

Revision of the ISO/TS 13136:2012 and ISO 16654:2001 Amd1. State of Play

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Outline

- Amendment 1 to the ISO 16654:2001 method on the detection of *E. coli* O157 in food and feed: State of play
- Revision of the standard ISO TS 13136:2012 on the detection Shiga toxin-producing *E. coli* in food and feed.

Revision of the ISO 16654:2001

History

- In 2006 WG6 assessed the equivalence between the ISO 16654 and the NMKL method No 164, 2. Ed. 2005
- NMKL method No 164 was validated in a collaborative study in 2002 (minced meat, raw milk and lettuce)
- Project for validation in mandate M/381 included a reduced study as agreed by CEN in 2007 in Cairo (only one epidemiologically relevant matrix, milk)
- The inter-laboratory study on milk was conducted in 2012
- An additional study on sprouts was agreed and run in 2014

Revision of the ISO 16654:2001

Results of the inter-laboratory studies

- Performance parameters calculated in the studies for the validation of NMKL method No 164
- Performance parameters calculated in the CEN Mandate study

Have been included in the Amd 1

- The draft Amendment was sent to CEN at the end of March 2015
- Comments and suggestions from CEN were returned in September 2015 (accepted by EURL VTEC in September 2015)
- A formal vote was launched and the results were returned to the project leader in February 2016
- Positive replies: 100 % approving at CEN level and 95% at ISO level

- The comments raised during the voting of the ISO 16654:2001 amendment have been discussed also with the newly nominated TAG 18 members (Rome 23-24 June 2016)
- Final draft has been prepared and submitted to CEN secretariat, together with the response to the comments received

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

Nov. 7, 2012: The Standard was published

3 years



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

Revision of the ISO/TS 13136:2012

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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: ☒

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

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 Enteraggative *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 ☒
- IDF: for information ☐ approval ☒

The first meeting of the newly appointed TAG 18 was held in Rome 23-24 June 2016

Twelve international experts were present and one participated via skype. Three EURL-VTEC staff took part in the meeting

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

Revision of the ISO/TS 13136:2012 – Enrichment Step

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

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

Data obtained in our lab showed that most of the non-O157 STEC (belonging to the top Five O-groups) show inhibition when grown in presence of the Supplements

Growth of Stressed Strains of Four Non-O157 Shiga Toxin–Producing *Escherichia coli* Serogroups in Five Enrichment Broths

BAVO VERHAEGEN,^{1,2} KOEN DE REU,^{2*} MARC HEYNDRICKX,^{2,3} INGE VAN DAMME,¹ AND LIEVEN DE ZUTTER^{1*}

Eight STEC strains belonging to O26, O111, O145, O103 tested in mTSB; BPW; BPW plus 1 g/liter sodium pyruvate (BPWp); Brila (Merck); and SEB.

low-nutrient nonselective enrichment medium BPW is the best option to resuscitate very or slightly stressed STEC cells

BPW as the most reliable enrichment

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Revision of the ISO/TS 13136:2012 – *stx*-genes targets

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Research

Whole-Genome Characterization and Strain Comparison of VT2f-Producing *Escherichia coli* Causing Hemolytic Uremic Syndrome

Laura Grande✉, **Valeria Michelacci**, **Roslen Bondi**, **Federica Gigliucci**, **Eelco Franz**, **Mahdi Askari Badouei**, **Sabine Schlager**, **Fabio Minelli**, **Rosangela Tozzoli**, **Alfredo Caprioli**, and **Stefano Morabito**

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

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 – Miscellaneous



EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità



**16th inter-laboratory study on the detection of Verocytotoxin-producing
E. coli (VTEC) in sprout spent irrigation water (PT16)**

Laboratory procedure



EU Reference Laboratory for *E. coli*
Department of Veterinary Public Health and Food Safety
Unit of Foodborne Zoonoses
Istituto Superiore di Sanità



CEN ISO/TS 13136:2012

**Report on the primary validation of the PCR screening reactions and the
determination of the performance parameters, based on the results of
the analytical tests carried out within the EU-RL VTEC proficiency
testing program (2009-2015)**



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- Reconsideration of serogroups;
- Expression of results;
- Performance characteristics;
- Isolation step.

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Revision of the ISO/TS 13136:2012 – Isolation

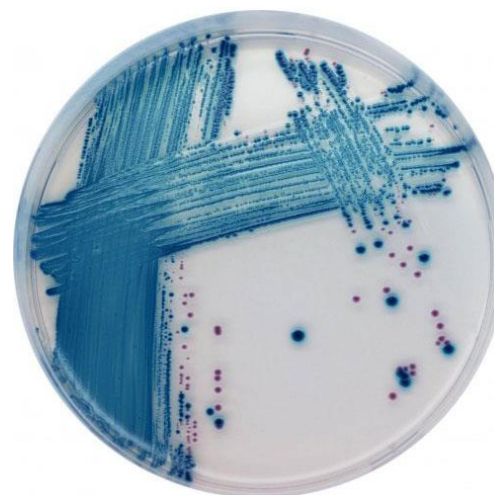
ACID SHOCK

- 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
- **Complex procedure**
- **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**



Chromogenic media?



Table 2. Growth and colony appearance of STEC and non-STEC *E. coli* strains on six chromogenic isolation media.

Strain	Serotype	<i>TerB</i>	TBX	RBA	RE	mMac	Chr ST	Chr ID
MB 5323	O5:H-	–	blue-green	grey-green	green	– *	–	purple
MB 5324	O5:H-	+	blue-green	grey-green	green	green	mauve	purple
MB 5321	O8:H-	–	blue-green	red	green	–	–	purple
MB 5322	O8:H9	–	blue-green	purple	green	–	–	–
MB 5325	O55:H12	+	blue-green	purple	green	grey-green	mauve	blue
MB 5312	O55:H7	–	blue-green	red	green	–	–	purple
MB 5342	O63:H6	–	blue-green	red	dark blue	–	–	purple
MB 5313	O63:H6	–	blue-green	red	dark blue	–	–	purple
MB 5334	O84:H-	+	blue-green	cream-white	yellow	yellow	mauve	white
MB 5333	O84:H28	+	blue-green	red-purple	green	green	mauve	purple
MB 5336	O91:H-	–	blue-green	purple	green	–	–	purple
MB 5335	O91:H21	–	blue-green	purple	green	–	–	purple
MB 5339	O113:H2	–	blue-green	red-purple	green	–	–	purple
MB 5338	O113:H21	+	blue-green	red-purple	green	grey-green	mauve	purple
MB 5950	O118:H16	+	blue-green	purple	green	red	mauve	purple
MB 5951	O118:H16	+	blue-green	purple	green	grey-green	mauve	purple
MB 5337	O121:H19	+	blue-green	red	yellow	red	mauve	red
MB 5326	O128:H-	–	blue-green	red-purple	green	–	–	purple
MB 5327	O128:H-	–	blue-green	red-purple	green	–	–	purple
MB 5329	O146:H28	+	blue-green	purple	green	green	mauve	purple
MB 5328	O146:H-	+	blue-green	purple	green	grey-green	mauve	purple
MB 5340	O182:H34	+	blue-green	purple	dark blue	green	mauve	purple
MB 5341	O182:H25	+	blue-green	purple	dark blue	green	mauve	purple
MB 5948	O26:H11	+	blue-green	purple	green	red	mauve	purple
MB 5316	O26:H11	+	blue-green	purple	green	red	mauve	purple
MB 2658	O26:H11	+	blue-green	purple	green	red	mauve	purple
MB 2775	O26:H11	+	blue-green	purple	green	red	mauve	green
MB 5307	O103:H2	–	blue-green	purple	green	blue-green	mauve	purple
MB 5308	O103:H2	–	blue-green	purple	green	blue-green	mauve	purple
MB 2654	O103:H2	–	blue-green	purple	green	–	–	purple
MB 2651	O103:H2	–	blue-green	purple	green	–	–	purple
MB 5949	O111:H2	+	blue-green	grey-green	green	grey-green	mauve	purple
MB 5310	O111:H8	+	blue-green	grey-green	green	grey-green	mauve	purple
MB 2679	O111:H-	+	blue-green	grey-green	green	grey-green	mauve	purple

**Verhaegen B, De Reu K,
Heyndrickx M, De Zutter L.
Int J Environ Res Public
Health. 2015 Jun
17;12(6):6965-78.**

Conclusions

ISO TS 13136:2012 is widely adopted at EU Level

Formal revision process started at CEN/ISO

Discussion concerns several points - improvement

Isolation is still the bottleneck

Thank you (again!) for your attention!

