

**14th Annual Worksop of the National Reference Laboratories for *E. coli***  
**Rome 4-5 November 2019**

## **Update on the activities of TAG18**

**Rosangela Tozzoli**

**EU Reference Laboratory for *E. coli***  
**Istituto Superiore di Sanità**



**Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria**  
Istituto Superiore di Sanità



## 25<sup>th</sup> meeting of CEN/TC 275/WG 6 Lausanne, 2018

### Recommendation N 487

**TAG18 “Shiga toxin producing *E. Coli*” – Group leader : Rosangela Tozzoli**  
EN ISO/PWI 13136-1 Microbiology of the food chain - Real-time polymerase chain reaction (PCR)-based method for the detection of foodborne pathogens -- Part 1: Horizontal method for the detection and isolation of Shiga toxin-producing *Escherichia coli* (STEC)

WG6 noted the progress of the work on this method and invited TAG 18 to provide a draft ISO/NP 13136-1 by April 2019.

This recommendation should be forwarded to:

- CEN/TC275 : for information ☒ approval ☐
- ISO/TC34/SC9: for information ☒ approval ☐
- IDF: for information ☒ approval ☐

A TAG 18 meeting was organized in Rome (17 and 18 of October 2018) to discuss with the experts on the recommendations taken at the general meeting held in Lausanne

Fifteen international experts and three EURL for *E. coli* staff members participated in the meeting



Main focus: addressing specific issues concerning EN ISO/PWI 13136-1, in order to prepare the draft by April 2019

The discussion concerning Part 1 led to the following decisions:

- Indicate BPW as the only enrichment broth
- Indicate 41.5°C as the enrichment temperature, with exceptions in cases of suspect that stressed bacteria may be present (for instance in case of frozen samples).
- Maintain the same reagents (primers and probes) for carrying out the initial screening for the virulence genes *stx* and *eae*.
- Indicate the method of enrichment dilution to aid isolation.
- Indicate the application of the acid shock in an informative annex for the isolation of STEC in vegetables
- Indicate that the use of two selective media is mandatory, one being TBX and the other of choice, using a different substrate and/or principle used in TBX. Specify the importance of using different selective/chromogenic media for improving the isolation of STEC.
- It was also proposed to indicate testing a minimum of 50 colonies with at least 30 or half from the first medium and then the remaining from the secondary medium of choice or any additional media used.
- Expression of the results on the basis of the isolation.

A draft of EN ISO/PWI 13136-1 was prepared by the EURL for *E. coli*

The draft was circulated among TAG18 members in March 2019



comments from the TAG experts were collected

EURL prepared the draft document that has been sent to WG6 by the end of April 2019

During the preparation of the draft to be proposed to CEN TC275 WG6, the opportunity to include the detection of *eae* gene in the screening step of Part 1 arose to be still pending:



The *eae* gene detection not to be included in Part 1, whereas indicated in the characterization scheme of the STEC isolates of Part 2?

# Recommendation

TAG 18 should discuss and prepare a document stating the advantages and disadvantages of retaining in Part 1/moving to Part 2 eae detection, including also information about the impact of eae gene detection in National/regional regulations

CEN TC 275 WG6 asked to prepare the document by the end of the year, which will be the basis of an online voting

## Some discussion about Part 2

The purity of the strains is a crucial pre-requisite of Part 2 and should be always verified.

The experts agreed to change the reagents for O145 serogroup detection, which are currently specific for serotype O145:H28.

Possibility of keeping Part 2 as flexible as possible: This could potentially be achieved by quoting specific EURL for *E. coli* procedures or including an annex containing the reagents to detect specific serogroups, and would be more easily revised compared to revising the standard.

Primers and probes sequences could be indicated in an informative annex or as examples in the main part of the standard

Include the characterization of the different *stx* subtypes as well as EAEC targets

Possibility for WGS

#### Recommendation N 512

**TAG18 “Shiga toxin producing *E. Coli*” – Group leader : Rosangela Tozzoli**

**EN ISO/PWI 13136-2 Part 2: Horizontal method for the characterization of Shiga toxin-producing *Escherichia coli* (STEC)**

WG6 agreed with the proposal to add an informative annex on primers and probes for the detection of serogroup-specific genes in part 2, to allow sufficient flexibility for further serogroups.

WG6 agreed that TAG18 should include characterization of the different *stx* subtypes as well as EAEC (Enterotoxigenic *E. coli*) targets in EN ISO 13136-2.

This recommendation should be forwarded to:

- CEN/TC275 : for information ☒ approval ☐
- ISO/TC34/SC9: for information ☐ approval ☒
- IDF: for information ☐ approval ☒

#### Recommendation N 513

**TAG18 “Shiga toxin producing *E. Coli*” – Group leader : Rosangela Tozzoli**

**Project EN ISO 16654:2001/AMD 2 *Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *E. coli* O157- Amendment 2* to include performance testing of the mTSE as enrichment broth**

WG6 invited to draft an EN ISO/NP 16654/AMD2 by April 2020. The project will be activated by correspondence.

This recommendation should be forwarded to:

- CEN/TC275 : for information ☒ approval ☐
- ISO/TC34/SC9: for information ☐ approval ☒
- IDF: for information ☒ approval ☐