:H56	O145:H34	O164:NT	40/60
L72	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	30/0
L73	O91:H21	O103:H2	O146:ND	O114:ND	O157:H7	O103:H25	O174:H8	ONT:ND	O145:H34	O145:ND	50/60

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score % O/H
Result	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	
L74	O91:H21	O103:H2	O146:H21	ONT:H31	O157:H7	O2:H25	O55, O22:H8	O139:H1	O145:H34	ONT:H2	70/100
L75	ONT:ND	ONT:ND	ONT:ND	ONT:ND	O157:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	20/0
L76	O91:H21	O103:H2	O146:H36	O154:H31	O157:H7	O2:H25	ONT:H8	O139:H1	O145:H34	O171:H2	90/90
L77	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:H7	O103:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	30/10
L78	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L79	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L80	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L81	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H-	O145:H34	O46:H-	90/80
L82	O91:H25	O103:H2	O146:H21	O154:H31	O157:H7	O2:H51	O22:H8	O139:H20	O145:H34	O171:H-	100/60
L84	O91:H21	O103:H2	ONT:H21	O154:H31	O157:H7	O2:H25	O22:H8	ONT:NT	O145:H34	ONT:H2	70/90
L85	ONT:ND	ONT:ND	O146:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	20/0
L86	O91:H21	O103:H2	O146:H21	ONT:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O46:H2	80/100
L87	ONT:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	30/0
L88	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L89	O91:ND	O103:ND	ONT:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	40/0
L90	O91:H21	O103:H2	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:H34	O171:H2	100/100
L91	O91:H21	ONT:H2	O146:H21	O154:H31	O157:H7	ONT:H25	O22:H8	O139:H56	O145:H34	O171:H-	80/70
L92	O91:H21	O103:H2	O146:H21	O125:H31	O157:H7	O2:H25	O22:H8	O139:H56	O55:H5	O111:H2	70/80
L93	O146:ND	ONT:ND	O146:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	O127a:ND	ONT:ND	20/0
L94	O91:ND	O103:ND	O146:ND	ONT:ND	O157:H7	O2:ND	ONT:ND	ONT:ND	O145:ND	ONT:ND	60/10
L95	O91:H21	O103:NT	O146:H21	O154:H31	O157:H7	O2:H25	O22:H8	O139:H1	O145:NT	O171:H-	100/70
L96	ONT:ND	ONT:ND	ONT:ND	ONT:ND	O157:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	ONT:ND	10/0
L97	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	ND:ND	0/0
L98	ONT:ND	O103:ND	O121:ND	O113:ND	O157:ND	O128:ND	O111:ND	O26:ND	O145:ND	ONT:ND	30/0
L99	O91:H21	O103:H2	ONT:H21	ONT:NT	O157:H7	ONT:NT	ONT:H8	ONT:NT	ONT:NT	ONT:H2	30/60

NM = non motile; ND = ND; ONT = O group reported as not typeable; NT = not typeable; HR = H rough

Table A3. Individual results for fermentation of sorbitol; all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	
L01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L100	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L12	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L17	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L19	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L22	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L24	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L28	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	
L42	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L45	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L51	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L53	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L54	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L55	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	90
L56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L57	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L61	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L62	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L63	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L64	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L65	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L66	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Positive	80
L67	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L68	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L69	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Negative	Positive	80
L70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L71	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L72	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L73	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L74	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L75	Positive	Negative	Positive	Negative	Positive	ND	ND	ND	ND	ND	100
L76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L77	Negative	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	90
L78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L79	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L81	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L82	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L84	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L86	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L87	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L89	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	70
L90	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L91	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L92	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L93	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Positive	Negative	Positive	90
L94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L95	Positive	Negative	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	100
L96	ND	ND	ND	Negative	ND	ND	ND	ND	ND	ND	10
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Results that are not in accordance with the results table are shaded grey.

Table A4. Individual results for β-glucuronidase production all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L100	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	90
L101	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L12	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L19	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L22	Positive	Positive	Positive	Positive	Negative	ND	ND	ND	ND	ND	100
L23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L24	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	80
L25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L28	Positive	Positive	Positive	Positive	Positive	ND	ND	ND	ND	ND	80
L29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L42	Positive	Positive	Positive	Positive	Negative	ND	ND	ND	ND	ND	100
L45	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L51	Positive	Positive	Positive	Positive	Negative	ND	ND	ND	ND	ND	100
L52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L53	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	90
L54	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L55	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	90
L56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L57	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	90
L61	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L64	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L65	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L67	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L71	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L73	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L74	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	90
L75	Positive	Positive	Positive	Positive	Negative	ND	ND	ND	ND	ND	100
L76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L77	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L79	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L81	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L82	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L84	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L86	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L87	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L89	Positive	ND	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	90
L90	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L91	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L92	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	100
L93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L95	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive	Positive	90
L96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

ND = Not done

Results that are not in accordance with the results table are shaded grey.

Table A5. Individual results for vero cytotoxin production all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
L01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L100	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	90
L101	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L12	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive	80
L13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L24	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L42	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L45	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L46	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
L51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L54	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L55	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L56	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive	80
L57	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L61	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L65	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L67	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L69	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	90
L70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L73	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L75	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L77	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Positive	80
L78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L79	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L81	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L82	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Negative	Positive	80
L84	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L85	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	90
L86	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L87	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L89	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L90	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L91	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L92	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L93	Positive	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Negative	Positive	50
L94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L95	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100
L96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

ND = Not done

Results that are not in accordance with the results table are shaded grey.

Table A6. Individual results for haemolysin production all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	
L01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	
L10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L100	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L12	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L22	Negative	Negative	Negative	Negative	Negative	ND	ND	ND	ND	ND	20
L23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L24	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L28	Negative	Negative	Negative	Negative	Negative	ND	ND	ND	ND	ND	20
L29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L34	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L42	Ent.	Negative	Negative	Negative	Negative	ND	ND	ND	ND	ND	40
L45	α	Negative	Negative	Negative	α	ND	ND	ND	ND	ND	20
L46	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L53	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L54	Ent.	α	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	90
L55	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L56	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Negative	90
L57	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	α	α	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	80
L61	Negative	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L63	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L64	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Negative	90
L65	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L67	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L71	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L72	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L73	Ent.	Ent.	Negative	Negative	Ent.	Negative	Ent.	α	Negative	Negative	90
L74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L75	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L77	Ent.	Negative	Negative	Negative	Ent.	Negative	Negative	Ent.	Negative	Negative	60
L78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L79	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L80	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L81	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	Negative	Negative	Negative	90
L82	α	Ent.	Negative	Negative	Ent.	Negative	Ent.	α	Negative	Negative	80
L84	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L85	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	
L86	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L87	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L89	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L90	Negative	Negative	Negative	Negative	Negative	Negative	Negative	α	Negative	Negative	50
L91	Ent.	α	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	90
L92	Ent.	Ent.	Negative	Ent.	Ent.	Negative	Ent.	α	Negative	Negative	100
L93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L94	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Ent.	Negative	Negative	40
L95	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Ent.: Entero haemolysin. α: alpha haemolysin

Results that are not in accordance with the results table are shaded grey.

Table A7. Individual results for gene detection of *vtx1*, *vtx2* and *eae* all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
	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-eae*
Result	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	
L01	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L02	-, +, -	+, -, +	-, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	80-100-100
L03	-, +, -	+, -, +	-, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, +	-, -, +	-, +, -	90-90-90
L07	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L08	-, +, -	+, -, +	+, +, -	-, -, -	-, +, +	ND	ND	ND	ND	ND	80-100-100
L10	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L100	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L101	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L11	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L12	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L13	-, +, +	+, -, +	+, +, +	+, -, +	-, +, +	ND	ND	ND	ND	ND	100-100-40
L14	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L15	-, +, -	+, -, -	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100-100-80
L17	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	+, +, -	-, +, +	-, +, -	90-100-100
L19	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L20	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L21	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L22	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L23	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L24	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L25	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L28	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L29	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L34	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L36	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L37	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L41	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L42	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L45	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L46	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L49	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L50	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L51	-, +, +	+, -, +	+, +, +	+, -, +	-, +, +	ND	ND	ND	ND	ND	100-100-40
L52	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L53	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L54	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, -, +	-, +, -	100-90-100

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
	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-eae*
Result	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	
L55	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L57	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L58	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L60	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L61	-, +, +	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100-100-90
L62	-, +, -	+, -, +	+, +, -	-, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	90-100-100
L63	-, +, -	+, -, +	+, +, -	-, -, -	-, +, +	-, -, -	+, +, -	-, -, -	-, -, +	-, +, -	90-70-100
L64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L65	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L66	-, +, -	+, -, +	-, +, -	+, -, -	-, +, +	+, +, -	+, +, -	-, +, -	-, +, +	-, +, -	80-100-100
L67	-, +, -	+, -, +	+, +, +	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100-100-90
L68	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L69	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L70	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L71	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L72	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L73	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L74	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L75	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	ND	ND	ND	ND	ND	100
L76	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, -, +	-, +, -	100-90-100
L77	-, +, -	+, -, -	+, +, -	+, -, -	-, +, -	-, +, -	+, +, -	-, -, -	-, -, -	-, +, -	100-80-70
L78	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L79	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L80	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L81	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L82	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L84	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L85	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, -, +	-, +, -	100-90-100
L86	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, -	-, +, -	100-100-90
L87	-, +, -	+, -, +	-, +, -	+, -, -	+, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	80-100-100
L88	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L89	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L90	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L91	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, -, +	-, +, -	100-90-100
L92	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L93	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L94	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L95	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100
L96	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	+, -, +	-, +, -	90-90-100
L97	-, +, ND	+, -, ND	+, +, ND	+, -, ND	-, +, ND	-, +, ND	+, +, ND	-, +, ND	-, +, ND	-, +, ND	100-100-0
L98	-, +, -	+, -, -	+, -, -	+, -, -	-, +, -	-, +, -	-, +, -	-, +, -	-, +, -	-, +, -	90-90-70
L99	-, +, -	+, -, +	+, +, -	+, -, -	-, +, +	-, +, -	+, +, -	-, +, -	-, +, +	-, +, -	100

*If all three genes (*vtx1, vtx2 *and* eae*) are correctly determined, only one score of 100% is given.*

Results that are not in accordance with the results table are shaded grey.

Table A8. 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	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	
L01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L02	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L03	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L07	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	
L101	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L17	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L21	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L22	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L23	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L24	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	alpha-hlyA	negative	negative	90
L25	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L28	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L29	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L34	ehxA	ehxA	negative	ehxA	ehxA	negative	negative	negative	negative	negative	90
L36	hlyA + (PCR)	hlyA + (PCR)	negative	hlyA + (PCR)	hlyA + (PCR)	negative	negative	negative	negative	negative	90
L37	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L41	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L42	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L45	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L46	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L50	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L52	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L53	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L54	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L55	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L57	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L61	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L62	hlyA	hlyA	negative	hlyA	hlyA	negative	negative	negative	negative	negative	100
L63	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L65	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L66	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L67	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L68	hly+	hly+	negative	hly+	hly+	negative	hly+	negative	negative	negative	100
L69	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L70	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L71	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	ehxA	90
L72	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L73	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	alfa-hly	negative	negative	90
L74	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L75	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L76	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L77	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L78	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L79	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L80	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L81	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L82	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L84	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	alfa-hlyA	negative	negative	90
L85	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L86	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L87	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	
L88	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L89	ehxA	ehxA	negative	ehxA	negative	negative	ehxA	negative	negative	negative	90
L90	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	hlyA	negative	negative	90
L91	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L92	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L93	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L94	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L95	ehxA	negative	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	90
L96	ehxA	ehxA	negative	ehxA	ehxA	negative	ehxA	negative	negative	negative	100
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0

Results submitted as Alfa-hly were considered as incorrect because the α-haemolysin is different from enterohaemolysin. Results submitted as hlyA and hly were considered as enterohaemolysin and hence correct but reflect the fact that the nomenclature is not universal. Results that are not in accordance with the results table are shaded grey.

Table A9. Individual results for *vtx* suptyping all participants

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	vtx1a	vtx1c, vtx2a	*vtx1d*	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	
L01	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	ND	ND	ND	ND	ND	80
L02	vtx1a	vtx2a	vtx1c, vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	40
L03	vtx1a	vtx2a, vtx2b	vtx1d	vtx2a, vtx2c, vtx2d	vtx2d	vtx2g	vtx1c, vtx2d	vtx2e	vtx2f	vtx2c, vtx2d	70
L07	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L08	vtx1a	vtx1a, vtx1c, vtx2a	ND	vtx2a, vtx2c, vtx2d	vtx2a, vtx2c, vtx2d	ND	ND	ND	ND	ND	20
L10	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	ND	ND	ND	ND	ND	60
L100	vtx1a	vtx1c, vtx2a, vtx2b, vtx2c	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L101	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L11	vtx1a	vtx1c, vtx2a	vtx1d	vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L12	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L13	vtx1a	vtx1a, vtx1c	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	40
L14	vtx1a	vtx1c, vtx2a, vtx2d	vtx1c, vtx1d	vtx2a, vtx2c	vtx2c	ND	ND	ND	ND	ND	40
L15	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	60
L17	vtx1a	vtx1c, vtx2b vtx2e	vtx1d	vtx2a, vtx2c	vtx2a, vtx2b, vtx2d	vtx2g	vtx1c, vtx2b	vtx1c, vtx2b	vtx2f	vtx2b, vtx2c, vtx2d	70
L19	vtx1a	vtx1c, vtx2a, vtx2c	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	vtx2g	vtx1c	vtx2e	vtx2f	vtx2c, vtx2d	80
L20	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L21	vtx1a, vtx1c	vtx1a, vtx1c	vtx1a, vtx1c, vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	0
L22	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L23	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L24	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L25	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L28	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	ND	ND	ND	ND	ND	80
L29	ND	vtx2b	ND	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	40
L34	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L36	vtx1a	vtx1c, vtx2a, vtx2c	vtx1d	vtx2a, vtx2c	vtx2a, vtx2c, vtx2d	ND	ND	ND	ND	ND	60
L37	vtx1a, vtx1c	vtx1c, vtx2a, vtx2c	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	60
L41	vtx1a	vtx1c, vtx2a, vtx2c	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	40

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	vtx1a	vtx1c, vtx2a	*vtx1d*	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	
L42	vtx1a	vtx1c, vtx2a, vtx2d	vtx1a,vtx1c,vtx1d	vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	20
L45	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	100
L46	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L49	vtx1a	vtx1c, vtx2a,vtx2c	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	ND	40
L50	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c, vtx2d	vtx2d	ND	ND	ND	ND	ND	80
L51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L52	vtx1a	vtx1c, vtx2a	vtx1a, vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	ND	ND	ND	ND	ND	60
L53	vtx1a	vtx1c, vtx2a,vtx2b	vtx1d	vtx2a, vtx2c	vtx2a, vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L54	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L55	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L56	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L57	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	70
L58	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L60	vtx1a	vtx1c, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c	90
L61	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L62	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L63	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L64	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L65	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	100
L66	vtx1a	vtx2a, vtx2b	vtx1c	vtx2a, vtx2c	vtx2c, vtx2d	vtx1d, vtx2g	vtx1c, vtx2b, vtx2f	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	50
L67	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L68	ND	ND	ND	ND	ND	vtx2g	ND	ND	vtx2f	ND	100
L69	vtx1a	vtx1c, vtx2a	vtx1d	vtx2a,-	vtx2a	ND	ND	vtx2a	ND	vtx2a	43
L70	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L71	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L72	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c, vtx2d	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L73	ND	ND	ND	ND	ND	ND	vtx2d	vtx2e	vtx2f	ND	67
L74	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L75	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	ND	ND	ND	ND	ND	80
L76	ND	vtx2c, vtx2d	ND	vtx2c, vtx2d	vtx2c, vtx2d	ND	ND	ND	ND	vtx2c, vtx2d	0
L77	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L78	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L79	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L80	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	70
L81	ND	ND	ND	ND	ND	ND	ND	ND	vtx2f	ND	100
L82	vtx1a	vtx1c, vtx2a, vtx2b, vtx2g	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L84	vtx1a	vtx1c, vtx2a, vtx2b, vtx2c	vtx1d	vtx2a, vtx2c	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	80
L85	vtx1a	vtx1c, vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c	90
L86	vtx1a	vtx1c, vtx2a, vtx2b	vtx1d	vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L87	vtx1a	-,vtx2a, vtx2b	vtx1d	vtx1c, vtx2a, vtx2c	vtx2c	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	70
L88	vtx1a	vtx1c, vtx2a,vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L89	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L90	vtx1a	vtx1c, vtx2a,vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90

Lab no.	Strain no.										
	AA1	BB2	CC3	DD4	EE5	FF6	GG7	HH8	ii9	JJ10	Score %
Result	vtx1a	vtx1c, vtx2a	*vtx1d*	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	
L91	ND	ND	ND	ND	ND	ND	ND	ND	vtx2f	ND	100
L92	vtx1a	vtx1c, vtx2a,vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L93	vtx1a	vtx1a, vtx2b	vtx1c	vtx2a, vtx2c	vtx2a, vtx2c	vtx2g	vtx1a, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, -	50
L94	vtx1a	vtx1c, vtx2a,vtx2b	vtx1d	vtx2a, vtx2c	vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	90
L95	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L96	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0
L98	vtx1a	*vtx1c*, -	vtx1d	vtx2a, -	vtx2d	vtx2b	vtx2g	vtx2e	vtx2f	-, vtx2c, -	60
L99	vtx1a	vtx1c, vtx2a,vtx2b, vtx2c	vtx1d	vtx2a, vtx2c, vtx2d	vtx2c, vtx2d	vtx2g	vtx1c, vtx2b	vtx2e	vtx2f	vtx2b, vtx2c, vtx2d	70

Scores are given for the combined subtyping of vtx1 *and* vtx2*.*

Results that are not in accordance with the results table are shaded grey.