

# *Inter-laboratory study on VTEC identification and typing - 2008*



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# *Inter-laboratory study on VTEC identification and typing – 2008: aim*

## **Evaluation of THE NRLs capability to correctly identify:**

- VTEC using the same PCR method:
  - *vtx1* gene
  - *vtx2* gene
  - *eae* gene (intimin-coding)
- Main VTEC serogroups by
  - serological assay
  - genotypic assay

# *Inter-laboratory study on VTEC identification and typing – 2008: aim*

## **Evaluation of THE NRLs capability to correctly identify VTEC by:**

- Using a shared real time PCR scheme for:
  - *vtx1* gene
  - *vtx2* gene
  - *eae* gene (intimin-coding)
  - Main VTEC serogroups

# *Materials and Methods*

## *Analysis of the results*

### *For every NRL:*

- *Availability of the assay*
- *No. of results provided by every NRL*
- *Performance of the NRL*

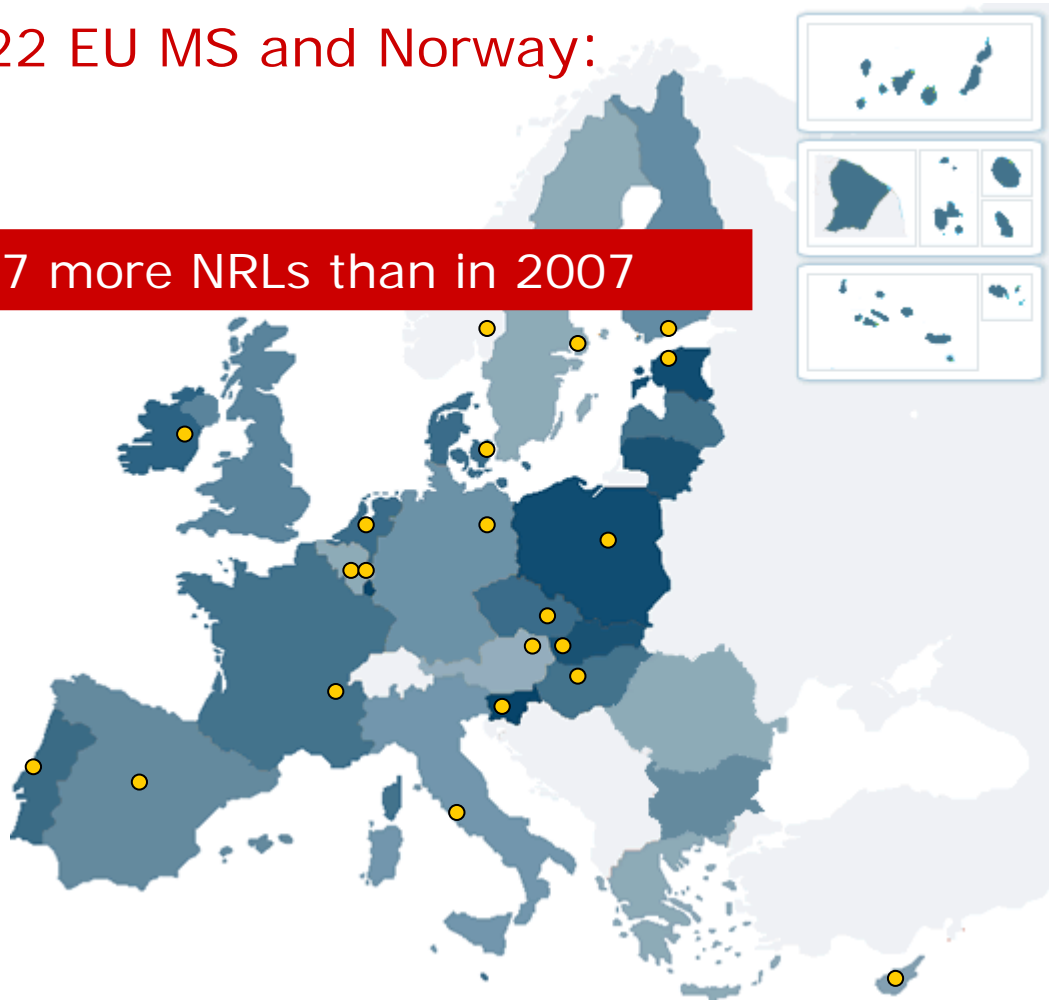
### *For the overall NRLs network:*

- *No. of strains that had a full characterization:*
  - *Serogroup*
  - *detection of vtx1, vtx2, eae*

# Materials and Methods - 1

28 NRLs from  
22 EU MS and Norway:

7 more NRLs than in 2007



- Austria
- Belgium (2)
- Cyprus
- Czech Republic
- Denmark
- Estonia
- Finland (2)
- France
- Germany
- Hungary (2)
- Ireland
- Italy
- Latvia
- Lithuania
- Norway
- Poland
- Portugal
- Slovakia (2)
- Slovenia
- Spain (2)
- Sweden (2)
- The Netherlands

# Materials and Methods - 2

- **7 strains** of *E. coli* tested for:  
O serogroup, VT production, presence of *vtx1*, *vtx2* and *eae* encoding genes
- Tests for virulence genes performed with protocols provided by CRL (PCR)
- Test for serogrouping and VT production performed with methods available

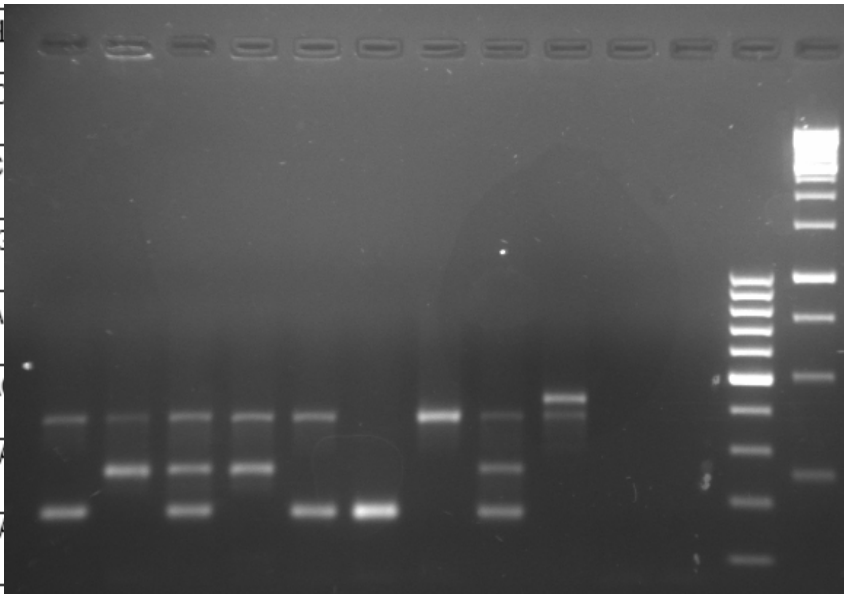
<i>strain</i>	<i>serogroup</i>	<i>VT</i>	<i>vtx1</i>	<i>vtx2</i>	<i>eae</i>
<b>A08</b>	<b>O145</b>	<b>+</b>	<b>+</b>	<b>-</b>	<b>+</b>
<b>B08</b>	<b>O121</b>	<b>+</b>	<b>-</b>	<b>+</b>	<b>+</b>
<b>C08</b>	<b>O111</b>	<b>The top 5 VTEC serogroup + O121 and O91</b>			
<b>D08</b>	<b>O157</b>				
<b>E08</b>	<b>O103</b>				
<b>F08</b>	<b>O91</b>	<b>+</b>	<b>+</b>	<b>-</b>	<b>-</b>
<b>G08</b>	<b>O26</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>+</b>

## Identification and characterisation of Verocytotoxin-producing *Escherichia coli* (VTEC) by PCR amplification of virulence genes

Annex 1.

A08 B08 C08 D08 E08 F08 G08 ED647 ED378

Target gene	Primer Name (ref.)	Primer Seq
eae	eaeAF (Paton & Paton, 1998)	GACCCGGCACAAGC
	eaeAR (Paton & Paton, 1998)	CCACCTGCAGCAAC
vtx1	stx1F (Paton & Paton, 1998)	ATAAATCGCCATTCC
	stx1R (Paton & Paton, 1998)	AGAACGCCCACTGA
vtx2 (group)	stx2F (Paton & Paton, 1998)	GGCACTGTCTGAAA
	stx2R (Paton & Paton, 1998)	TCGCCAGTTATCTGA
vtx2f	128-1 (Schmidt et al. 2000)	AGA TTG GGC GTC A TTG
	128-2 (Schmidt et al. 2000)	TAC TTT AAT GGC CGC CCT GTC TCC



# Materials and Methods

## Analysis of the results

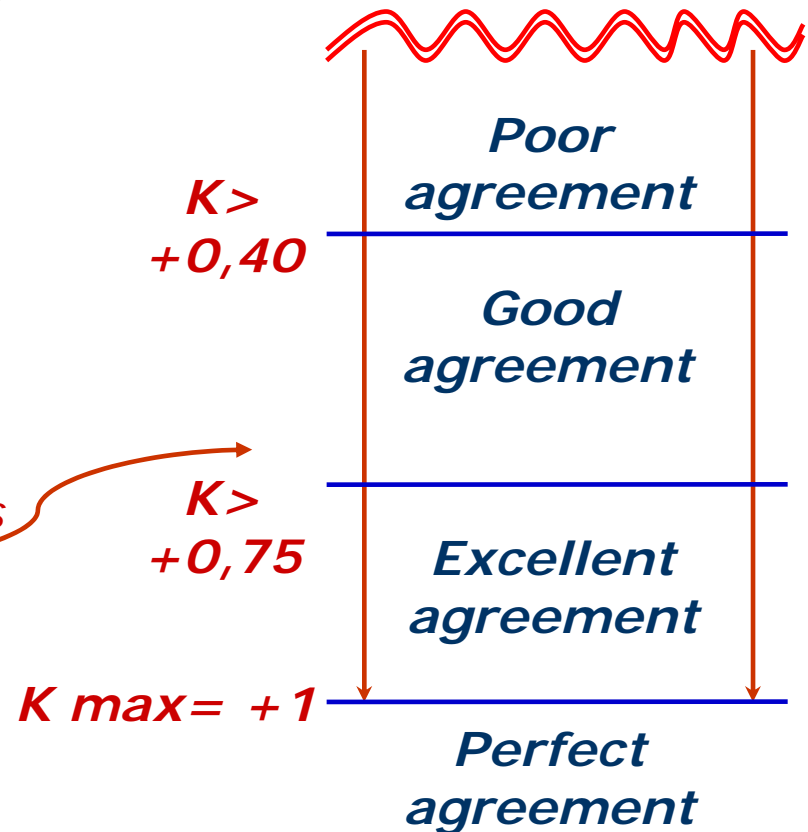
- **Agreement** of results with the true values (Gold Standard): **K test** (95% C.I.)

- **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

strain/lab		L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28
A08	O145	t	t	t	t	na	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na
B08	O121	na	na	na	na	na	t	false	na	t	false	na	na	na	t	na	false	na	false	t	na	t	na	t	na	na	t	na	na
C08	O111	t	t	t	t	na	t	t	t	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
D08	O157	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
E08	O103	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false
F08	O91	na	na	na	na	na	na	t	na	t	t	t	na	na	t	t	na	t	na	t	na	t	t	t	na	na	t	t	na
G08	O26	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false
A08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
B08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
C08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
D08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
E08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
F08	VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
G08	VT-	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
A08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
B08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
C08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
D08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	na	na	t	t	t	t	t	na	t
E08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
F08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	na	na	t	t	t	t	t	na	t
G08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
A08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
B08	vtx2+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
C08	vtx2+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
D08	vtx2+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
E08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
F08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
G08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
A08	eae+	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
B08	eae+	t	t	t	t	t	t	t	t	t	t	false	t	false	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
C08	eae+	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
D08	eae+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
E08	eae+	t	t	t	false	t	t	t	t	t	t	t	t	false	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
F08	eae-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	na	t
G08	eae+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t	na	na	t	t	t	t	t	na	t

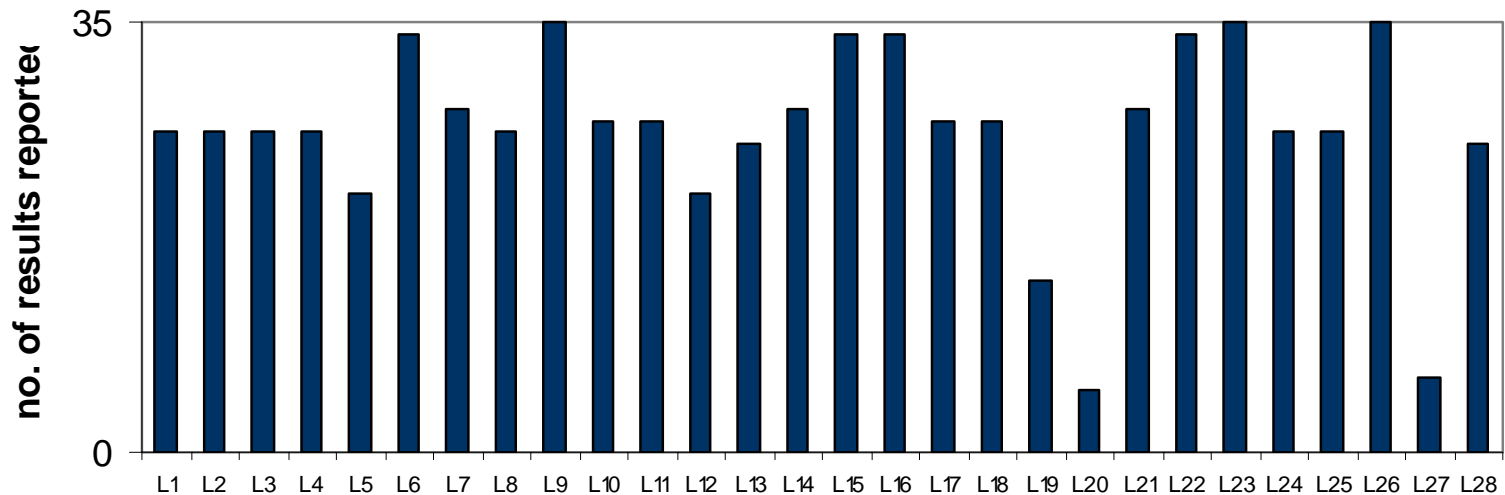
**3 NRLs did not perform conventional PCR but only Real Time**

- 28 NRLs (7 more than 2007)
- 732 out of 980 expected results were provided (75%)
- 15 false results were reported (2%)

# Results – Number of test

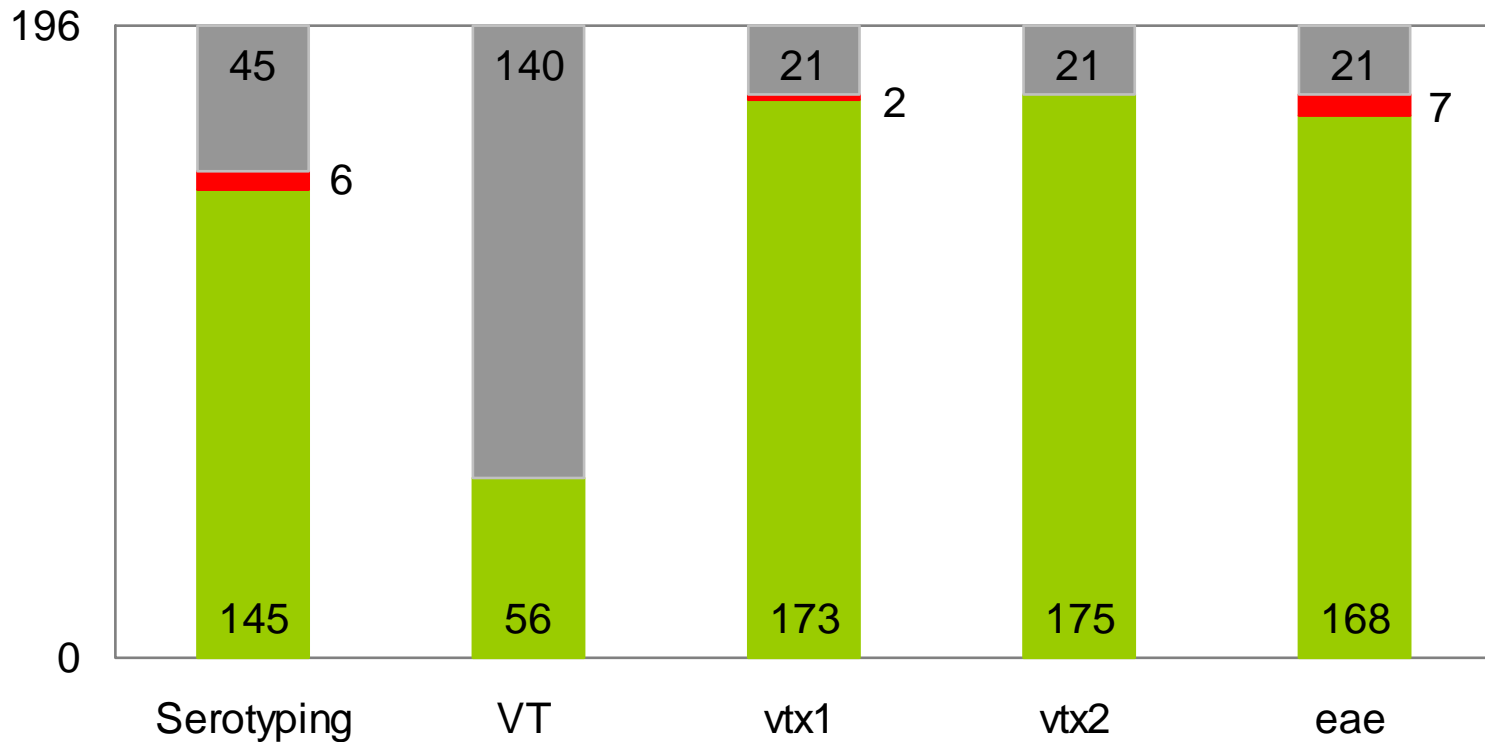
<b>No. of results provided</b>	<b>NRLs</b>	<b>%</b>
all (35)	3	11%
30-34	4	14%
25-29	16	57%
20-25	2	7%
< 20	3	11%

No. of results reported for NRL



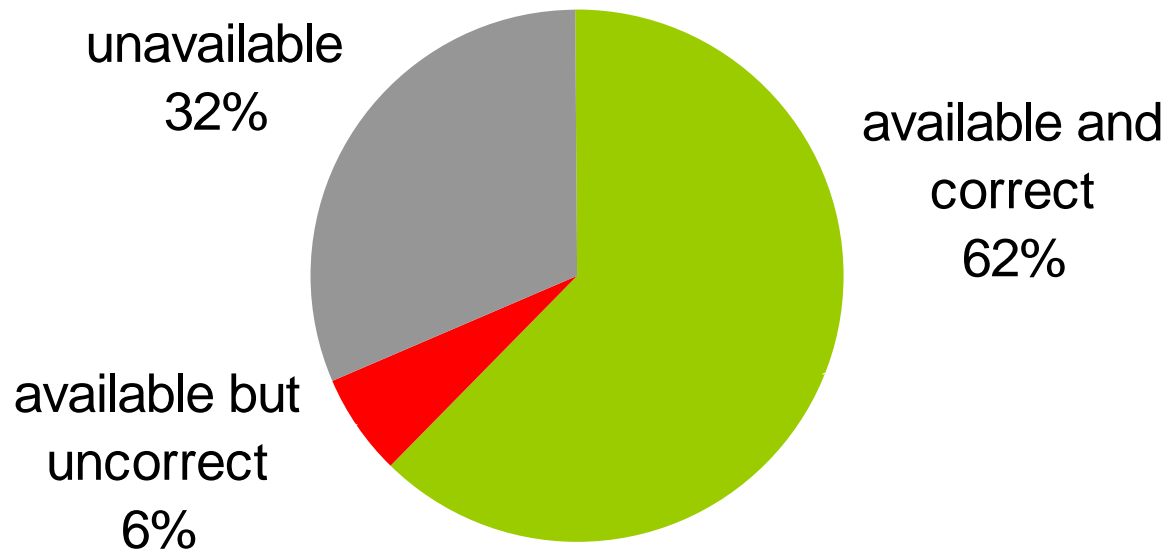
# Results – NRL network capabilities

correct, uncorrect and lacking results for assay



# *Results – NRL network capabilities*

**full characterization of the VTEC strains (N=196)**



# Results 1 – Serogroup

strain	lab	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28
A08	O145	t	t	t	t	na	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na
B08	O121	na	na	na	na	na	t	false	na	t	false	na	na	na	t	na	false	na	false	t	na	t	na	t	na	na	t	na	na
C08	O111	t	t	t	t	na	t	t	t	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
D08	O157	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
E08	O103	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false
F08	O91	t	t	t	t	na	t	t	t	t	t	t	na	na	t	t	na	t	na	t	na	t	t	t	na	na	t	t	na
G08	O26	t	t	t	t	na	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false

**1 NRL did not perform serogrouping**

**Methods:** *Slide agglutination and or molecular serogrouping*

**Number of laboratories:** **27**

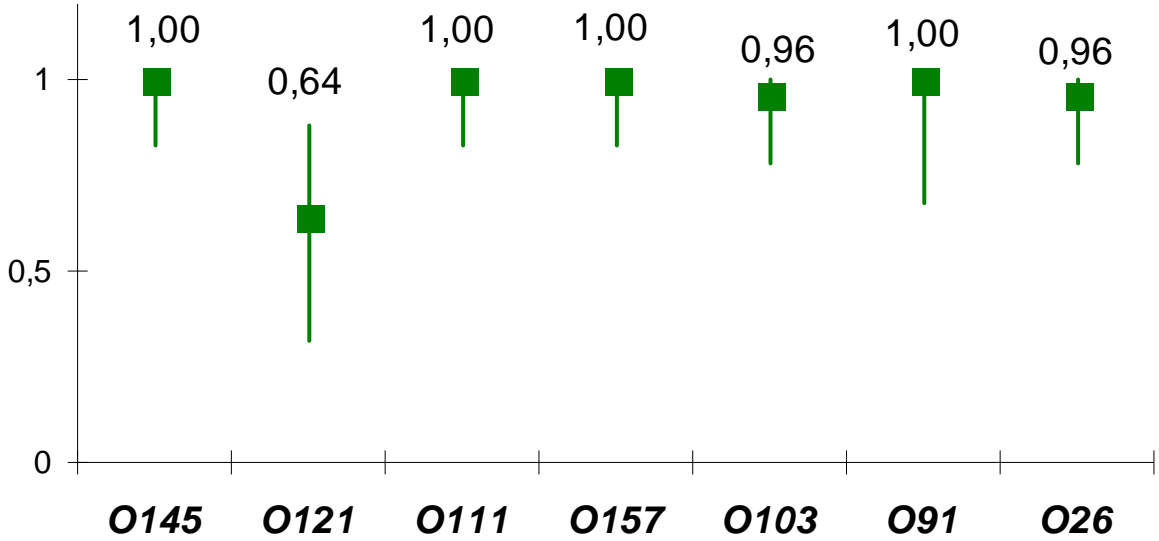
- **O145, O111, O157, O91** were always correctly identified
- 4 labs mis-identified **O121** as **O103**
- 1 lab failed to identify **O103** and **O26**

**Overall:**

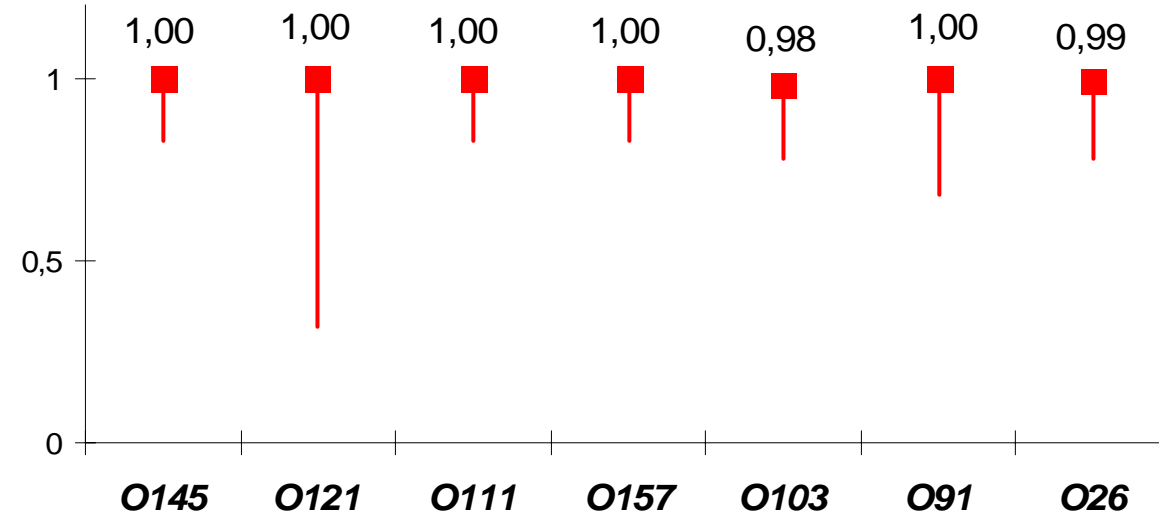
- Agreement (K): **0.94 (CI: 0.91 – 1)**
- Sensibility: **0.96 (CI: 0.98 – 1)**
- Specificity: **0.99 (CI: 0.91 – 1)**

# *Results – Sensibility and Specificity for serogroups*

*Sensibility*



*Specificity*



# Results 1 – vtx genes detection

strain	lab	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28
A08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
B08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
C08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
D08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	na	na	t	t	t	t	t	t	na	t
E08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
F08	vtx1+	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	na	na	t	t	t	t	t	t	na	t
G08	vtx1-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
A08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
B08	vtx2 +	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
C08	vtx2 +	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
D08	vtx2 +	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
E08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
F08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
G08	vtx2-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t

**Number of laboratories: 25**

- **All the strains were correctly identified for vtx2**
- **1 false negative and 1 false positive results for vtx1**

**Overall vtx1:**

- Agreement (K): **0.97 (CI: 0.94 – 1)**
- Sensibility: **0.99 (CI: 0.94 – 1)**
- Specificity: **0.99 (CI: 0.92 – 1)**

**Overall vtx2:**

- Agreement (K): **1.0 (CI: 0.98 – 1)**
- Sensibility: **1.0 (CI: 0.94 – 1)**
- Specificity: **1.0 (CI: 0.95 – 1)**

# Results 1 – eae gene detection

strain	lab	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28
A08	eae +	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
B08	eae +	t	t	t	t	t	t	t	t	t	t	false	t	false	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
C08	eae +	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
D08	eae +	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
E08	eae +	t	t	t	false	t	t	t	t	t	t	t	t	false	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
F08	eae-	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	na	na	t	t	t	t	t	t	na	t
G08	eae +	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	false	t	t	na	na	t	t	t	t	t	t	na	t

**Number of laboratories: 25**

- **All the eae- strains were correctly identified**
- **7 false negative results were reported**

**Overall eae detection:**

- **Agreement (K): 0.85 (CI: 0.81 – 0.95)**
- **Sensibility: 0.86 (CI: 0.81 – 0.91)**
- **Specificity: 0.96 (CI: 0.91 – 0.98)**

**The performance values for eae are lower than those for the other characteristics, mainly due to the high number of errors from one NRL**



# Results 1 – VT production

<i>strain</i>	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20	L21	L22	L23	L24	L25	L26	L27	L28
A08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
B08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
C08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
D08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
E08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
F08 VT+	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na
G08 VT-	na	na	na	na	na	t	na	na	t	na	na	na	na	na	t	t	na	na	t	na	na	t	t	na	na	t	na	na

**Number of laboratories: 8**

- **All the strains** were correctly identified

**Overall VT:**

- Agreement (K): **1.00**
- Sensibility: **1.00 (CI: 0.81 – 0.91)**
- Specificity: **1.00 (CI: 0.91 – 0.98)**

## *Conclusions*

- An excellent performance from all the NRLs
- The NRLs Network demonstrated an excellent capability to identify the top five VTEC serogroups and the virulence genes
- The NRLs Network demonstrated a good capability to identify the additional serogroups included in the study

but...

- A few NRLs did not participate in the part III (mandatory) but performed only Real-time PCR
- 1 laboratory did not perform serotyping even for O157