

Update on the revision of ISO TS 13136:2012

EU Reference Laboratory for E. coli
Istituto Superiore di Sanità

Revision of the 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

22nd 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- Amendment 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:

Revision of the ISO TS 13136:2012

23rd meeting of CEN/TC 275/WG 6
11-13 May 2016 AFNOR La Plaine Saint
Denis – France

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Recommendation N 421

TAG18 “*Shiga toxin producing E. Coli*” – Group leader : Stefano Morabito

(CEN) ISO TS 13136:2012 *Microbiology of food and animal feed - Real-time polymerase chain reaction-based method for the detection of foodborne pathogens - Horizontal method for the detection of Shiga toxin-producing E. coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups*

WG6 confirmed the revision of CEN ISO TS 13136:2012 as a full EN ISO Standard and register as a PWI.

WG6 noted that TAG18 experts will meet on 23-24 June 2016 to discuss at least the following aspects:

- Enrichment
- Revision of the *Stx*-gene sub-types detected;
- Possible addition of Enteroaggregative *E.coli* (EAEC) virulence genes;
- Inclusion of a protocol for spent irrigation water from sprouting seeds;
- Reconsideration of serogroups;
- Expression of results;
- Performance characteristics;
- Isolation step.

This recommendation should be forwarded to:

- CEN/TC275 : for information approval
- ISO/TC34/SC9: for information approval
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Revision of the ISO TS 13136:2012

First TAG 18 meeting in Rome: Twelve international experts + three EURL-VTEC staff were present and one participated via skype.

A point by point discussion on the proposals for revisions was carried out.

 <p>EU Reference Laboratory for <i>E. coli</i> Department of Veterinary Public Health and Food Safety Unit of Foodborne Zoonoses Istituto Superiore di Sanità</p>  <p>Meeting of TAG18 on Shiga toxin-producing <i>Escherichia coli</i> 23-24 June 2016</p> <p>Aula "Zampieri" Istituto Superiore di Sanità Via Giano della Bella 34, Rome, Italy</p> <p>PROVISIONAL AGENDA</p> 	<p>Thursday 23 June</p> <p>14.00 Registration of participants 14.15 Welcome, Introduction, and Housekeeping 14.30 Introduction on the purpose of the meeting 14.45 Discussion on the amendment 1 to the ISO 16654:2001 method on the detection of <i>E. coli</i> O157 in food and feed 15.15 Coffee break 15.45 Discussion on the revision of the method for the detection of Shiga toxin-producing <i>E. coli</i> in food and feed 17.30 Closure of the first day</p> <p>Friday 24 June</p> <p>9.30 Discussion on the revision of the standard ISO TS 13136 on the detection Shiga toxin-producing <i>E. coli</i> in food and feed 11.00 Coffee Break 11.30 General Discussion and concluding remarks 13.00 Closure of the meeting</p>
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24th meeting of CEN/TC 275/WG 6
Rosangela Tozzoli
22-23 June 2017
MITA KAIGISHO Auditorium, Tokyo, JAPAN

Discussion during the first meeting of TAG 18 was held in Rome 23-24 June 2016

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This recommendation should be forwarded to:

BPW as the most reliable enrichment

23rd meeting of CEN/TC 275/WG 6

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Survey to the NRLs for *E. coli* in the EU on the presence of Stx2f subtype in *E. coli* isolated from food

- ISO/TC34/SC9: for information approval

- IDF: for information approval

3 MS perform routine testing for *stx2f*. No positive findings as of today

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Revision of the ISO TS 13136:2012 – Inclusion of the EAEC targets

SCIENTIFIC OPINION



ADOPTED: 2 December 2015

PUBLISHED: 16 December 2015

doi:10.2903/j.efsa.2015.4330

Public health risks associated with Enteroaggregative *Escherichia coli* (EAEC) as a food-borne pathogen

EFSA Panel on Biological Hazards (BIOHAZ)

From the conclusions:

“Current evidence indicates that in EU MSs EAEC are primarily non-zoonotic in origin and that transmission mainly occurs by person-to-person spread and by the contamination of foods by asymptomatic carriers.”

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Revision of the ISO/TS 13136:2012 – Isolation

ACID SHOCK

- Based on *E. coli* acid-tolerance
- Reduces background microflora without addition of supplements
- Acid Treatment: treatment with low pH (2) for 1 h
- It can be applied independently from IMS or as a post-IMS treatment
- **Not verified with all STEC types**

Revision of the ISO/TS 13136:2012 – Isolation

DILUTION

- Dilution of the enrichment procedure before plating
- **Easy to do**
- **Good outcome**
- **Easily adoptable by the labs**

Possibility to split the ISO/TS 13136:2012 in two parts

Two proposals

First Proposal of repartition ISO/TS 13136 in Part I and Part II

Scope Part I:

Part I of the Standard: detection and identification of the major virulence genes of STEC and the genes associated with the serogroups O157, O111, O103, O26, and O145.

Scope part II:

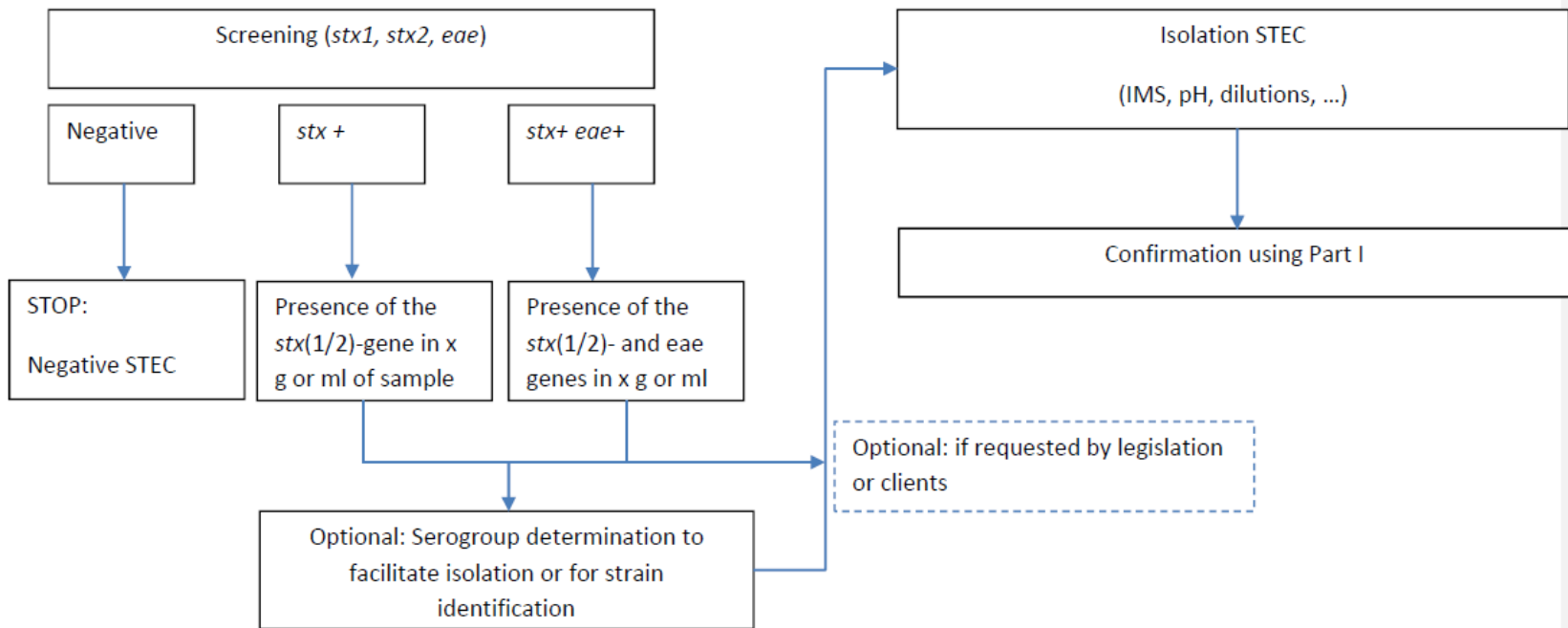
Part II of the Standard describes the isolation of STEC from samples positive for the presence of the *stx* genes

Isolation can be facilitated by using serogroup-specific enrichment techniques (e.g. immunomagnetic separation, IMS) or by means of selective isolation media. Part I of this Standard can be used for confirmation of isolated STEC strains.

Scheme of the first proposal

Part I Screening for STEC and serogroups

Part II Isolation of STEC



Possibility to split the ISO/TS 13136:2012 in two parts

Second Proposal of repartition ISO/TS 13136 in Part I and Part II

Scope of Part I:

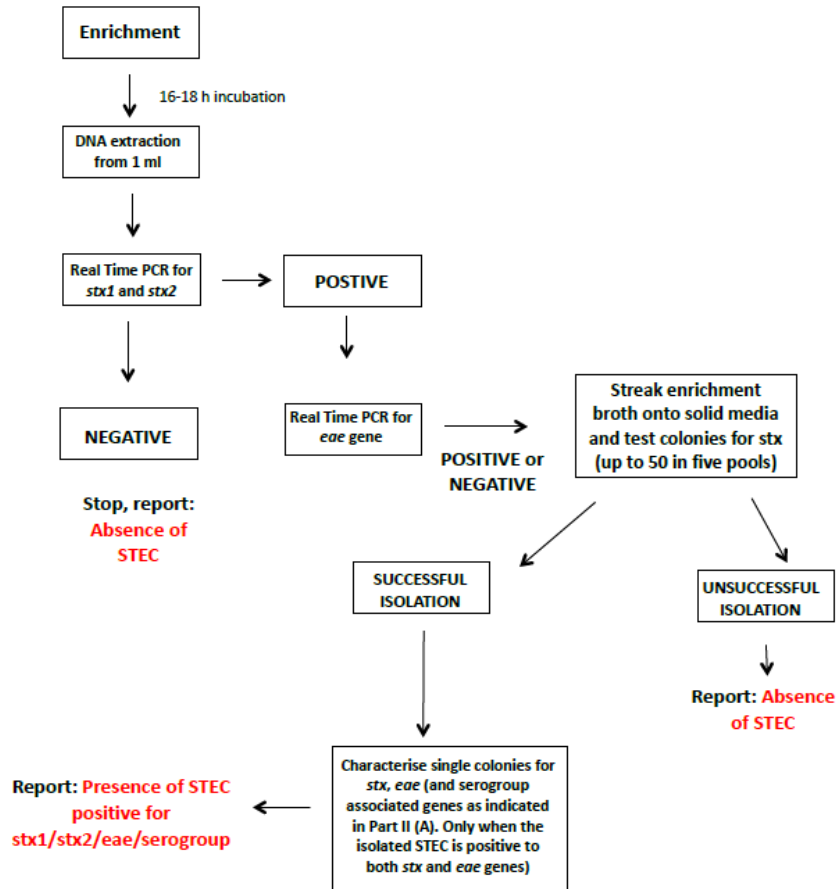
Part I of the Standard describes the detection and identification of the major virulence genes of STEC, namely *stx1*, *stx2* and *eae* genes and the isolation of the STEC strain in *stx*-positive samples.

Scope of Part II:

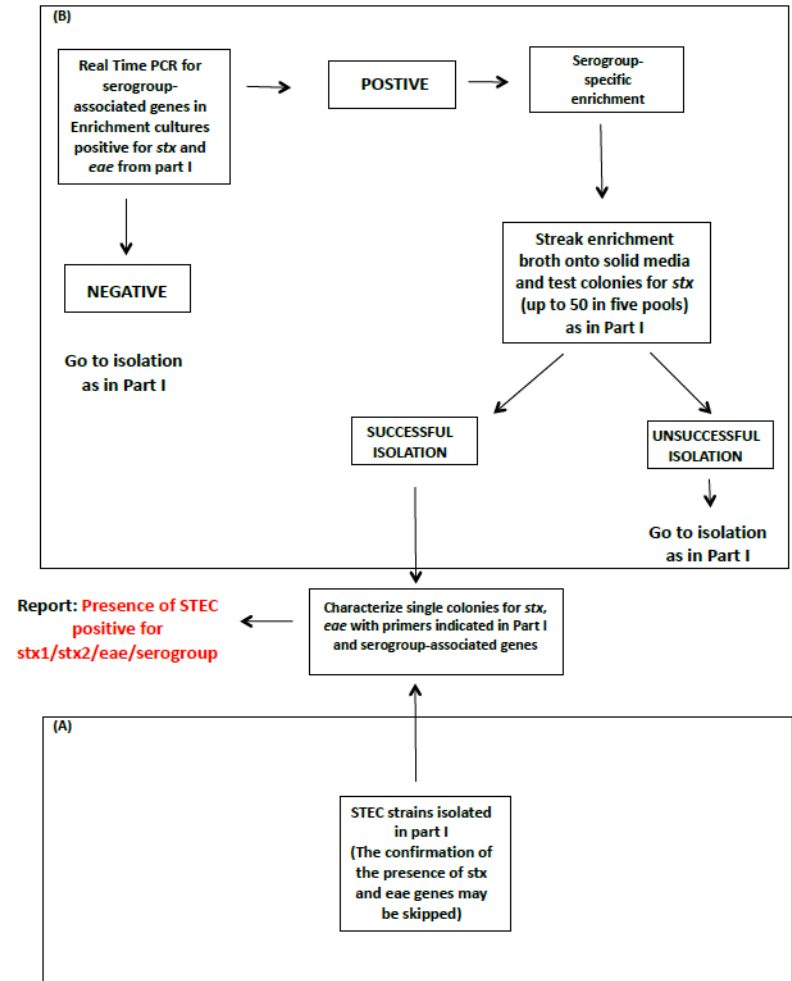
Part II describes the identification of the O-groups genes in the STEC isolated in Part I as well as the optional use of IMS to aid the isolation of the STEC under certain circumstances. In particular, when the enrichment culture is positive for both *stx* and *eae* genes, the IMS-based protocol may be applied to samples positive to one of the serogroups O157, O111, O103, O26, and O145, when positivity to one of the O-groups genes is detected.

Scheme of the second proposal

Part I: Detection and isolation of STEC from food and feed (Mandatory)



Part II: Identification of serogroup-associated genes in isolated STEC from part I (A) and IMS-aided isolation of STEC in Food (B – Optional)



Outcome of the discussion at the 24th CEN/TC275/WG6 meeting

Recommendation N 467

TAG18 “Shiga toxin producing E. Coli” – Group leader : Stefano Morabito
(CEN) ISO TS 13136:2012 *Microbiology of food and animal feed - Real-time polymerase chain reaction (PCR)-based method for the detection of foodborne pathogens - Horizontal method for the detection of Shiga toxin-producing Escherichia coli (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups.*

1) WG6 recommended that the decisions of TAG18 following Recommendation N 421 (Paris 2016) were accepted, including how to split the method into two parts:

Part 1 : Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the detection **and isolation** of Shiga toxin-producing

Escherichia coli (STEC) ~~and the determination of O157, O111, O26, O103 and O145 serogroups~~

Part 2: Real-time polymerase chain reaction (PCR)-based method for the detection of food-borne pathogens -- Horizontal method for the **characterization** of Shiga toxin-producing *Escherichia coli* (STEC)

WG6 recommended that ISO/TC34/SC9 registers an EN ISO/PWI 13136 Part 2.

2) WG6 asked TAG 18 to consider the inclusion of performance testing of culture media in prEN ISO 13136 parts 1 & 2 according to EN ISO 11133:2014 with the support of ISO/TC34/SC9 JWG5.

3) WG6 recommended that TAG18 no longer consider Enteroaggregative *E.coli* (EAEC) for inclusion in the revised standard.

4) WG 6 agreed to nominate Rosangela Tozzoli (EURL *E.coli*) as Group leader to replace Stefano Morabito.

A second meeting of TAG 18 is planned for December 2017

Starting the draft of the new standard

Thank you for your attention 😊