Update on the activities of TAG18

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Revision of the ISO TS 13136:2012

Announced in late 2015

Three TAG 18 meetings in Rome (last one on the 17th and 18th of October 2018)

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: ☒

New structure of the standard

Part 1

+ enrichment broth

↓

Incubation

↓

Real Time PCR for *stx* and *eae* genes

↓

Positive to *stx* genes:
Streak enrichment culture onto solid media to isolate the STEC (up to 50 colonies tested)

Based on isolation results: presence/absence of STEC in XX g

Part 2

↓

STEC serotyping
virulotyping

ISO TS 13136 revision: Part 1

TAG 18 agreed in maintaining the same primers and probes for *stx1*, *stx2*, *eae* genes

BPW as the most reliable enrichment

How to reduce the growth of background microflora in the absence of supplements

Discussion about the appropriateness of raising the incubation temperature to 41.5°C

Experiences of the TAG 18 participants were shared

Results of a survey conducted by EURL *E. coli* in the network of NRLs for *E. coli*

Do you have any data collected in your lab about the use of the enrichment temperature of 41.5°C for the detection of VTEC other than O157?

20 No, 5 Yes

Would you be willing to voluntarily participate in an inter-laboratory study to assess the effect of the increased enrichment temperature on the outcome of the analysis (this could be an optional step in one of the next PTs on food matrices)

6 No, 19 Yes

Voluntary inter-laboratory study on the effect of the enrichment temperature of 41.5 °C on the detection and isolation of STEC in sprouts

A total of 48 Laboratories including 31 NRLs and 17 Italian OLs participated in the voluntary study

The participants received two sets of three samples (25 g red radish sprouts each containing 0, 2 and 20 CFU/g of STEC O26)

Contaminant (*Genotype*)	**Contamination level in:**		
	Sample 1	**Sample 2**	**Sample 3**
Strain C1188-02, STEC O26 ***(stx1+, stx2+, eae+)*** UoM 0.209 log CFU/ml	-	**Low: 2 CFU/g**	**High: 20 CFU/g**

One set to be analized applying ISO TS 13136 as such (enrichment at 37°C in BPW)

One set to be analized by ISO TS 13136 but enrichment carried out at 41.5°C in BPW

Sensitivity

Real Time PCR

	Enrichment at 37 ° C				Enrichment at 41.5 ° C			
	stx1	***stx2***	***eae***	***wzx$_{O26}$***	***stx1***	***stx2***	***eae***	***wzx$_{O26}$***
Se (low level)	93.9 %*	97.9 %*	100 %	100 %	100 %	100 %	100 %	100 %
Se (high level)	97.9 %	97.9 %	100 %	100 %	100 %	100 %	100 %	100 %

Isolation

	Enrichment at 37 ° C	Enrichment at 41.5 ° C
Se (low level)	73.3 %	92.2 %
Se (high level)	80.7 %	94 %

LOD 37°C

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1		0,005	0,169	5,435	3,880	7,615	23,491	16,767	32,911	0,000
Combined data		0,005	0,169	5,435	3,880	7,615	23,491	16,767	32,911	0,000

LOD 41.5°C

No. of matrix	Name of matrix	Matrix effect	SD of log matrix effect	$LOD_{50\%}$ [1]			$LOD_{95\%}$ [2]			Test statistic matrix effect
				Detection limit	Lower conf. limit	Upper conf. limit	Detection limit	Lower conf. limit	Upper conf. limit	
i	$matrix_i$	F_i	s_{fi}	$d_{0.5,i}$	$d_{0.5,iL}$	$d_{0.5,iU}$	$d_{0.95,i}$	$d_{0.95,iL}$	$d_{0.95,iU}$	$\lvert z_i \rvert$
1		0,016	0,197	1,692	1,141	2,509	7,312	4,929	10,845	0,000
Combined data		0,016	0,197	1,692	1,141	2,509	7,312	4,929	10,845	0,000

Conclusions from the interlaboratory study results

The network of Laboratories working in the field of pathogenic *E. coli* is made up of very collaborative participants

the sensitivity of the method in the Real Time PCR screening step slightly increased when the enrichment temperature applied was 41.5 °C

the isolation step was strongly improved when the enrichment temperature was raised at 41.5 °C, being particularly beneficial for the low level contamination samples

LOD_{50} for the isolation step was much lower when the enrichment was carried out at 41.5 °C (1.692 CFU/g), compared to the enrichment at 37 °C (5.435 CFU/g)

Improvement of 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
- Succesfully applied for the isolation of STEC from sprouts during a PT organized by the EURL-VTEC
- **Not verified with all STEC types and any food matrices**

Quoted as an informative step in Part 1 for aiding the isolation of STEC in vegetables and run in parallel with direct plating

DILUTION

Improvement of isolation

The use of a single medium (TBX) is currently quoted in the ISO TS 13136

More selective media (at least two) should be indicated

Facultative use of alternative media, as chromogenic agars, SHIBAM

CT-SMAC and RMAC may aid the isolation of O157 and O26

ISO TS 13136 revision: Part 2

TAG 18 agreed in maintaining the same primers and probes for the genes associated to O157, O111, O103 and O26

Change the O145-serogroup associated gene (ihp1) which targets O145:H28 serotype only

Not to include O104;H4 detection: seems to have been a punctual event; Current regulation referes to the EURL-VTEC method

Include the detection of serogroups O45 and O121 (O121 is listed in the top-20 STEC serogroups associated with human infection in the EU- EFSA and ECDC joint SR 2016) – under discussion

Include further characterization of STEC isolated strains (i.e. additional virulence genes detection and/or stx genes subtyping?) – under discussion

Discussion about the field of application of ISO TS 13136:2012

This Technical Specification is applicable to:

1) products intended for human consumption and the feeding of animals;
2) environmental samples in the area of food production and food handling;
3) environmental samples in the area of primary production.

Animal faecal samples fall in point 3

Recommendation N 476

Part 2 Horizontal method for the characterization of STEC

WG6 invited TAG 18 to include a horizontal validation (including irrigation water) as part of the revision of CEN ISO/TS 13136:2012.

This recommendation should be forwarded to:
- CEN/TC275 : for information ☒ approval ☐
- ISO/TC34/SC9: for information ☒ approval ☐
- IDF: for information ☒ approval ☐

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 ☐

Recommendation N 488

WG6 agreed to include serogroups O45, and O121 as used in the US regulatory framework for analyses of trimmed beef and to consider within TAG 18 the inclusion of serogroup O174.

WG6 agreed to exclude serotype O104:H4.

This recommendation should be forwarded to:
- CEN/TC275 : for information ☒ approval ☐
- ISO/TC34/SC9: for information ☐ approval ☒
- IDF: for information ☒ approval ☐

Resolution N 829
Systematic Review

- SC9 noted the comments and ballot results from the Systematic Review of ISO 21527 parts 1 and 2:2008, and resolved to confirm these standards for an additional five years.
SC9 requested WG16 to take into account these comments for the revision of ISO 21527-1&-2.

- SC9 agreed to launch a second amendment to ISO 16654:2001 to include the culture media performance testing for mTSB enrichment. A project leader will be sought.

- SC9 noted the comments and ballot results from the Systematic Review of ISO 6887-6:2013, and resolved to confirm this standard for an additional five years.
SC9 invited WG8 to respond to the comments received during the Systematic Review and then discuss whether revision is needed.
SC9 agreed a resolution by correspondence would be launched if a revision was needed and a Project Leader for Part 6 would be requested.

- SC9 noted the comments and ballot results from the Systematic Review of ISO 13307:2013, and resolved to confirm this standard for an additional five years. SC9 invited CEN/TC275/WG6 to consider whether a revision is necessary to take into account the comments made during the Systematic Review.

This resolution should be forwarded to:
- CEN/TC275/WG6: for info ☐ approval ☒
- ISO/TC34/SC5 IDF: for info ☒ approval ☐

with the help of experts from ISO/TC34/SC9 WG5 “Culture media” (EN ISO 11133:2014 Preparation, production, storage and performance testing of culture media)

Preparation of the draft of Part I of the standard

TAG18 experts agreed that there is no need of adding the detection of O174 serogroup in Part II

Part II is aiming at characterising the STEC isolated strains, in terms of serogroups (top-5+O121 and O45) but also including additional characterization as the pathogenic potential of each STEC strain is not predicted by the serogroup they belong to