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Results of the 6th joint PT on VTEC and *vtx* genes typing

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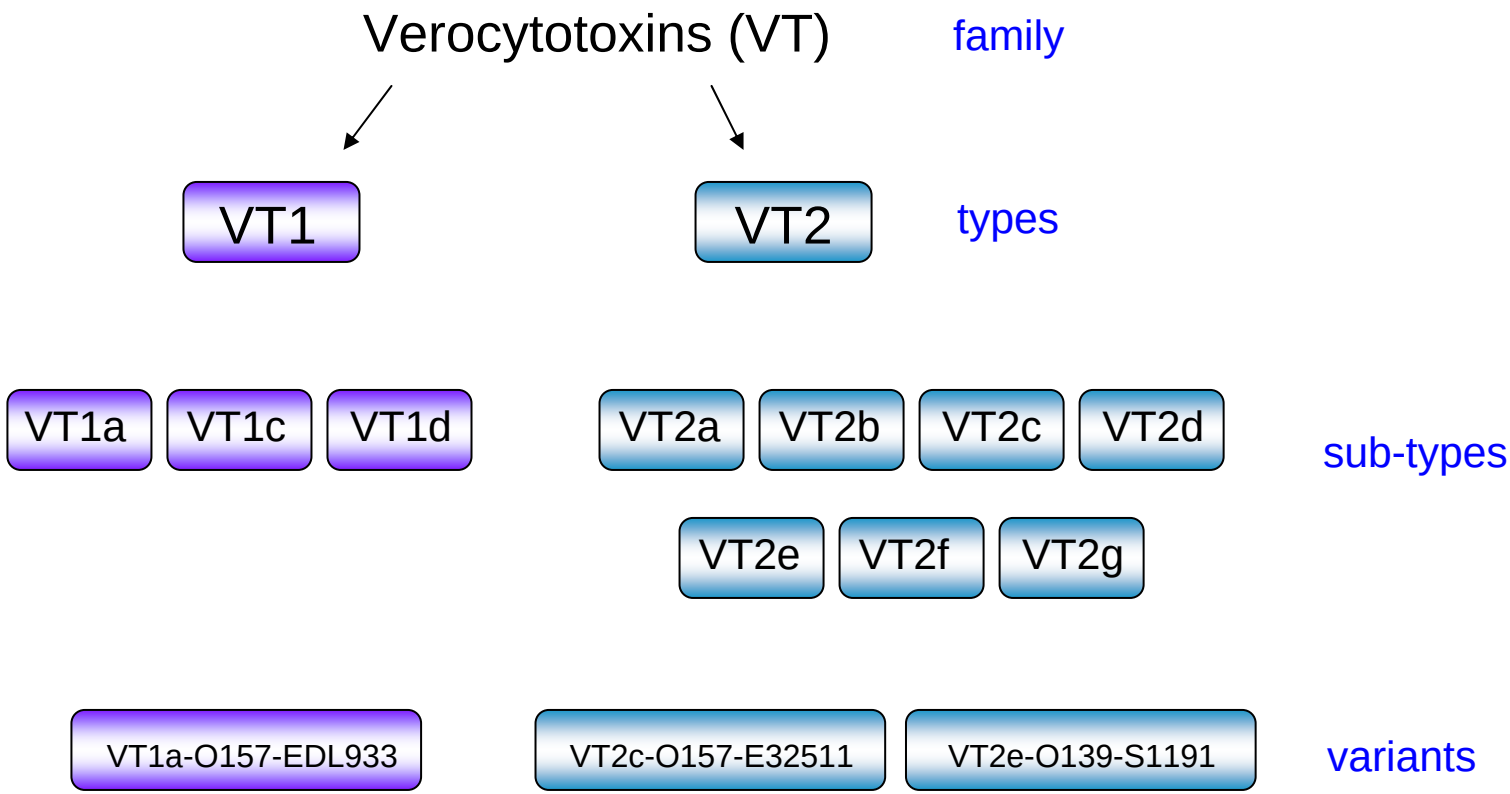
Conducted jointly with the network of medical National Reference Laboratories for VTEC referring to the European Center for Disease Prevention and Control

Objective of the 6th PT: VTEC characterization and *vtx* genes subtyping

A total of 80 labs participated
NRLs EU-RL network (882/04)
ECDC-FWD network
Other laboratories

Verocytotoxins classification



Aim of the study:

✓Assess the proficiency of NRLs in VTEC strains characterisation and typing

Identification of main virulence genes: *vtx1* group, *vtx2* group, *eae*
Identification of the O antigens of test strains (top eleven serogroups)

✓Assess the performance of a recently developed PCR-based method for VT-coding genes subtyping

Six *vtx* subtypes were selected for the interlaboratory study, those mostly involved in severe human infections
vtx1 group: *vtx1a*, *vtx1c*, *vtx1d*
vtx2 group: *vtx2a*, *vtx2c*, *vtx2d*

Participants: 30 NRLs from 25 EU MS, Croatia, Norway and Switzerland

Characteristics of the five *E. coli* sent to the laboratories

Strain	Serogroup	*vtx1* group gene (subtype)	*vtx2* group gene (subtype)	*eae* (intimin) gene
1	O103	+ (*vtx1a*)	-	+
2	O146	+ (*vtx1c*)	+ (*vtx2a*)	-
3	O154	+ (*vtx1d*)	-	-
4	O157	-	+ (*vtx2a* + *vtx2c*)	+
5	O91	-	+ (*vtx2d*)	-

Subtyping protocol, agreed with SSI, was provided to the NRLs, and a set of control strains was sent together with the test strains

Bacterial cultures were seeded in soft-agar

Samples were labelled with randomly generated numerical codes

The results were submitted on-line

Results (I): NRLs performance

The NRL performance was evaluated in respect with:

- ✓detection of *vtx*1 and *vtx2* genes group and *eae* (altogether)
- ✓identification of the O-group (within the expected panel of 11 serogroups - O157, O26, O103, O111, O145, O91, O121, O113, O128, O55, O146)

For each of the item the NRL performance was evaluated by calculating:

- ✓Agreement (Cohen's Kappa, values > 0.75 "excellent" agreement, values between 0.45 and 0.75 "good" agreement and values <0.45 "poor" agreement)
- ✓Sensitivity
- ✓Specificity

Detection of the main virulence genes

NRL	Detection of genes in:																
	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5				
	vtx1	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*	*vtx1*	*vtx2*	*eae*		
True value	+	-	+	+	+	-	+	-	-	-							
L01																	
L02				-													
L03				-													
L08							-										
L10																	
L12																	
L14																	
L15			-														
L17																	
L19																	
L20																	
L21																	
L22																	
L23																	
L24																	
L25																	
L28																	
L29																	
L34																	
L36																	
L37																	
L41																	
L42																	
L45																	
L46																	
L49																	
L50																	
L51						+			+								+
L75																	

One NRL received the samples but couldn't perform the PT - excluded from analysis

24 NRLs correctly identified the presence/absence of all the target genes in the test samples.

Three NRLs failed to detect the presence of *vtx1* gene in one sample.

One NRL failed to detect the *eae* gene in one sample an another one reported three false positive results for *eae*

Detection of the main virulence genes: agreement (Cohen's Kappa)

NRL	K values		
	K value	95% lower limit	95% upper limit
L01	1	0.49	1
L02	0.87	0.37	1
L03	0.87	0.37	1
L08	0.87	0.37	1
L10	1	0.49	1
L12	1	0.49	1
L14	1	0.49	1
L15	0.87	0.37	1
L17	1	0.49	1
L19	1	0.49	1
L20	1	0.49	1
L21	1	0.49	1
L22	1	0.49	1
L23	1	0.49	1
L24	1	0.49	1
L25	1	0.49	1
L28	1	0.49	1
L29	1	0.49	1
L34	1	0.49	1
L36	1	0.49	1
L37	1	0.49	1
L41	1	0.49	1
L42	1	0.49	1
L45	1	0.49	1
L46	1	0.49	1
L49	1	0.49	1
L50	1	0.49	1
L51	0.59	0.13	1
L75	1	0.49	1
Overall	0.97	0.88	1

The overall agreement between the results reported by the 29 NRLs and the true values of the samples was considered excellent, as well as the single agreements of all but one the NRLs

Detection of the main virulence genes: sensitivity and specificity

	Sensitivity (Se) and Specificity (Sp) for each NRL									
NRL	**L01**	**L02**	**L03**	**L08**	**L10**	**L12**	**L14**	**L15**	**L17**	**L19**
Se	100	87,5	87,5	87,5	100	100	100	87,5	100	100
Sp	100	100	100	100	100	100	100	100	100	100
NRL	**L20**	**L21**	**L22**	**L23**	**L24**	**L25**	**L28**	**L29**	**L34**	**L36**
Se	100	100	100	100	100	100	100	100	100	100
Sp	100	100	100	100	100	100	100	100	100	100
NRL	**L37**	**L41**	**L42**	**L45**	**L46**	**L49**	**L50**	**L51**	**L75**	
Se	100	100	100	100	100	100	100	100	100	
Sp	100	100	100	100	100	100	100	57,1	100	

Results (II): performance of *vtx* subtyping protocol - subtyping of *vtx1*

27 NRLs performed *vtx1* subtyping - one NRL used an alternative method thus not included in the analysis.

✓21/27 NRLs correctly identified the vtx1subtypes

✓6 labs reported a total of 11 errors

NRL	Detection of the gene subtype in:								
	Sample 1			Sample 2			Sample 3		
	vtx1a	*vtx1c*	*vtx1d*	*vtx1a*	*vtx1c*	*vtx1d*	*vtx1a*	*vtx1c*	*vtx1d*
True Value	+	-	-	-	+	-	-	-	+
L01									
L02								+	
L03									
L08				+					-
L10									
L12									
L14								+	
L15									
L17									
L19									
L20									
L21		+		+			+	+	
L22									
L23									
L24									
L25									
L28									
L29									
L34									
L36									
L37		+							
L41									
L42							+	+	
L45									
L46									
L49									
L50									
L51									
L75									

Performance characteristics of the method for *vtx1* subtyping

The performance characteristics have been evaluated on the basis of the **24 non-outlier NRLs** - those who did not report errors in PCR detection of the main virulence genes

Analyte	Performance characteristics		
	Sensitivity	Specificity	Accuracy
***vtx1a* gene**	**100 %**	**93.5 %**	**95.7 %**
***vtx1c* gene**	**100 %**	**89.1 %**	**92.8 %**
***vtx1d* gene**	**100 %**	**100 %**	**100 %**
Overall typing	**100 %** (95 %CI 100 % - 100 %)	**94.2 %** (95 %CI 90.3 % - 98.1 %)	**96.1 %** (95 %CI % 93.5 – 98.7 %)

Results (III): performance of *vtx* subtyping protocol - subtyping of *vtx2*

28 NRLs performed *vtx2* subtyping

✓10/28 NRLs correctly identified the vtx2 subtypes

✓18 labs reported a total of 37 errors (32 false positive)

NRL	Detection of the gene subtype in:								
	Sample 2			Sample 4			Sample 5		
	vtx2a	*vtx2c*	*vtx2d*	*vtx2a*	*vtx2c*	*vtx2d*	*vtx2a*	*vtx2c*	*vtx2d*
True Value	+	-	-	+	+	-	-	-	+
L01								+	
L02						+		+	
L03						+			
L08						+	+	+	
L10								+	
L12									
L14			+					+	-
L15						+		+	
L17	-						+		
L19		+						+	
L20									
L21	-					+		+	
L22									
L23									
L24									
L25									
L28								+	
L29	-								
L34									
L36		+					+	+	
L37		+							
L41		+				+		+	
L42			+	-		+		+	
L45									
L46									
L49		+				+		+	
L50						+			
L51									
L75									

Performance characteristics of the method for *vtx2* subtyping

Analyte	Performance characteristics		
	Sensitivity	**Specificity**	**Accuracy**
***Vtx2a* gene**	**93.3 %**	**90.9 %**	**92.5 %**
***Vtx2c* gene**	**100 %**	**68.9 %**	**79.1 %**
***Vtx2d* gene**	**95.5 %**	**84.8 %**	**88.1 %**
Overall typing	**95.5 %** (95 % CI 90.3 % - 99.3 %)	**79.5 %** (95 % CI 72.8 % - 87.2 %)	**86.6 %** (95 % CI 82.0 % - 91.1 %)

Results (IV): Identification of O-groups

Availability of reagents to the detect the 11 serogroups

Number (%) of NRLs potentially able to identify serogroup:										
O157	**O26**	**O103**	**O145**	**O111**	**O91**	**O121**	**O55**	**O128**	**O113**	**O146**
29	29	29	29	28	27	21	20	17	16	14
(100 %)	(100 %)	(100 %)	(100 %)	(97 %)	(93 %)	(72 %)	(69 %)	(59 %)	(55 %)	(48 %)

The performance was evaluated on the basis of this indication

Serogroup identification

NRL	Serogroup identification in:				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
True Value	O103	O146	O154 (O N.T.)	O157	O91
L01	O103	O N.T.	O N.T.	O157	O91
L02	O103	O N.T.	O N.T.	O157	O N.T.
L03	O103	O N.T.	O N.T.	O157	O91
L08	O103	O26	O26	O157	O91
L10	O103	O N.T.	O N.T.	O157	O91
L12	O103	O146	O154	O157	O91
L14	O103	O146	O N.T.	O157	O91
L15	O103	O146	O N.T.	O157	O91
L17	O103	O146	O154	O157	O91
L19	O103	O146	O N.T.	O157	O91
L20	O103	O N.T.	O N.T.	O157	O N.T.
L21	O103	O N.T.	O121	O157	O121
L22	O103	O N.T.	O N.T.	O157	O91
L23	O103	O N.T.	O N.T.	O157	O91
L24	O103	O146	O154	O157	O91
L25	O103	O N.T.	O N.T.	O157	O91
L28	O103	O N.T.	O N.T.	O157	O91
L29	O103	O146	O N.T.	O157	O91
L34	O103	O146	O55	O157	O N.T.
L36	O103	O146	O154	O157	O91
L37	O103	O N.T.	O N.T.	O157	O91
L41	O103	O N.T.	O154	O157	O91
L42	O103	O146	O N.T.	O157	O91
L45	O103	O146	O N.T.	O157	O91
L46	O103	O146	O N.T.	O157	O91
L49	O103	O146	O N.T.	O157	O91
L50	O103	O N.T.	O N.T.	O157	O91
L51	O111	O N.T.	O N.T. *	O157	O91
L75	O N.T.	O N.T.	O N.T.	O157	O N.T.

Sample 3 consisted in a VTEC O154 not comprised among the top 11

✓23/29 NRLs correctly identified all the serogroups they declared to be able to detect

✓6 labs reported a total of 11 errors

Serogroup identification: Analytical Performance of NRLs

NRL	K values		
	K value	95 % lower limit	95 % upper limit
L01	1,00	0,71	1,00
L02	1,00	0,61	1,00
L03	1,00	0,78	1,00
L08	0,60	0,03	1,00
L10	1,00	0,71	1,00
L12	1,00	0,81	1,00
L14	1,00	0,74	1,00
L15	1,00	0,72	1,00
L17	1,00	0,81	1,00
L19	0,88	0,69	1,00
L20	1,00	0,60	1,00
L21	0,52	0,20	0,85
L22	1,00	0,60	1,00
L23	1,00	0,69	1,00
L24	1,00	0,81	1,00
L25	1,00	0,64	1,00
L28	1,00	0,67	1,00
L29	1,00	0,69	1,00
L34	0,73	0,45	1,00
L36	1,00	0,74	1,00
L37	1,00	0,69	1,00
L41	1,00	0,71	1,00
L42	1,00	0,71	1,00
L45	1,00	0,74	1,00
L46	1,00	0,81	1,00
L49	1,00	0,72	1,00
L50	0,85	0,59	1,00
L51	0,64	0,33	0,95
L75	0,48	0,25	0,72
Overall	0,93	0,88	0,99

Sensitivity (Se) and Specificity (Sp) for each NRL										
NRL	L01	L02	L03	L08	L10	L12	L14	L15	L17	L19
Se	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %
Sp	100 %	100 %	100 %	95 %	100 %	100 %	100 %	100 %	100 %	100 %
NRL	L20	L21	L22	L23	L24	L25	L28	L29	L34	L36
Se	100 %	67 %	100 %	100 %	100 %	100 %	100 %	100 %	75 %	100 %
Sp	100 %	94 %	100 %	100 %	100 %	100 %	100 %	100 %	98 %	100 %
NRL	L37	L41	L42	L45	L46	L49	L50	L51	L75	
Se	100 %	100 %	100 %	100 %	100 %	100 %	75 %	67 %	33 %	
Sp	100 %	100 %	100 %	100 %	100 %	100 %	100 %	97 %	100 %	

Capability of the NRL network to identify the serogroups of the test strains

NRL	Serogroup identification in:				
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
True Value	O103	O146	O154	O157	O91
L01					
L02					
L03					
L08		O26	O26		
L10					
L12					
L14					
L15					
L17					
L19					
L20					
L21			O121		O121
L22					
L23					
L24					
L25					
L28					
L29					
L34			O55		O N.T.
L36					
L37					
L41					
L42					
L45					
L46					
L49					
L50		O N.T.			
L51	O111		O148		
L75	O N.T.				O N.T.

Concluding remarks:

The present study was conducted jointly with the network of medical National Reference Laboratories for VTEC referring to the ECDC. This represents an important achievement in the light of harmonizing the typing methods used by both the NRL networks

Evaluation of NRL proficiency

✓ 24 NRLs (83%) correctly identified the presence/absence of the main virulence genes (*vtx1* group, *vtx2* group, *eae*) in all the test samples.

✓ 23 NRLs (79 %) typed correctly all the serogroups that they had claimed to be able to identify.

- all the NRLs identified correctly the O157 strain included among the test samples
- all but two detected the O103 strain
- O91 was identified by 24/27 NRLs that had claimed to be able to identify this O-group.

Performance of the *vtx* subtyping method

✓The performance parameters of the proposed subtyping method were calculated using the data from **24 non-outlier NRLs**, defined as those who did not report errors in the PCR detection of the main virulence genes.

✓Sensitivity, specificity and accuracy were satisfactory for *vtx1* subtyping (100 %, 94.2 %, 96.1 %, respectively) **but not for *vtx2* subtyping (95.5 %, 79.5 %, 86.6 %, respectively), which will require further adjustment.**

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Thank you for your attention!