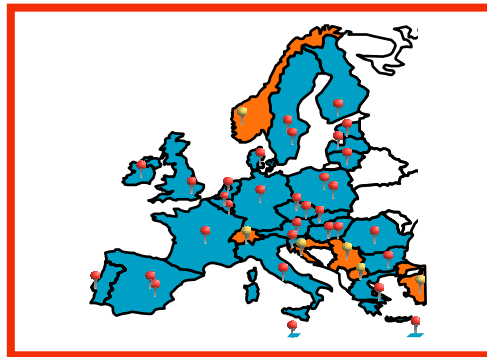


## Results of the 7<sup>th</sup> PT on the detection of VTEC in vegetables



Rosangela Tozzoli, Gaia Scavia, and Clarissa Ferreri



# 7<sup>th</sup> PT on the detection of VTEC in vegetables

## Samples: raw spinach spiked with:

Contaminant	Sample A	Sample B	Sample C
VTEC O157, <i>vtx1, vtx2, eae</i>	40 CFU/gr	0	0
VTEC O145, <i>vtx1, eae</i>	0	40 CFU/gr	0



Uncertainty of measurement (ISO TS 19036:2006)  
VTEC O157: **0.27 logCFU**  
VTEC O145: **0.37 logCFU**

# Detection of pathogenic non-O157 VTEC in food



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## ISO/DTS 13136



**Microbiology of food and animal feeding stuffs – Real-time polymerase chain reaction (PCR)-based method for the detection of food borne pathogens – Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) belonging to O157, O111, O26, O103 and O145 serogroups**

### General information

**Number of Pages:**

**Edition:** 1 (Monolingual)

**ICS:** [07.100.30](#)

**Status:**  Under development

**Stage:** [30.99](#) (2011-07-14)

**TC/SC:** [TC 34/SC 9](#)

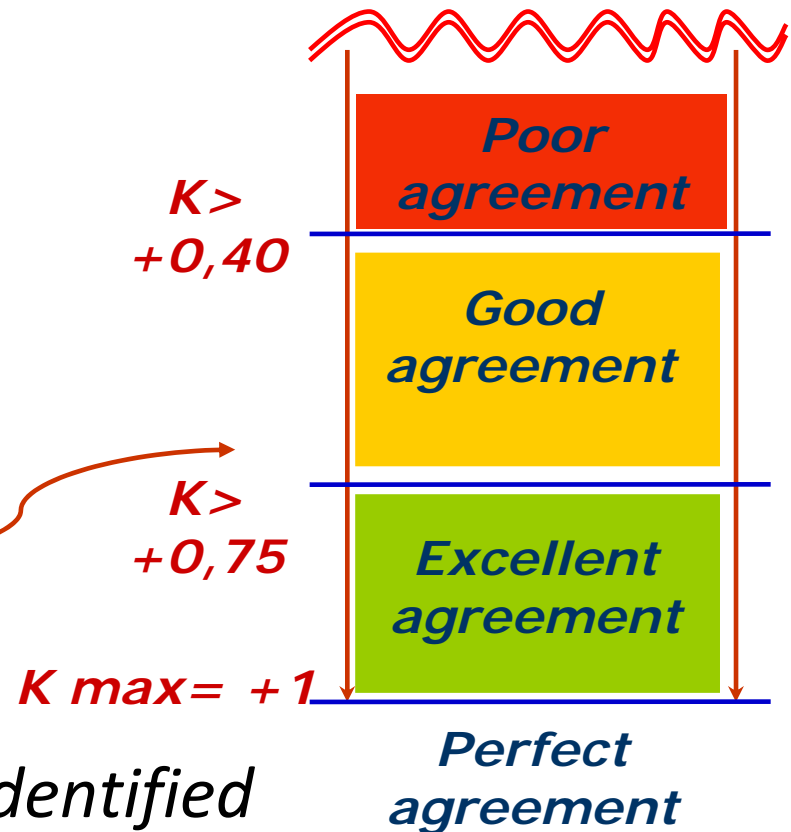


# Evaluation of the laboratory analytical performances

- **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**
- **Proportion of results correctly identified**

# 7<sup>th</sup> PT - results: participation

Laboratories receiving samples of the 7<sup>th</sup> PT: **41**

- ✓ 26 of 32 E.coli EU-NRLs: 81%
- ✓ 22 of 27 EU Member States: 81%
- ✓ 4 NRL non EU countries
- ✓ 11 other public labs involved in food control (10 in Italy)

## *Number of laboratories*

	<i>Receiving Samples</i>	<i>Providing Results</i>
	26	
	4	
other	11	10
<b>overall</b>	<b>41</b>	<b>38</b>

# 7<sup>th</sup> PT - results: step 1 – Real Time PCR screening

Lab	Sample 1								Sample 2								Sample 3							
	vtx1	vtx2	eae	O157	O26	O103	O111	O145	vtx1	vtx2	eae	O157	O26	O103	O111	O145	vtx1	vtx2	eae	O157	O26	O103	O111	O145
GS	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L01	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L02	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L04	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L05	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L14	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L15	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L26	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

22 Labs provided correct results for all the samples

L51	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L53	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L55	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L57	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L58	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L62	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L69	-	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L70	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L75	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-
L77	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L78	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L82	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L87	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L91	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L94	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L95	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
L98	+	+	+	+	-	-	-	-	+	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

# 7<sup>th</sup> PT - results: **step 1 – Real Time PCR screening**

## **Sample 1**

false negative results for: *vtx1* (3 labs), *vtx2* (2 labs), *eae* (2 labs)

## **Sample 2**

False negative results for *vtx1* (2 labs) and *eae* (2 labs)

## **Sample 3**

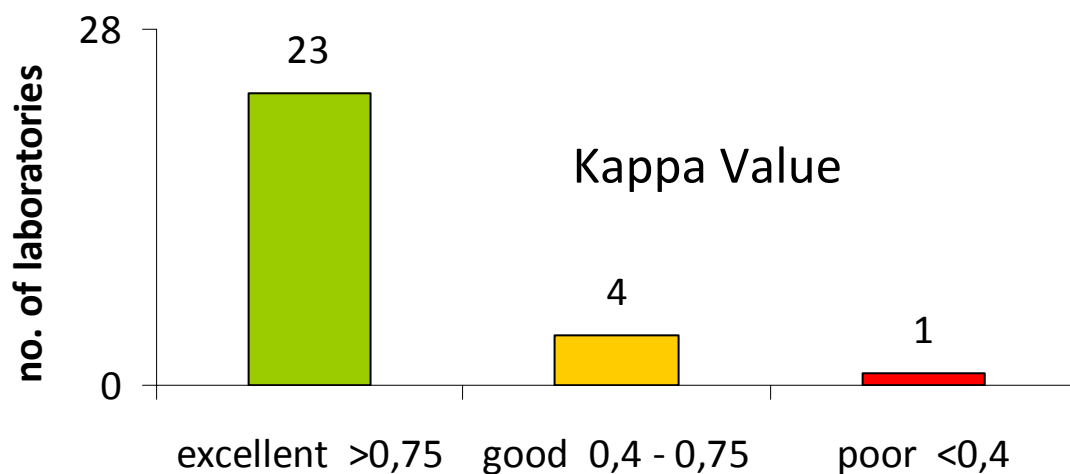
False positive *vtx1*, *vtx2*, *eae* and O157 serogroup specific antigens (cross contamination of the PCR reaction??)

# 7<sup>th</sup> PT - results: step 1 - PCR screening

Kappa	Se	Sp
1	100	100
na	na	na
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
0,88	85,7	100
0,53	50	100
0,65	100	76,5
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
1	100	100
0,7	66,6	100
1	100	100
1	100	100

## Performances of the Real Time PCR assay

- Overall Kappa: 0.94 (95% CI: 0.85 – 1)
- Sensitivity: 94,3% (95% CI: 90,1% - 97,6%)
- Specificity: 98,8% (95% CI: 97,6% - 100%)





# 7<sup>th</sup> PT - results: step 2 – Isolation and genotyping

Lab	sample 1				sample 2				sample 3		
	O157	vtx1	vtx2	eae	O145	vtx1	vtx2	eae	vtx1	vtx2	eae
L01	+	+	+	+	+	+	-	+			
L02											
L04	+	+	+	+	+	+	-	+			
L05	+	+	+	+	+	+	-	+			
L14	+	+	+	+	+	+	-	+			
L15											
L26					+	+	-	+			
L31	+	+	+	+	+	+	-	+			

21 Labs provided correct results for all the samples

L53	+	+	+	+	+	+	-	+			
L55	+	+	+	+	+	+	-	+			
L57	+	+	+	+	+	+	-	+			
L58	+	+	+	+	+	+	-	+			
L62	+	+	+	+	+	+	-	+			
L69	+	-	+	+	+	+	-	+			
L70											
L75	+	+	+	+	+	+	-	+			
L77	+	+	+	+	+	+	-	+			
L78	+	+	+	+	+	+	-	+			
L82	+	+	+	+	+	+	-	+			
L87	+	+	+	+	+	+	-	+			
L91					+	+	-	+			
L94	+	+	+	+							
L95	+	+	+	+	+	+	-	+			
L98	+	+	+	+	+	+	-	+			

# 7<sup>th</sup> PT - results: step 2 – Isolation and genotyping

Lab	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98	
sample 1	Green	Red	Green	Green	Green	Red	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red	Green	Green	Green	Green	Green	Red	Green	Green	Green

## Sample 1: O157, vtx1+, vtx2+, eae+

22 of the 28 labs (78%) correctly isolated and genotyped the O157 strain

- 1 lab isolated the O157 but genotyped the strain as **vtx1- (false negative)** (L69)
- 1 lab **did not report results or did not perform the isolation step** (L15)
- 2 labs **could not isolate the O157 strain** despite a correct result in the PCR screening step (L26, L91)
- 2 lab **did not isolate the strain as a consequence of errors** made in the PCR screening step (L2, L70)

# 7<sup>th</sup> PT - results: step 2 – Isolation and genotyping

Lab	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98
sample 2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Sample 2: O145, vtx1+, vtx2-, eae+

24 of the 28 NRLs (86%) correctly identified the sample

- 2 labs **did not report results or did not perform the isolation step** (L70, L15)
- 2 labs did not isolate the strain as a consequence of errors made in the PCR screening step (L2, L94)

# 7<sup>th</sup> PT - results: step 2 – Isolation and genotyping

Lab	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98	
sample 3																													

## Sample 3: negative result (PCR: vtx1-; vtx2-; eae-)

28 of the 28 laboratories (100%) correctly identified the sample

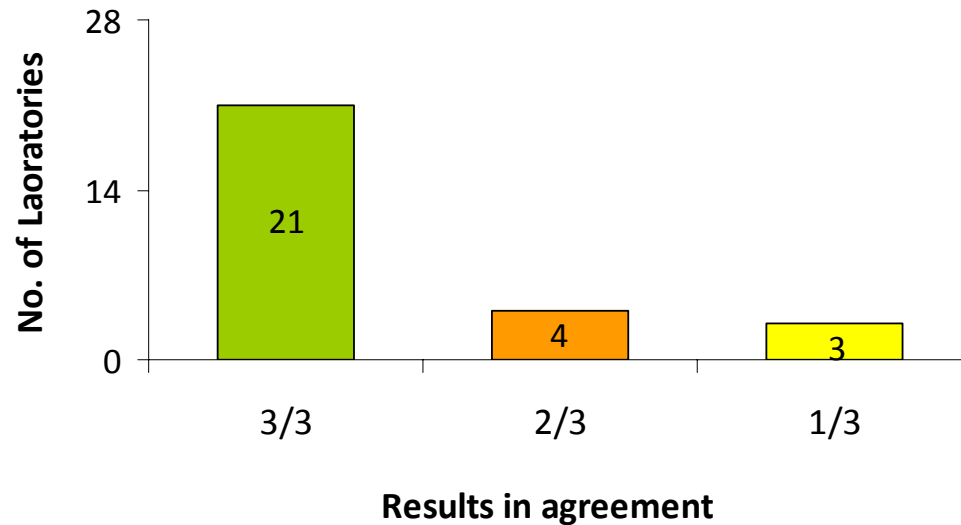
- 1 lab (L75) reported the positive detection of vtx1, vtx2, eae and O157 serogroup specific antigens in PCR (possibly due to cross contamination of Real Time PCR reaction) but didn't report the isolation of the strain

# 7<sup>th</sup> PT - results: step 2 – Isolation and genotyping

Lab	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98		
sample 1	Green	Red	Green	Green	Green	Red	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red	Green	Green	Green	Green	Green	Green	Red	Green	Green	Green	
sample 2	Green	Red	Green	Green	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red	Green	Green	Green	Green	Green	Green	Red	Green	Green	Green
sample 3	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

Of the 28 participating labs:

- 21 (75%) correctly isolated the two strains
  - 7 labs made errors



# 7<sup>th</sup> PT : Overall performance - 1

## Step 1 – Real Time PCR screening

	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98	
sample 1																													
sample 2																													
sample 3																													

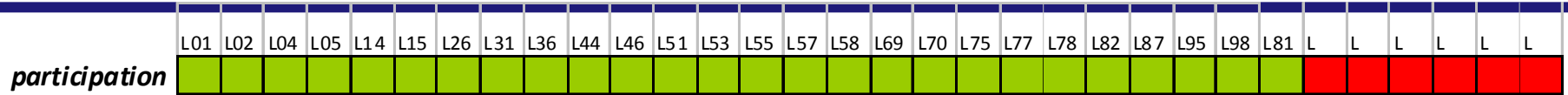
## Step 2 – Strain Isolation and genotyping

Lab	L01	L02	L04	L05	L14	L15	L26	L31	L36	L44	L46	L51	L53	L55	L57	L58	L62	L69	L70	L75	L77	L78	L82	L87	L91	L94	L95	L98	
sample 1																													
sample 2																													
sample 3																													

Of the 28 participating labs:

- **20 (71%)** did not report errors in both screening and isolation steps
- **19** of the 25 EU-RLs (**76%**) reported correct results for all the samples
- Of the 22 EU Member States represented in the PT 7, **16 (73%)** correctly characterized all the samples

# 7<sup>th</sup> PT : Summary of the performances of the 32 EU-NRLs



26 of 32 NRLs: 81%



25 of 32 NRLs: 78%



**19 of 32 NRLs: 60%**



16 of 27 Member States (63%) had an NRL able to participate in the PT 7 and to identify correctly all the samples

# Thank you!



For your attention

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