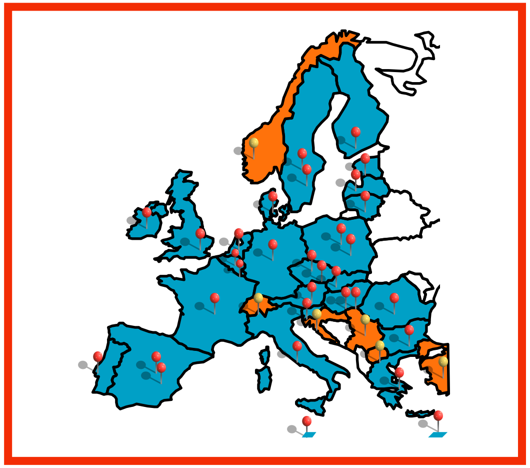
# Joint ECDC-FWD (4th EQA) – EU-RL (PT10) PT on the identification and typing of VTEC: the EU-RL network results



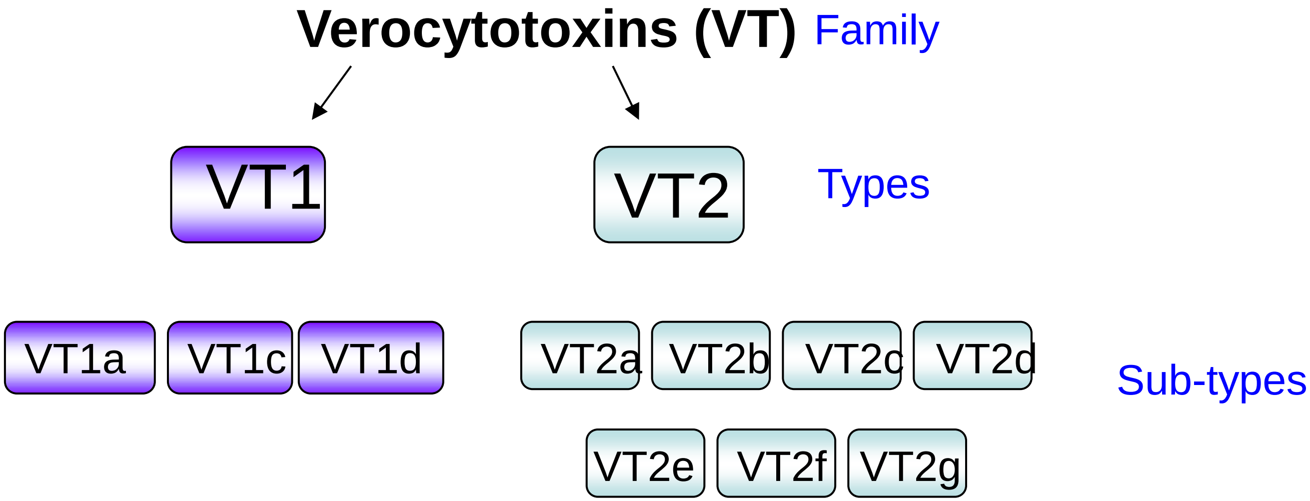
# OBJECTIVES (1)

## To assess the proficiency of the NRLs in:

- ✓ The detection of the main VTEC virulence genes: *vtx1* group, *vtx2* group and *eae*.
- ✓ The detection of the target genes of Enteroaggregative *E. coli* (*aggR* and *aaiC*)
- ✓ The identification of a range of 12 VTEC serogroups, selected on the basis of their relevance in human infections in Europe
- ✓ PFGE Typing (1st EQA round, facultative) in view of the collection of molecular typing data on VTEC by EFSA

# OBJECTIVES (2)

**To assess the performance of the revised version of the PCR-based method for the subtyping of VT-coding genes previously adopted in PT6 (J Clin Microbiol. 2012; 50: 2951-63)**



**Verocytotoxins (VT)** Family

VT1

VT2

Types

VT1a VT1c VT1d

VT2a VT2b VT2c VT2d

VT2e VT2f VT2g

Sub-types

# PARTICIPANTS

**31 NRLs representing**

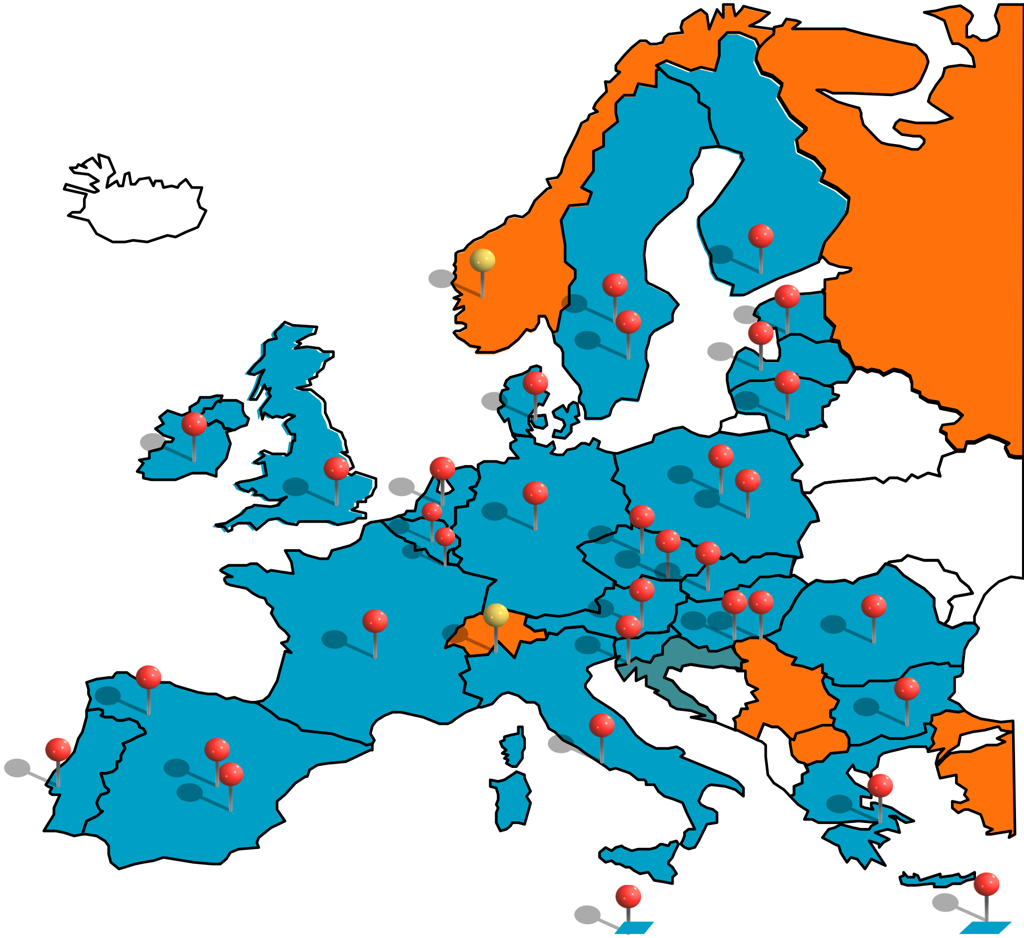
● **26 EU countries**

● **+ the NRLs of**

**Norway**

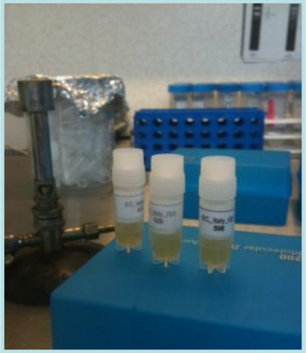
**Switzerland**

**Egypt**



# METHODS (1)

- ✓ Standard operating procedures available at the EU-RL web site
- ✓ Test and control strains provided as cultures in soft-agar
- ✓ Samples labelled with randomly generated numerical codes
- ✓ Results submitted on-line via the web site

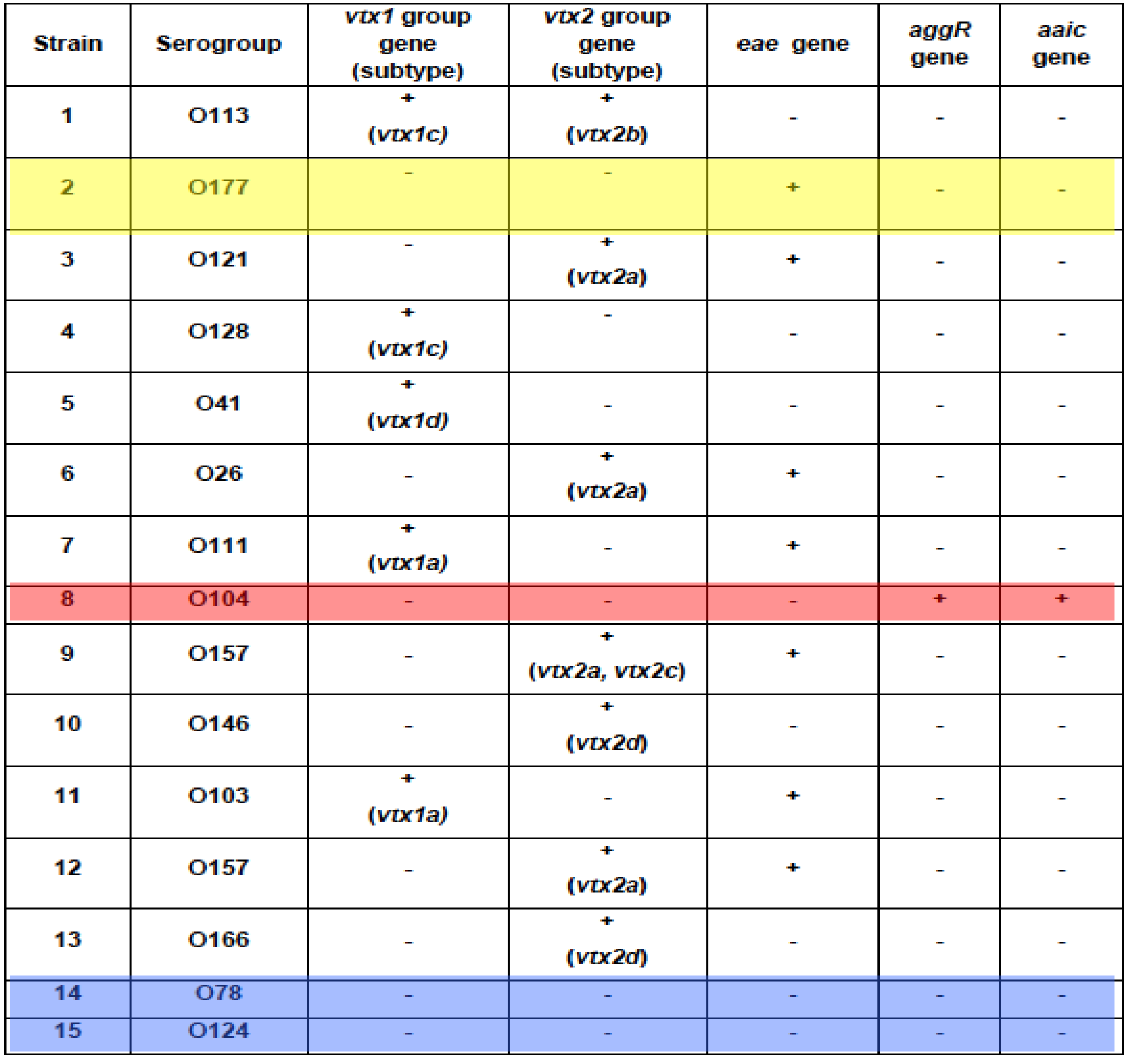


# METHODS (2)

## Evaluation of the results

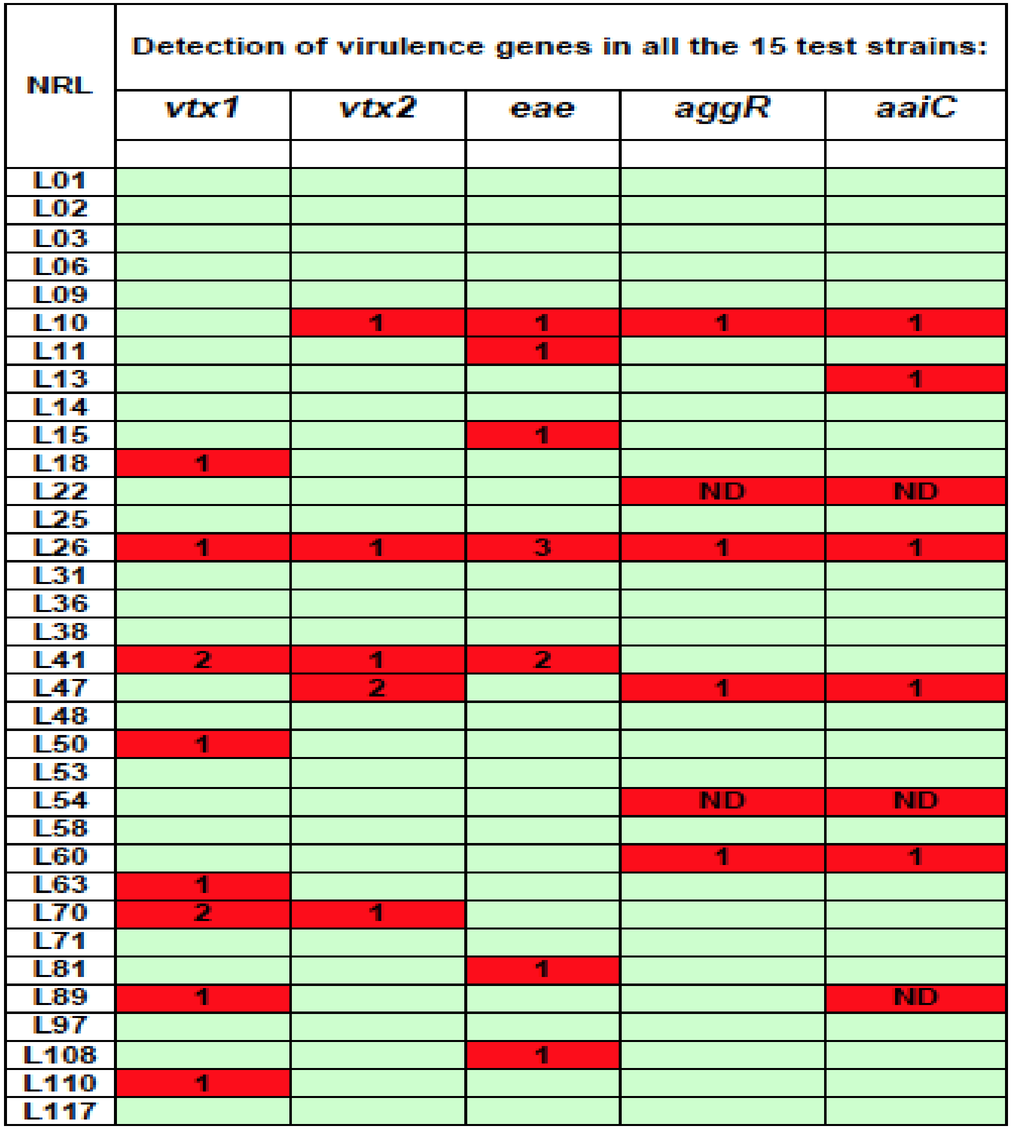
- **Detection of virulence genes:**
  - Agreement (Cohen’s Kappa), Sensitivity, Specificity
- **O serogroup identification**
  - Assignement of penalty points for strains typed incorrectly
  - Under-performance threshold: **4 points**
- **PCR method for *vtx*-genes sub-typing**
  - Agreement (Cohen’s Kappa), Sensitivity, Specificity

# Characteristics of the test strains



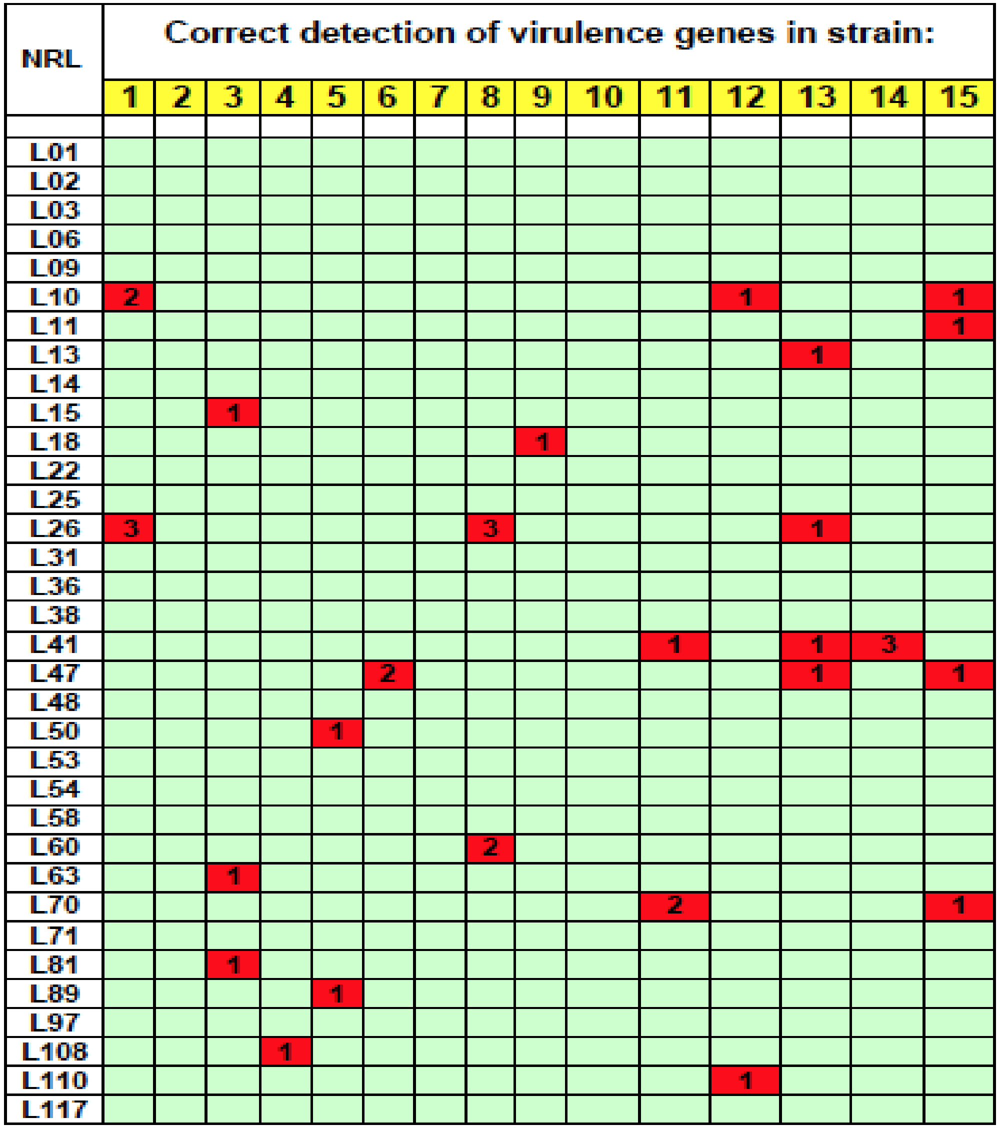
| Strain | Serogroup | *vtx1* group gene (subtype) | *vtx2* group gene (subtype) | *eae* gene | *aggR* gene | *aaic* gene |
|---|---|---|---|---|---|---|
| 1 | O113 | +<br>(*vtx1c*) | +<br>(*vtx2b*) | - | - | - |
| 2 | O177 | - | - | + | - | - |
| 3 | O121 | - | +<br>(*vtx2a*) | + | - | - |
| 4 | O128 | +<br>(*vtx1c*) | - | - | - | - |
| 5 | O41 | +<br>(*vtx1d*) | - | - | - | - |
| 6 | O26 | - | +<br>(*vtx2a*) | + | - | - |
| 7 | O111 | +<br>(*vtx1a*) | - | + | - | - |
| 8 | O104 | - | - | - | + | + |
| 9 | O157 | - | +<br>(*vtx2a*, *vtx2c*) | + | - | - |
| 10 | O146 | - | +<br>(*vtx2d*) | - | - | - |
| 11 | O103 | +<br>(*vtx1a*) | - | + | - | - |
| 12 | O157 | - | +<br>(*vtx2a*) | + | - | - |
| 13 | O166 | - | +<br>(*vtx2d*) | - | - | - |
| 14 | O78 | - | - | - | - | - |
| 15 | O124 | - | - | - | - | - |

# RESULTS: detection of virulence genes



| NRL | Detection of virulence genes in all the 15 test strains: | | | | |
|---|---|---|---|---|---|
| | *vtx1* | *vtx2* | *eae* | *aggR* | *aaiC* |
| | | | | | |
| L01 | | | | | |
| L02 | | | | | |
| L03 | | | | | |
| L06 | | | | | |
| L09 | | | | | |
| L10 | | 1 | 1 | 1 | 1 |
| L11 | | | 1 | | |
| L13 | | | | | 1 |
| L14 | | | | | |
| L15 | | | 1 | | |
| L18 | 1 | | | | |
| L22 | | | | ND | ND |
| L25 | | | | | |
| L26 | 1 | 1 | 3 | 1 | 1 |
| L31 | | | | | |
| L36 | | | | | |
| L38 | | | | | |
| L41 | 2 | 1 | 2 | | |
| L47 | | 2 | | 1 | 1 |
| L48 | | | | | |
| L50 | 1 | | | | |
| L53 | | | | | |
| L54 | | | | ND | ND |
| L58 | | | | | |
| L60 | | | | 1 | 1 |
| L63 | 1 | | | | |
| L70 | 2 | 1 | | | |
| L71 | | | | | |
| L81 | | | 1 | | |
| L89 | 1 | | | | ND |
| L97 | | | | | |
| L108 | | | 1 | | |
| L110 | 1 | | | | |
| L117 | | | | | |

# RESULTS: detection of virulence genes



| NRL | Correct detection of virulence genes in strain: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| L01 | | | | | | | | | | | | | | | |
| L02 | | | | | | | | | | | | | | | |
| L03 | | | | | | | | | | | | | | | |
| L06 | | | | | | | | | | | | | | | |
| L09 | | | | | | | | | | | | | | | |
| L10 | 2 | | | | | | | | | | | 1 | | | 1 |
| L11 | | | | | | | | | | | | | | | 1 |
| L13 | | | | | | | | | | | | | 1 | | |
| L14 | | | | | | | | | | | | | | | |
| L15 | | | 1 | | | | | | | | | | | | |
| L18 | | | | | | | | | 1 | | | | | | |
| L22 | | | | | | | | | | | | | | | |
| L25 | | | | | | | | | | | | | | | |
| L26 | 3 | | | | | | | 3 | | | | | 1 | | |
| L31 | | | | | | | | | | | | | | | |
| L36 | | | | | | | | | | | | | | | |
| L38 | | | | | | | | | | | | | | | |
| L41 | | | | | | | | | | | 1 | | 1 | 3 | |
| L47 | | | | | | 2 | | | | | | | 1 | | 1 |
| L48 | | | | | | | | | | | | | | | |
| L50 | | | | | 1 | | | | | | | | | | |
| L53 | | | | | | | | | | | | | | | |
| L54 | | | | | | | | | | | | | | | |
| L58 | | | | | | | | | | | | | | | |
| L60 | | | | | | | | 2 | | | | | | | |
| L63 | | | 1 | | | | | | | | | | | | |
| L70 | | | | | | | | | | | 2 | | | | 1 |
| L71 | | | | | | | | | | | | | | | |
| L81 | | | 1 | | | | | | | | | | | | |
| L89 | | | | | 1 | | | | | | | | | | |
| L97 | | | | | | | | | | | | | | | |
| L108 | | | | 1 | | | | | | | | | | | |
| L110 | | | | | | | | | | | | 1 | | | |
| L117 | | | | | | | | | | | | | | | |

# RESULTS: detection of virulence genes

- ✓ **18 NRLs (53 %) correctly identified the presence/absence of the target genes in the test strains**

- ✓ **16 NRLs provided a total of 35 incorrect results (25 false positive and 10 false negative)**
  - ✓ **10 incorrect results for *vtx1* (8 NRLs)**
  - ✓ **6 incorrect results for *vtx2* (5 NRLs)**
  - ✓ **10 incorrect results for *eae* (7 NRLs)**
  - ✓ **4 incorrect results for *aggR* (4 NRLs)**
  - ✓ **5 incorrect results for *aaiC* (5 NRLs)**

# Agreement (Cohen's Kappa) for the detection of virulence genes

**Overall K value:**

**VTEC: 0.96**

***AggR*: 0.93**

***Aaic*: 0.91**

**None of the NRLs who did the tests had a poor agreement**

| NRL | K values for the detection of genes: | | |
|---|---|---|---|
| | *vtx1, vtx2, eae* | *aggR* | *aaic* |
| L01 | 1 | 1 | 1 |
| L02 | 1 | 1 | 1 |
| L03 | 1 | 1 | 1 |
| L06 | 1 | 1 | 1 |
| L09 | 1 | 1 | 1 |
| L10 | 0.91 | 0.63 | 0.63 |
| L11 | 0.95 | 1 | 1 |
| L13 | 1 | 1 | 0.63 |
| L14 | 1 | 1 | 1 |
| L15 | 0.97 | 1 | 1 |
| L18 | 0.95 | 1 | 1 |
| L22 | 1 | ND | ND |
| L25 | 1 | 1 | 1 |
| L26 | 0.78 | 0.63 | 0.63 |
| L31 | 1 | 1 | 1 |
| L36 | 1 | 1 | 1 |
| L38 | 1 | 1 | 1 |
| L41 | 0.78 | 1 | 1 |
| L47 | 0.91 | 0.63 | 0.63 |
| L48 | 1 | 1 | 1 |
| L50 | 0.95 | 1 | 1 |
| L53 | 1 | 1 | 1 |
| L54 | 1 | ND | ND |
| L58 | 1 | 1 | 1 |
| L60 | 1 | 0.63 | 0.63 |
| L63 | 0.95 | 1 | 1 |
| L70 | 0.86 | 1 | 1 |
| L71 | 1 | 1 | 1 |
| L81 | 0.95 | 1 | 1 |
| L89 | 0.95 | 1 | ND |
| L97 | 1 | 1 | 1 |
| L108 | 0.95 | 1 | 1 |
| L110 | 0.95 | 1 | 1 |
| L117 | 1 | 1 | 1 |
| | | | |
| **Overall** | 0.96 | 0.93 | 0.91 |

| | |
|---|---|
| 🟩 | excellent agreement |
| 🟨 | good agreement |
| 🟥 | poor agreement |

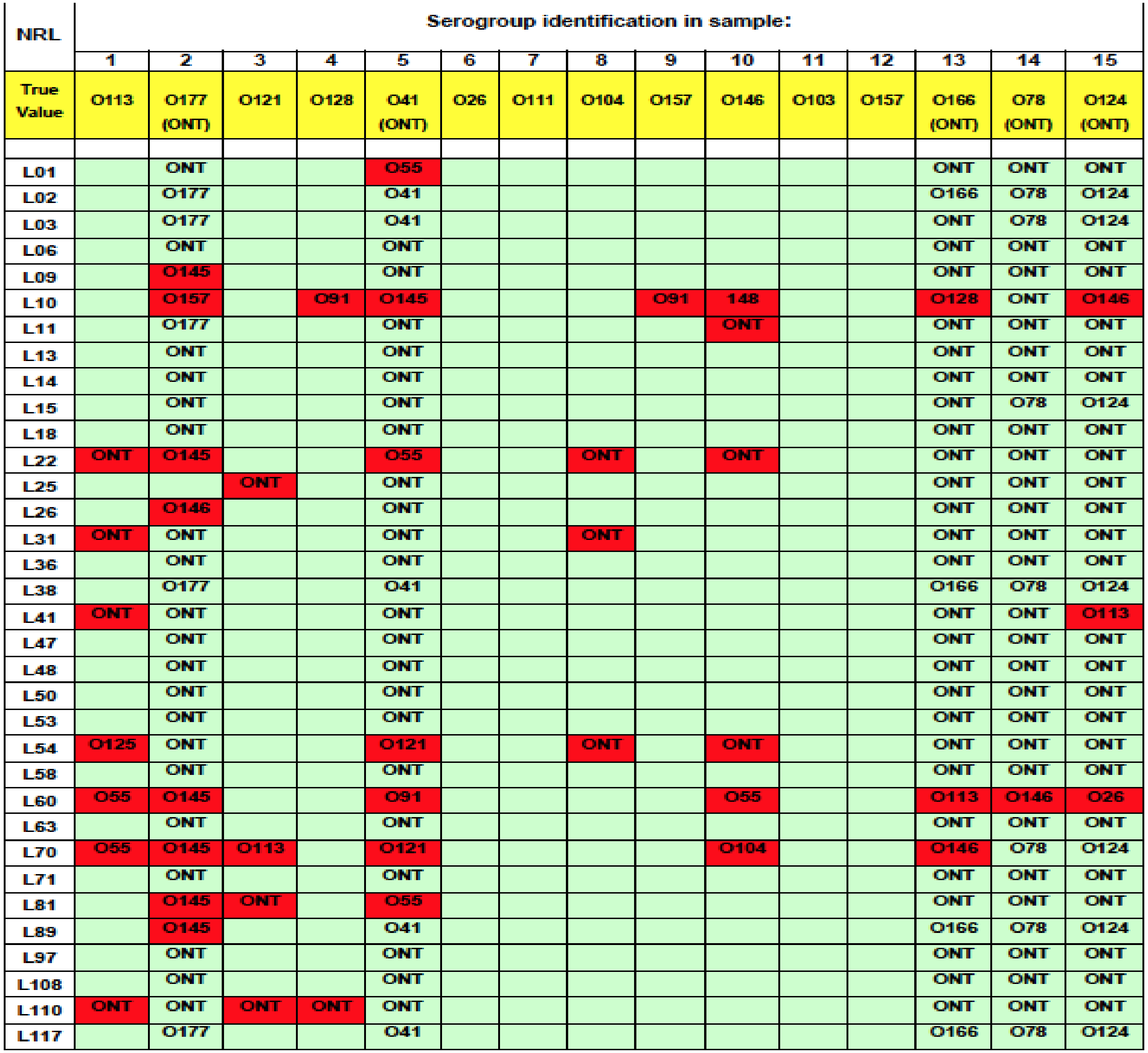
# RESULTS - O serogroup identification

## O Serogroups in the scope of the study

- ✓ **The "top 5" (O157, O26, O103, O111, O145)**
  - ✓ Assignement of 4 penalty points for each strain typed incorrectly

- ✓ **O104 + other 6 serogroups of public health relevance (ECDC-FWD data)**
  - ✓ Assignement of 2 penalty points for each strain typed incorrectly
- ✓ **Other strains:** ONT considered as a correct result
  - ✓ Assignement of 1 penalty point for each strain typed incorrectly

# RESULTS - O serogroup identification



| NRL | Serogroup identification in sample: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| True Value | O113 | O177 (ONT) | O121 | O128 | O41 (ONT) | O26 | O111 | O104 | O157 | O146 | O103 | O157 | O166 (ONT) | O78 (ONT) | O124 (ONT) |
| L01 | | ONT | | | O55 | | | | | | | | ONT | ONT | ONT |
| L02 | | O177 | | | O41 | | | | | | | | O166 | O78 | O124 |
| L03 | | O177 | | | O41 | | | | | | | | ONT | O78 | O124 |
| L06 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L09 | | O145 | | | ONT | | | | | | | | ONT | ONT | ONT |
| L10 | | O157 | | O91 | O145 | | | | O91 | 148 | | | O128 | ONT | O146 |
| L11 | | O177 | | | ONT | | | | | ONT | | | ONT | ONT | ONT |
| L13 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L14 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L15 | | ONT | | | ONT | | | | | | | | ONT | O78 | O124 |
| L18 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L22 | ONT | O145 | | | O55 | | | ONT | | ONT | | | ONT | ONT | ONT |
| L25 | | | ONT | | ONT | | | | | | | | ONT | ONT | ONT |
| L26 | | O146 | | | ONT | | | | | | | | ONT | ONT | ONT |
| L31 | ONT | ONT | | | ONT | | | ONT | | | | | ONT | ONT | ONT |
| L36 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L38 | | O177 | | | O41 | | | | | | | | O166 | O78 | O124 |
| L41 | ONT | ONT | | | ONT | | | | | | | | ONT | ONT | O113 |
| L47 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L48 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L50 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L53 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L54 | O125 | ONT | | | O121 | | | ONT | | ONT | | | ONT | ONT | ONT |
| L58 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L60 | O55 | O145 | | | O91 | | | | | O55 | | | O113 | O146 | O26 |
| L63 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L70 | O55 | O145 | O113 | | O121 | | | | | O104 | | | O146 | O78 | O124 |
| L71 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L81 | | O145 | ONT | | O55 | | | | | | | | ONT | ONT | ONT |
| L89 | | O145 | | | O41 | | | | | | | | O166 | O78 | O124 |
| L97 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L108 | | ONT | | | ONT | | | | | | | | ONT | ONT | ONT |
| L110 | ONT | ONT | ONT | ONT | ONT | | | | | | | | ONT | ONT | ONT |
| L117 | | O177 | | | O41 | | | | | | | | O166 | O78 | O124 |

# RESULTS - O serogroup identification

**Correct identification for the other serogroups:**

**O128: 32 NRLs (94%)**

**O121: 30 NRLs (88%)**

**O146: 28 NRLs (82%)**

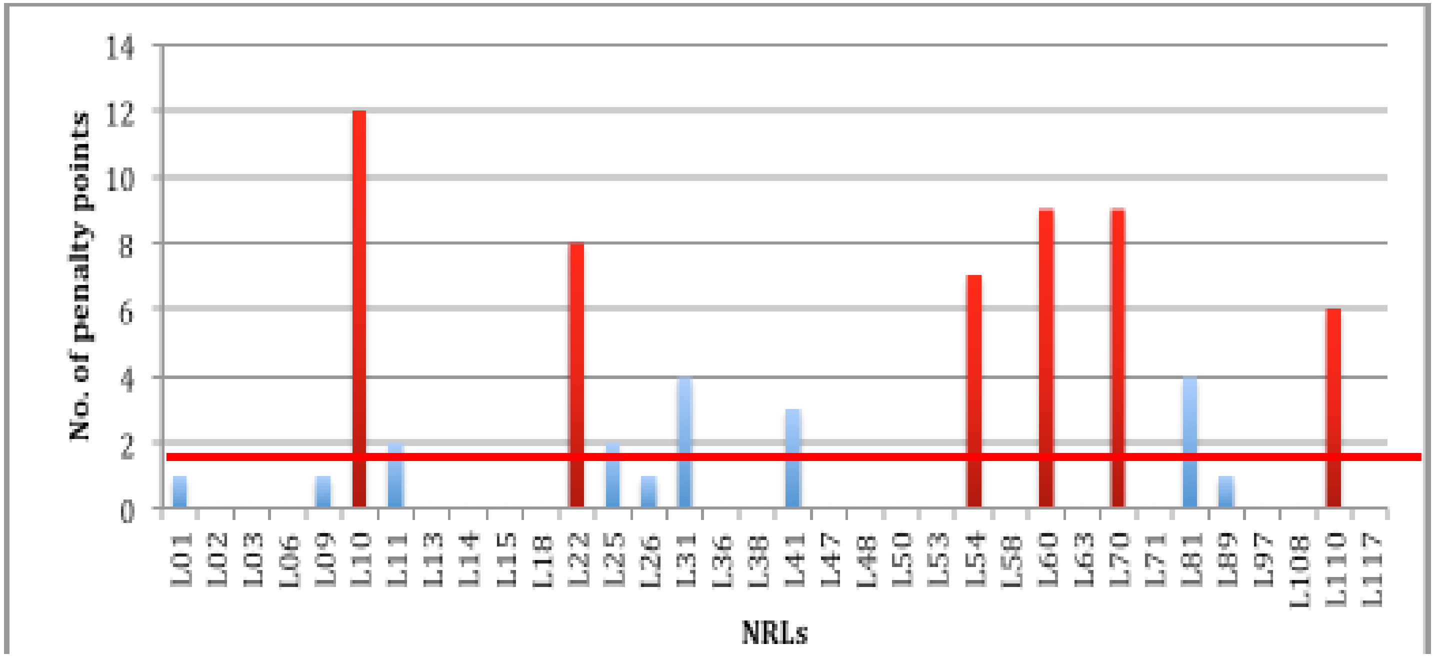
**O113: 27 NRLs (79%)**

**Serogroups out of the PT scope (5 strains)**

**11 NRLs reported 22 incorrect results, assigning these strains to other serogroups**

# O-group identification: penalty points

## Score achieved by each NRL

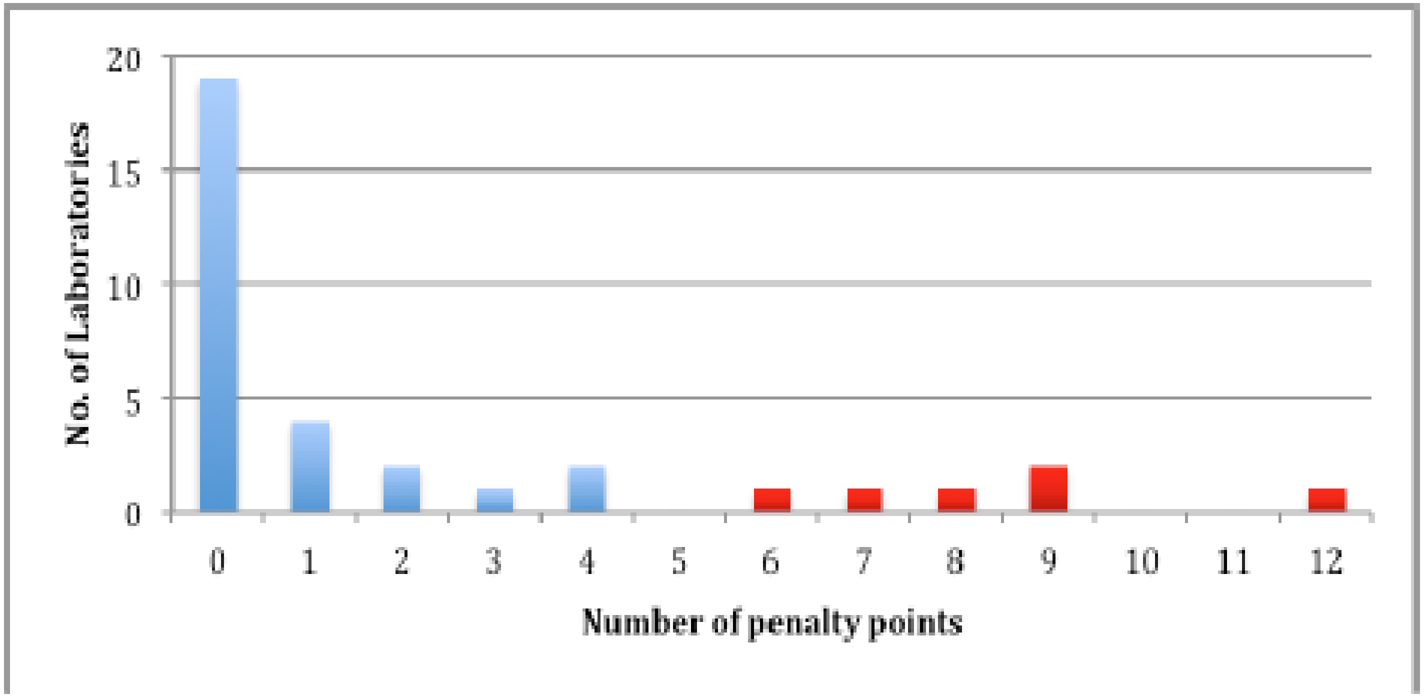


**6 NRLs obtained a score > 4 (not satisfactory)**

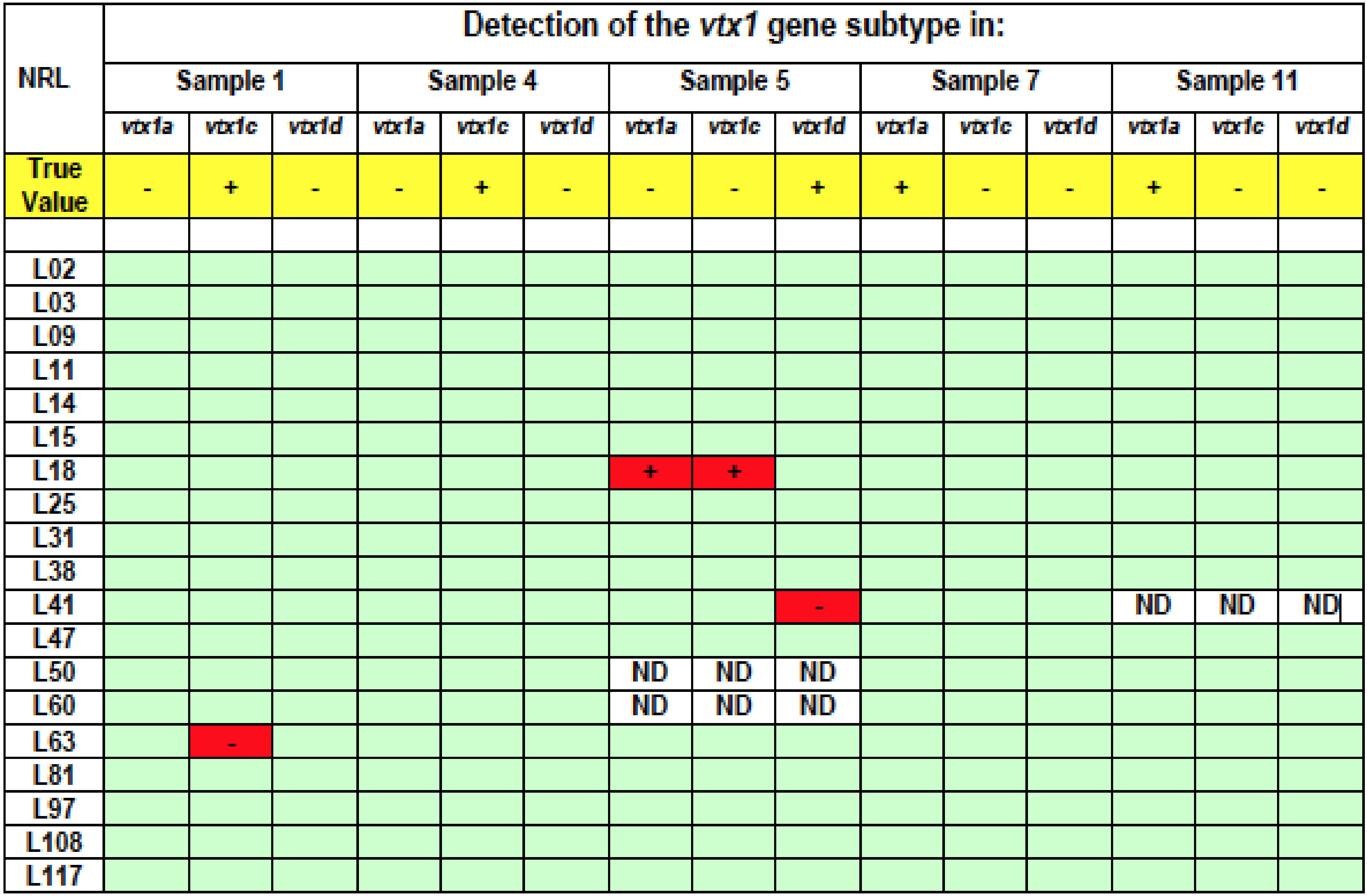
**2 NRLs obtained a score = 4, but their performance was considered as satisfactory since they did not make errors with the "top 5" serogroups**

# O serogroup identification: penalty points

## NRLs grouped by the score



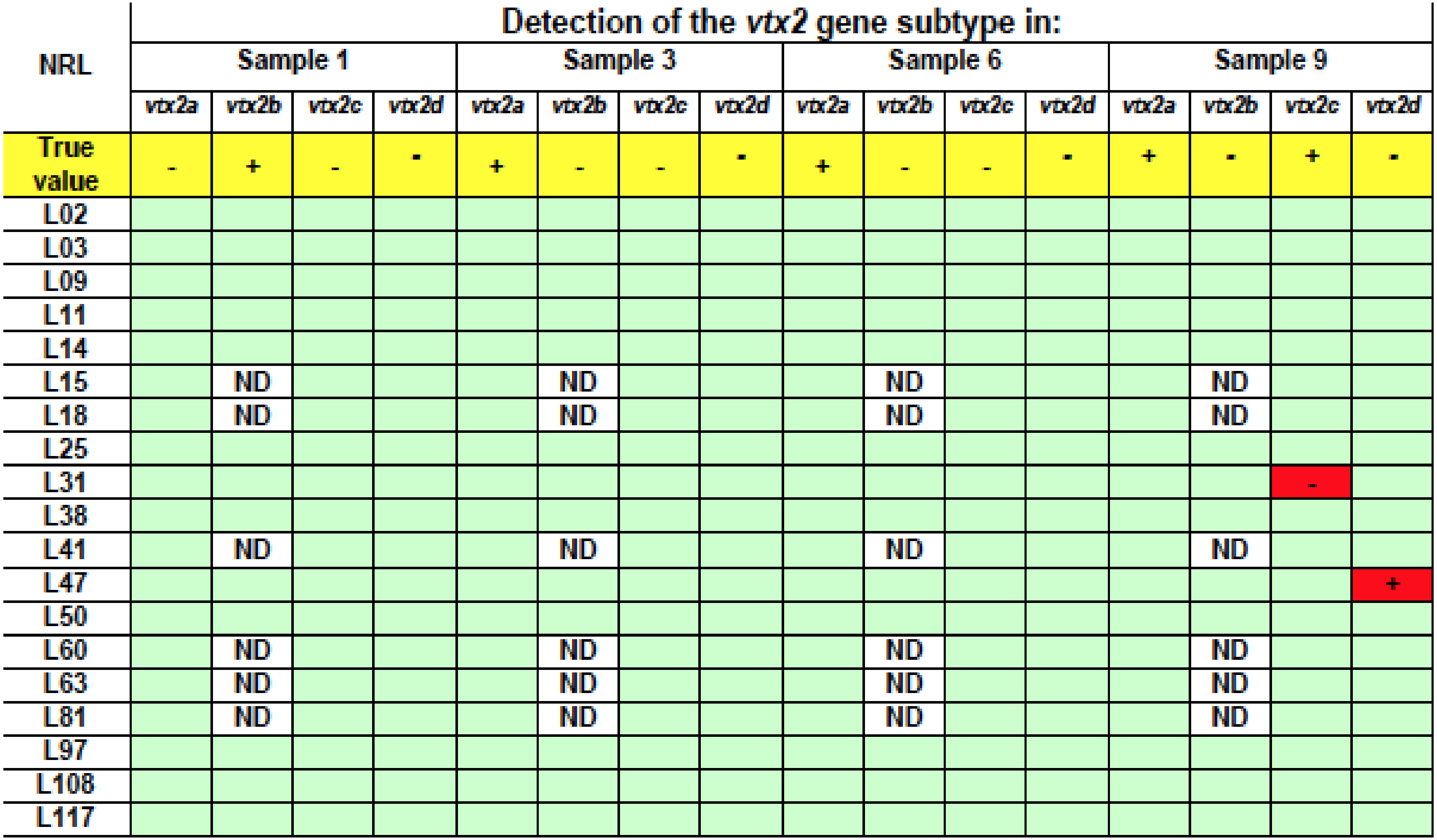
# RESULTS: Sub-typing of *vtx1* genes (5 strains)



| NRL | Detection of the *vtx1* gene subtype in: | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | | | Sample 4 | | | Sample 5 | | | Sample 7 | | | Sample 11 | | |
| | vtx1a | vtx1c | vtx1d | vtx1a | vtx1c | vtx1d | vtx1a | vtx1c | vtx1d | vtx1a | vtx1c | vtx1d | vtx1a | vtx1c | vtx1d |
| True Value | - | + | - | - | + | - | - | - | + | + | - | - | + | - | - |
| | | | | | | | | | | | | | | | |
| L02 | | | | | | | | | | | | | | | |
| L03 | | | | | | | | | | | | | | | |
| L09 | | | | | | | | | | | | | | | |
| L11 | | | | | | | | | | | | | | | |
| L14 | | | | | | | | | | | | | | | |
| L15 | | | | | | | | | | | | | | | |
| L18 | | | | | | | + | + | | | | | | | |
| L25 | | | | | | | | | | | | | | | |
| L31 | | | | | | | | | | | | | | | |
| L38 | | | | | | | | | | | | | | | |
| L41 | | | | | | | | | - | | | | ND | ND | ND |
| L47 | | | | | | | | | | | | | | | |
| L50 | | | | | | | ND | ND | ND | | | | | | |
| L60 | | | | | | | ND | ND | ND | | | | | | |
| L63 | | - | | | | | | | | | | | | | |
| L81 | | | | | | | | | | | | | | | |
| L97 | | | | | | | | | | | | | | | |
| L108 | | | | | | | | | | | | | | | |
| L117 | | | | | | | | | | | | | | | |

**The *vtx1* subtype was identified correctly by 15 of the 19 NRLs (79 %)**

# RESULTS: Sub-typing of *vtx2* genes (7 strains)

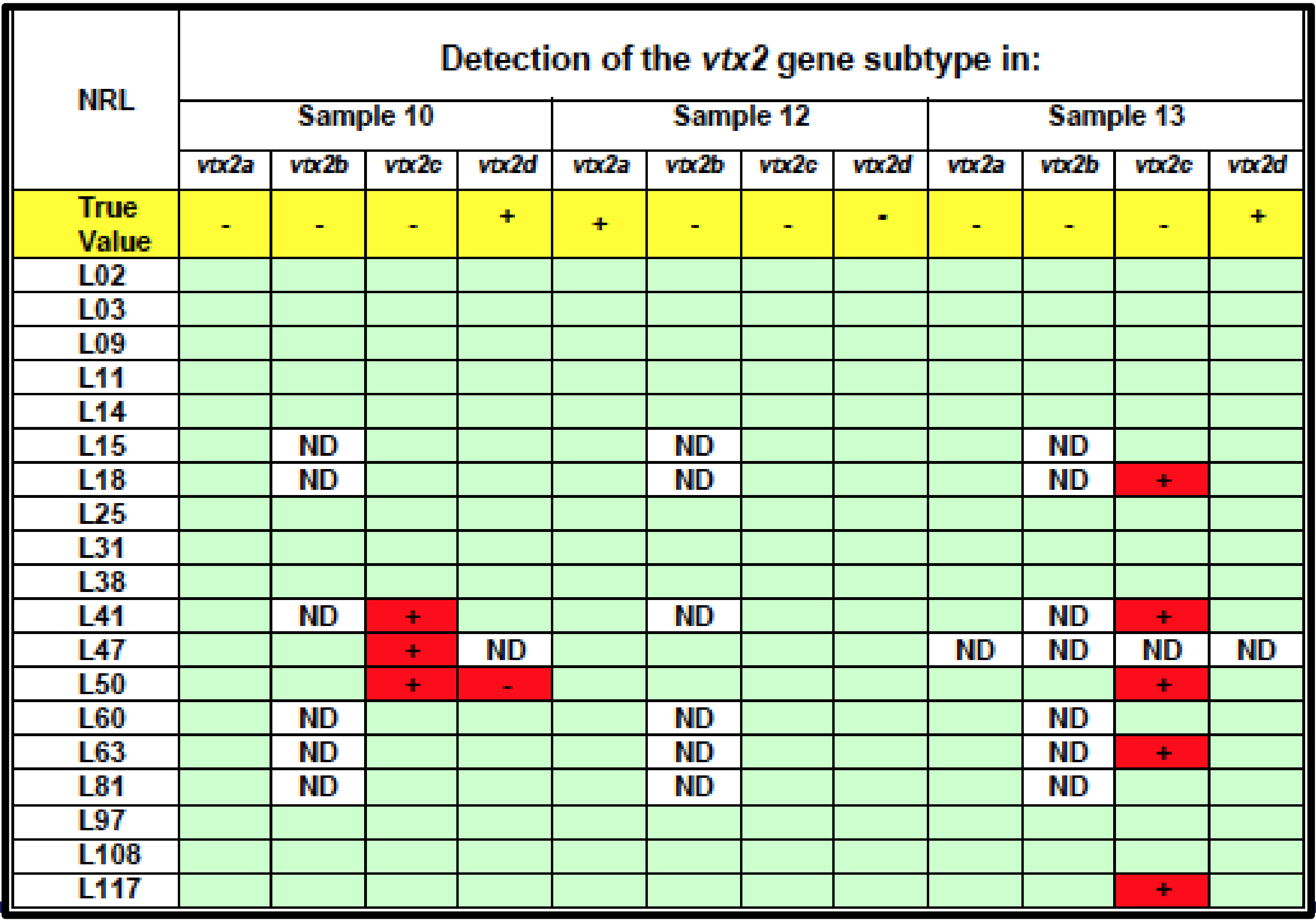


| NRL | Detection of the *vtx2* gene subtype in: | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | | | | Sample 3 | | | | Sample 6 | | | | Sample 9 | | | |
| | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* |
| True value | - | + | - | - | + | - | - | - | + | - | - | - | + | - | + | - |
| L02 | | | | | | | | | | | | | | | | |
| L03 | | | | | | | | | | | | | | | | |
| L09 | | | | | | | | | | | | | | | | |
| L11 | | | | | | | | | | | | | | | | |
| L14 | | | | | | | | | | | | | | | | |
| L15 | | ND | | | | ND | | | | ND | | | | ND | | |
| L18 | | ND | | | | ND | | | | ND | | | | ND | | |
| L25 | | | | | | | | | | | | | | | | |
| L31 | | | | | | | | | | | | | | | - | |
| L38 | | | | | | | | | | | | | | | | |
| L41 | | ND | | | | ND | | | | ND | | | | ND | | |
| L47 | | | | | | | | | | | | | | | | + |
| L50 | | | | | | | | | | | | | | | | |
| L60 | | ND | | | | ND | | | | ND | | | | ND | | |
| L63 | | ND | | | | ND | | | | ND | | | | ND | | |
| L81 | | ND | | | | ND | | | | ND | | | | ND | | |
| L97 | | | | | | | | | | | | | | | | |
| L108 | | | | | | | | | | | | | | | | |
| L117 | | | | | | | | | | | | | | | | |

# RESULTS: Sub-typing of *vtx2* genes (7 strains)

**The *vtx2 subtype* was identified correctly by 9 of the 19 NRLs (47 %)**

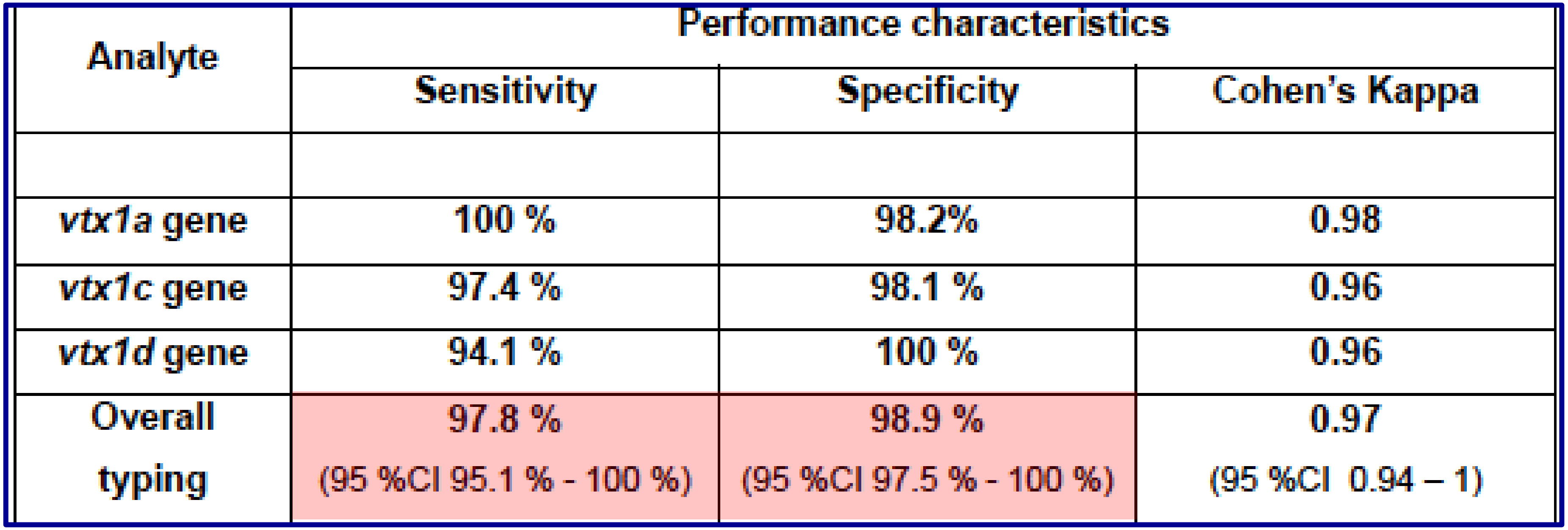
**(8 false positive results for *vtx2c* for *vtx2d*-positive strains )**



| NRL | Detection of the *vtx2* gene subtype in: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 10 | | | | Sample 12 | | | | Sample 13 | | | |
| | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* | *vtx2a* | *vtx2b* | *vtx2c* | *vtx2d* |
| True Value | - | - | - | + | + | - | - | - | - | - | - | + |
| L02 | | | | | | | | | | | | |
| L03 | | | | | | | | | | | | |
| L09 | | | | | | | | | | | | |
| L11 | | | | | | | | | | | | |
| L14 | | | | | | | | | | | | |
| L15 | | ND | | | | ND | | | | ND | | |
| L18 | | ND | | | | ND | | | | ND | + | |
| L25 | | | | | | | | | | | | |
| L31 | | | | | | | | | | | | |
| L38 | | | | | | | | | | | | |
| L41 | | ND | + | | | ND | | | | ND | + | |
| L47 | | | + | ND | | | | | ND | ND | ND | ND |
| L50 | | | + | - | | | | | | | + | |
| L60 | | ND | | | | ND | | | | ND | | |
| L63 | | ND | | | | ND | | | | ND | + | |
| L81 | | ND | | | | ND | | | | ND | | |
| L97 | | | | | | | | | | | | |
| L108 | | | | | | | | | | | | |
| L117 | | | | | | | | | | | + | |

# Sub-typing of *vtx1* genes

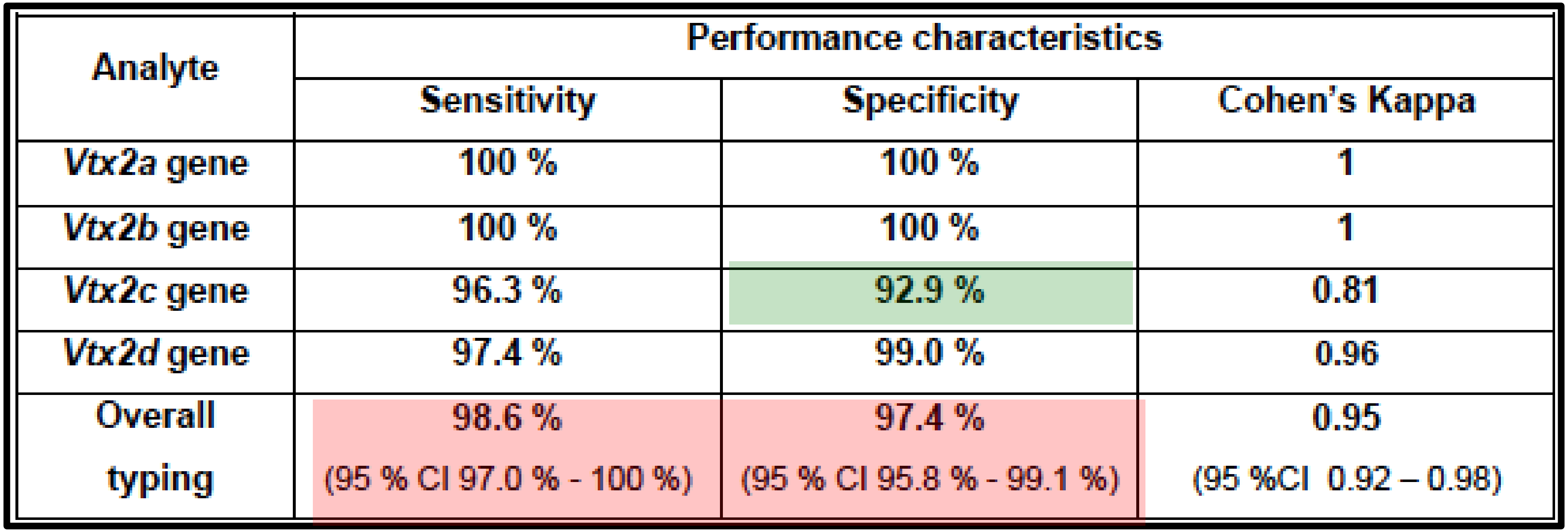
## Performance characteristics



| Analyte | Performance characteristics | | |
|---|---|---|---|
| | Sensitivity | Specificity | Cohen's Kappa |
| | | | |
| *vtx1a* gene | 100 % | 98.2% | 0.98 |
| *vtx1c* gene | 97.4 % | 98.1 % | 0.96 |
| *vtx1d* gene | 94.1 % | 100 % | 0.96 |
| Overall typing | 97.8 %<br>(95 %CI 95.1 % - 100 %) | 98.9 %<br>(95 %CI 97.5 % - 100 %) | 0.97<br>(95 %CI 0.94 – 1) |

# Sub-typing of *vtx2* genes

## Performance characteristics



| Analyte | Performance characteristics | | |
|---|---|---|---|
| | Sensitivity | Specificity | Cohen’s Kappa |
| *Vtx2a* gene | 100 % | 100 % | 1 |
| *Vtx2b* gene | 100 % | 100 % | 1 |
| *Vtx2c* gene | 96.3 % | 92.9 % | 0.81 |
| *Vtx2d* gene | 97.4 % | 99.0 % | 0.96 |
| Overall typing | 98.6 %<br>(95 % CI 97.0 % - 100 %) | 97.4 %<br>(95 % CI 95.8 % - 99.1 %) | 0.95<br>(95 %CI 0.92 – 0.98) |

| | Sensitivity | Specificity |
|---|---|---|
| | **95.5%** | **79.5%** |

**Results obtained in PT6 in 2010**

# CONCLUDING REMARKS

- ✓ 31 NRLs (26 EU MS) participated in the study
- ✓ Excellent agreement for all the lab in the detection of the VTEC target genes
- ✓ Good results for the detection of the “new” target genes of Enteroaggregative *E. coli*
- ✓ Six NRLs under-performed in the O serogroup identification and will need a follow-up PT
- ✓ Substantial improvement in the performance of the *vtx2* subtyping method with respect to the results obtained in PT6