

Shiga Toxin-producing *Escherichia coli*: connecting standardization, public health and food safety regulatory bodies

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EU Food safety regulations

What

EC Reg 178/2002

Competent authority

FOOD BUSINESS OPERATORS

Who

EC Reg 882/2004
Official controls for food
conformity assessment
EC Reg 854/2004
Official controls on food of
animal origin

EC Reg 852/2004
General food hygiene rules

EC Reg 853/2004
General hygiene rules for
food of animal origin

How

EC Reg 2073/2005
Microbiological criteria

EC Reg 2074/2005
Fishery products,
biotoxins, calcium in
meat and meat
products...

EC Reg 2075/2005
Trichinella spp in meat

Food safety assessment follows the classical food microbiology approach

- ✓ 5 Cultural Standards for food safety assessment (1441/07)
 - ✓ 1HPLC (1441/07 Histamin)
- ✓ 12 Cultural Standards for process hygiene assessment (1441/07)



Emergence of a new food safety paradigm

New threats!

- ✓ Non cultivable microorganisms (NoV, HAV)
- ✓ Microorganisms that cannot be distinguished from non-pathogenic strains belonging to the same species (STEC)
- ✓ Microorganisms evolving new or shuffled virulence traits (STEC)

Shiga Toxin-producing *E. coli*

One of the most dangerous foodborne pathogens !



Life-threatening clinical manifestation

Very low infectious dose (**10-100 CFU !**)

Large community outbreaks



STEC: Clinical manifestation

- **Intestinal**
 - Asymptomatic
 - Watery Diarrhoea
 - Hemorrhagic Colitis



- **Systemic**
 - Hemolytic Uremic Syndrome (HUS)
 - Neurological involvement





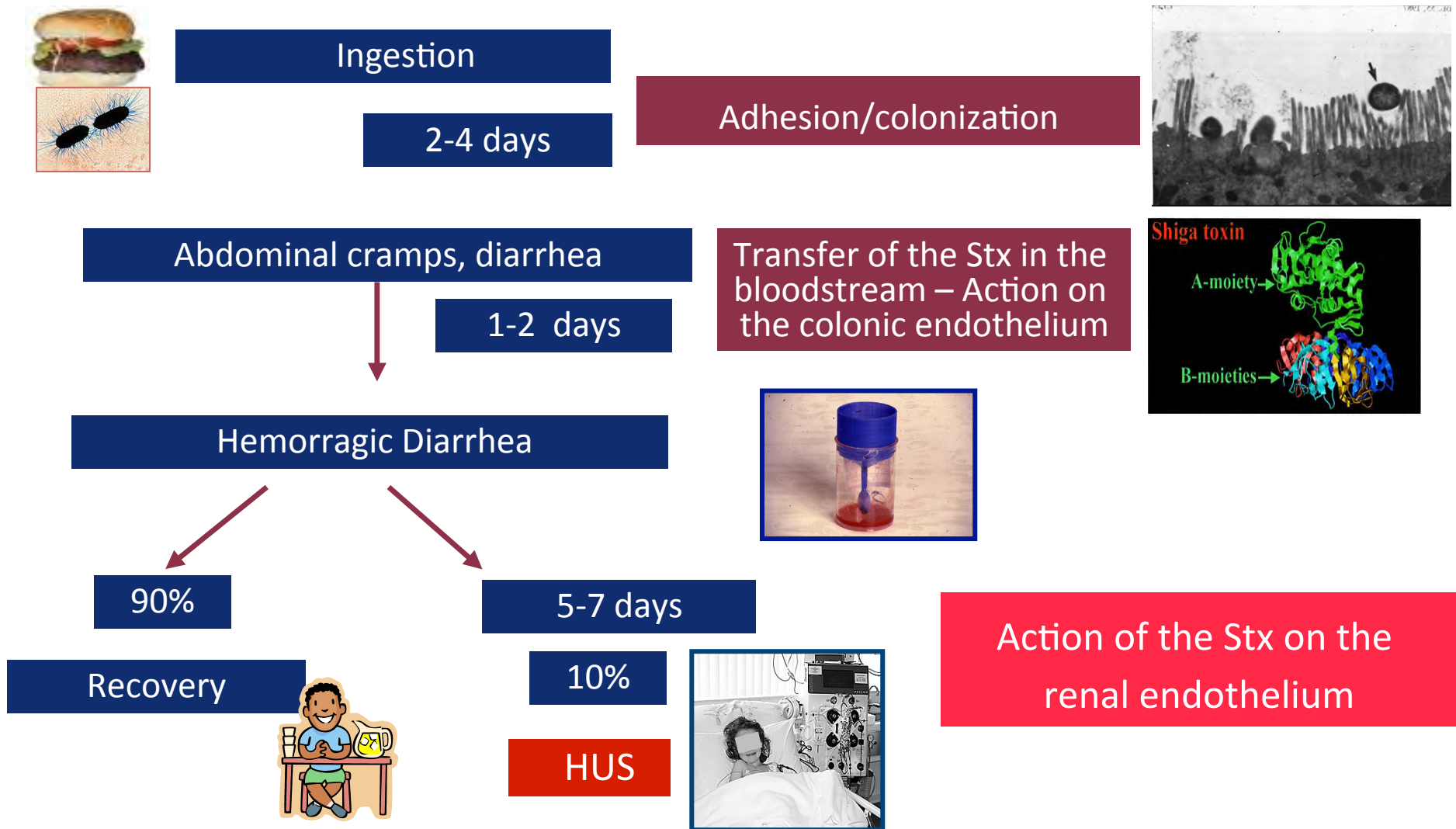
Hemolytic Uremic Syndrome (HUS)

- Hemolytic Anemia
- Thrombocytopenia
- Acute Renal failure



- Main cause of renal failure in childhood and usually requires dialysis
- Antibiotic treatment is not effective and is not recommended

Pathogenesis of STEC infections



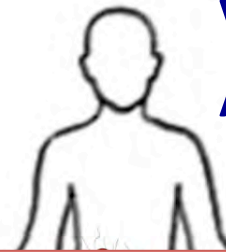
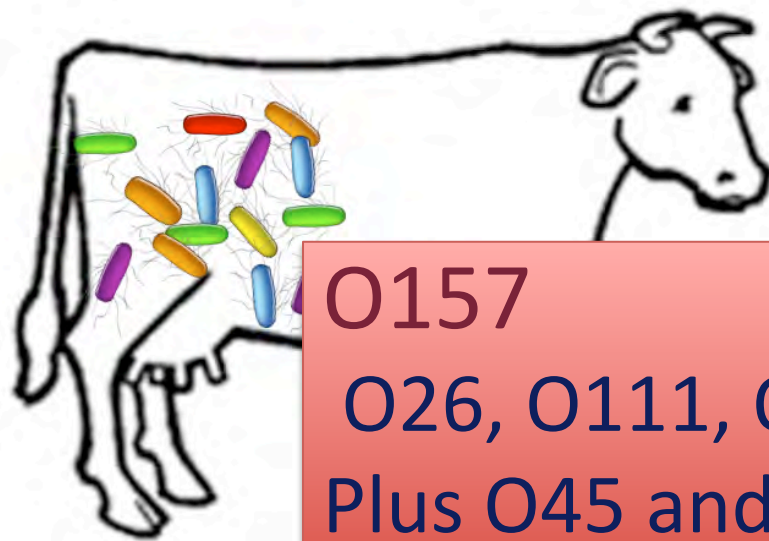
STEC identification: A challenge!

Cannot be differentiated phenotypically from commensali di *E. coli*



Identification based on the presence of virulence genes

STEC: Public health considerations



VTEC
ASSOCIATED WITH (HC, HUS)

O157

O26, O111, O103, O145,

Plus O45 and O121 in the US

...ence features
...cing adhesion

STEC

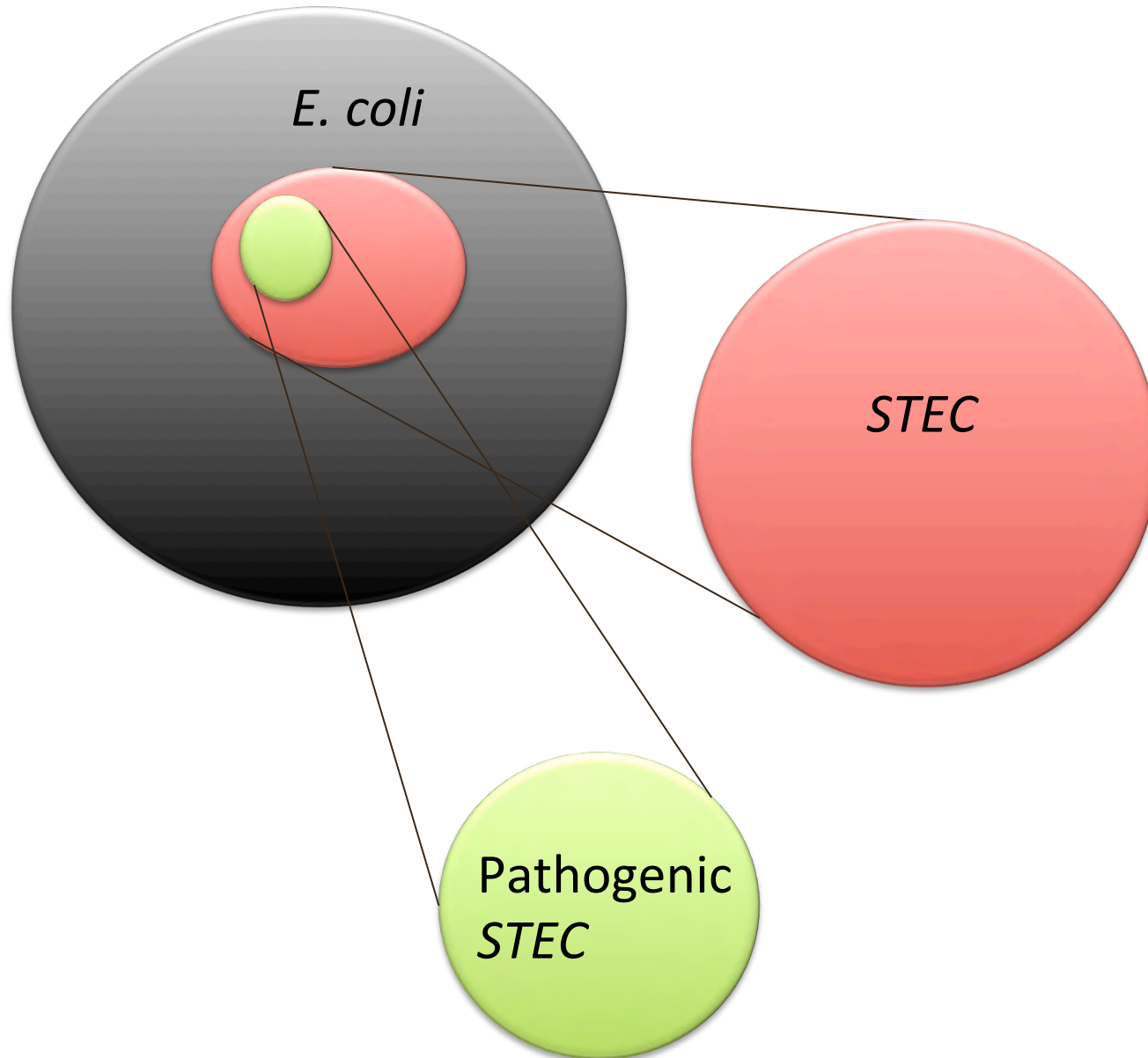
>100 sierogroups

In the reservoir



EFSA Definition (opinions 2007 and 2009)

STEC: Standardization challenges



STEC: Diagnostic Complexity

Clinical Diagnosis: Any STEC in a stool specimen from a patient with compatible symptoms is pathognomonic

What about the predictive value of the *stx genes* in the vehicles of infection?

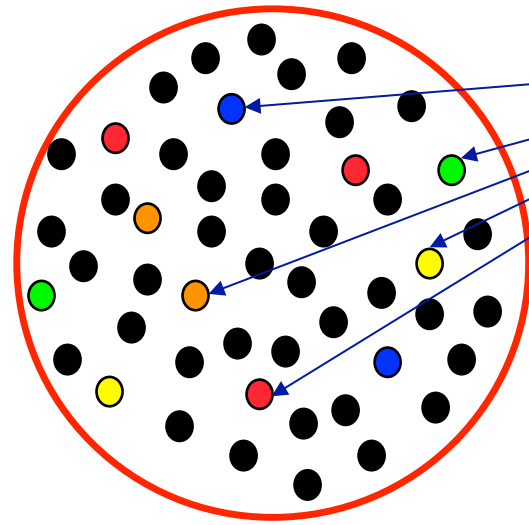
Presence of *stx* genes do not necessarily implies the presence of a pathogenic STEC in the food sample

Presence of *eae* gene in together with the *stx* genes is a good cue, but is not sufficient yet

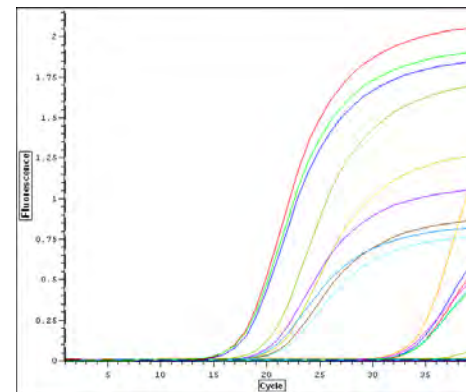
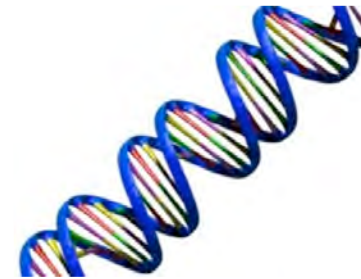
Serogroup must be determined

STEC: Standardization reaction

- STEC O157
- STEC O26
- STEC O103
- STEC O111
- STEC O145



Food Sample
Enrichment culture



stx genes
eae gene
O26
O103
O111
O145
O157

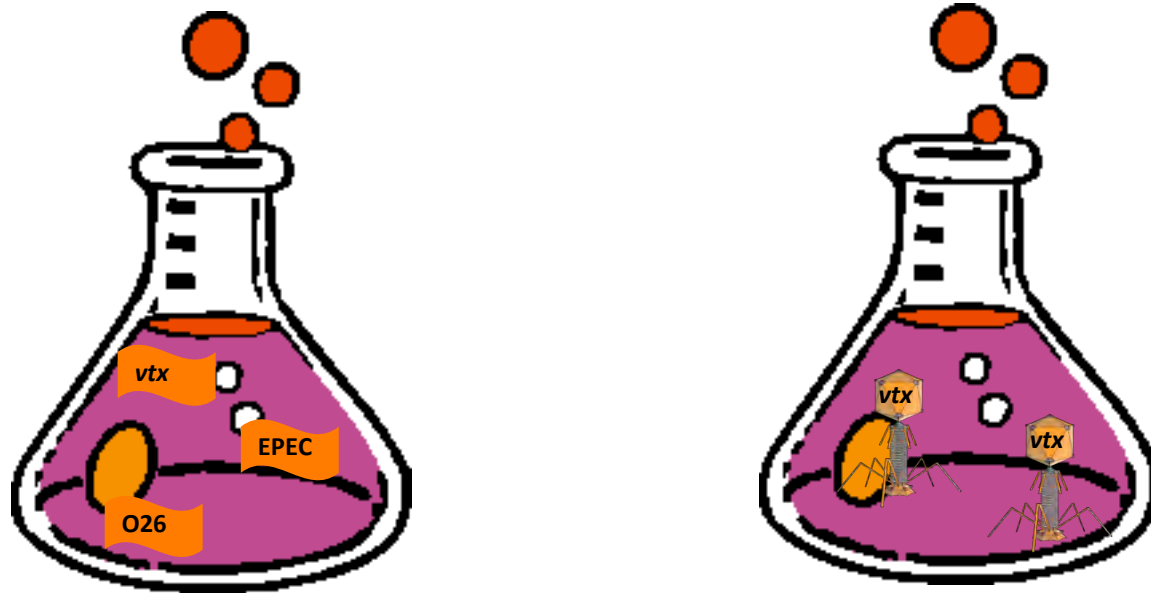
} genes

**CEN/ISO/PRF 13136 for the detection of STEC
belonging to the top five serogroups**

Multi-Target Molecular Screening

Virulence and serogroup-associated genes might be present in different bacterial cells

Presence of free *stx*-phages in the enrichment culture



ISOLATION IS REQUIRED TO DEMONSTRATE THE PRESENCE OF ALL THE GENES IDENTIFIED IN THE SAME LIVE BACTERIAL CELL

May-June 2011: Germany struck by the largest STEC outbreak in the EU

3,842 cases

2987
non-HUS STEC

855
HUS

18 deaths
(0.6%)

35 deaths
(4.1%)

Of HUS cases,

- 68% women

- **Median age 42 years (0-91 years)**

- Bloody diarrhoea in 79%

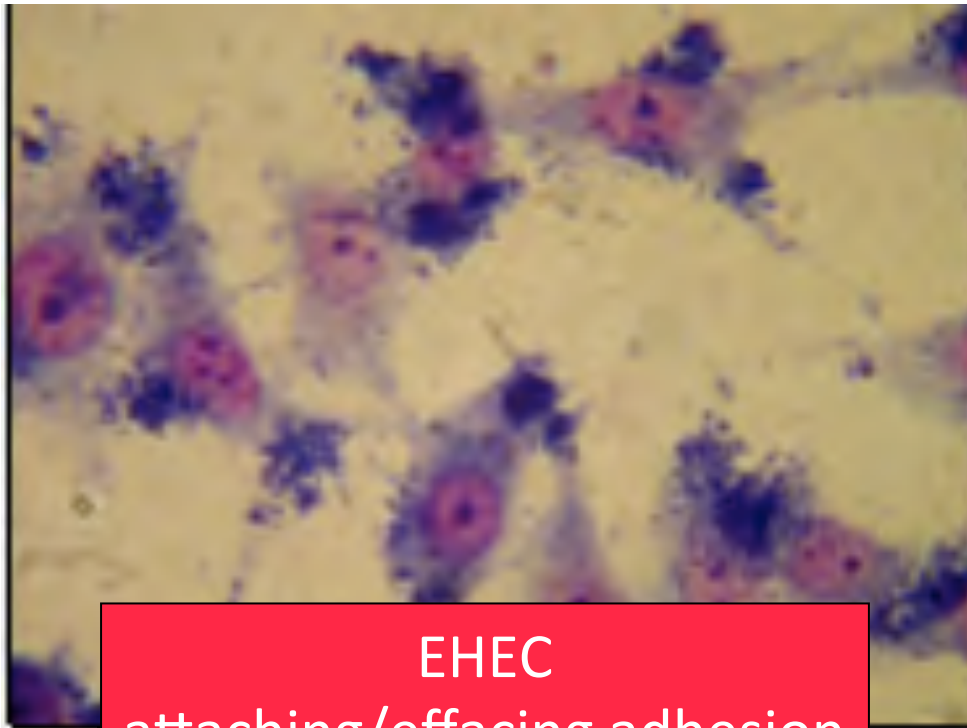
A brand new type of STEC?

E-ALERT

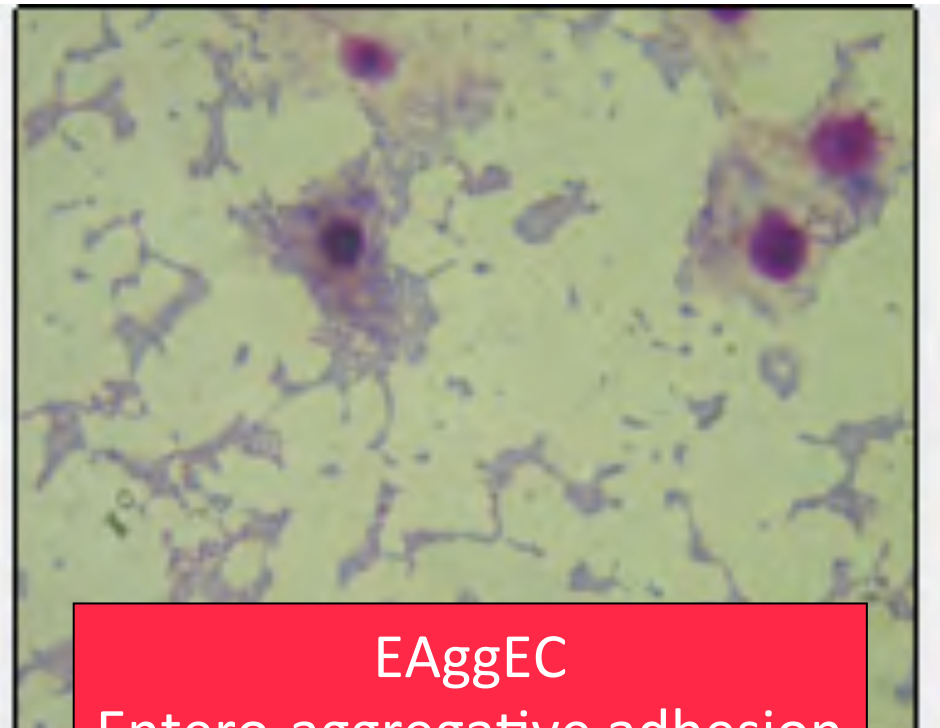
Characteristics of the enteroaggregative Shiga toxin/ verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011

F Scheutz (fsc@ssi.dk)^{1,2}, E Møller Nielsen², J Frimodt-Møller^{1,3}, N Boisen^{1,2}, S Morabito⁴, R Tozzoli⁴, J P Nataro⁵, A Caprioli⁴

1. World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, Department of Microbiological Surveillance and Research, Copenhagen, Denmark
2. Food-borne pathogens and typing, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark
3. Department of Clinical Microbiology, Hillerød Sygehus, Hillerød, Denmark
4. European Union Reference Laboratory for *Escherichia coli*, Department of veterinary public health and food safety, Istituto Superiore di Sanità, Rome, Italy
5. University of Virginia School of Medicine, Charlottesville, United States



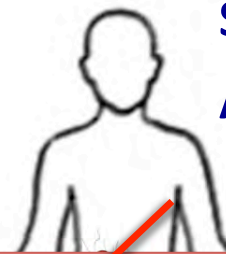
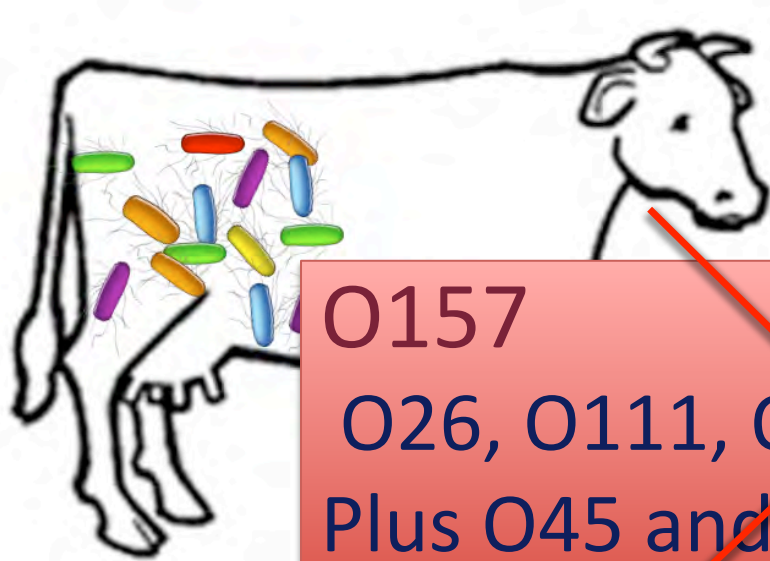
EHEC
attaching/effacing adhesion



EAggEC
Entero-aggregative adhesion



The infecting agent was not in the definition



STEC
ASSOCIATED WITH (HC, HUS)

O157

O26, O111, O103, O145,

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VTEC

>100 sierogroups

In the reservoir



STEC O104:H4

After the “STEC O104” outbreak the need to rethink on the method emerged

- ✓ More STEC serogroups beside the “top five” cause severe disease
- ✓ *eae*-negative STEC are not necessarily a “second-grade” hazard
- ✓ Diarrhoea is a disease anyway

Request for a revision of the pathogenic STEC definition and the associated detection methodology

DRAFT SCIENTIFIC OPINION

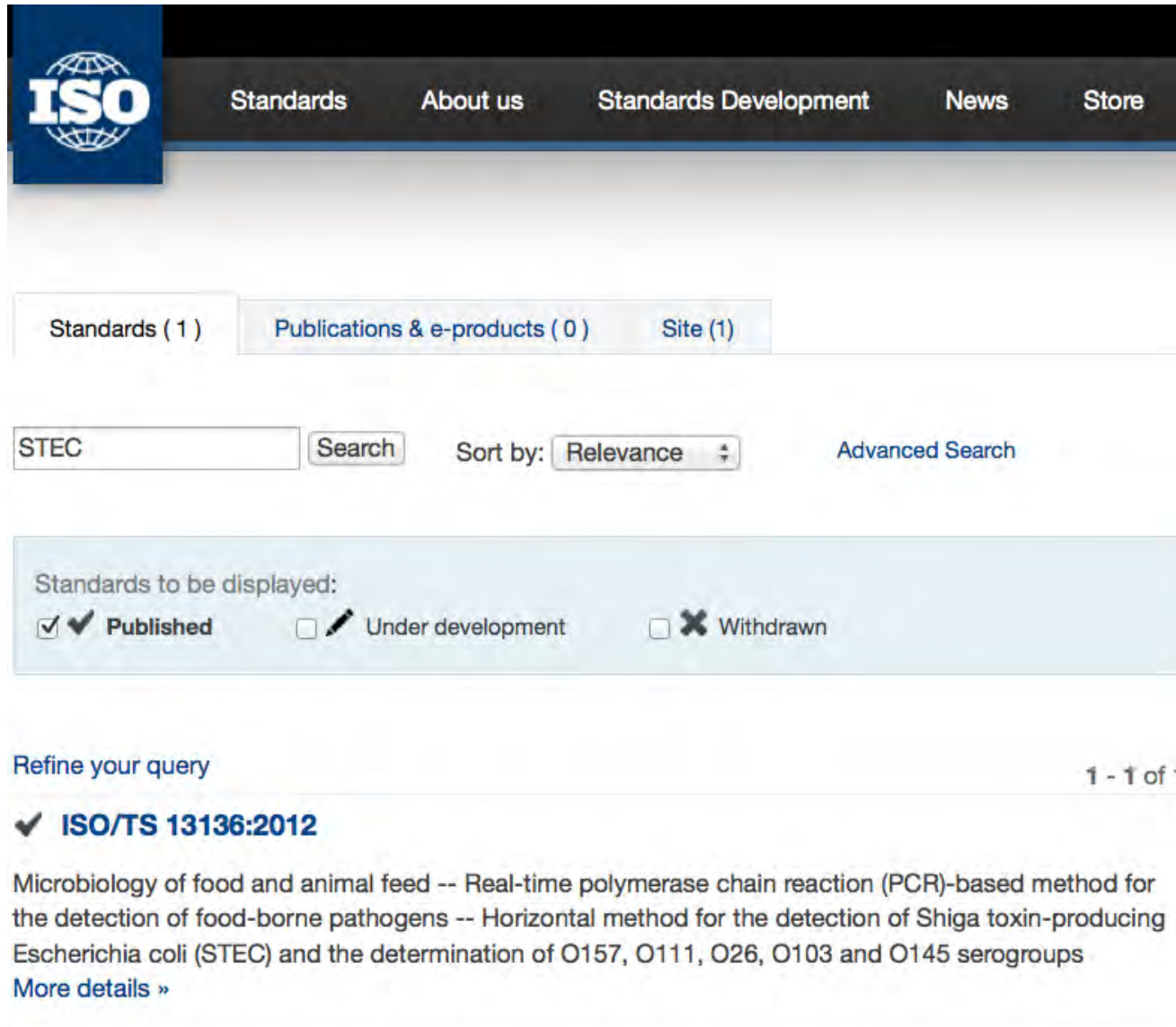
Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment

Published on April the 9th, 2013

EFSA Panel on Biological Hazards (BIOHAZ)

- ✓ ...Pathogenicity can neither be excluded nor confirmed for a given STEC serogroup or serotype based on the seropathotype concept or analysis of the public health surveillance data.
- ✓ ...There is no single or combination of marker(s) that defines the potential of a STEC strain to cause human disease. While *vtx2*- and *eae*-positive strains are associated with a high risk of more serious illness other virulence gene combinations and/or serotypes may also be associated with serious disease, including HUS.
- ✓ ...Patient-associated (e.g., age, immune status, antibiotic therapy in the pre-infection period), and dose-related factors may also be of importance.

ISO/TS 13136:2012 for the detection of STEC in food published Nov., 7th 2012



The screenshot shows the ISO website's search interface. At the top left is the ISO logo. A navigation bar contains links for Standards, About us, Standards Development, News, and Store. Below the navigation bar are three tabs: Standards (1), Publications & e-products (0), and Site (1). The search bar contains the text 'STEC' and a 'Search' button. To the right of the search bar is a 'Sort by:' dropdown menu set to 'Relevance' and a link for 'Advanced Search'. Below the search bar is a filter section titled 'Standards to be displayed:' with three options: 'Published' (checked), 'Under development' (unchecked), and 'Withdrawn' (unchecked). At the bottom left, there is a 'Refine your query' link. At the bottom right, it shows '1 - 1 of 1'. The search results list one item: 'ISO/TS 13136:2012' with a checkmark icon. The description for this item is: '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'. A link for 'More details »' is provided below the description.

ISO

Standards About us Standards Development News Store

Standards (1) Publications & e-products (0) Site (1)

STEC Search Sort by: Relevance Advanced Search

Standards to be displayed:
 Published Under development Withdrawn

Refine your query 1 - 1 of 1

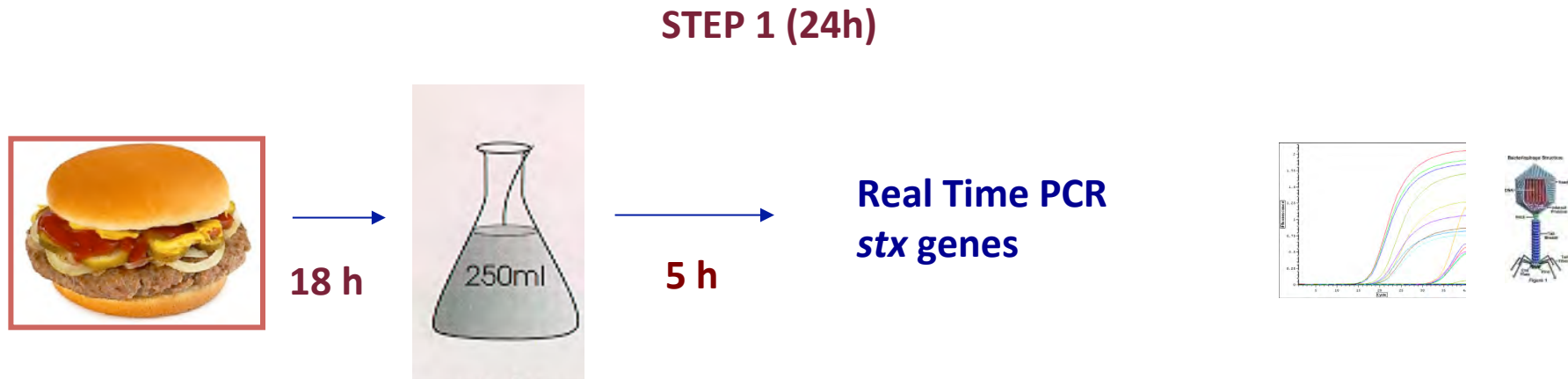
✓ **ISO/TS 13136:2012**

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
[More details »](#)

ISO TS 13136: Detection of STEC in food and identification of the “top five serogroups”

...any STEC must be considered pathogenic to humans and potentially causing severe disease depending on both the risk profile of the food commodity (ready-to-eat foods vs foods intended to be consumed after technological treatment such as pasteurization, cooking etc. used to reduce any bacteria present in the food) and the health status of the subject ingesting the food.

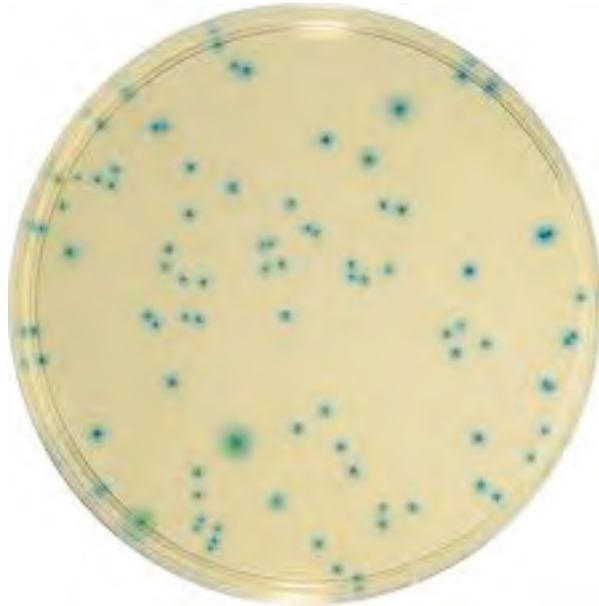
ISO TS 13136: Detection of STEC in food **and** identification of the “top five serogroups”



Negative specimens: release!

**Positive specimens: Presumptive identification
of VTEC - **Isolation- Hold!****

Isolation of VTEC from food enrichment cultures



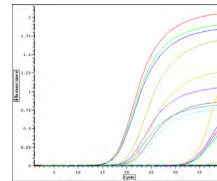
Up to 50 colonies. PCR Screening for the *stx* genes

The scope of the ISO TS 13136 is the detection of STEC in food. Any risk evaluation of has been removed from the standard 'cause it is prerogative of the **risk manager!!!**

ISO TS 13136: Detection of STEC in food **and** identification of the “top five serogroups”

STEP 2 (2h)

Real Time PCR
eae gene

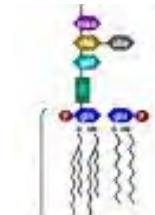
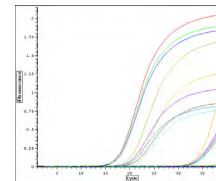


Attaching/Effacing adhesion



STEP 3 (2h)

Real Time PCR
Top five serogroups-associated
genes



Step 2 and 3: Drive the selection of reagents to ease the isolation. Informative for the Competent Authority

ISO/TS 13136: Reference method for conformity assessment of sprouts

12.3.2013

EN

Official Journal of the European Union

L 68/19

COMMISSION REGULATION (EU) No 209/2013

of 11 March 2013

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

(Text with EEA relevance)

(c) the following row 1.29 and the corresponding footnotes 22 and 23 are added:

1.29 Sprouts ⁽²³⁾	Shiga toxin producing <i>E. coli</i> (STEC) O157, O26, O111, O103, O145 and O104:H4	5	0	Absence in 25 grams	CEN/ISO TS 13136 ⁽²²⁾	Products placed on the market during their shelf-life
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⁽²²⁾ Taking into account the most recent adaptation by the European Union reference laboratory for *Escherichia coli*, including Verotoxigenic *E. coli* (VTEC), for the detection of STEC O104:H4.

⁽²³⁾ Excluding sprouts that have received a treatment effective to eliminate *Salmonella* spp. and STEC.;

ISO/TS 13136: Reference method for conformity assessment of sprouts

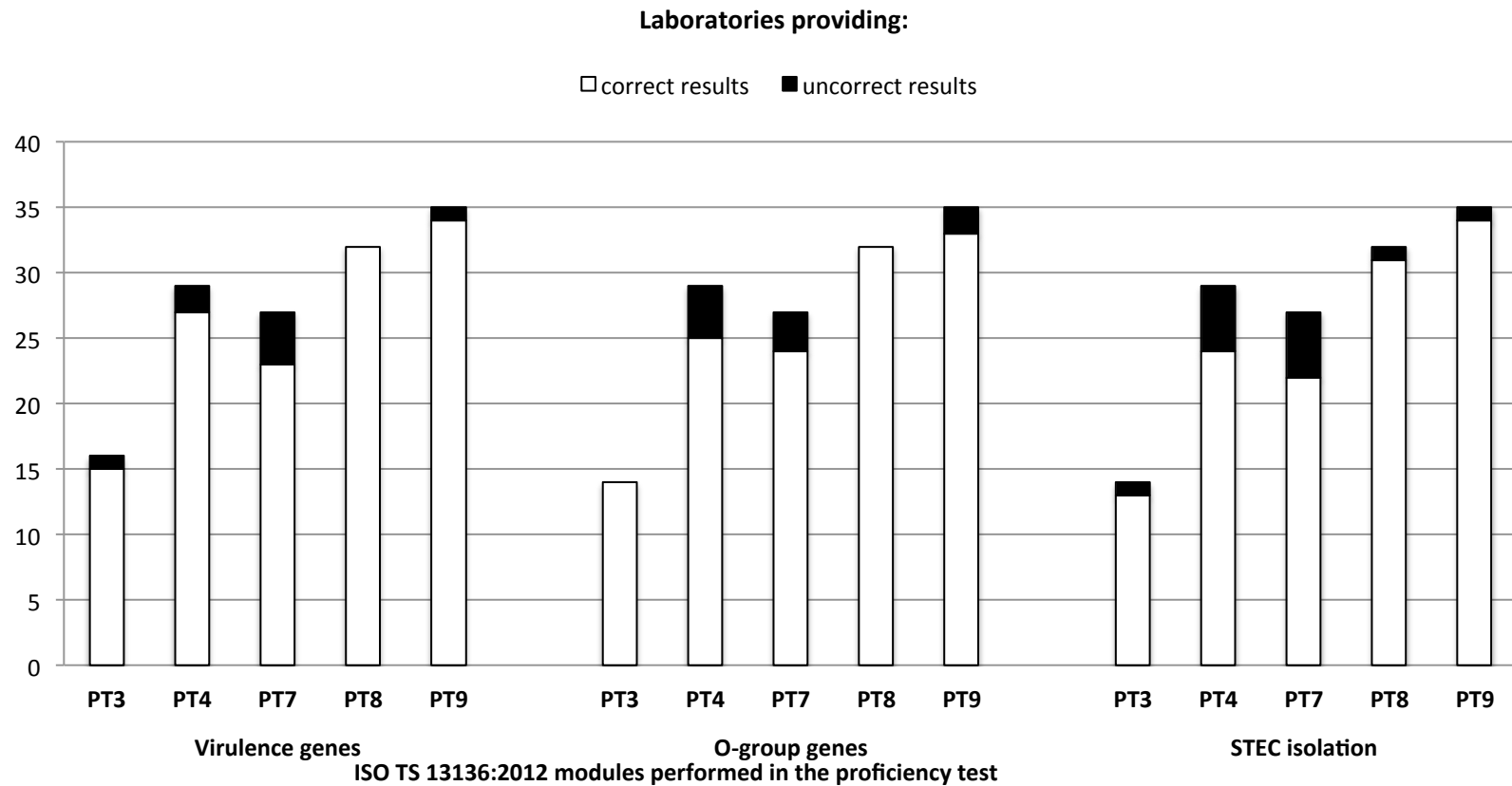
Recital 12

Certain STEC serogroups (namely O157, O26, O103, O111, O145 and O104:H4) are recognized to be those causing the most of the Haemolytic Uremic Syndrome (HUS) cases occurring in the EU. Furthermore serogroup O104:H4 caused the outbreak in May 2011 in the Union. Therefore microbiological criteria should be considered for these six serotypes. **It cannot be excluded that other STEC serogroups may be pathogenic to humans as well. In fact, such STEC may cause less severe forms of disease such as diarrhoea and or bloody diarrhoea or may also cause HUS and therefore represent a hazard for the consumer's health.**

Verotoxigenic *E. coli* (VTEC), for the detection of STEC O104:H4.

(²³) Excluding sprouts that have received a treatment effective to eliminate *Salmonella* spp. and STEC.;

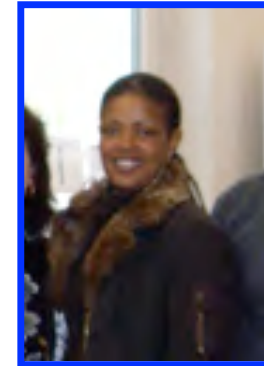
STEC detection in food: are we prepared?



Concluding remarks

- The global food market multiplied the sources of food commodities and raw materials
- The genomic plasticity of microorganisms like *E. coli* favours the emergence of new pathogenic variants
- Cross-talking between public health, standardization and regulatory bodies can be the “magic bullet”
- The EU RLs play a pivotal role by acting as trait d’union between all the actors involved and by establishing the preparedness

Thank you for your attention



The EU-RL VTEC folks