

Inter-laboratory study on VTEC identification and typing - 2007



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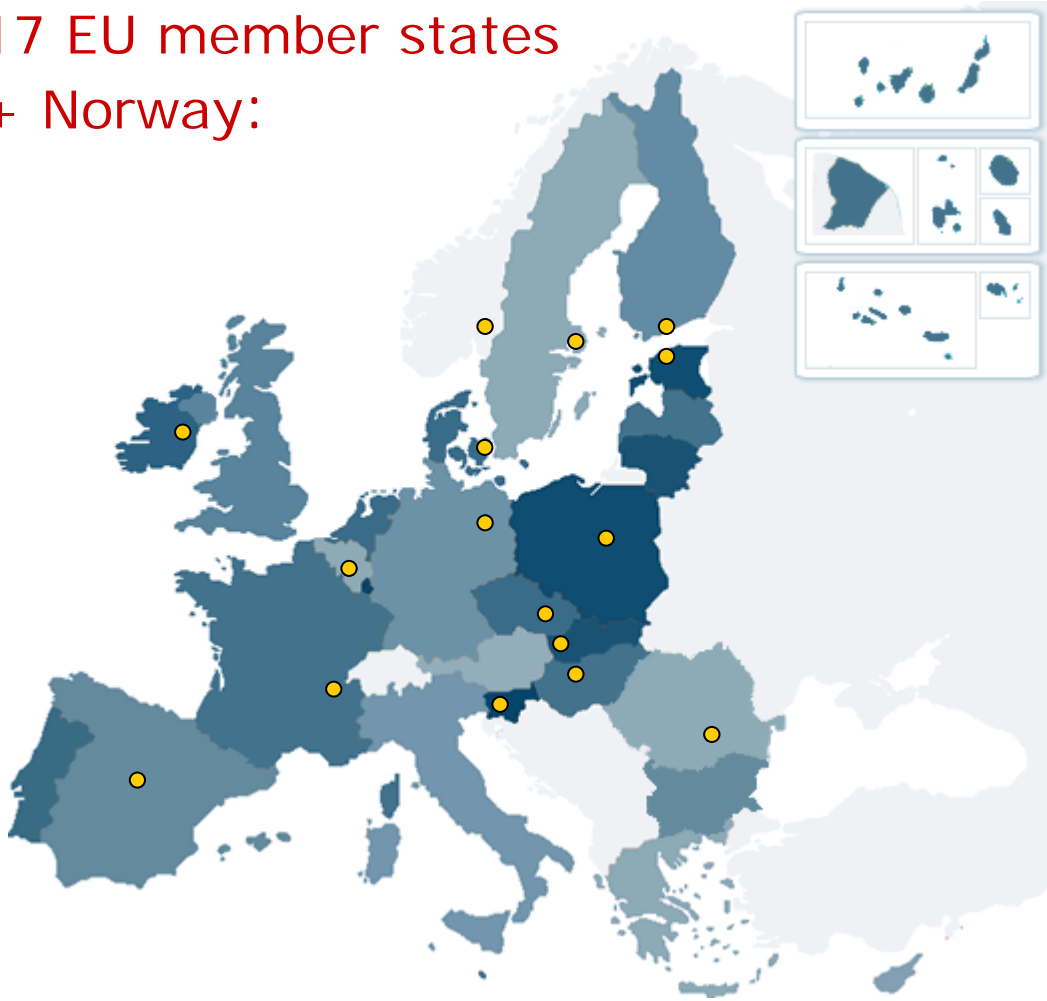
Inter-laboratory study on VTEC identification and typing – 2007: aim

Evaluation of THE NRLS capability to identify:

- the most common pathogenic serogroups
- VT production
- *vtx1* gene
- *vtx2* gene
- *eae* gene (intimin-coding)

Materials and Methods - 1

21 NRLs from
17 EU member states
+ Norway:



- Belgium (2)
- Czech Republic
- Denmark
- Estonia
- Finland (2)
- France
- Germany
- Hungary (2)
- Ireland
- Norway
- Poland
- Romania
- Slovakia
- Slovenia
- Spain
- Sweden (2)
- The Netherlands

Materials and Methods - 2

- 5 strains of *E. coli* tested for:
O serogroup, VT production, presence of *vtx1*, *vtx2* and *eae* encoding genes
- Tests were performed with methods available in each NRL

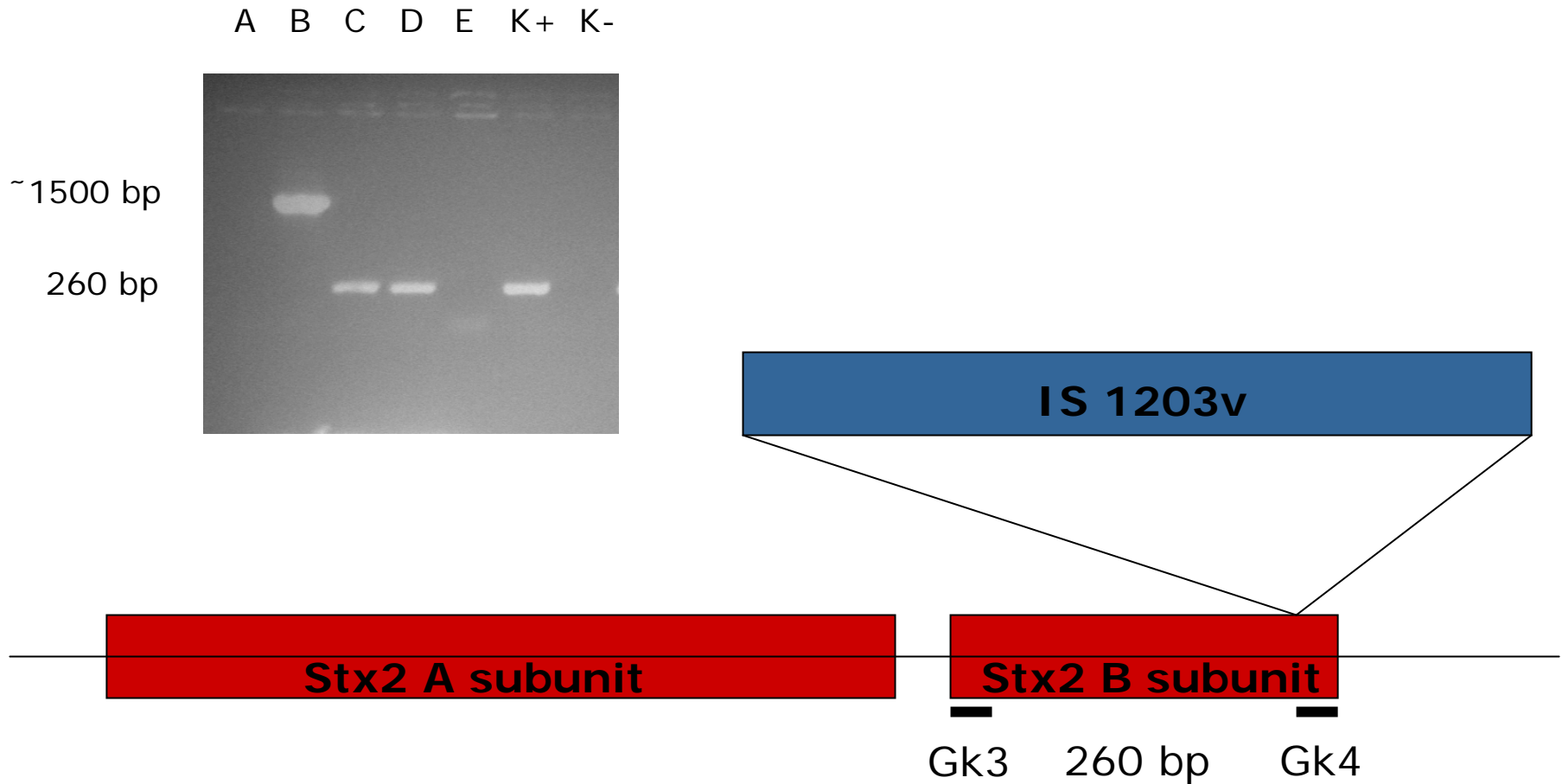
<i>strain</i>	<i>serogroup</i>	<i>VT</i>	<i>vtx1</i>	<i>vtx2</i>	<i>eae</i>
A07	O111	-	-	-	+
B07	O103	-	-	+ (*)	+
C07	O157	+	+	+	+
D07	O26	+	+	+	+
E07	O145	+	+	-	+

Materials and Methods - vtx2 identification

Primers used:

LP43/LP44	A subunit	(Cebula et al. 1995)
Mk1/Mk2	A subunit	(Karch and Meyer 1989)
Stx2 fwd/rev	A subunit	(Paton et al. 1998)
StxII /F/R	A subunit	(China et al. 1996)
VT2F/VT2R/VT2P	A subunit	(Nielsen and Andersen 2003)
GK3/GK4	B subunit	(Russmann et al. 1995)
5'II/3'II	A subunit	(Brian et al. 1992)
VT2F/R/P	A subunit	No reference given
Vtx2-F/R	A subunit	No reference given

Materials and Methods - vtx2 identification-Strain B07



Materials and Methods- vtx2 identification

Primers used:

Result assignement criterium

LP43/LP44	A subunit	(Cebula et al. 1995)
Mk1/Mk2	A subunit	(Karch and Meyer 1989)
Stx2 fwd/rev	A subunit	(Paton et al. 1998)
StxII /F/R	A subunit	(China et al. 1996)
VT2F/VT2R/VT2P	A subunit	(Nielsen and Andersen 2003)
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VT2F/R/P	A subunit	No reference given
Vtx2-F/R	A subunit	No reference given

Materials and Methods – 3

Analysis of the results

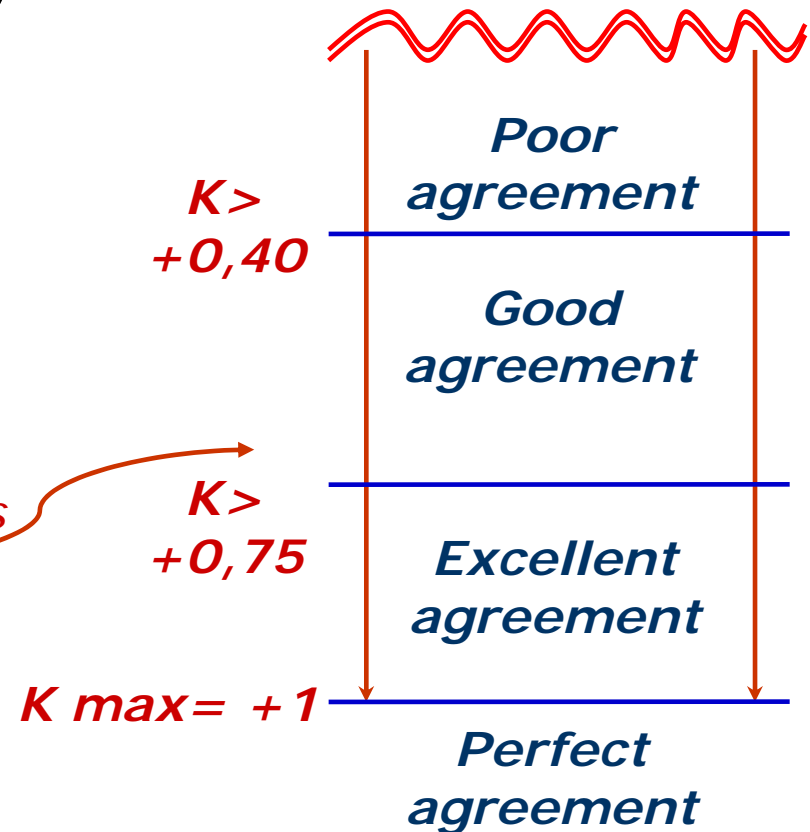
- **Agreement** of results with the true values (Gold Standard): **K test** (95% C.I.)

- **Kappa test.** Cohen's kappa is a measure of association between two measurements of the same item.

- K test estimates the level of agreement **beyond chance**

- How to interpret K value:

Fleiss J.L. (Statistical methods for rates and proportions, 1981)



- **Accuracy:** **Sensibility and Specificity** (95% C.I.)

Results

403 tests

9 false results (2,2%)

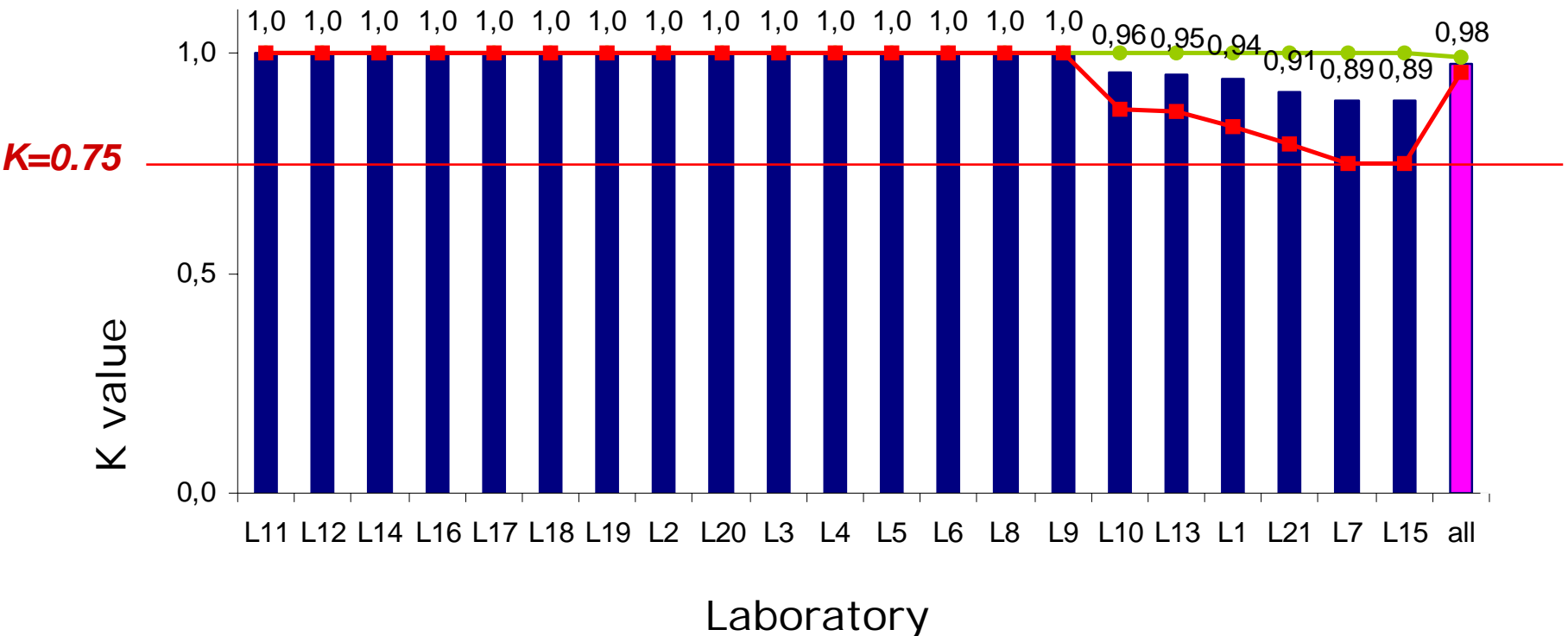
	<i>no. of test</i>	<i>no. of Labs</i>	<i>%</i>
	25 (all)	5	24%
	20-24	8	38%
	15-19	6	29%
	10-14	1	5%
	5-9	1	5%

<i>test</i>	<i>serotyping</i>					<i>VT detection</i>					<i>vtx1 detection</i>					<i>vtx2 detection</i>					<i>eae detection</i>				
	A07 O111	B07 O103	C07 O157	D07 O26	E07 O145	A07 VT-	B07 VT-	C07 VT+	D07 VT+	E07 VT+	A07 vtx1-	B07 vtx1-	C07 vtx1+	D07 vtx1+	E07 vtx1+	A07 vtx2-	B07 vtx2+ (*)	C07 vtx2+	D07 vtx2+	E07 vtx2-	A07 eae+	B07 eae+	C07 eae+	D07 eae+	E07 eae+
L1	t	t	t	t	t	t	false	t	t	t	t	t	t	t	t	t	t	t	t	t	-	-	-	-	-
L2	-	-	-	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L3	-	-	-	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	-	-	-	-	-
L4	t	t	t	t	t	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L5	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L6	t	-	t	t	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L7	t	t	t	t	t	-	-	-	-	-	t	t	t	t	false	t	false	t	t	t	t	t	t	t	t
L8	-	-	-	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L9	t	t	t	t	t	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L10	t	false	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L11	t	t	t	t	t	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L12	t	t	t	t	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L13	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t
L14	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L15	t	false	t	t	false	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L16	t	t	t	t	t	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L17	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L18	-	-	-	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L19	-	-	t	-	-	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L20	t	t	t	t	t	-	-	-	-	-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
L21	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	false	t

Results 1 – Overall agreement

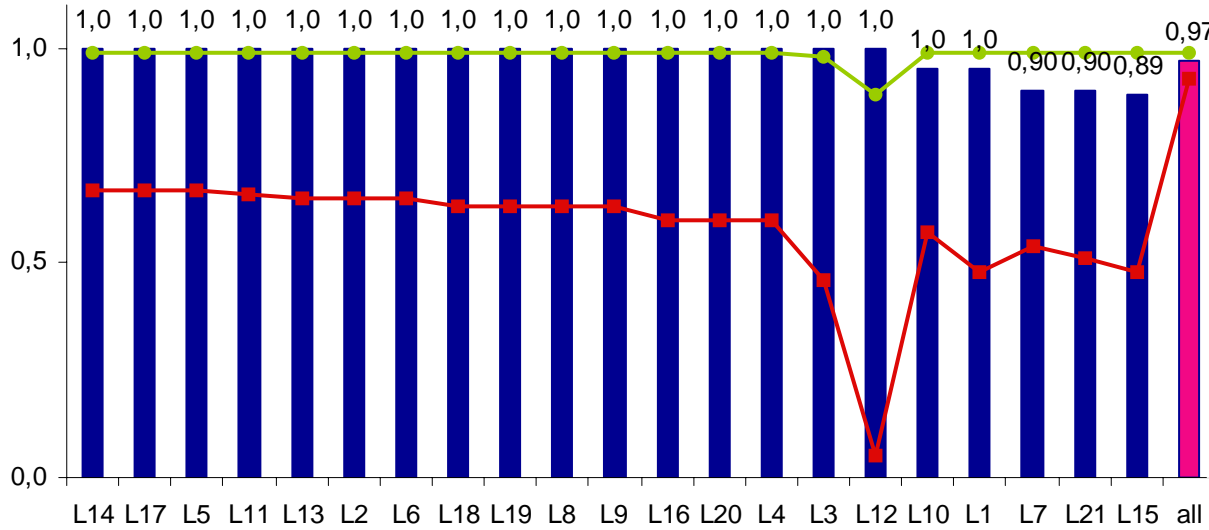
- All the labs had excellent agreement with the gold standard
- 15 labs had a perfect agreement

Overall K (agreement)



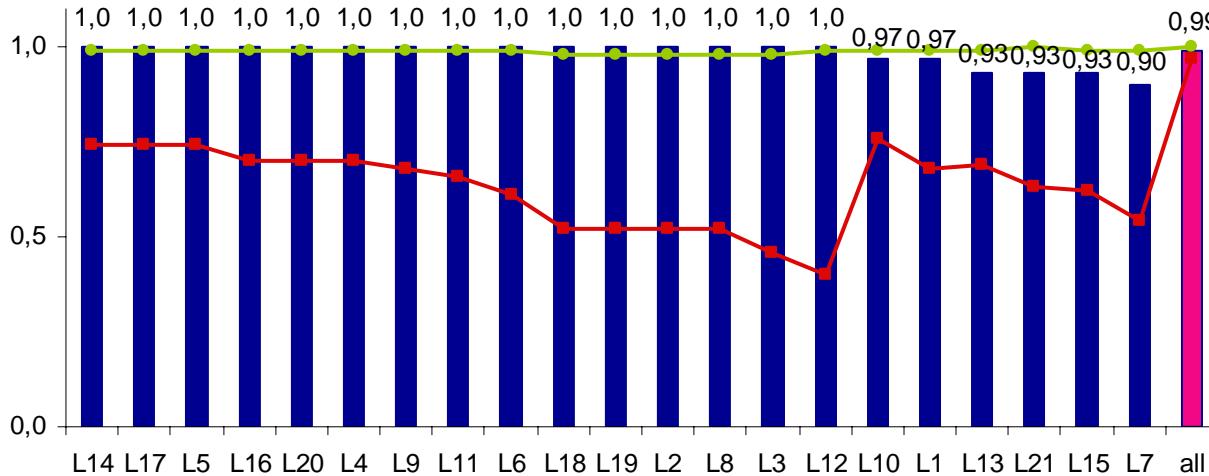
Results 2 – Overall Se and Sp

Overall sensibility



Overall **Sensibility**:
 0.97
 (95% CI: 0.93 – 0.99)

Overall specificity



Overall **Specificity**:
 0.99
 (95% CI: 0.97 – 1.00)

Results 3 – Serogroup

test Strain Labs	serotyping				
	A07 O111	B07 O103	C07 O157	D07 O26	E07 O145
L1	t	t	t	t	t
L2	-	-	-	-	-
L3	-	-	-	-	-
L4	t	t	t	t	t
L5	t	t	t	t	t
L6	t	-	t	t	-
L7	t	t	t	t	t
L8	-	-	-	-	-
L9	t	t	t	t	t
L10	t	false	t	t	t
L11	t	t	t	t	t
L12	t	t	t	t	t
L13	-	t	t	t	t
L14	t	t	t	t	t
L15	t	false	t	t	false
L16	t	t	t	t	t
L17	t	t	t	t	t
L18	-	-	-	-	-
L19	-	-	t	-	-
L20	t	t	t	t	t
L21	t	t	t	t	t

Methods: **Slide agglutination**

Number of laboratories: **17 (81%)**

Serogroups of the strains:

O111, O103, O157, O26, O145

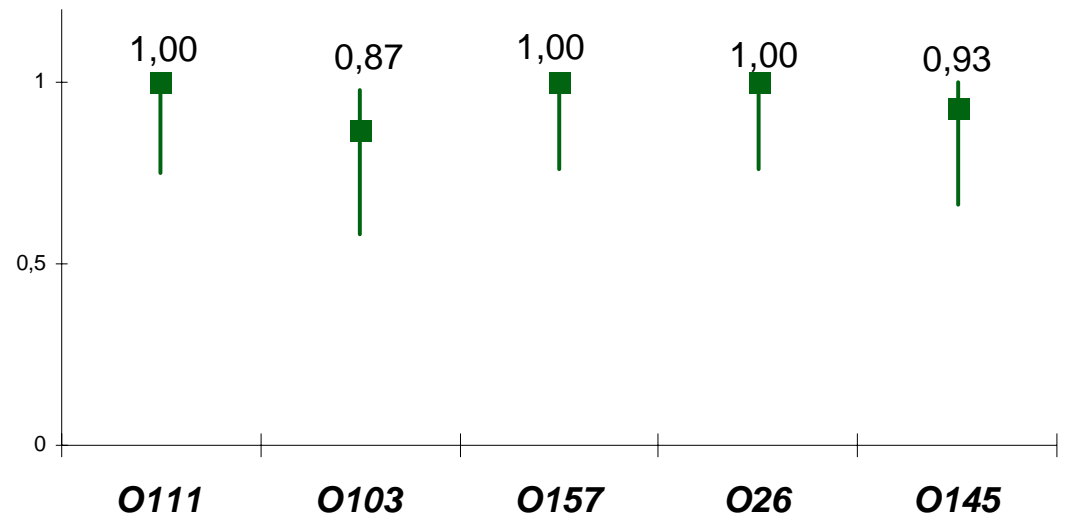
- **O111, O157 and O26** strains were correctly identified from all the labs
- 2 labs failed to identify **O103** (O111 and O NT)
- 1 lab failed to identify **O145** (O111)

Overall:

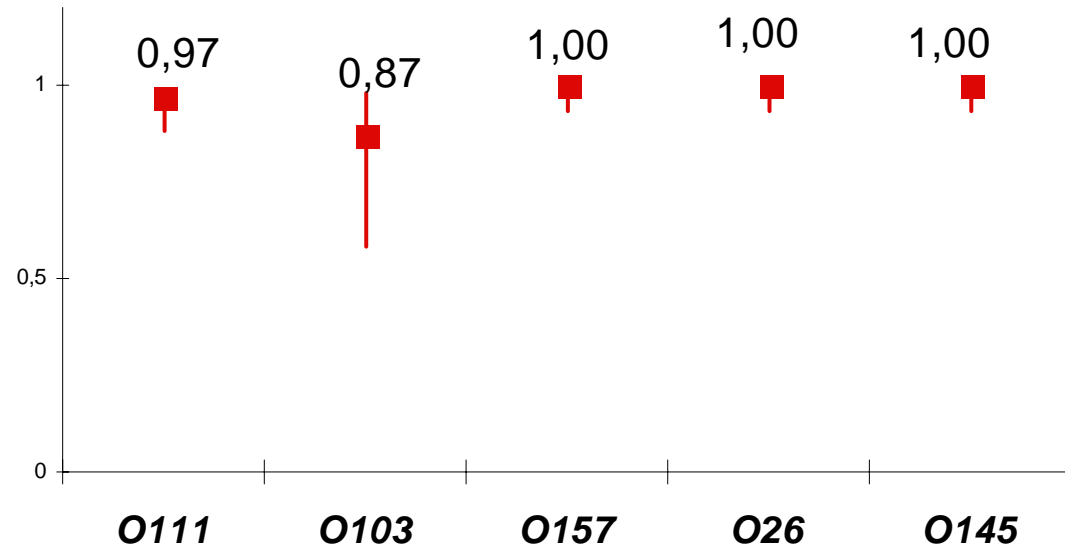
- Agreement (K): **0.95 (CI: 0.86 – 1)**
- Sensibility: **0.96 (CI: 0.86 – 1)**
- Specificity: **0.99 (CI: 0.96 – 1)**

Results 4 – Sensibility and Specificity for serogroups

Sensibility



Specificity



Results 5 – VT production

test Strain Labs	VT detection				
	A07 VT-	B07 VT-	C07 VT+	D07 VT+	E07 VT+
L1	t	false	t	t	t
L2	-	-	-	-	-
L3	-	-	-	-	-
L4	-	-	-	-	-
L5	t	t	t	t	t
L6	-	-	-	-	-
L7	-	-	-	-	-
L8	-	-	-	-	-
L9	-	-	-	-	-
L10	t	t	t	t	t
L11	-	-	-	-	-
L12	-	-	-	-	-
L13	t	t	t	t	t
L14	t	t	t	t	t
L15	-	-	-	-	-
L16	-	-	-	-	-
L17	t	t	t	t	t
L18	-	-	-	-	-
L19	-	-	-	-	-
L20	-	-	-	-	-
L21	t	t	t	t	t

Methods: *Vero cells assay*
XXX

Number of laboratories: *7 (33%)*

Strains to be tested: *3 VT+, 2 VT-*

- *All the VT + strains* were correctly identified
- *1 VT - strain* were tested positive from *1 lab*

Overall:

- Agreement (K): *0.93 (CI: 0.79 – 1)*
- Sensibility: *1.00 (CI: 0.83 – 1)*
- Specificity: *0.90 (CI: 0.54 – 0,99)*

Results 6 - vtx1 gene detection

test Strain Labs	vtx1 detection				
	A07 vtx1-	B07 vtx1-	C07 vtx1+	D07 vtx1+	E07 vtx1+
L1	t	t	t	t	t
L2	t	t	t	t	t
L3	t	t	t	t	t
L4	t	t	t	t	t
L5	t	t	t	t	t
L6	t	t	t	t	t
L7	t	t	t	t	false
L8	t	t	t	t	t
L9	t	t	t	t	t
L10	t	t	t	t	t
L11	t	t	t	t	t
L12	-	-	-	-	-
L13	t	t	t	t	t
L14	t	t	t	t	t
L15	t	t	t	t	t
L16	t	t	t	t	t
L17	t	t	t	t	t
L18	t	t	t	t	t
L19	t	t	t	t	t
L20	t	t	t	t	t
L21	t	t	t	t	t

Methods: **PCR**

• **primers: XXX**

Number of laboratories: **20 (95%)**

Strains to be tested: **3 vtx1+, 2 vtx1-**

- **a vtx1 + strain** were tested negative from 1 lab
- **All the vtx1 - strains** were correctly identified

Overall:

- Agreement (K): **0.98 (CI: 0.93 – 1)**
- Sensibility: **0.98 (CI: 0.90 – 1)**
- Specificity: **1.00 (CI: 0.89 – 1.00)**

Results 7 – vtx2 gene detection

test Strain Labs	vtx2 detection				
	A07 vtx2-	B07 vtx2+ (*)	C07 vtx2+	D07 vtx2+	E07 vtx2-
L1	t	t	t	t	t
L2	t	t	t	t	t
L3	t	t	t	t	t
L4	t	t	t	t	t
L5	t	t	t	t	t
L6	t	t	t	t	t
L7	t	false	t	t	t
L8	t	t	t	t	t
L9	t	t	t	t	t
L10	t	t	t	t	t
L11	t	t	t	t	t
L12	-	-	-	-	-
L13	t	t	t	t	t
L14	t	t	t	t	t
L15	t	t	t	t	t
L16	t	t	t	t	t
L17	t	t	t	t	t
L18	t	t	t	t	t
L19	t	t	t	t	t
L20	t	t	t	t	t
L21	t	t	t	t	t

Methods: **PCR**

- primers: **XXX**

Number of laboratories: **20 (95%)**

Strains to be tested: **3 vtx2+ (one of them did not react with some primers), 2 vtx2-**

- **All the (C07, D07) vtx2 + strains were correctly identified**
- **The B07 strain (O103 vtx2+) was correctly identified, accordingly with the primers used, from all the labs but one**
- **All the vtx2 - strains were correctly identified**

Overall:

- Agreement (K): **0.98 (CI: 0.93 – 1)**
- Sensibility: **0.98 (CI: 0.97 – 1.00)**
- Specificity: **1.00 (CI: 0.79 – 1.00)**

Results 8 – eae gene detection

test Strain Labs	eae detection				
	A07 eae+	B07 eae+	C07 eae+	D07 eae+	E07 eae+
L1	-	-	-	-	-
L2	t	t	t	t	t
L3	-	-	-	-	-
L4	t	t	t	t	t
L5	t	t	t	t	t
L6	t	t	t	t	t
L7	t	t	t	t	t
L8	t	t	t	t	t
L9	t	t	t	t	t
L10	t	t	t	t	t
L11	t	t	t	t	t
L12	-	-	-	-	-
L13	false	t	t	t	t
L14	t	t	t	t	t
L15	t	t	t	t	t
L16	t	t	t	t	t
L17	t	t	t	t	t
L18	t	t	t	t	t
L19	t	t	t	t	t
L20	t	t	t	t	t
L21	t	false	t	false	t

Methods: **PCR**

• primers: **XXX**

Number of laboratories: **18 (86%)**

- All the strains were eae +
- 17 labs correctly tested all the strains
- 1 lab failed to identify eae gene in two cases and 1 lab in one case

Overall:

- Agreement (K): **Not available**
- Sensibility: **0.96 (CI: 0.86 – 1)**
- Specificity: **Not available**

Conclusions

- An excellent overall (0.98) agreement between results from the labs and the true values (GS) was obtained

- A good accuracy of the tests was obtained in terms of sensibility and specificity

but...

- NRL from “only” 17 MS + Norway participated to the study

- 4 laboratories did not perform serotyping even for O157

- 2 false results with O103 and 1 with O145 were recorded