

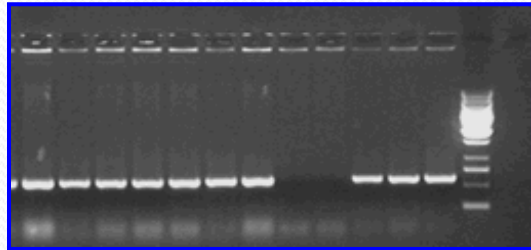
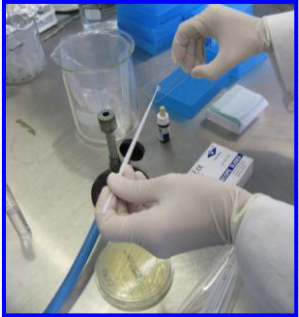
7<sup>th</sup> Annual workshop of the EU Reference Laboratories for *E. coli*, Rome 8-9 November 2011

**The upcoming Proficiency Tests:  
PT9 on the detection of VTEC in seeds  
and PT10 on strain typing**

**Rosangela Tozzoli**

# Functions and duties of EU-RLs (882/2004)

Development of methods for the detection of VTEC in food and animal samples and the organisation of proficiency tests (PT) on those methods for the designated NRLs for *E. coli* in the EU and EFTA Member States, EU Candidate Countries, and certain third countries.



# 9<sup>th</sup> inter-laboratory study on VTEC detection in seeds intended for sprout production (PT9)



**EU Reference Laboratory for *E.coli***  
*Department of Veterinary Public Health and Food Safety*  
*Unit of Foodborne Zoonoses*  
**Istituto Superiore di Sanità**



**9<sup>th</sup> inter-laboratory study on the detection of Verocytotoxin-producing *E. coli* (VTEC) belonging to the serogroups most involved in human infections (O157, O111, O26, O103, and O145) in samples of seeds intended for sprout production**

**Laboratory Guideline**

Summer 2011

Inter-laboratory study on naturally contaminated seeds

Three samples of beet seed lots sent to 8 Labs (July 2011)

Sample A *	Sample B	Sample c
Real Time PCR results expected		
<i>vtx2+</i> , <i>ehly+</i> <i>vtx1-</i> , <i>eae-</i>	Negative	Negative

\* VTEC O74, *vtx2+*, *ehly+* and *vtx1-*; *eae-*  
Isolated from the seed lot

Inter-laboratory study on naturally contaminated seeds  
**CONFLICTING RESULTS**

Sample (Expected results)	No. Positive / No. of Labs			
	<i>vtx1</i>	<i>vtx2</i>	<i>eae</i>	<i>e-hly</i>
<b>A</b> ( <i>vtx2+</i> , <i>e-hly+</i> )	0/8	0/8	2/6*	0/4
<b>B</b> ( <i>negative</i> )	0/8	1/8	0/6	0/4
<b>C</b> ( <i>negative</i> )	0/8	0/8	0/6	0/4

**\* The *eae* + strain isolated by both Labs !!!!**

# Studies on naturally contaminated seeds

- ✓ Another lot of beet seeds
- ✓ **VTEC O74**, ***vtx2+*** isolated on 12 June 2011
- ✓ Testing of other 8 aliquots (50g) on 14 July 2011

Date of testing	Real Time PCR for <i>vtx2</i> (Ct)								
	Aliquots								
	1	2	3	4	5	6	7	8	9
12 June 2011	<b>+</b> <b>(Ct=23)</b>								
14 July 2011		<b>+</b> <b>(Ct=36)</b>	<b>-</b>	<b>-</b>	<b>+</b> <b>(Ct=37)</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>

## Studies on naturally contaminated seeds

### Conflicting results: **why** ????

- ✓ **Low level of contamination (*but strains isolated .....*)**
- ✓ **Death of bacteria (*but short interval .....*)**
- ✓ **Not homogeneous contamination of lots (*most likely .....*)**

# Troubles with seeds testing....



European Food Safety Authority

EFSA Journal 2011;9(10):2390

## SCIENTIFIC REPORT OF EFSA

### Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Europe: Taking Stock<sup>1</sup>

European Food Safety Authority<sup>2,3</sup>

European Food Safety Authority (EFSA), Parma, Italy

...heterogeneously distributed within the food matrix. This is particularly true when dealing with seeds: the matrix is made up by particles, which may become **singularly contaminated** and dispersed in lots of large dimensions. Moreover, the contamination is likely to occur at a **very low level**.



# Spiking of seeds test portions

Seeds contaminated by soaking in a bacterial liquid culture for 1 hour @ RT



Evaluation of the level of contamination of the seeds:  
1 seed put in 5 ml of PBS (ten replicates), vortex,  
10 min @RT, vortex, diluted and counted

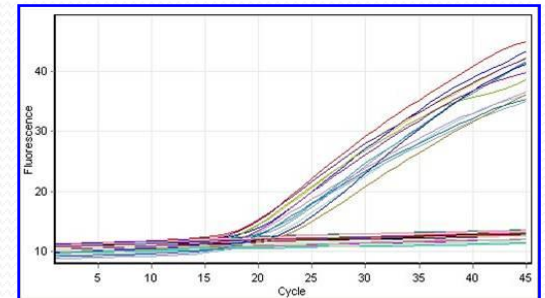
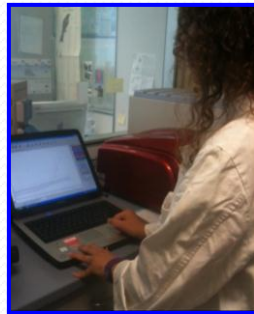
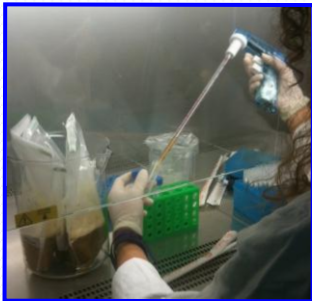
**$10^6$ - $10^7$  CFU/seed**

A single  
contaminated  
seed added to  
each test portion



# Be ready.....

Samples will be sent on the 19<sup>th</sup> of November 2012



# 10<sup>th</sup> inter-laboratory study on strain typing, including PFGE (PT10)

Conducted jointly with the network of  
medical NRLs for VTEC referring to the  
ECDC FWD Network

Invitation to participate to:

- ✓ NRLs EU-RL network (882/04)
- ✓ ECDC-FWD network
- ✓ Other laboratories

# PT10 design

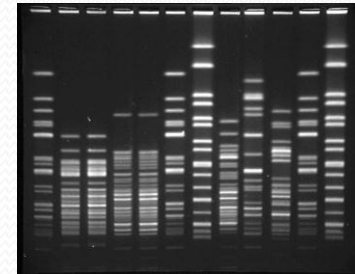
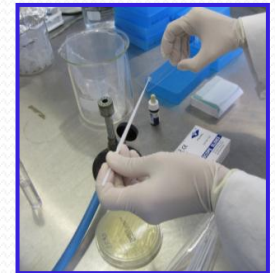
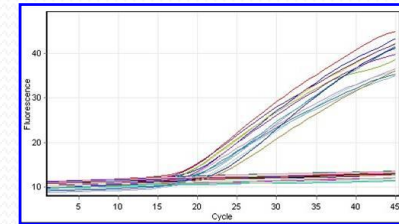
## **Samples:**

15 strains in soft agar, labelled with randomly-generated codes



## **Requests:**

- + Detection of virulence genes (*eae*, *vtx1*, *vtx2*, *aggR*, *aaiC*) by PCR or RT-PCR **MANDATORY**
- + Detection of *vtx*-genes subtypes **FACULTATIVE**
- + Serogroup identification **MANDATORY** for serogroups: O157, O26, O103, O111, O145, O91, O121, O113, O128, O55, O146, O104
- + PFGE on a subset of test strains **FACULTATIVE**





**Samples will be sent by SSI in December 2012**

..... but the deadline for results submission will be  
**March 2013**

Protocols for PCR detection of:

- Virulence genes
- Serogroups associated genes

Will be provided

Protocol for PFGE:

PulseNet Protocol, available at

[http://www.pulsenetinternational.org/SiteCollectionDocuments/pfge/5%201%205%202%205%204\\_PNetStand\\_Ecoli with Sflexneri.pdf](http://www.pulsenetinternational.org/SiteCollectionDocuments/pfge/5%201%205%202%205%204_PNetStand_Ecoli_with_Sflexneri.pdf)

The labs participating will be asked to provide the PFGE images as tiff files (profile generation EQA)



Thank you for your collaboration!!!!