Revision of the ISO TS 13136

EU RL for *E. coli* Annual Workshop 2015

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

Nov. 7, 2012: The Standard was published

2014: 70% of MS adopted the method.
41.4% of the totality of food samples analysed in the EU with the ISOTS 13136

Nov. 6, 2015: The Revision of the Standard is announced

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

22th meeting of CEN/TC 275/WG 6 22-24 June 2015 Delft – NETHERLANDS

Recommendation N 393

TAG 18 "*Shiga toxin producing E. Coli*" – Group leader : Stefano Morabito

Project EN ISO 16654/AMD1 *Microbiology of the food chain -- Horizontal method for the detection of* Escherichia coli *O157- Amendement 1*

WG6 agreed with the EURL STEC proposal to prepare a revision of CEN ISO/TS 13136:2012 *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* as a full standard, but keeping the development of the amendment to EN ISO 16654:2001 as TAG 18 priority.

TAG 18 was asked to consider, in particular, methods used outside Europe in the revision.

WG6 asked the Secretariat to launch a call for experts and also request proposals for the revision, without waiting for comments received after ISO Systematic Review.

WG6 invited SC9 to develop an EN ISO standard, CEN lead, and notify ISO/TC147/SC4 "Water quality - Microbiological methods" that the scope would be broadened to include analysis of irrigation water for vegetables including sprouts.

This Recommendation should be approved by:
- CEN/TC275: ☐
- ISO/TC34/SC9: ☒
- ISO/TC34/SC5 IDF: ☒

Some points to be considered for the revision of the ISO/TS 13136:2012

mTSB (1,5 g/lt Bile Salts)+ 16 mg/lt Novobiocine

mTSB (1,5 g/lt Bile Salts)+ 12 mg/lt Acriflavine
(Milk and dairy products)

Is it really necessary?

Is it appropriate?

Some points to be considered for the revision of the ISO/TS 13136:2012

Revision of the array of Stx-genes subtypes detected by the method

Is it appropriate?
Which subtypes?

Some points to be considered for the revision of the ISO/TS 13136:2012

ISOLATION PROCEDURE!!!

Can we improve it?

Some preliminary data:

Susceptibility of STEC to Novobiocine and Acriflavine

Materials and Methods

- 54 STEC strains

O157 (5); **O26** (11); **O145** (6); **O111** (11); **O103** (4); **O113** (6); **O91** (3); **O45** (3); **O121** (2); **O152** (1); **O18** (1); **O75** (1)

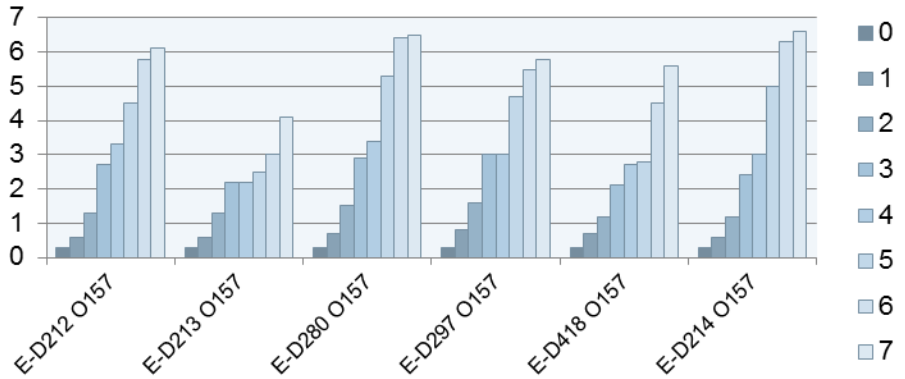
+ STEC O157 EDL 933 As reference

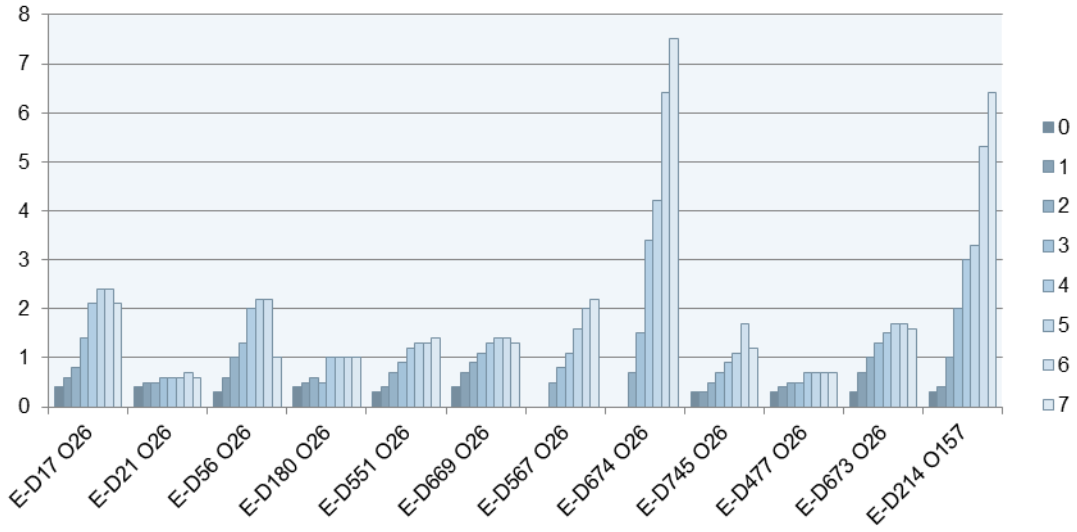
- **mTSB +N (16 mg/l) or A (12 mg/l).**

- **Standardized cultures of STEC**

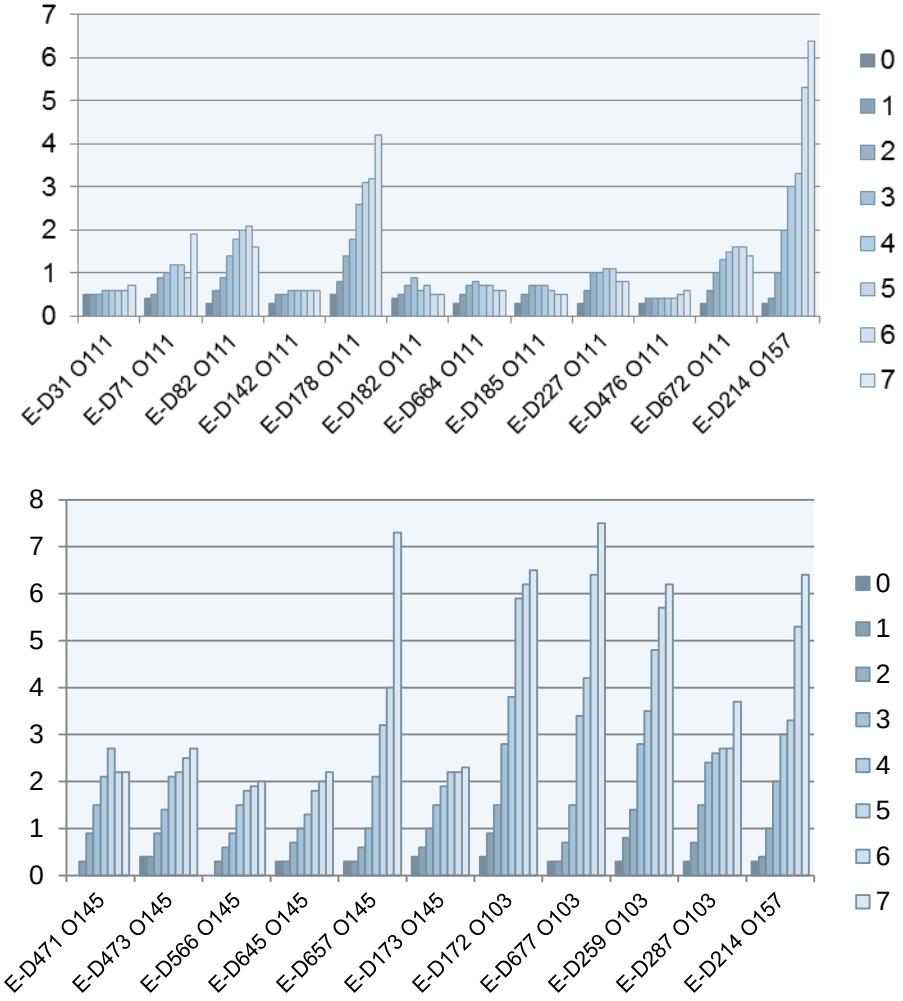
- **Readings (Mac Farland) at 0-1-2-3-4-5-6-7 hrs**

Novobiocin -I

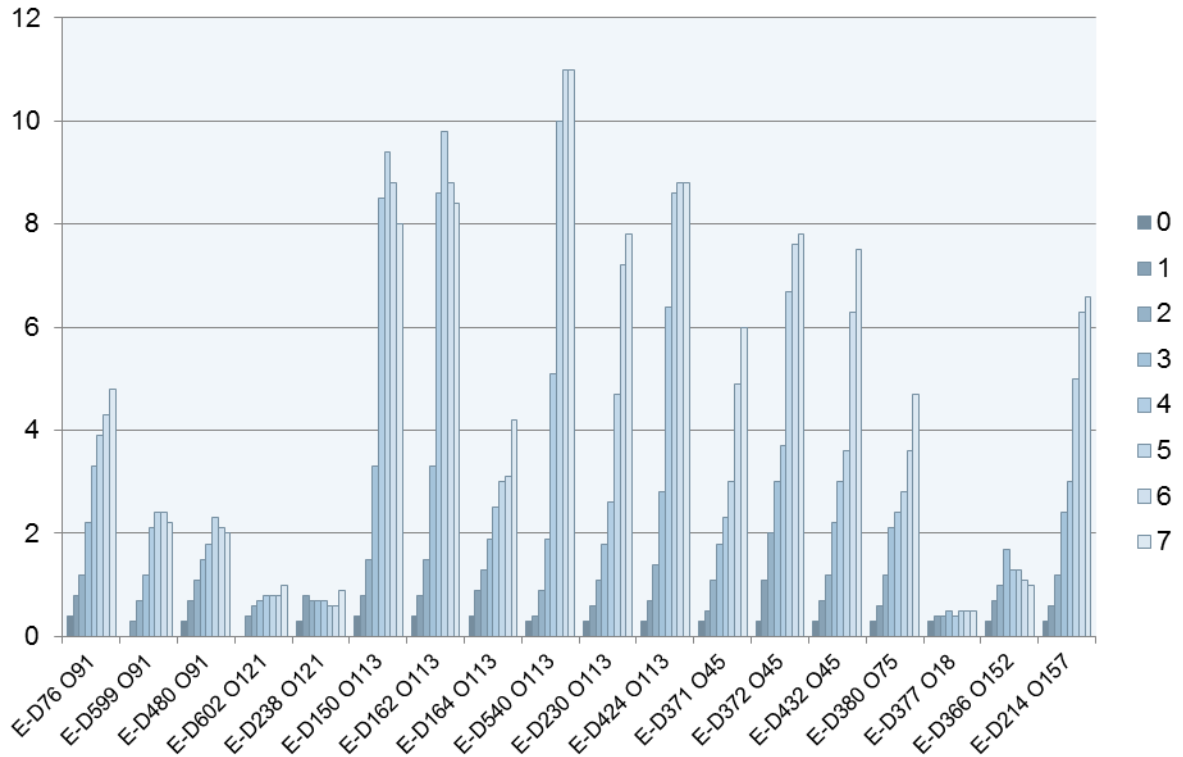




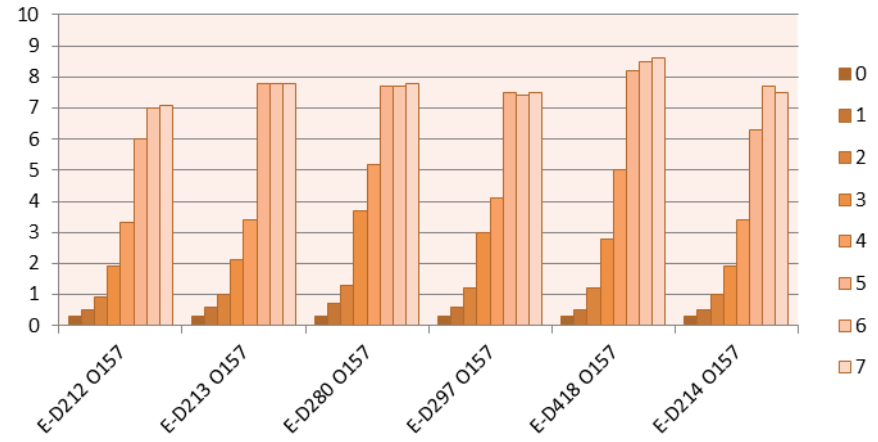
Novobiocin -II

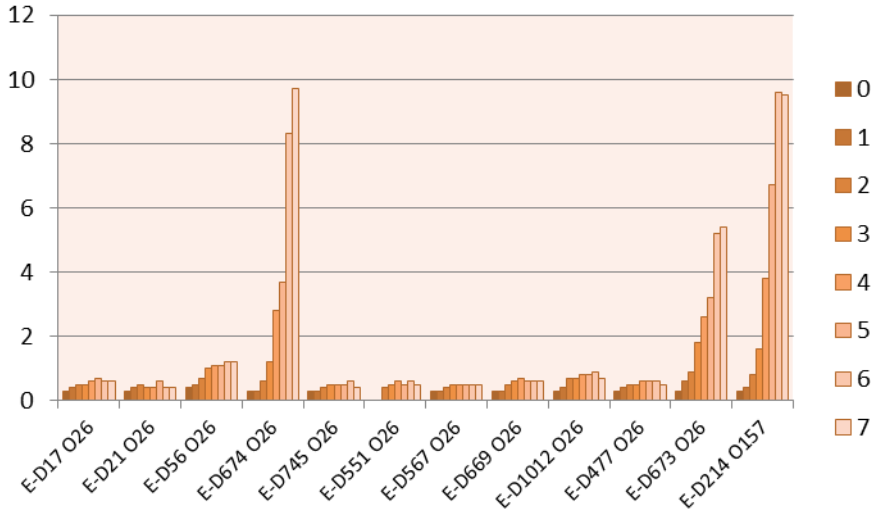


Novobiocin -III



Acriflavin-I





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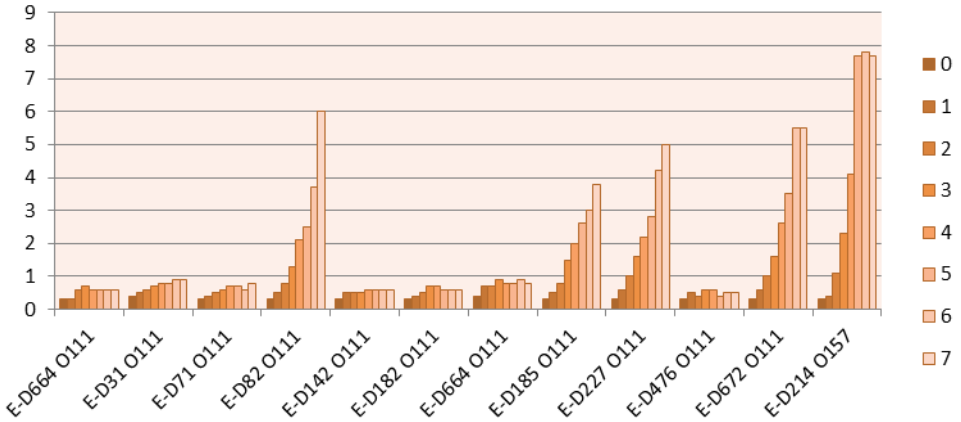
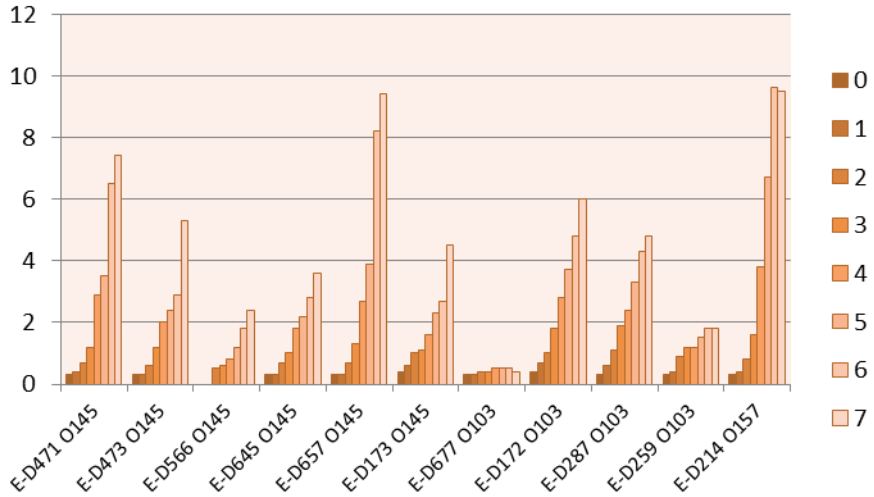
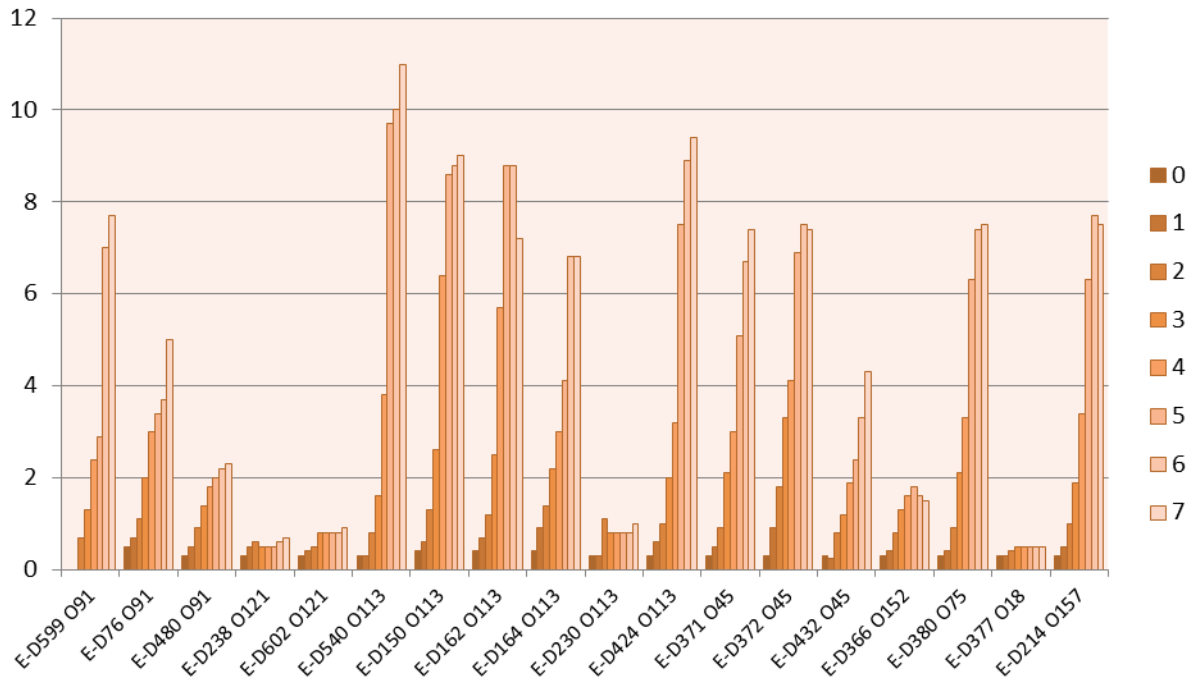




Acriflavin-III



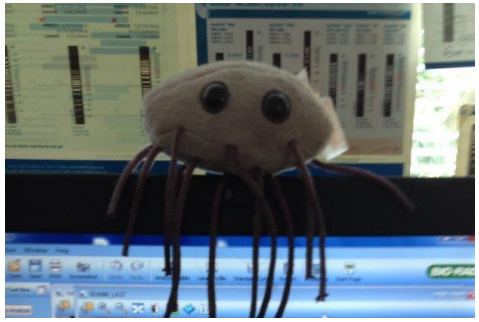
Conclusions

Most of the non-O157 STEC (belonging to the top Five O-groups) show inhibition when grown in presence of the Supplements

Our data did not take into account (yet) the matrix effect and the competitive microflora

Experiments on spiked food samples coming soon

Road Map for the revision process



An E-mail has been sent to trigger the launch of the call for experts

Hopefully the enrollment process will start early next year

Our intention is to have a first physical meeting in Rome around mid 2016

We'll keep you posted!!!!!