

A standard method for the detection of STEC in foodstuffs



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The road map to a standard method for the detection of STEC in foodstuffs

STEC “ad hoc” group meetings:

- **1st meeting, AFNOR, 31/01 and 01/02/05.**
- **2nd Meeting, September 2005 Copenhagen, Denmark**

CRL chairmanship- July 2006

- **Rome, ISS March the 5th, 2007**
- **Rome, ISS November the 22nd, 2007**

15th meeting of CEN/TC 275/WG 6 May 2008. Helsinki – Finland

Resolution 165: Shiga toxin producing E.coli

WG6 agreed to launch a TS on this topic, VA, CEN lead.

Stefano Morabito (CRL VTEC) was asked to send the draft method to the Secretariat before the end of July. The draft will include the choice between the 2 enrichment broths depending on food matrices. Then the Secretariat will launch an informal consultation of 3 months among WG6 members as soon as possible.

15th meeting of CEN/TC 275/WG 6 May 2008. Helsinki – Finland

Resolution 165: Shiga toxin producing E.coli

WG6 recommended to ISO TC34 SC9 to launch the NWIP vote as soon as possible (VA, CEN lead), together with the draft mentioned before.

The comments received will be considered by the ad'hoc group which will then prepare the draft to be submitted to the 3 month vote (acceptance of proposal and of draft) before publication.

This resolution should be approved:

- at the next CEN/TC275 meeting
- at the next ISO/TC34 SC9 meeting

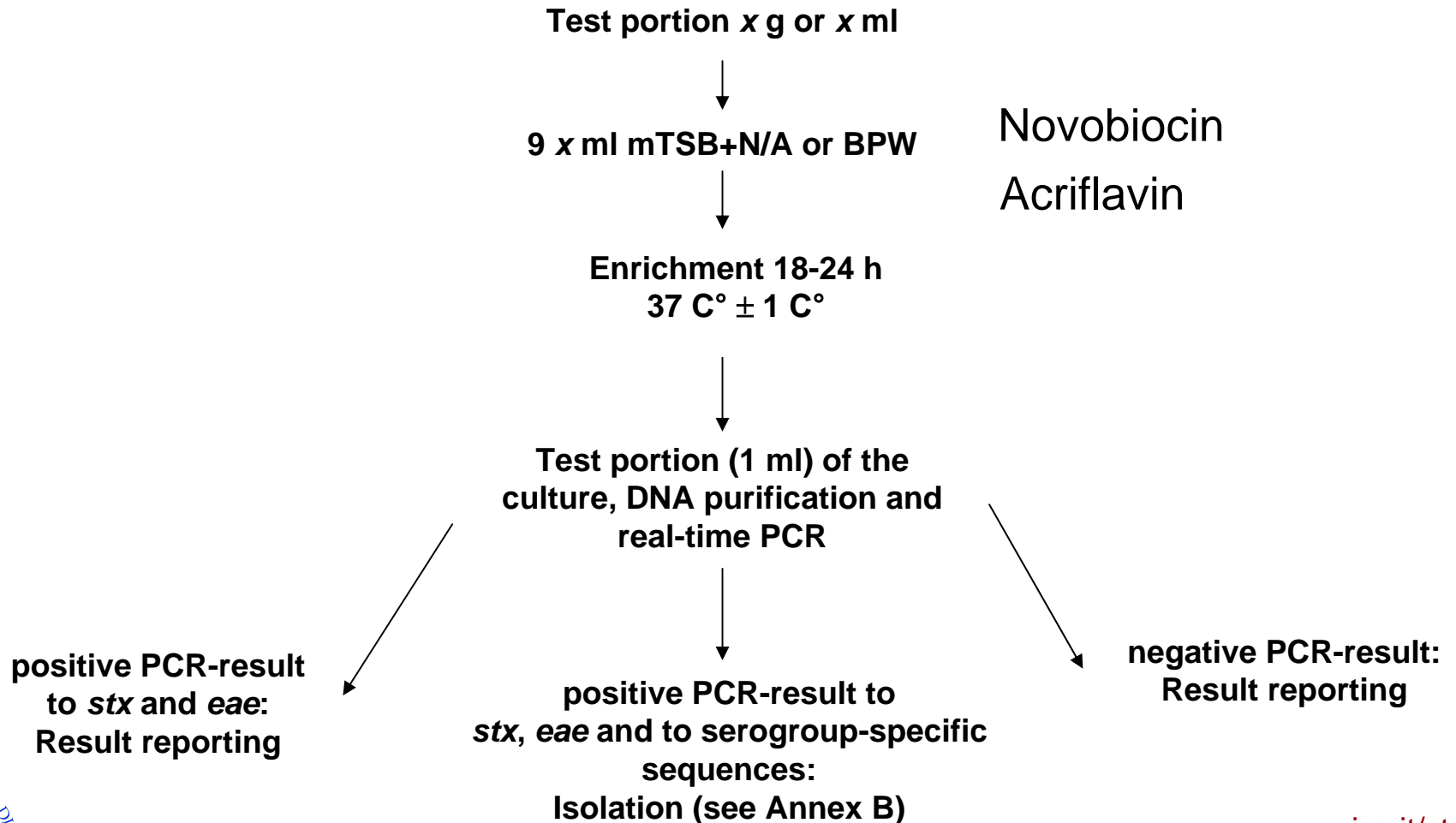
Next meeting will be on April 2009 in Spain

ISO/TS XXXXX:200X

Microbiology of food and animal feeding stuffs – Horizontal method for the detection of Shiga toxin-producing *Escherichia coli* (STEC) belonging to O157, O111, O26, O103 and O145 serogroups - Qualitative Real-time polymerase chain reaction (PCR)-based Method

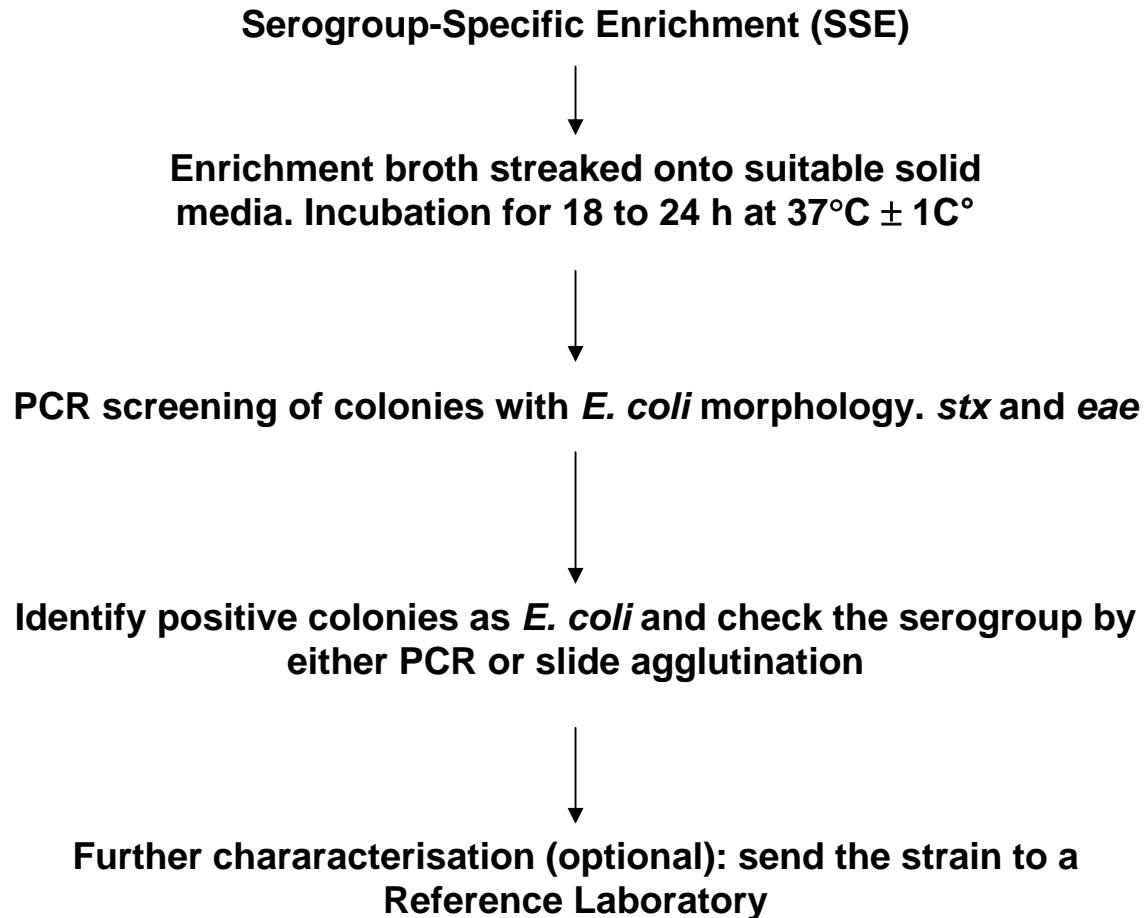
Annex A (normative)

Flow-diagram of the screening PCR procedure



Annex B (normative)

Flow-diagram of the isolation procedure



Annex C (informative)

Identification of Shiga toxin-producing *Escherichia coli* (STEC) by multiplex PCR amplification of virulence genes and detection of PCR products by agarose gel electrophoresis

Real Time PCR Primers and Probes: *stx* and *eae* (normative)

Target gene	Forward primer, reverse primer and probe sequences (5'-3') ^a	Amplicon size (bp)	Location within sequence	GenBank accession number
<i>stx1</i> [§]	TTTGTYACTGTSACAGCWGAAGCYTTACG CCCCAGTTCARWGTRAGRTCMACRTC Probe- CTGGATGATCTCAGTGGGCGTTCTTATGTAA	131	878–906 983–1008 941–971	M16625
<i>stx2</i> [§]	TTTGTYACTGTSACAGCWGAAGCYTTACG CCCCAGTTCARWGTRAGRTCMACRTC Probe-TCGTCAGGCACTGTCTGAAACTGCTCC	128	785–813 785–813 838–864	X07865
<i>eae</i> [*]	CAT TGA TCA GGA TTT TTC TGG TGA TA CTC ATG CGG AAA TAG CCG TTA Probe- ATAGTCTCGCCAGTATTCGCCACCAATACC	102	899-924 1000-979 966-936	Z11541

Real Time PCR Primers and Probes: Serogrouping (normative)

Target gene (serogroup)	Forward primer, reverse primer and probe sequences (5'-3')	Amplicon size (bp)	Location within sequence	GenBank accession number
[§] <i>rfbE</i> (O157)	TTTCACACTTATTGGATGGTCTCAA CGATGAGTTTATCTGCAAGGTGAT Probe- AGGACCGCAGAGGAAAGAGAGGAATTAAGG	88	348-372 412-435 381-410	AF163329
[§] <i>wbdI</i> (O111)	CGAGGCAACACATTATATAGTGCTTT TTTTTGAATAGTTATGAACATCTTGTTTAGC Probe- TTGAATCTCCAGATGATCAACATCGTGAA	146	3464-3489 3579-3609 3519-3548	AF078736
[§] <i>wzx</i> (O26)	CGCGACGGCAGAGAAAATT AGCAGGCTTTTATATTCTCCAACCTTT Probe- CCCCGTTAAATCAATACTATTTTACGAGGTTGA	135	5648-5666 5757-5782 5692-5724	AF529080
[§] <i>ihp1</i> (O145)	CGATAATATTTACCCACCAGTACAG GCCGCCGCAATGCTT Probe- CCGCCATTCAGAATGCACACAATATCG	132	1383-1408 1500-1514 1472-1498	AF531429
[*] <i>wzx</i> (O103)	CAAGGTGATTACGAAAATGCATGT GAAAAAAGCACCCCGTACTTAT Probe- CATAGCCTGTTGTTTTAT	99	4299-4323 4397-4375 4356-4373	AY532664

Real Time PCR Primers and Probes: References

- [1] **Nielsen EM, Andersen MT.** Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5' nuclease PCR assay. **2003 J Clin Microbiol. 41(7):2884-2893.**
- [2] **Perelle S, Dilasser F, Grout J, Fach P.** Detection by 5'-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world's most frequent clinical cases. **2004 Mol Cell Probes. 18(3):185-192.**
- [3] **Perelle S, Dilasser F, Grout J, Fach P.** Detection of *Escherichia coli* serogroup O103 by real-time polymerase chain reaction. **2005 J Appl Microbiol 98(5):1162-1168.**

Results reporting

1) Samples negative to *stx* genes: Absence of STEC in the test portion of x g or x ml (see ISO 7218).

2) Samples positive to *stx* genes only: Presumptive presence of STEC in the test portion of x g or x ml .

3) If isolation is achieved:

3.1) Samples positive to *stx* and *eae* genes only: Presence of STEC potentially pathogenic to humans in the test portion of x g or x ml.

3.2) Samples positive to *stx* and *eae* genes and belonging to one of the serogroups O157, O111, O26, O103 and O145: Presence of STEC highly pathogenic to humans in the test portion of x g or x ml .

Results reporting

4) If no isolation is achieved:

4.1) Samples positive to *stx* and *eae* genes: Presumptive presence of STEC potentially pathogenic to humans in the test portion of x g or x ml.


4.2) Samples positive to *stx* and *eae* genes and belonging to one of the serogroups O157, O111, O26, O103 and O145: Presumptive presence STEC highly pathogenic to human in the test portion of x g or x ml.

Where are we now, exactly?

ISO Global Directory II - ISO/NP TS 13136

https://directory.iso.org/ISOCD2/portals/std/index_portal.jsp

Portale applicazioni X ISO Global Directory II - ISO/NP TS... X



Committees **Projects** **Member Bodies** **Organizations** **People** **Roles** **Country**

Welcome Dott. Stefano Morabito - stefano.morabito@iss.it 2008-12-01 MB Ad

Projects - ISO/NP TS 13136

Reference: [ISO/NP TS 13136](#)

Title: [Microbiologia of food and animal feeding stuffs -- Horizontal method for the detection of Shiga toxin-producing Escherichia coli \(STEC\) belonging to 0157, 0111, 026, 0103 and 0145 serogroups -- Qualitative Real-time polymerase chain reaction \(PCR\) based method](#)

Member of Committee: [ISO/TC 34/SC 9](#)

Current stage: 10.20

Stage date: 2008-11-24

Project leader: [Morabito Stefano Dott. @ ISS - Roma](#)

Maintenance agency:

Registration authority:

Project submitter:

Source Application: PMDB

Project ID: 53328

Serial Number: 2072325

International harmonized stage codes

STAGE	SUBSTAGE						
	90 Decision Substages						
	00 Registration	20 Start of main action	60 Completion of main action	92 Repeat an earlier phase	93 Repeat current phase	98 Abandon	99 Proceed
00 Preliminary stage	00.00 Proposal for new project received	00.20 Proposal for new project under review	00.60 Close of review			00.98 Proposal for new project abandoned	00.99 Approval to ballot proposal for new project
10 Proposal stage	10.00 Proposal for new project registered	10.20 New project ballot initiated	10.60 Close of voting	10.92 Proposal returned to submitter for further definition		10.98 New project rejected	10.99 New project approved
20 Preparatory stage	20.00 New project registered in TC/SC work programme	20.20 Working draft (WD) study initiated	20.60 Close of comment period			20.98 Proposal for new project under review	20.99 Close of review
30 Committee stage	30.00 Committee draft (CD) registered	30.20 CD study/ballot initiated	30.60 Close of voting/comment period	30.9 CD back Work			
40 Enquiry stage	40.00 DIS registered	40.20 DIS ballot initiated: 5 months	40.60 Close of voting	40.9 Full circu refer to TC		10.20 New project ballot initiated	10.60 Close of voting
50 Approval stage	50.00 FDIS registered for formal approval	50.20 FDIS ballot initiated: 2 months. Proof sent to secretariat	50.60 Close of voting. Proof returned by secretariat	50.9 FDIS back SC			
60 Publication stage	60.00 International Standard under publication		60.60 International Standard published			20.20 Working draft	20.60 Close of
90 Review stage		90.20 International Standard under periodical review	90.60 Close of review	90.92 International Standard to be revised	90.93 International Standard confirmed		90.99 Withdrawal of International Standard proposed by TC or SC
95 Withdrawal stage		95.20 Withdrawal ballot initiated	95.60 Close of voting	95.92 Decision not to withdraw International Standard			95.99 Withdrawal of International Standard

Time schedule to complete the road map

- The draft has been sent in the due time (July 31 2008) to CEN secretariat
- Comments from CEN/ISO to CRL ???
- Discussion of comments among ad hoc group members, production of a final draft and resubmission to CEN secretariat **about 45 days**
- Three months voting (...)
- Final approval and sending for printing ???

Thank you for your attention!