5th Annual workshop of the EU Reference Laboratories for E. coli

Rome, 8 October 2010

# 4th and 5th inter-laboratory study on the detection and typing of VTEC - 2010

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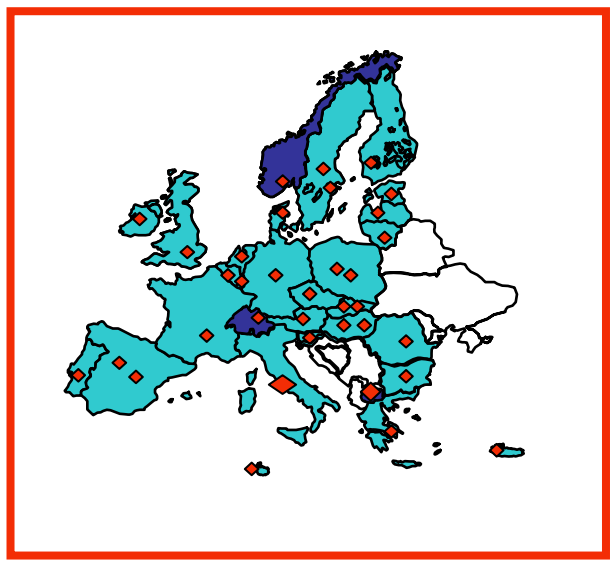


**Istituto Superiore di Sanità - Dipartimento Sanità Pubblica Veterinaria e Sicurezza Alimentare**

**European Union Reference Laboratory for *E.coli***

4th PT: detection of VTEC non-O157 in Food

5th PT: VTEC Typing

# 4th PT: detection of VTEC non-O157 in Food

Milk Samples (25 ml) spiked with

Sample 1
40 cfu/ml VTEC O103 *eae+; vt2+*
$10^2$ cfu/ml *E. faecium* ATCCL565
$10^2$ cfu/ml *E. coli* ATCC35218

Sample 2
$10^2$ cfu/ml *E. faecium* ATCCL565
$10^2$ cfu/ml *E. coli* ATCC35218

Uncertainty of Measurement (ISO TS 19036:2006)

VTEC O103 *eae+; vt2+* **0,24** log cfu/ml
*E. faecium* ATCCL565 **0,38** log cfu/ml
*E. coli* ATCC35218 **0,22** log cfu/ml

# 4th PT: detection of VTEC non-O157 in Food

***Stability assessment (17043:2010):***

Samples prepared on April the 27th and stored @ 4°C
Samples assayed on April, the 27th; 29th; May the 3rd; 5th; 10th; 12th; 14th.

***Homogeneity assessment (17043:2010):***

Samples prepared for shipment on May the 14th and stored @ 4°C.
Three sets of samples randomly selected immediatly after the preparation and assayed on the same day.

# 4th PT: detection of VTEC non-O157 in Food

**Samples shipped in refrigerated boxes on May the 17th.**

All Labs received the parcels between 18 and 19 of May .

**24 Labs received the samples at temperatures compliant with the ISO 7218:2007 (range 2-8°C).**

4 Labs received the samples at temperatures >8°C.
4 Labs received the samples at temperatures <2°C.
2 Labs did not report temperature upon arrival.

# 5th PT: VTEC Typing

Isolated VTEC strains seeded in soft-agar borosilicate glass vials

| Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|
| VTEC O121 | VTEC O91 | VTEC O113 | VTEC O145 | O111 |
| *vt2+, eae+* | *vt1+* | *vt1+, vt2+* | vt1+, eae+ | *eae* |

**Sample codes have been randomly assigned to each laboratory**

# 5th PT: VTEC Typing

***Sample preparation:***

*Reference stock*s stored in Microbanks under nitrogen vapours.
Selected samples revitalized on nutrient Agar on May the 3rd
Single colonies picked and streaked in replicates onto Nutrient Agar on May the 4th.
One replica used for strain checks on May the 5th.

***Homogeneity assessment (17043:2010):***

Samples prepared from the other replica on May the 6th and stored @ RT until shipment.
Three sets of samples randomly selected on May the 10th and assayed in the following two days.

# 5th PT: VTEC Typing

**Samples shipped in the same parcel of the 4th PT samples on May the 17th.**

Stability assessment not required.

Some vials of Samples 1 and 2 cross-contaminated.

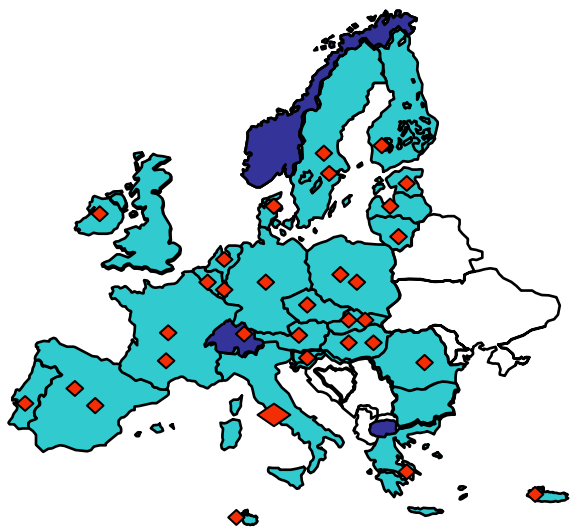
Rate of contamination below 1 out of three (not detected by the Homogeneity assessment).

Contamination Occurred during the preparation of vials (different combination of reportings).

# Upload of PTs results via web


# Partecipants to PT4 and/or PT5

- **Total participating labs to PT4 and/or PT5: 32**
- **E. coli NRLs**: 29/ 32 appointed NRLs (NRL Finland participated with two labs)
- **EU Member States**: 24 / 27 (Luxembourg participated via Belgium)
- **Non-EU countries** : Norway, Switzerland



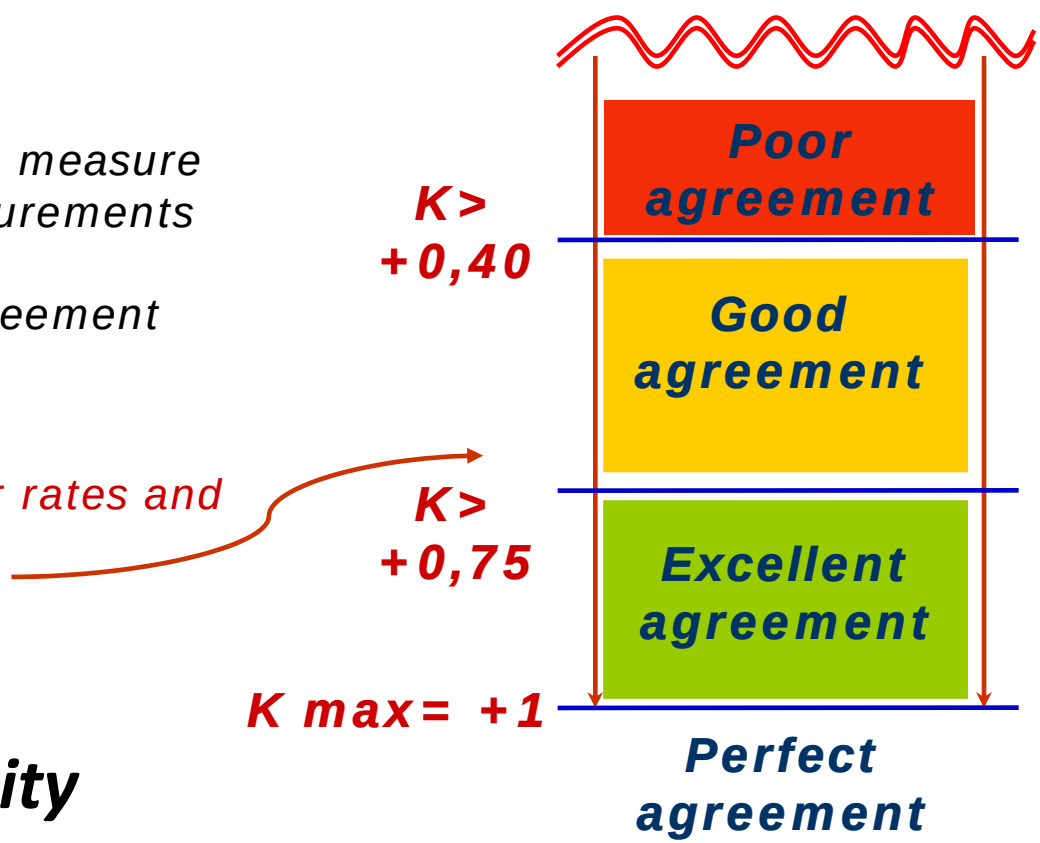
# Evaluation of the laboratory analytical perfomances

- ***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)*



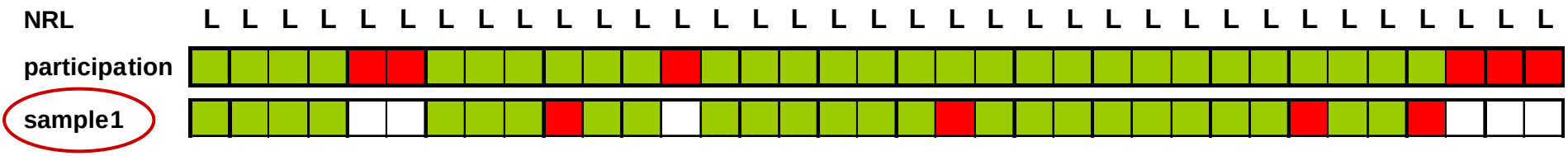

- ***Sensitivity*** *and* ***Specificity***

# Results: participation to 4th & 5th PT


PT 4th

- **Total participating labs to PT4: 29**
- **26 of 32 E.coli NRLs: 81%**
- **22 of 27 EU Member States: 81%**
- **2 countries outside EU**

# 4th PT : Results – Gold standard value and lab reports - 1

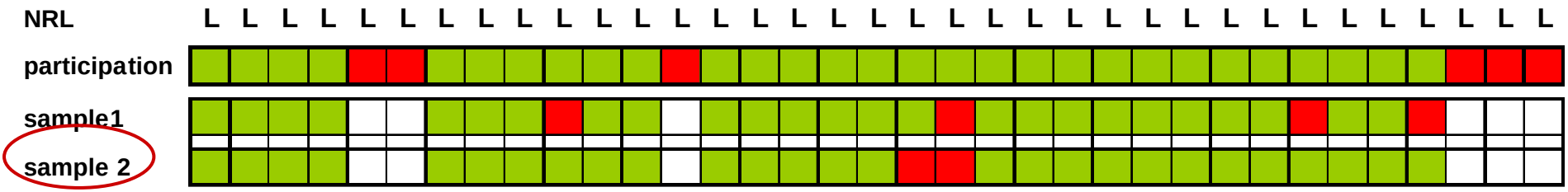



## Sample 1: O103, vtx1-, vtx2+, eae+

25 of the 29 (86%) participating labs correctly identified the results

- 2 labs failed the identification of the **O103 serogroup** specific gene in PCR and couldn't therefore isolate the strain
- 1 lab reported a **false positive vtx1** detection in PCR and genotyped as vtx1+ the isolated strain as well
- 1 lab failed to **isolate the strain** after correct detection by PCR of the virulence and serogroup specific antigens

# 4th PT : Results – Gold standard value and lab reports - 2

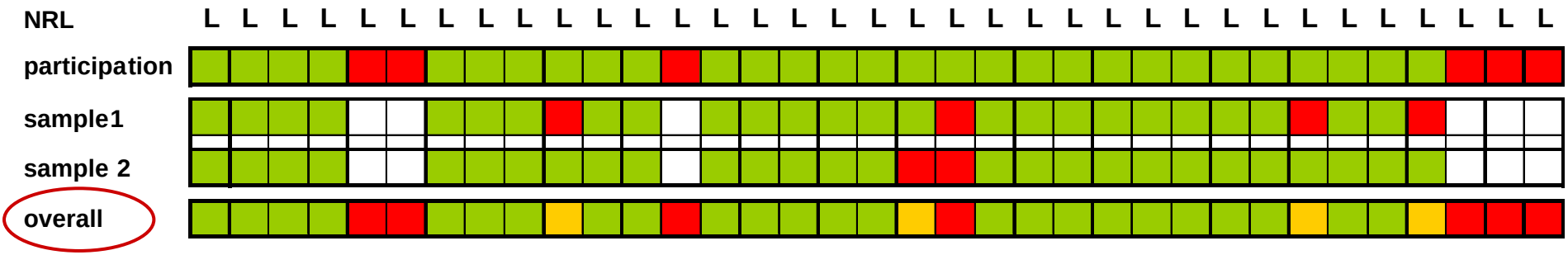



## Sample 2: negative result (PCR: vtx1-; vtx2-; eae-)

27 of the 29 participating labs (93%) correctly identified the sample

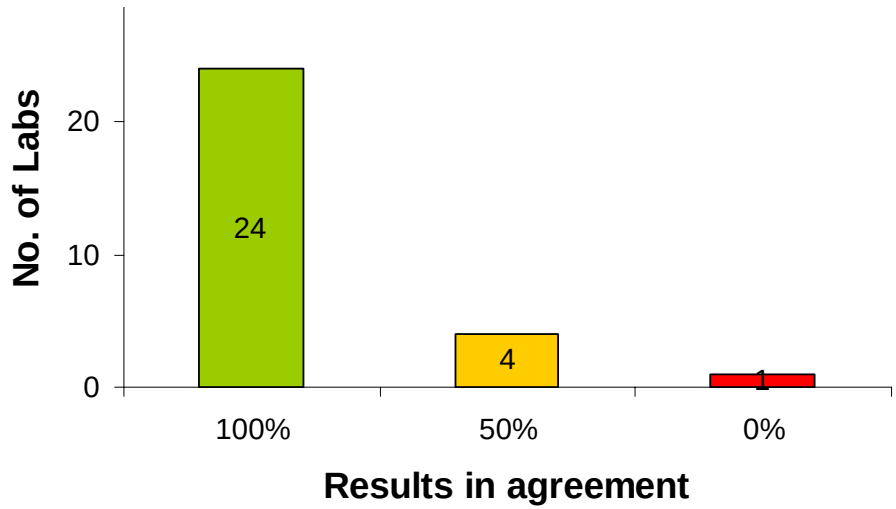
- 2 labs reported the **isolation of a O103 strain, vtx1+, vtx2-, eae+** and reported the false positive detection in PCR of O103 serogroup specific gene, vtx1 and eae genes
- possibly due to cross contamination during the analysis.

# 4th PT : Overall performances - 1




Of the 29 **participating labs**:

- 24 (83%) correctly identified both the samples
- 4 (14%) reported errors for one of the two samples
- 1 reported errors for both the samples




Of the 27 EU **Member States**:

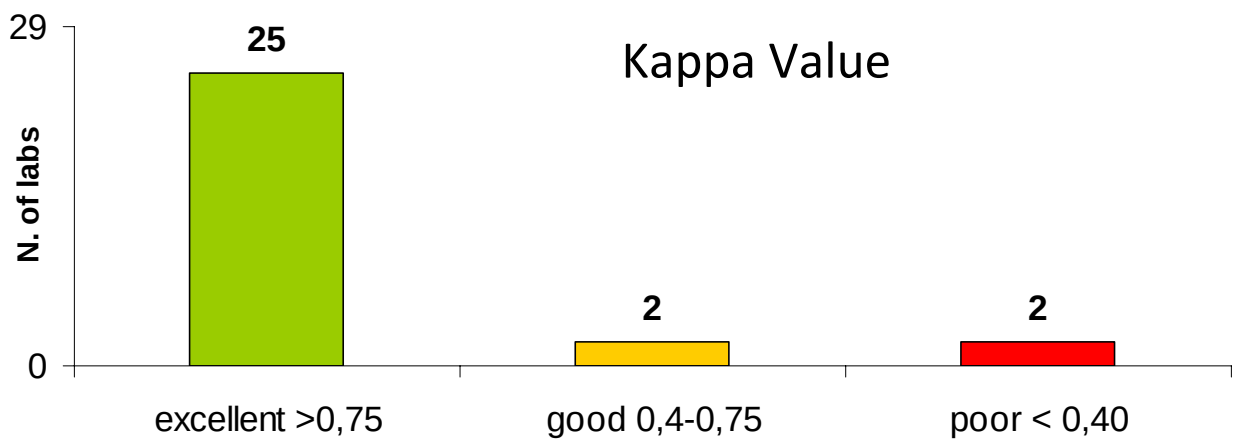
- 19 (70%) had at least one NRL able to participate in the PT 4 and correctly characterize the samples

# 4th PT : Laboratory performances - 2

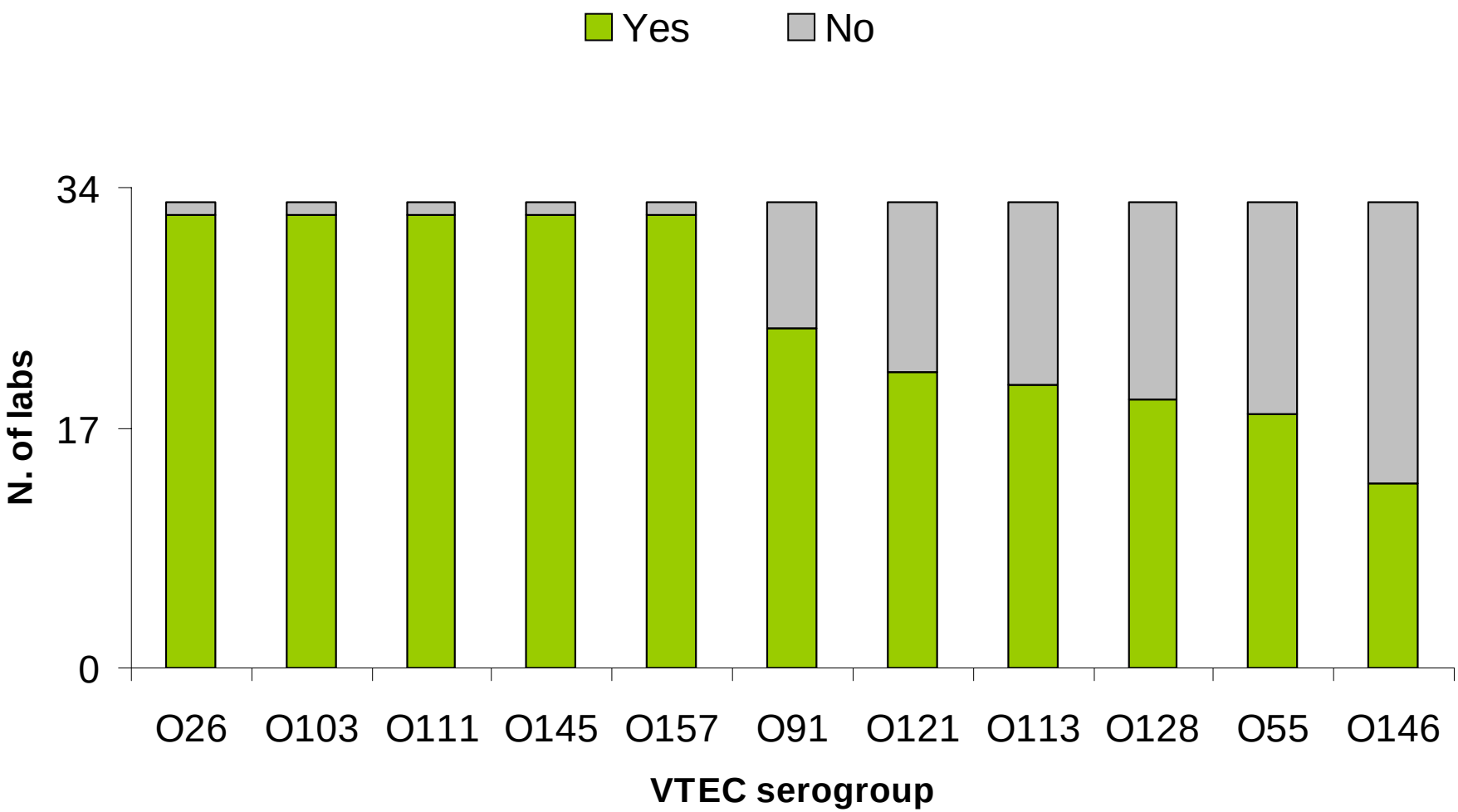
| | kappa | se | sp |
|---|---|---|---|
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 0,75 | **67%** | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 0,14 | 100% | **73%** |
| L | 0,2 | 100% | **64%** |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 0,75 | **67%** | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |
| L | 1 | 100% | 100% |

## Performances of the Real Time PCR assay

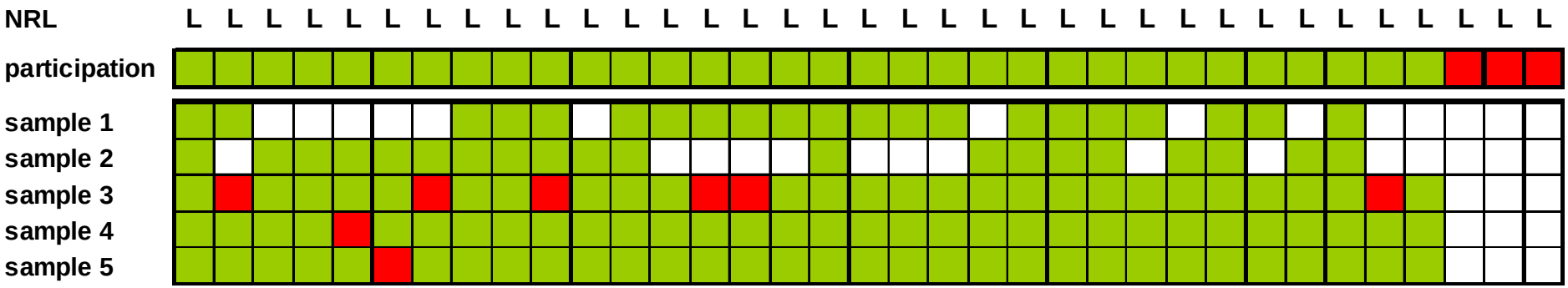
- Overall Kappa: 0.936
- Sensitivity: 97,7% (95% CI: 94,5% - 100%)
- Specificity: 97,8% (95% CI: 96,2% - 99,4%)



# 5th PT Results – Serotyping capability of the participating



# 5th PT Results – Gold standard value and lab reports - 1

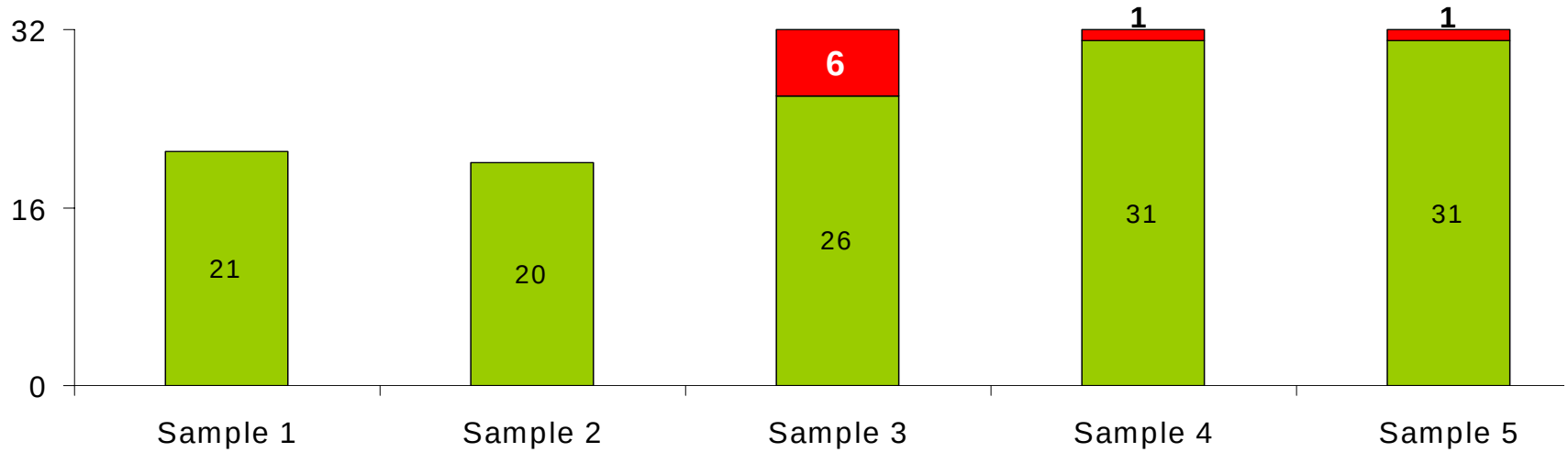



- Sample 1: **O121**, vtx1-, vtx2+, eae+
- Sample 2: **O91**, vtx1+, vtx2-, eae-
- Sample 3: **O113**, vtx1+, vtx2+, eae-
- Sample 4: **O145**, vtx1+, vtx2-, eae+
- Sample 5: **O111**, vtx1-, vtx2-, eae+

Assessed cross contamination of samples 1 & 2

For samples 1 & 2 false results weren't taken into account !!

# 5th PT Results – Gold standard value and lab reports - 2




**Considering only samples 3,4,5**

- Correct results 88 (91%)
- False results: 8 (8,3%)

**Sample 3**:

- 3 labs failed to detect serogroup O113
- 2 labs reported a false negative for vtx1
- 1 lab reported a false positive for eae

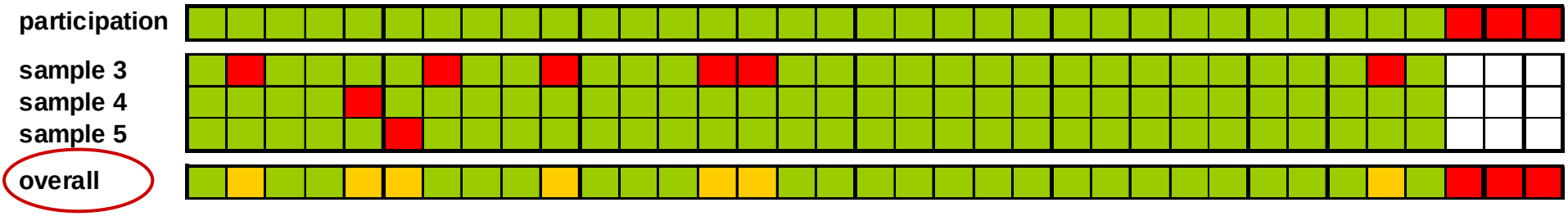
**Sample 4**:

- 1 lab failed to detect serogroup O145

**Sample 5**:

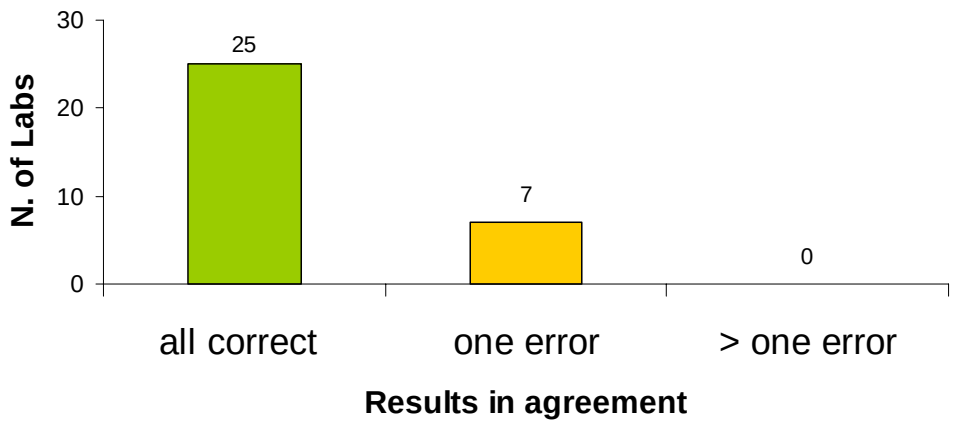
- 1 lab reported a false positive vtx1

# 5th PT : Overall performances (only samples 3, 4, 5)




Of the 32 **participating labs**:

- 25 (76%) correctly identified all the samples
- 7 (21%) reported false results for one samples



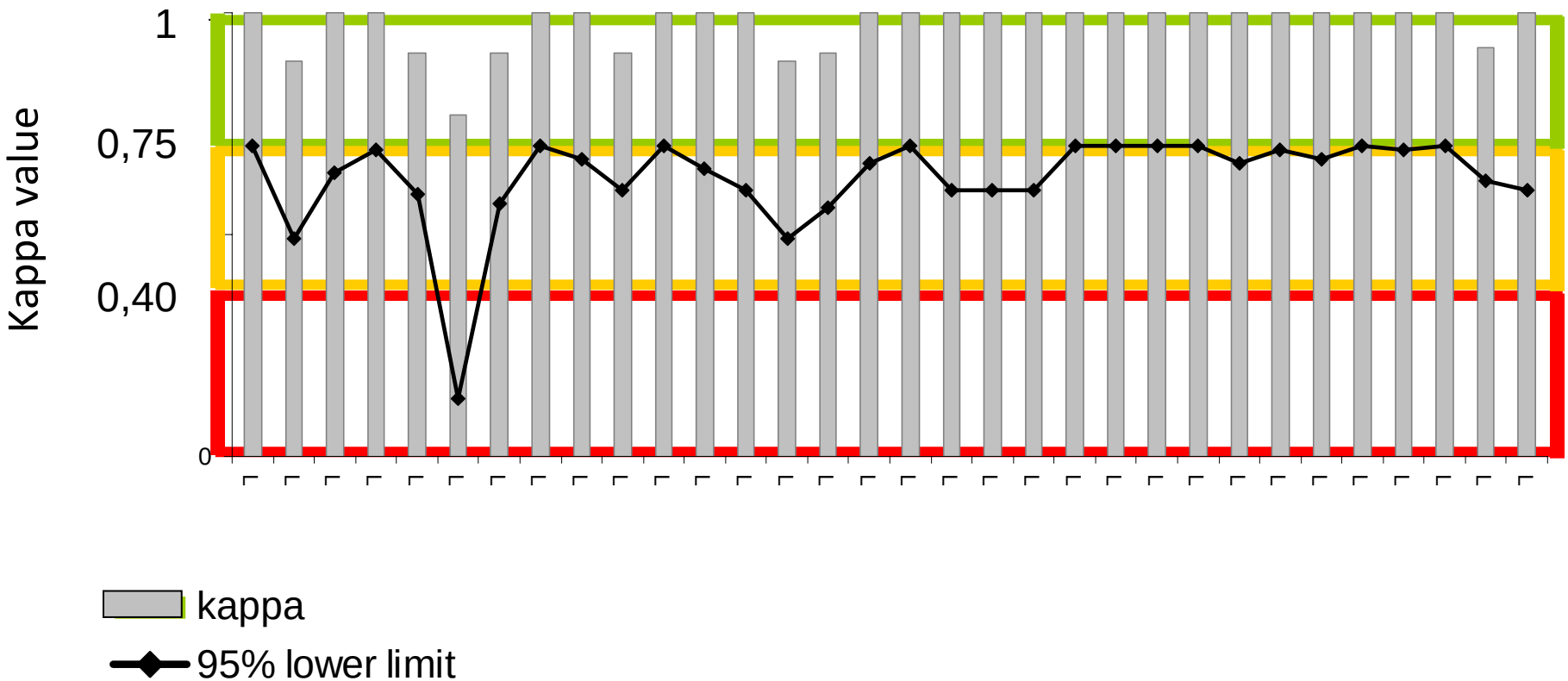

Of the 27 EU **Member States**:

- 19 (70%) had at least one NRL able to participate in the PT 5 and correctly typed all the strains

# 5th PT : Lab performances (only samples 3, 4 ,5)

## Agreement – Kappa value

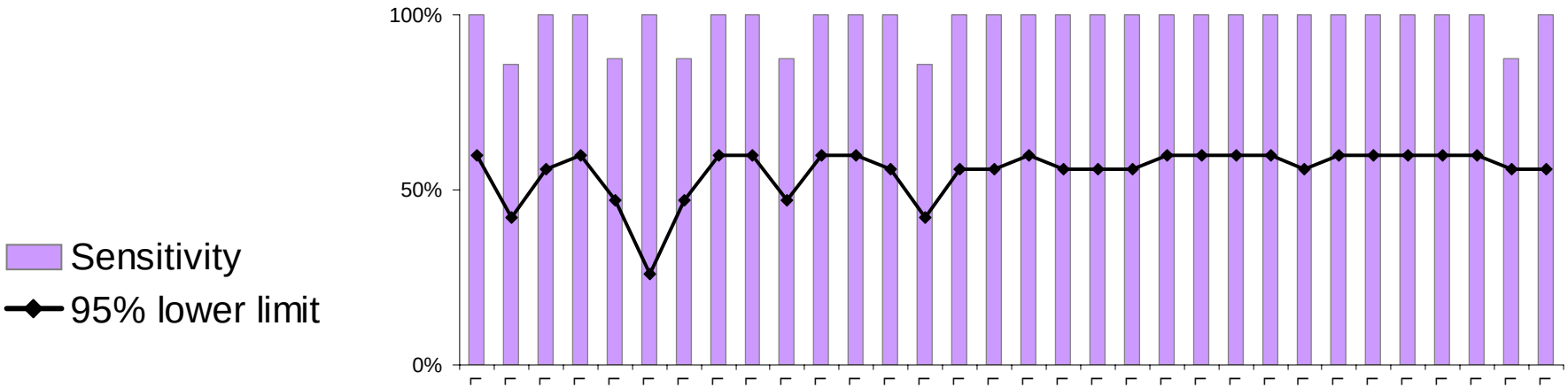
**Overall Kappa: 0,98** (95% CI 0,92 - 1,0)

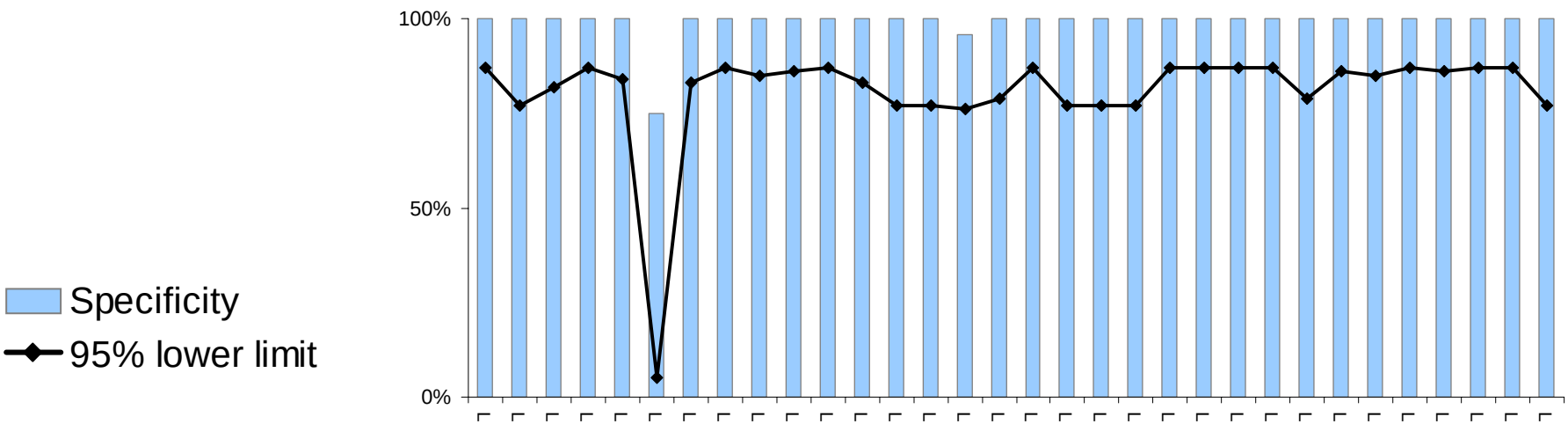


# 5th PT : Lab performances (only samples 3, 4, 5)

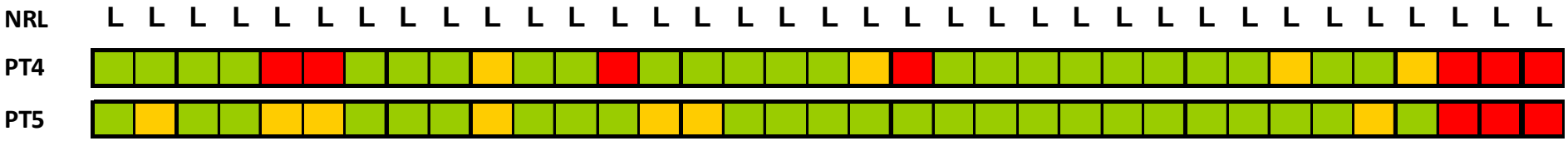
**Overall Sensitivity: 97,5%** (95% CI 95,5% – 99,4%)

**Overall Specificity: 99,7%** (95% CI 99,4% – 100%)



# 4th & 5th PT - Summary




- 20 labs participated and fully performed to both PT 4th & 5th
- 8 labs participated to both the PTs but reported errors in one of the two PTs
- 1 lab fully performed but partecipated only to PT 5th
- 3 labs had unsatisfactory performances in both PT 4th & 5°
- 3 labs didn't partecipate in both PTs

# 4th & 5th PT – Concluding remarks

## Strenghts

- Web-based reporting
- Further increase in the participation and improvement in performances
- Acquisition of the International Standard for VTEC detection in food
- Most NRLs can identify VTEC genotypes
- Most NRLs can identify the “top-five” VTEC serogroups

## Weakness

- 3 NRLs did not participate
- 1 NRL did not perform serotyping even for O157
- cross-contamination of samples for the 5th

# Lessons learnt from the 4th and 5th PTs

**Corrective actions on the EU-RL VTEC side**

- Set the temperature reporting as mandatory in the web portal.
- Prepare vials for each strain in different days/spaces to avoid cross-contaminations.
- Increase the number of tests for homogeneity assessment in order to reveal problems.

# Under-proficiency management

- Advice and support continuously provided to NRLs by individual contacts (Phone, E-mail)
- The training program for the NRLs will continue.
- A standard 1-week training program has been developed.
- *Ad hoc* visits will be done to the Labs with recurrent under-proficiency

# One week-training program at EU RL-VTEC

**VTEC identification and characterisation by molecular methods-Hands-on Approach-**

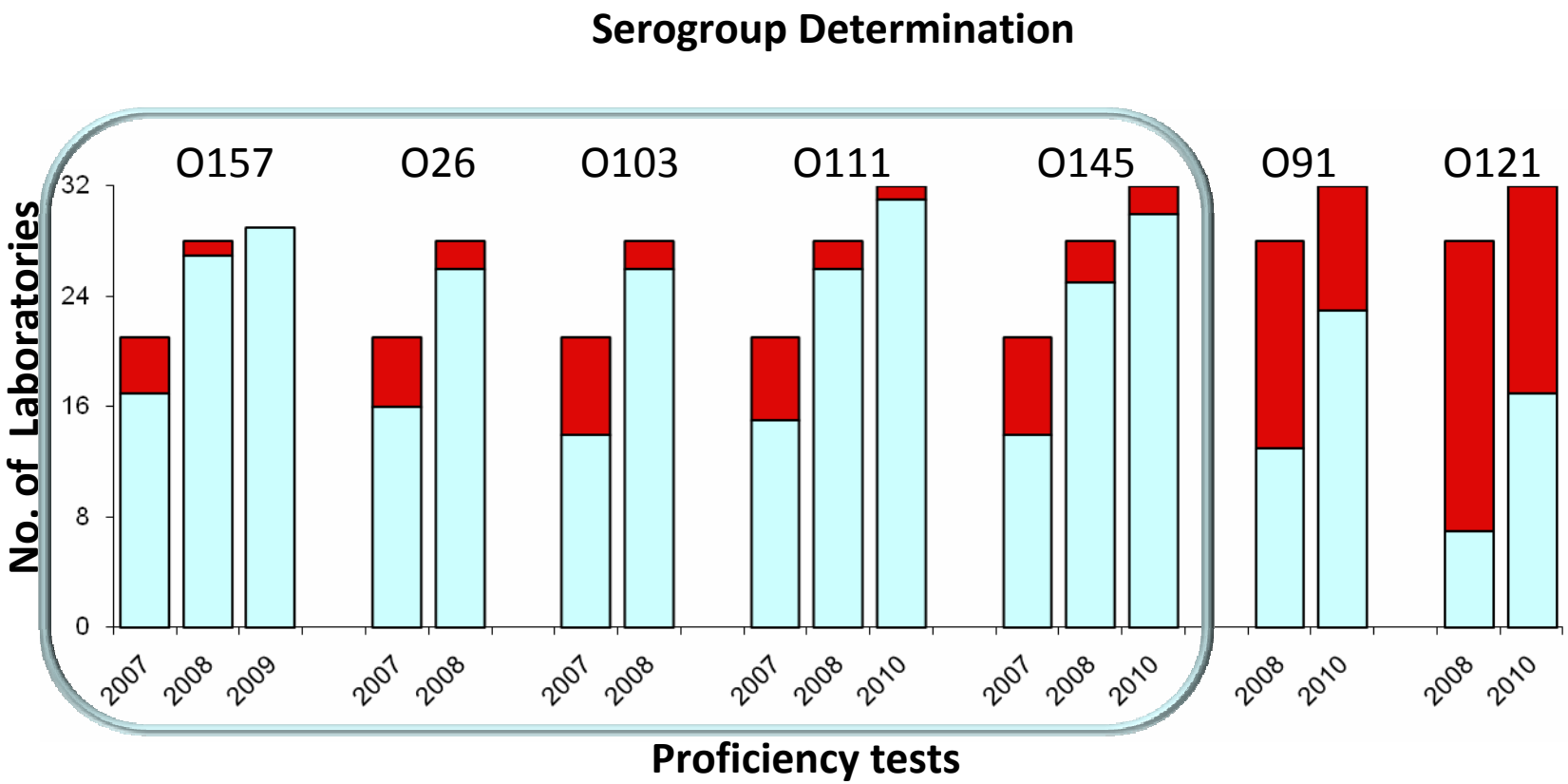
- **Day 1**
  - introduction to conventional PCR.
  - Inocula of test strains and control strains
  - Overview on the activities of the EU RL-VTEC
- **Day 2**
  - Template preparation and introduction to Real Time PCR
  - Conventional PCR for STEC detection and typing (CRL_Method 01 28_04_08)
  - Molecular serogrouping by conventional PCR
- **Day 3**
  - Agarose gel electrophoresis
  - discussion on the conventional PCR results
  - Test and control strains DNA preparation for Real Time PCR
- **Day 4**
  - Real Time PCR according to the ISO TS for the identification and characterisation of using **different** Real Time technologies.
- **Day 5**
  - Evaluation of the results obtained and general discussion on the activities carried out

# Trend analyses after a four-year activity period

**EU RL main objectives in the 1st mandate:**

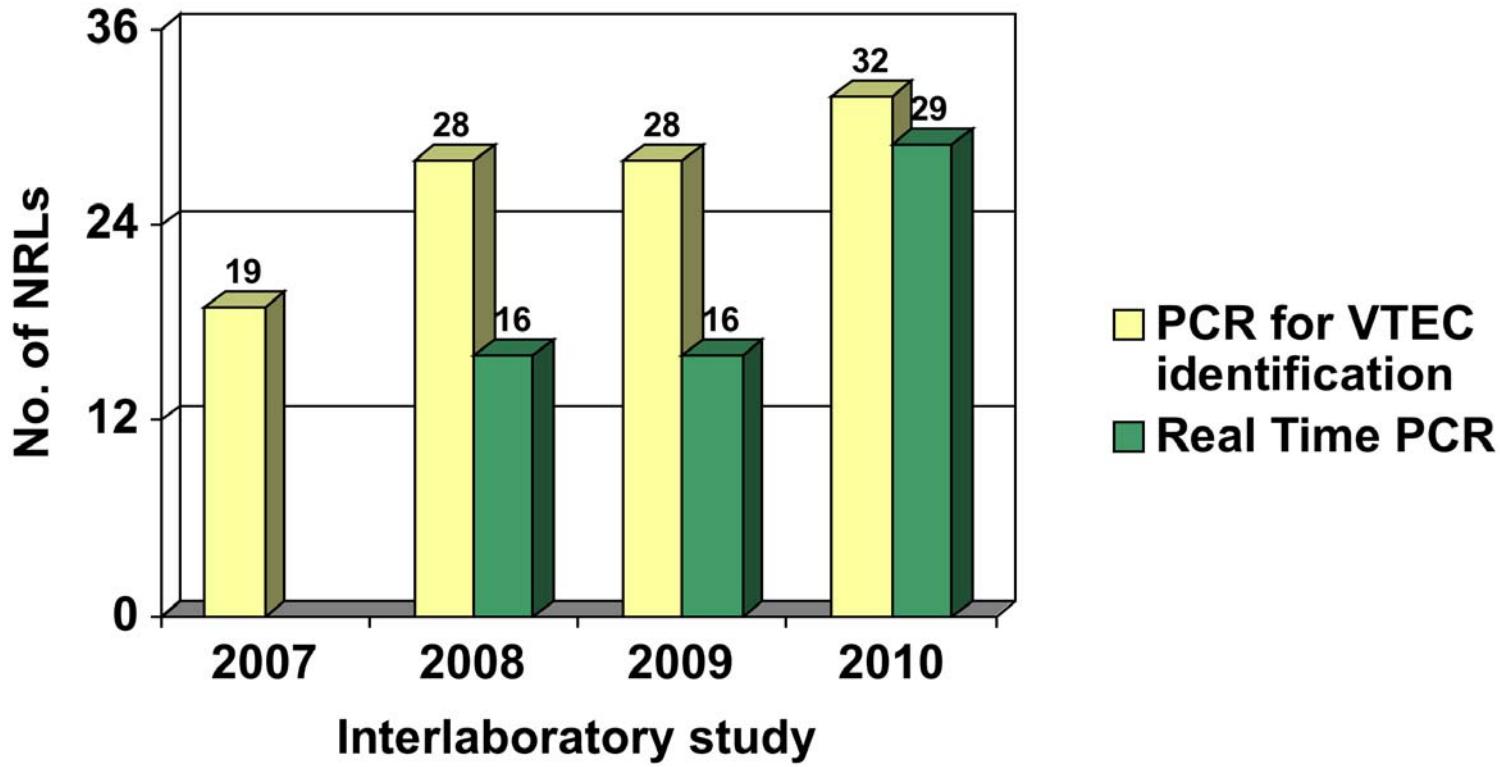
- Establish and consolidate the network of NRLs
- Spread knowledge and technical skill on VTEC detection, identification and typing, particularly non-O157 VTEC
- Increase the coverage in the EU with respect to VTEC detection in food
- Build up an harmonized system for monitoring of VTEC in food and animal populations (zoonoses directive).

# Trend analyses after a four-year activity period

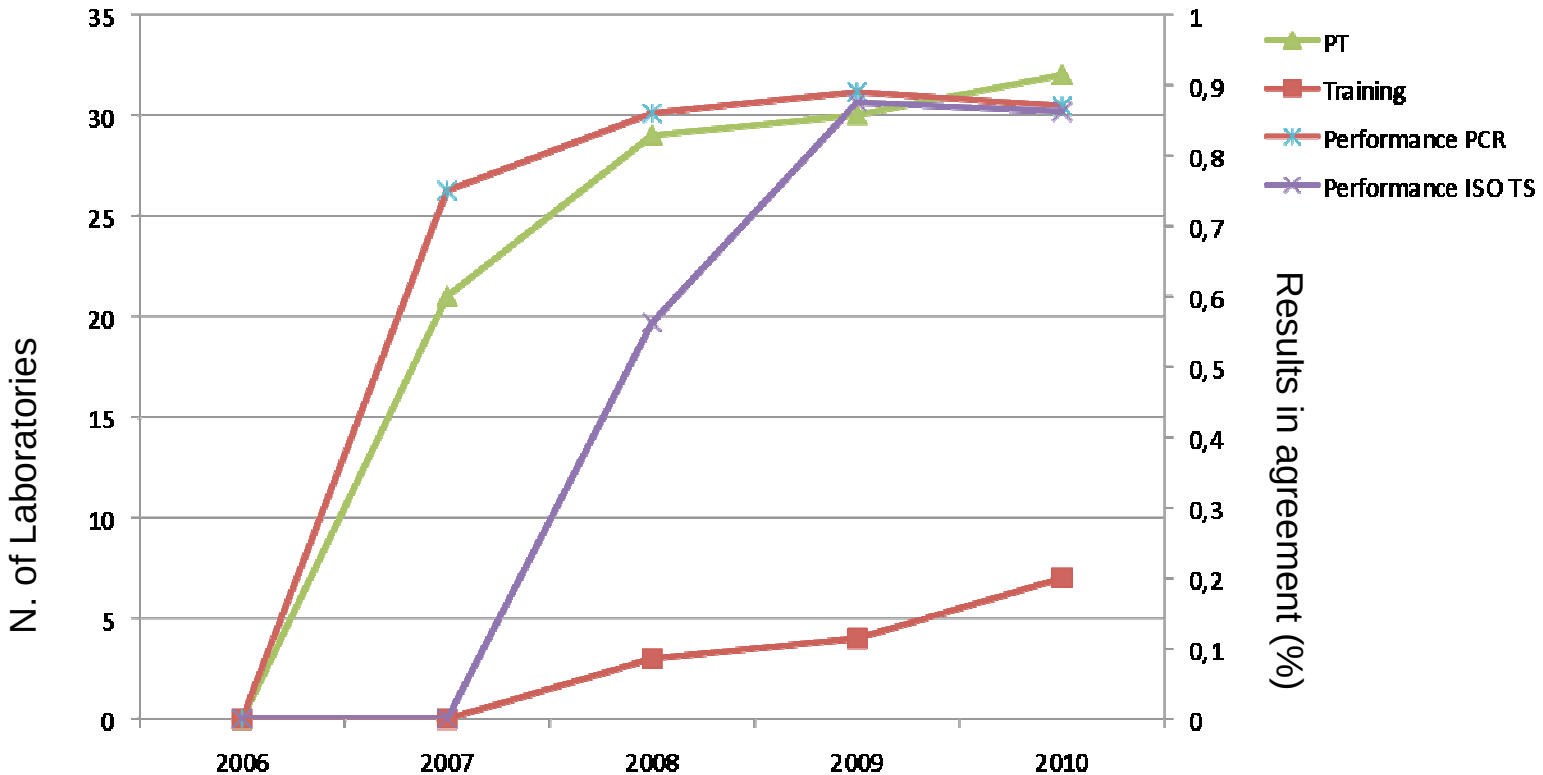


# Trend analyses after a four-year activity period

Molecular Methods



# Trend analyses after a four-year activity period



# Thank you!

For your attention

And for the efforts devoted to the success of the VTEC Network!