

# Detection methods for VTEC: update and perspectives in the EU and USA

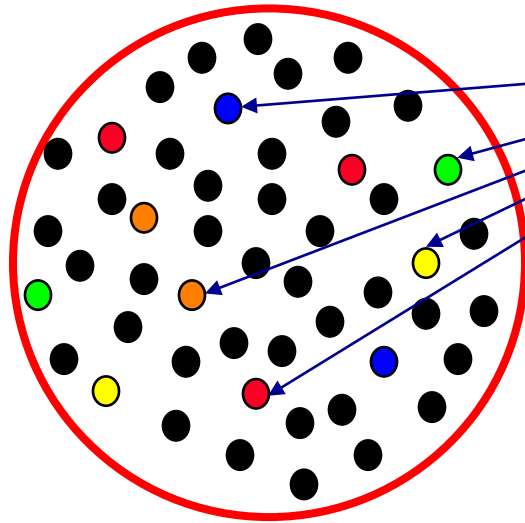
Stefano Morabito

EU Reference Laboratory for *E. coli* including VTEC;  
Food-borne Zoonose unit

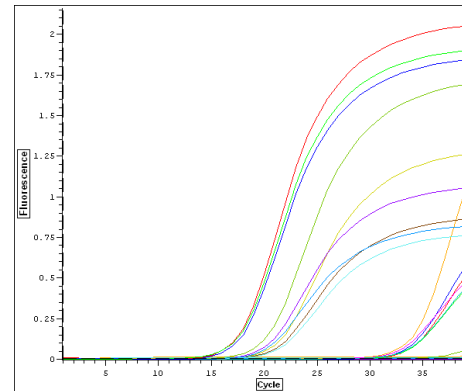
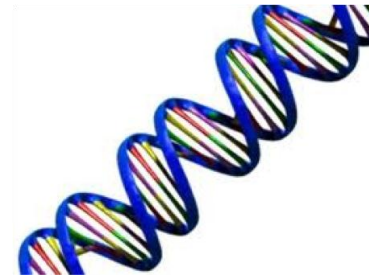
*Veterinary Public Health and Food Safety Department;  
Istituto Superiore di Sanità, Rome, Italy*

# CEN ISO PRF 13136 A MULTI-STEP SEQUENTIAL PROCEDURE

- VTEC O157
- VTEC O26
- VTEC O103
- VTEC O111
- VTEC O145



Food Sample  
Enrichment culture

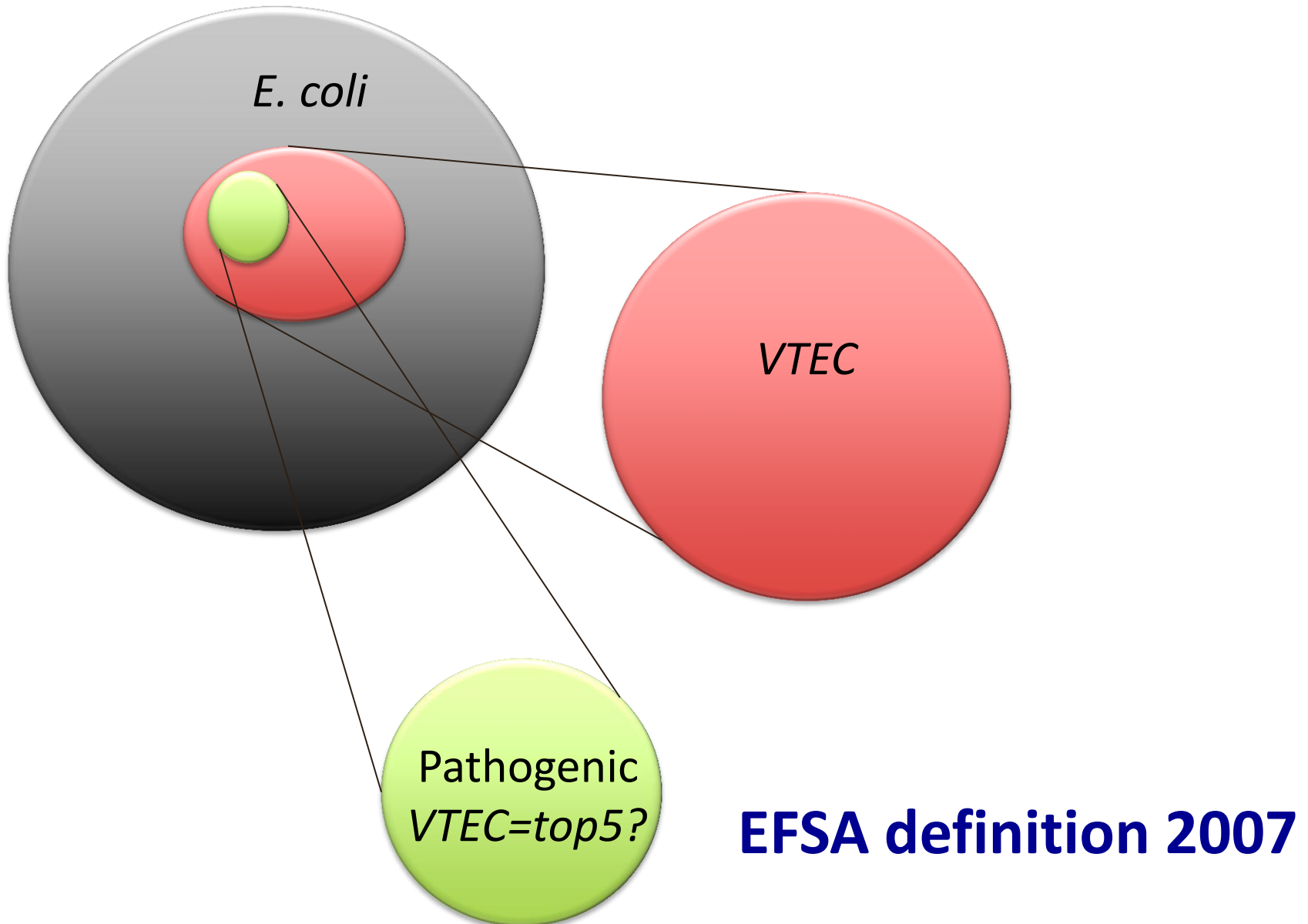


*vtx* genes  
*eae* gene  
O26  
O103  
O111  
O145  
O157

genes

Positivity to *stx*+*eae*+one of O-group associated genes: **ISOLATION!!!!**

# VTEC: Diagnostic Complexity



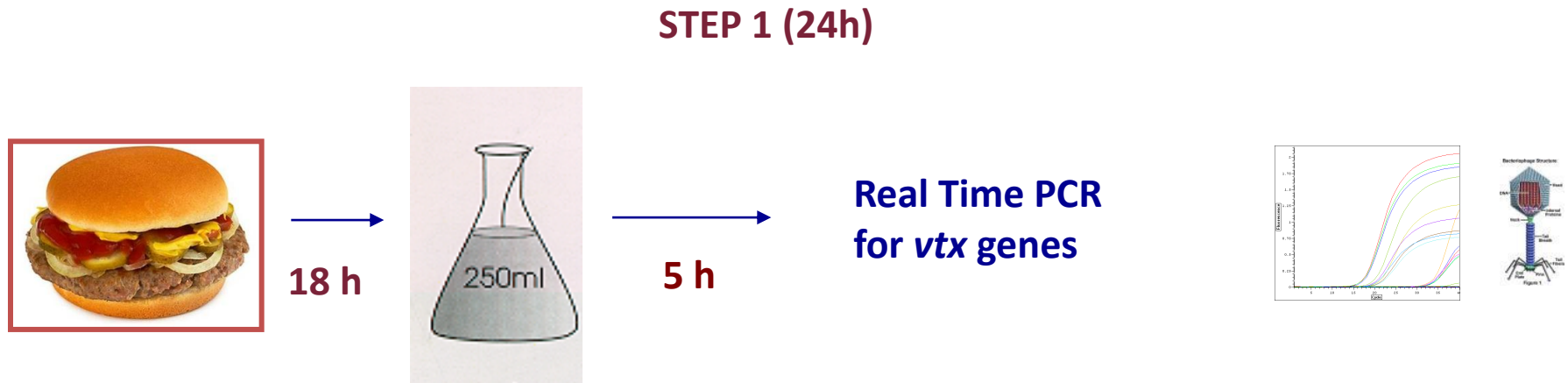
# Major issues towards the adoption of a broader view for testing food for VTEC

- ✓ Some VTEC serogroups may fall outside the top five and still cause disease in humans
- ✓ It may be worth identifying *eae*-negative VTEC in particular matrices
- ✓ VTEC causing diarrhoea are pathogens!!!

**VT-producing EAEC O104:H4!!!!**



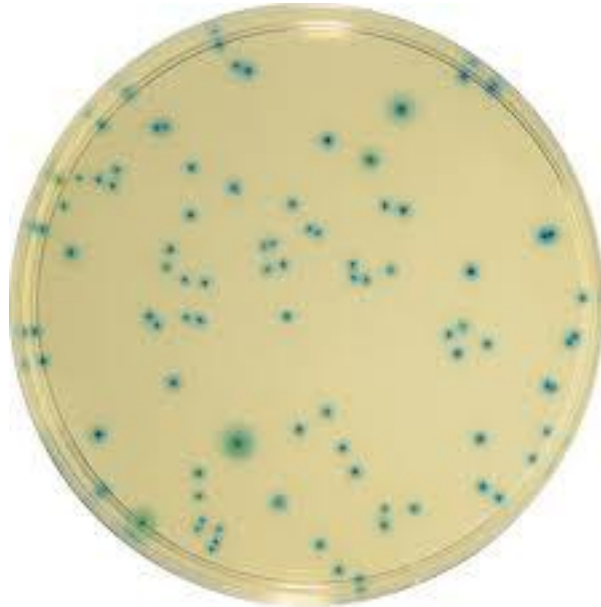
# ISO TS 13136: Detection of VTEC in food and identification of the top five sergroups



Negative samples: released

Positive samples: presumptive detection of VTEC go to isolation

# Isolation of pathogenic VTEC from food samples



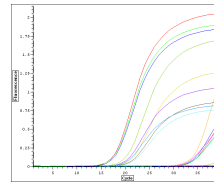
Up to 50 colonies screened by PCR for the presence of stx genes

Isolation of VTEC that do not belong to one of the serogroups identified in the PCR screening is accomplished by general screening of colonies from an *E. coli* agar medium (e.g. TBX)

# ISO TS 13136: Detection of VTEC in food and identification of the top five sergroups

## STEP 2 (2h)

Real Time PCR  
for *eae* gene

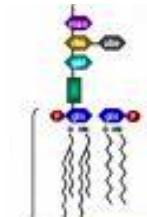
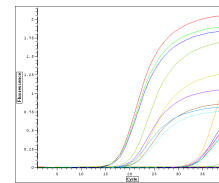


*Attaching/Effacing adhesion*



## STEP 3 (2h)

Real Time PCR for serogroup-  
associated  
genes

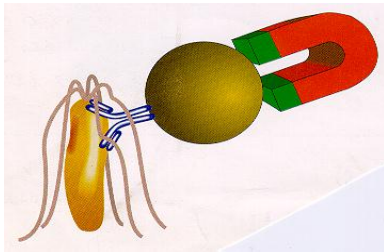


**Step 2 and 3 are carried out to drive the selection of reagents to ease the isolation of the bug. Informative to the risk manager**

# Isolation of pathogenic VTEC from food samples

STEP 4 (18 - 20 h)

## O-specific IMS



Isolation of VTEC belonging to one of the serogroups identified in the PCR screening is accomplished by IMS





Standards ( 1 )

Publications & e-products ( 0 )

Site (null)

13136

**AVAILABLE IN 2012!**

Standards to be displayed:

Published

Under development

Withdrawn

Refine your query

**(HOPEFULLY)**

1 - 1 of 1

**ISO/TS 13136**

Microbiology of food and animal feed -- 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) and the determination of O157, O111, O26, O103 and O145 serogroups

Hide details »

Edition	Stage	TC	ICS	Current stage date
1	60.00	TC 34/SC 9	07.100.30	2012-07-27

# From methodology to Regulations

- ✓ **The two sides of the Atlantic ocean started to implement regulations for VTEC**
- ✓ **EU approved microcriteria for VTEC in sprouts in October 2012 (enforced from March 2013)**
- ✓ **USA enforced rules on beef since June 2012**

# ISO TS 13136: Reference method for microcriteria assessment in sprouts



Brussels, **XXX**  
SANCO/10038/2012R3 D018801/09  
[...](2012) **XXX** draft

**COMMISSION REGULATION (EU) No .../..**

**of **XXX****

**amending Regulation (EC) No 2073/2005 as regards microbiological criteria for sprouts and the sampling rules for poultry carcasses and fresh poultry meat**

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# DEPARTMENT OF AGRICULTURE



## Food Safety and Inspection Service

PA/QC Division  
Station Road  
30605

### 9 CFR Parts 416, 417, and 430

[Docket No. FSIS–2010–0023]

Aimed at the “top 7” serogroups

Chapter **0157, 026, 045, 0111, 0121, 0103, 0145, ...**

Title: D **AGENCY:** Food Safety and Inspection Service, USDA.

*Shiga toxin-producing E. coli*

Effective **ACTION:** Final determination and request for comments.



58157

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## Proposed Rules

Federal Register

Vol. 76, No. 182

Tuesday, September 20, 2011

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# Data from the US (as of Oct 28 2012)

**Table 1: Results from Analysis of Raw Ground Beef Component Samples for non-O157 STEC**

<b>Results from Analysis of Raw Ground Beef Component Samples for non-O157 STEC<sup>1</sup></b>			
<b>Raw Ground Beef Components (RGBC)<sup>2</sup></b>			
<b>Source</b>	<b>As of Oct 28, 2012</b>		
	<b>Number Analyzed</b>	<b>Number Positive<sup>3</sup></b>	<b>Percent Positive</b>
<b>Federal Plants</b>	<b>1,491</b>	<b>31</b>	<b>2.08</b>
Trim Verification	1,159	12	1.04
Follow-up to Raw Ground Beef (RGB) Positive	49	0	0.00
Follow-up to RGBC Positive	283	19	6.71
<b>Imports</b>	<b>149</b>	<b>0</b>	<b>0.00</b>

# Data from the US (as of Oct 28 2012)

**Table 2: Non-O157 STEC (by serogroup) and *E. coli* O157:H7**

Non-O157 STEC (by serogroup) and <i>E. coli</i> O157:H7 YTD <sup>1</sup>				
Raw Ground Beef Components (RGBC) <sup>2</sup>				
Target STEC <sup>3</sup>	As of Oct 28, 2012			
	Federal Plants			Import
	Trim Verification Percent Positive (Number)	Follow-up to RGB Positive at Supplier Percent Positive (Number)	Follow-up to RGBC Positive Percent Positive (Number)	Verification/ Intensified Percent Positive (Number)
<b>O157:H7</b>	<b>0.80%</b> <b>(15/1,881)</b>	<b>0.61%</b> <b>(1/165)</b>	<b>1.07%</b> <b>(4/374)</b>	<b>1.26%</b> <b>(8/637)</b>
<b>Total non-O157 STEC</b>	<b>1.04%</b> <b>(12/1,159)</b>	<b>0.00%</b> <b>(0/49)</b>	<b>8.48%</b> <b>(24/283)</b>	<b>0.00%</b> <b>(0/149)</b>
O26	0.09% (1/1,159)	0.00% (0/49)	1.77% (5/283)	0.00% (0/149)
O45	0.09% (1/1,159)	0.00% (0/49)	0.71% (2/283)	0.00% (0/149)
O103	0.52% (6/1,159)	0.00% (0/49)	4.95% (14/283)	0.00% (0/149)
O111	0.26% (3/1,159)	0.00% (0/49)	0.35% (1/283)	0.00% (0/149)
O121	0.00% (0/1,159)	0.00% (0/49)	0.00% (0/283)	0.00% (0/149)
O145	0.09% (1/1,159)	0.00% (0/49)	0.71% (2/283)	0.00% (0/149)

# Concluding remarks

- ✓ **ISO TS 13136 Enlarged scope (all VTEC)**
- ✓ **Top five serogroups determination is still in the method but all VTEC+ samples must be subjected to isolation**
- ✓ **A new pathogenic VTEC definition is under discussion at EFSA**
- ✓ **USDA MLG 5B:00 in use since June 2012 (Beef)**
- ✓ **ISO TS 13136 should be available in 2012**
- ✓ **EU Testing will be implemented on sprouts by March 2013**
- ✓ **Data on beef from USDA indicate that prevalence may not be too high (PCR-based adoption of measure?)**

# Thank you for your attention!



## The EU-RL VTEC folks