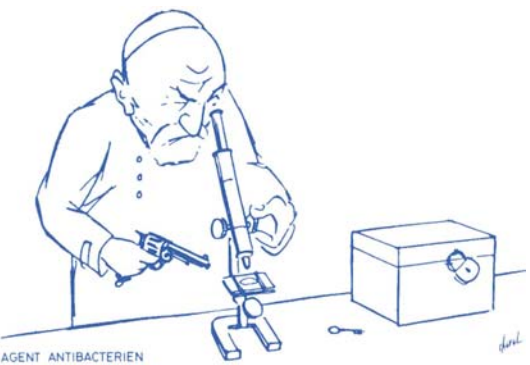
Assessment of a real-time PCR for the identification and characterisation of Verocytotoxigenic *E. coli* (VTEC)

GA Smith, C Jenkins, AJ Lawson, N Perry, T Cheasty
Microbiology Services, HPA Colindale, UK

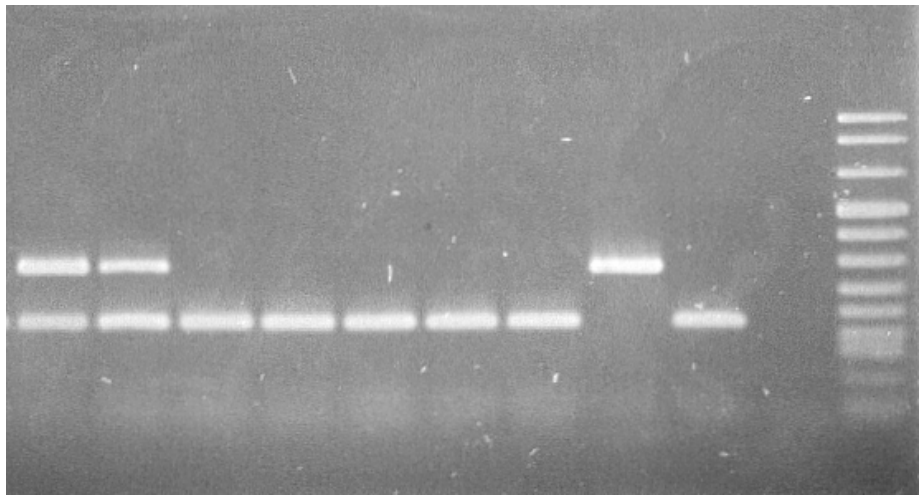


geraldine.smith@hpa.org.uk

Previous block-based PCR for *vtx*1 and *vtx*2 genes

*vtx*1
282bp

*vtx*2
164bp



*vtx*1
*vtx*2
controls
neg

Detection of the *eae* (conserved) was by a separate block-based PCR

Real-time PCRs for the identification of VTEC: guidelines from the Community Reference Laboratory (CRL) (http://www.iss.it/binary/vtec/cont/VTEC_RealTime.pdf)

- Vero cytotoxin genes (*vtx1& vtx2*)
- Conserved region of intimin (*eae*)
- *rfb*E encoding the O antigen of *E. coli* O157.

- validation study using VTEC cultures
- use of the assay in rapid identification of UK cases during the outbreak of VTEC O104 outbreak in Germany

Organisation of the real time assays

- Two duplex PCRs on 5-plex Rotorgene-Q (Qiagen)
- Duplex 1 = *vtx*1 + *eae*; duplex 2 = *vtx*2 + *rfb*O157
- Amplification parameters: 95°C for 5m, followed by 95°C for 15s and 60°C for 60s. The cycle threshold was 0.025 for *vtx*1and 0.05 for the others

YakY/ Joe →

Cy5 →

Duplex#1	Conc	Vol ul	Duplex#2	Conc	Vol ul
Fast blue	X1	12.5	Fast blue	X1	12.5
eae F primer	10uM	0.5	vtx2 F primer	10uM	0.5
eae R primer	10uM	0.5	vtx2 R primer	10uM	0.5
eae probe	1uM	0.5	vtx2 probe	1uM	0.5
vtx1 F primer	10uM	0.5	O157 F primer	10uM	0.5
vtx1 R primer	10uM	0.5	O157 R primer	10uM	0.5
vtx1 probe	1uM	0.5	O157 probe	1uM	0.5
Water		7.0	Water		7.0
DNA target		2.5	DNA target		2.5
Total		25	Total		25

← YakY/ Joe

← FAM

VTEC isolates for validation (545)

Source	Strain types	Number
England and Wales in 2010	O157	169
(i) UK and Ireland 2004-2010 (ii) UK and abroad pre-2004	Non-O157[1]	310
VTEC EQA isolates from ring trials	Non-O157	66

[1] Includes clinically-important serogroups from human disease

Concordance of previous block and real time methods

vtx genes	RT Agree	RT Disagree	Reason
Block O157	169 (100%)	0	
Block non-O157	372 (99%)	4 (1%)	*vtx*1d subtype

- 100% of the *E. coli* O157 strains were detected in the O157 *rfb*E PCR
- None of the *E. coli* of other serogroups was positive in the O157 *rfb*E PCR
- Real time *eae* PCR detected 100% isolates positive by block-based assay

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Ability to detect subtypes of *vtx* genes

vtx type	Subtype	Real time	Routine block
*vtx*1	**a**, b, c	Yes	Yes
*vtx*1	d	Yes	No
*vtx*2	**a**, b, **c**, **d**, e, g	Yes	Yes
*vtx*2	f	No	No

Most *vtx2*f strains produce intimin, so all *eae*-positive, *vtx*-negative strains can be tested with a specific *vtx*2f-PCR

Application to faeces samples

214 specimens: cases with diarrhoeal symptoms and possible links to Germany for detection of VTEC O104 between 25th May and 24th June 2011

- Plated on MacConkey agar, Sorbitol MacConkey agar with and without cefixime and tellurite (CT-SMAC and SMAC)
- Enriched (6h and18h) in modified tryptone soya broth (mTSB); DNA extracted from enrichment with Instagene (Bio-Rad Catalog# 732-6030

VTEC cases detected

7 specimens positive by PCR for *vtx* genes confirmed by culture

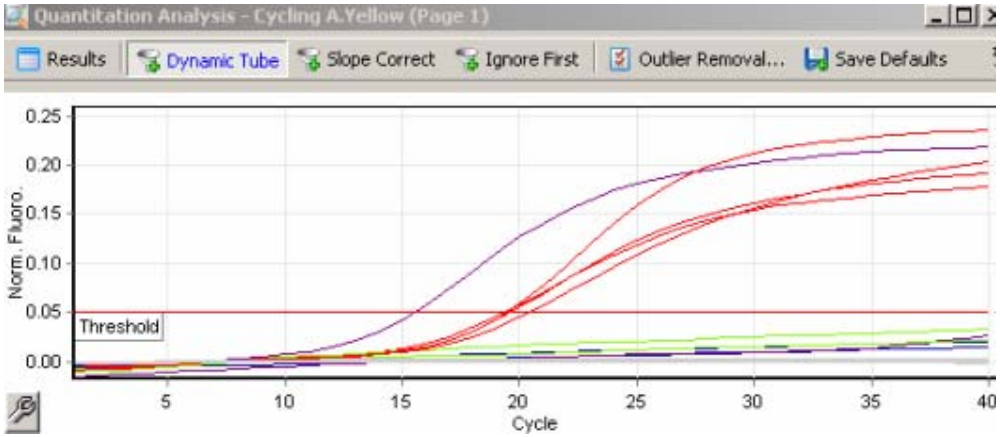
VTEC O104

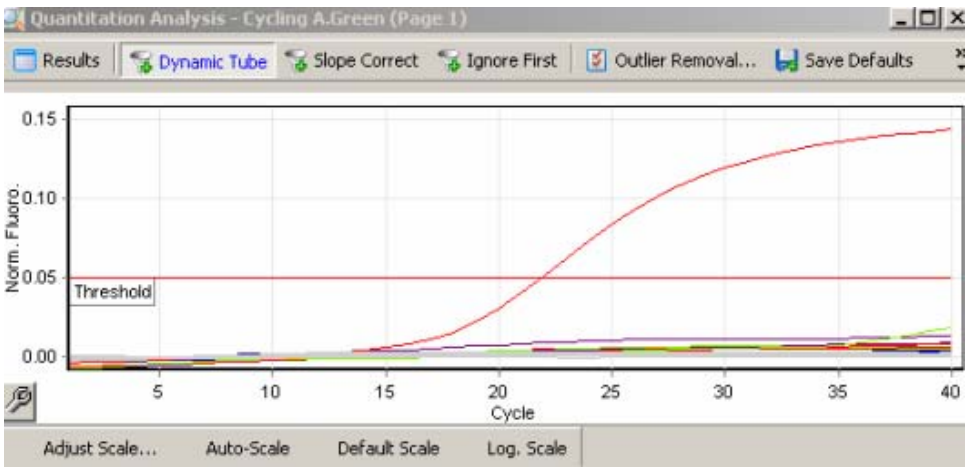
5 specimens positive for *vtx*2, negative *eae*: 8-24 h after receipt
*vtx*2 gene positive isolates obtained by colony picking: 24-30h after receipt

Biochemical ID and serotyping confirmed *E. coli* O104:H4
PCR positive for the *agg*R gene of enteroaggregative E. coli
Properties were consistent with the VTEC O104 strains isolated in Germany

Other VTEC

1 isolate *E. coli* O103 (*vtx*1,*eae*).
1 isolate *E. coli* O157 (*vtx*2, *eae, rfb*O157)





O104: *vtx*2 +ve

Negative for *vtx*1, *eae*, *rfb*O157

In total 7 cases of VTEC O104:H4 were confirmed in the UK during the period of the outbreak

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Conclusions and further work

- Confirmation of *vtx* genes in a wide range of VTEC
- Presence of *eae* gene in many clinically-important VTEC; enables routine detection of EPEC strains
- Molecular serotype for O157 < 4-h of culture receipt

During the VTEC O104 outbreak, the PCR was a rapid and reliable screening method for the detection of cases subsequently confirmed microbiologically by culture

- Generic detection of VTEC in faeces: current analysis of data on >500 samples
- Beginning evaluation of direct nucleic acid extraction from faeces on automated platform
- BUT isolation of VTEC from positive samples will still be time-consuming!!!