



5th external quality assurance (EQA-5) scheme for typing of VTEC in 2013-2014

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ECDC contracts for 4 years

ECDC framework contracts with the overall purpose of

- Performing surveillance of foodborne infections based on isolate characteristics (subtyping) → outbreak detection
- Ensure that comparable methods are used in all countries
 - Pathogens covered:
 - Salmonella
 - Listeria
 - Vero cytotoxin-producing E. coli



EQA-5 includes the following methods

- molecular typing: Pulsed Field Gel Electrophoresis (PFGE)
- serotyping O:H
- virulence determination:
 - genotyping of VTEC virulence genes: vtx1, vtx2, eae, aaiC and aggR
 - subtyping of vtx1 (vtx1a, vtx1c and vtx1d) and vtx2 (vtx2a to vtx2g)
 - phenotypic testing
 - production of Verocytotoxin/Shiga toxin
 - production of Extended Spectrum Beta Lactamases (ESBL)
 - production of β-glucuronidase
 - production of enterohaemolysin
 - fermentation of sorbitol



29 Participants enrolled

Austria **Belgium Bulgaria** Cyprus **Czech Republic** Denmark Estonia Finland France Germany Greece Hungary Iceland Ireland Italy

Latvia Lithuania Luxembourg **Netherlands** Norway Poland Portugal Romania Serbia Slovenia **Spain** Sweden Turkey **United Kingdom**

Submission of results



Form 1: Submission of results in the EQA-5 VTEC 2013-2014 PFGE, Serotyping, Phenotyping

Please fill in the form to submit your EQA-5 PFGE, serotyping and phenotypic data. Genotyping and subtyping results can be submitted by using form 2. Any comments can be written at the end of the form.

Let us know if something is not working properly.

Contact ecoli.eqa@ssi.dk or phone +45 3268 8341

Please note that all mandatory results (marked*) must be filled in before you can continue.

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QA provider		Form 1: Submission of results in the EQA-5 VTEC 2013-2014 PFGE, Serotyping, Phenotyping
	STATENS	*Required
	SERUM	INFORMATION
	INSTITUT	Name of the participating laboratory *
Continue »		Funded * ECDC funded Self-funded
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	Report Abuse - Terms of Service - Additional Terms	Laboratory ID: *
		Email address - to control the submitted data *
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Form 2: Submission of results in the EQA-5 VTEC 2013-2014 Genotyping and Subtyping

"Required

vtx subtyping

Strain 6 (Sub)

Strain 7 (Sub)

0

0

0

0

vtx1 (vtx1a, vtx1c and vtx1d) *

		vtx1a	vtx1c	vtx1d	vtx1a and vtx1c	vtx1a and vtx1d	vtx1c and vtx1d
Strain 1 (Sub)	0	0	0	0	0	0	0
Strain 2 (Sub)	0	0	0	0	0	0	0
Strain 3 (Sub)	0	0	0	0	0	0	0
Strain 4 (Sub)	0	0	0	0	0	0	0
Strain 5 (Sub)	0	0	0	0	0	0	0
Strain 6 (Sub)	0	0	0	0	0	0	0
Strain 7 (Sub)	0	0	0	0	0	0	0
Strain 8 (Sub)	0	0	0	0	0	0	0
Strain 9 (Sub)	0	0	0	0	0	0	0
Strain 10 (Sub)	0	0	0	0	0	0	0

Form 1: Submission of results in the EQA-5 VTEC 2013-2014 PFGE, Serotyping, Phenotyping

* Required

O Group Serotyping

ND	^	pup)"
01		ough: Orough, NT: NON-typable (if you only use a limited panel to analyse O-group
02		negative, please use NT as the result).
03		
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023		ough: Orough, NT: NON-typable (if you only use a limited namel to analyse O-group
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025		negauve, piease use ivit as the result.
026		

vtx2 (vtx2a, vtx2b, vtx2c, vtx2d, vtx2e, vtx2f and vtx2g) *

Please note the scrollbar below the strains - to select all possible combinations

vtx2a vb vtx2a vtx2b vtx2c vtx2d vtx2e vtx2f vtx2g and a vtx2b vb Strain 1 (Sub) 0 Strain 2 (Sub) 0 0 Strain 3 (Sub) 0 0 Strain 4 (Sub) 0 0 0 0 Strain 5 (Sub)

0

0

0

0

Form 1: Submission of results in the EQA-5 VTEC 2013-2014 PFGE, Serotyping, Phenotyping

"Required

SUBMITTED PFGE





PFGE Submission

- Tiff file (Quality of gel)
 - Please remember to use a correct tiff format
 - (not Raw data Gel Doc, not 16 bit -only 8 bit)

- XML export (both Quality of gel and evaluation of analysis in BioNumerics)
 - Please make sure your tiff file is included in the export, and if sent additionally – please make sure that it is exactly the same as used in the analysis in Bionumerics

Certificate and Evaluation





EVALUATION REPORT

STATENS SERUM

INSTITUT

EXTERNAL QUALITY ASSESSMENT SCHEME FOR SHIGATOXIN/VEROCYTOTOXIN–PRODUCING ESCHERICHIA COLI (STEC/VTEC) MAY 2014

FOODBORN INFECTIONS STATENS SERUM INSTITUT DENMARK

Dear Eva Møller Nielsen

In this evaluation report your individual results and scores are presented for the fifth international external quality assessment scheme (EQA) for VTEC funded by the European Food- and Waterborne Diseases and Zoonoses Network, European Centre for Disease Prevention and Control (ECDC).

Please find enclosed with this document your own submitted results and your scores and comments. Please study the results carefully and do not hesitate to contact us if you have any further question or suggestions.

Thank you very much for your participation. Your participation in this EQA programme will improve and maintain international harmonisation of typing of VTEC. Later this year, a short report on the results from this EQA programme will be published on the ECDC website.

Please find your results (enclosed):

- Pulsed Field Gel Electrophoresis
- Serotyping
- Genotyping
- Phenotyping

Yours sincerely,

EQA team Denmark

EQA-administrator STATENS SERUM INSTITUT T +45 3268 8341 | <u>E ecoll.eqa@bssl.dk</u> |Artiller/vej 5 | DK-2300 Copenhagen S | Denmark|



Page 1 of



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Service Servic

European Food- and Waterborne Diseases and Zoonoses network

STATENS SERUM INSTITUT

Certificate2.jpg

CERTIFICATE OF PARTICIPATION awarded to

FOODBORN INFECTIONS STATENS SERUM INSTITUT



to attest completion of

EXTERNAL QUALITY ASSESSMENT SCHEME FOR SHIGATOXIN/VEROCYTOTOXIN-PRODUCING *ESCHERICHIA COLI* (STEC/VTEC) 2013-2014

Covering the following:

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Pulsed Field Gel Electrophoresis - Gel Quality
 Pulsed Field Gel Electrophoresis - Gel analysis
 (BloNumerics)
 · Serotyping H type

Detection of *vbl*, *vbl* and *eae* Verocytotoxin/Shiga toxin
 Detection of *aggR* and *vbl* genes
 Subtyping of *vbl* and *vbl* genes
 Beta-glucuronidase
 Solutiol

Organised by: European Centre for Disease Prevention and Control Johan Giesecke, Chief Scientist Performed under ECDC/2012/039 contract by: Statens Serum Institut Eva Møller Nielsen, Head, Unit of foodborne infections

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KK11	9 3870 3155	078	H type	+ Vero Cell a	ESBL prod.	+ ⁺ Haemolysi	+ + Beta-glucu prod.	+ + Sorbitol fe	+ - eae gene	+ + ehx4 gene	+ ' vtx1 gene	· · ·	- vtx1a	vtx Subtyp	+ + aggR	+	aatA	Additional v genes
KK11 LL12 MM13	2 3870 3155 3485	078 0111 026	H type	+ + Vero Cell a	ESBL prod.	+ + Haemolysi	+ + Beta-glucu prod.	+ + + Sorbitol fe	- + + +	· + · <i>ehx4</i> gene	+ + +		- vbcla vbcla	· · · · ·	- +	- + + aaiC	aatA	Additional v genes
KK11 LL12 MM13 NN14	2 3870 3155 3485 3232	078 0111 026 0103	H2 H1 H11 H2	+ + + Vero Cell a	ESBL prod.	+ + + Haemolysi	+ + + Beta-glucu prod.	+ + + Sorbitol fe	+ + + + + - +	+ + + + ehx4 gene	+ + + + +		- vbcla vbcla	· · · ·	+	+ + +	aatA	Additional v genes
KK11 LL12 MM13 NN14 O015	ý 3870 3155 3485 3232 3390	078 0111 026 0103 055	H2 H- H11 H2 H7	+ + + + Vero Cell a	ESBL prod.	· + · + · Haemolysi	+ + + + Beta-glucu	- + + + Sorbitol fe	+ + + + + + + + + + + + + + + + + +	· + · + · ehx4 gene	+ + + + + + + + + + + + + + + + + + +		- vbcla vbcla vbcla vbcla	· · · ·	266e + - -		aatA	Additional v genes
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Pos.: Positive, Neg. Negative.

* Incorrect result or a gene is missing.

Other additional virulence genes will be described in the ECDC report.

Page 5 of 6



etA:

aatA:

Gene abbreviations

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

ehxA: CVD419. Plasmid encoded O157-enterohaemolysin.

- vtx1: NTP705. Verotoxin1; Almost identical with the Shiga toxin.
- vtx2: DEP28. Verotoxin2; Variants exist. Approx. 60% homology to vtx1.
- aggR: Gene encoding the master regulator in Enteroaggregative E. coli.
- aaiC: Chromosomal gene marker for Enteroaggregative E. coli.

G119. Heat labile enterotoxin (LT). Almost identical to cholera toxin. PCR fragment. The gene encodes the dispersin (aap) transporter protein, which is a good plasmid marker for Enteroaggregative *E. coli*.

VTEC & DEC – 10 STRAINS



RESULTS TABLE: Characteristics of strains

Strains/method	O group	H type	Vero cell assay	ESBL production	Haemolysin production	Beta-glucuronidase production	Sorbitol fermentation	eae gene	ehxA gene	vtx1 gene	vtx2 gene	vtx Subtypes			Additional virulence genes	Pathogenic group
KK11	078 ^{a)}	H2	-	-	-	+	+	-	-	-	-				aggR aaiC aatA	EAggEC
LL12	0111	H- /H8	+	-	+	+	+	+	+	+	-	vtx1a				STEC/VTEC
MM13	O26	H11	+	-	-	+	+	+	-	+	-	vtx1a				STEC/VTEC
NN14	O103	H2	+	-	+	+	+	+	+	+	-	vtx1a				STEC/VTEC
0015	O55	H7	+	-	-	+	-	+	-	+	-	vtx1a				STEC/VTEC
PP16	O121 ^{a)}	H19	+	-	+	+	+	+	+	-	+		vtx2a			STEC/VTEC
QQ17	0157	H7	+	-	+	-	-	+	+	-	+		vtx2a	vtx2c		STEC/VTEC
RR18	O 91	H14	+	-	+	+	+	-	+	-	+		vtx2b			STEC/VTEC
SS19	0166	H15	+	+	-	+	+	-	-	-	+		vtx2d		eltA	STEC/VTEC- ETEC
TT20	0145	H34	+	-	-	+	-	+	-	-	+		vtx2f			STEC/VTEC

a) Lactose negative



Excellent PFGE gel with high scores in all 7 parameters

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PFGE RESULTS EQA-4&5



First year

Second year



Figure 1: Results of the grading of the gels for the parameters Image Acquisition and Running Conditions (a) and Bands (b). The results from both first and second EQA are shown as average scores (in percentages) for all participating laboratories in categories: 'poor', 'fair'- 'excellent'.



PFGE RESULTS EQA-4&5



Figure 2: Results of the grading of the gels for the parameters 'Normalization' (a) and 'Band assignment' (b). The results of both first and second EQA are shown as average scores (in percentages) for all participating laboratories in categories: 'poor', 'fair' -'excellent'.

O grouping – 26 participant's scores



_	
Cc	orrect
11:	100%
3:	90%
3:	80%
2:	70%
2:	60%
4:	50%
1:	10%

Incorrect result

Strains included from the

	Strain, Lab	/ 0i	, 19	34	80	88	94	100	108	114	123	124	125	127
	KK11	078	078	078	078	078	078	078	078	078	078	NT	078	078
	LL12	011	1 011	1 0111	011	1 0111	0111	0111	0111	0111	0111	0111	0111	0111
	MM13	026	026	O26	026	O26	026	O26	O26	026	O26	O26	O26	O26
	NN14	O103	3 010	3 0103	3 O102	3 NT	O128	O103	O103	0103	O103	O103	O103	O103
	0015	055	055	055	055	NT	055	NT	055	055	055	055	055	055
	PP16	012	1 012	1 012	012	1 NT	NT	0121	0121	0121	0121	0121	NT	0121
	QQ17	015	7 015	7 0157	7 015	7 0157	0157	0157	0157	0157	0157	0157	0157	0157
	RR18	091	091	091	091	032	0114	091	091	091	091	NT	NT	091
	SS19	016	6 016	6 0166	5 NT	0110	NT	NT	O166	0166	O166	NT	0166	O166
	TT20	014	5 014	5 014	5 014	5 014	5 NT	0145	0145	0145	0145	0145	0145	0145
	128	129	130	131	132	133	134	135	136	137	138	139	153	222
	NT	078	NT	078	ND	NT	078	078	078	078	NT	078	NT	0119
	0111	0111	0111	0111	ND	0111	0111	0111	0111	0111	0111	0111	0111	0111
	O26	026	026	026	ND	026	O26	026	026	026	026	026	026	026
	O103	O103	O103	O103	ND	O103	O103	O103	0103	0103	O103	0103	0103	O103
	NT	055	055	055	ND	NT	055	055	055	055	055	055	NT	055
	0121	0121	NT	0121	ND	NT	0121	0121	0121	0124	0121	0121	0121	0121
	0157	0157	0157	0157	0157	0157	0157	0157	0157	0157	0157	0157	0157	0157
	NT	091	091	091	ND	NT	091	091	091	091	NT	091	NT	091
	NT	0166	NT	O166	ND	NT	NT	0166	0166	0166	NT	0166	055	NT
EQA-4	NT	0145	0145	0145	ND	0145	0145	0145	0145	0145	0103	0145	0145	0145

H typing – 19 participant's scores



	Strain Labor	/	Original	1	9	34	80	94	100	108	114	123
	KK11		H2	н	2	H2	H2	NT	ND	H2	H2	H2
	LL12		H-/H8	н	-	H-	H8	NT	ND	H8	H-	H8
	MM13		H11	н	11	H11	H11	NT	ND	H11	H11	H11
	NN14		H2	Н	2	H2	H2	NT	ND	H2	H2	H2
	0015		H7	н	7	H7	H7	NT	ND	H7	HS	H7
	PP16		H19	н	19	H19	H19	NT	ND	H19	H19	H19
	QQ17		H7	н	7	H7						
	RR18		H14	н	14	H14	NT	NT	ND	H14	H14	H14
	SS19		H15	Н	15	H15	NT	NT	ND	H15	H15	H15
	TT20		H34	Н	34	H34	NT	NT	ND	H34	H28	H34
6 0												
200	124	125	128	129		131	134	135	136	137	138	139
961	NT	NT	ND	H2		H2	H2	H2	H2	H2	ND	H2
el.	H-	NT	ND	H8		H8	NT	H8	H8	H8	ND	H8
	NT	NT	ND	H11		H11	H11	H11	H11	H11	ND	H11
	NT	NT	ND	H2		H2	H2	H2	H2	H2	ND	H2
	H7	NT	ND	H7		H7						
	NT	NT	ND	H19		H19	H19	H19	H19	H19	ND	H19
	H7	H7	H7	H7		H7						
	NT	NT	ND	H14		H14	H14	H14	H14	H14	ND	H14
	NT	NT	ND	H15		H15	H15	H15	H15	H15	ND	H15
	NT	NT	ND	H34		Н34	H34	H34	H34	H34	H7	H34

10: 100% 1: 90%

Correct

- 1: 80%
- 1: 70%
- 1: 30%
- 1: 20%
- 4: 10%

Incorrect result

Progress? O:H typing Comparison between EQA-4 and EQA-5





■eae vtx1 ■vtx2 ■ehxA

vtx subtyping results determined by 20/22 participants in EQA-4/EQA-5



The subtypes and maximum of correct results

vtx subtyping results determined by 20/22 participants in EQA-4/EQA-5



The subtypes and maximum of correct results



Other virulence genes

Strain No	False positive results (n)	agaR	aaiC	aatA	eltA
KK11		20	16	6	
LL12	aaaR(1)				
MM13					
NN14					
MM15					
PP16					
QQ17	aaaR(1)				
RR18					
SS19					13
TT20	aaaR(1)				



Phenotypic detection



participation



- Full O:H serotyping
 β-glucuronidase
- haemolysin
- Verocytotoxin
- Fermentation of sorbitol
- eae
- vtx1
- vtx2
- vtx subtyping
- ehxA gene
- ESBL
- aggR
- aaiC

EQA-5 66% 52% 48% 28% 90% 90% 97% 97% 76% 66% 62% **69%** 55%



Conclusions



O:H serotype correctly
β-glucuronidase
haemolysin
Verocytotoxin
Fermentation of sorbitol
ESBL

Genotypic detection

(4th)

(93%)

(89%)

(80%)

(87%)

(95%)

(100%)

EQA-5

69%

90%

94%

86%

97%

100%

(94%) 98% eae (96%) vtx1 subtyping: 98% 98% (89%) (97%) vtx2 subtyping: 94% (87%) 94% (99%) 98% ehxA (100%) aggR (20/17 participants) 100% (65%) aaiC (20/17 participants) 80% ETEC gene elt 65% (89%)



questions

- Is this the relevant number of tests?
 - Additions
 - Deductions
- These data can (and should!) be included in the molecular surveillance database?