# Validation of the detection and isolation of verocytotoxigenic *E. coli* (VTEC) belonging to the serogroups O26, O103, O111 and O145 in beef meat, carcass swabs, fresh vegetables and raw milk according to ISO/TS 13136:2012

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## INTRODUCTION

VTEC is a group of pathogenic *E. coli* bacteria that can cause bloody diarrhoea (BD) and haemolytic uremic syndrome (HUS) in humans, a serious condition that can lead to kidney failure and be fatal. Although the most reported VTEC serotype involved in severe human disease is *E. coli* O157:H7, other serotypes that have been associated to outbreaks and HUS include O26:H11, O103:H2, O111:NM, O121:H19 and O145:NM. The recently published ISO/TS13136:2012 describes the European standardized method for the detection of VTEC in foodstuff, including the determination of the top-5 serogroups (O157, O26, O111, O103, O145). In this work, the validation results for VTEC detection in beef meat, carcass swabs, carrots and raw milk according to ISO/TS13136:2012 is described.

## METHODS

### ISO/TS 13136:2012

Detection *stx1* and/or *stx2* (qPCR) GeneDiscCycler (Pall Genesystems)

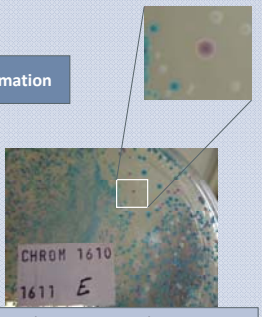
Sampling → 25g carrots/bovine meat Or 25 ml raw milk Or impregnated swab

Enrichment 37°C - 18H → 225ml BPW or mTSB + Acriflavine (raw milk)

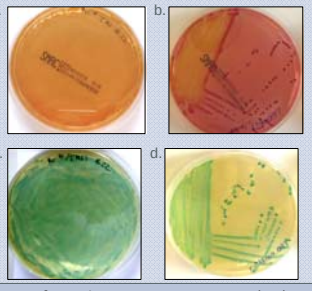
DNA Extraction

Detection *(Screening)*

Isolation /confirmation

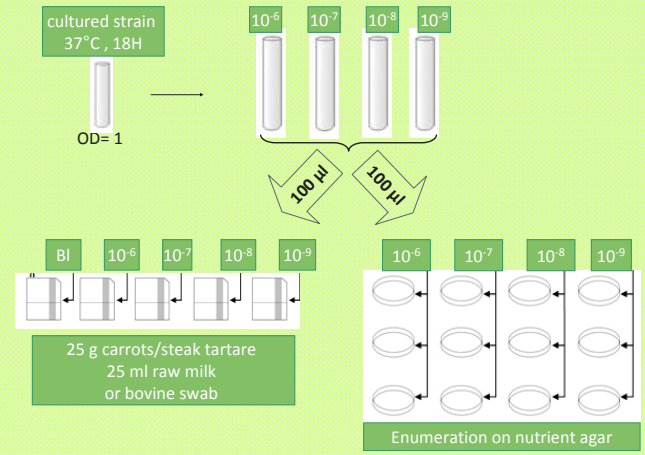



Characteristic colonies (pink/purple) on ChromagarSTEC®

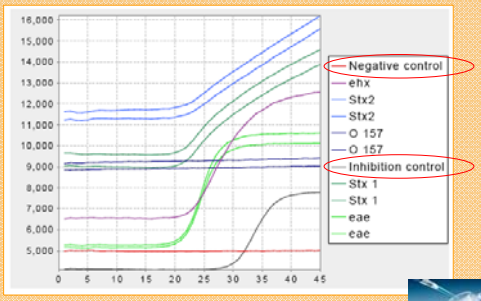



Isolation of *E. coli* O26 strain using IMS (a,c) or direct plating (b,d) on CT-SMAC (a,b) and TBX (c,d)

### Experimental set-up




### Screening result *E. coli* O26 *stx1 stx2 eae* on carrots




| Target | Ct | Results |
|---|---|---|
| eae | 22.955 | Presence |
| ehx | 24.451 | Presence |
| O 157 | 0.0 | Absence |
| Stx 1 | 22.709 | Presence |
| Stx2 | 23.466 | Presence |

Strains used in this study: *E. coli* O26:H11 (*eae stx1 stx2*), O103:H2 (*eae stx2*), O111:H8 (*eae stx1 stx2*), O145:H28 (*eae stx1*)

## RESULTS

| | Bovine meat bovine swabs | Carrots | Raw milk |
|---|---|---|---|
| LOD of the method | 1-54 cfu/25g or /swab | 1-6 cfu/ 25g | 1-2 cfu/25ml |
| Repeatability | 100% | 100% | 100% except *E. coli* O111 |
| Intra/Inter- Reproducibility | 100% | 100% | 100 % |
| LOD GeneDiscCycler | 1 cfu/PCR reaction | | |
| Matrix effect on qPCR | Absence | Absence | Inhibition (fat) |
| Effect of background flora on qPCR | Absence | Absence | Inhibition (lactic acid bacteria) |

**Matrix effect**

25g sample + *E. coli* O26 → Extraction → Analyse qPCR

**Effect background flora**

25g sample 37°C, 18H → *E. coli* O26 + → Extraction → Analyse qPCR

## CONCLUSION

- The LOD for both screening and isolation modules was between 1-40 cfu /25 grams (or 25 ml for raw milk) of matrix
- Levels starting from $4.10^4$ cfu/ml could be detected in the enriched sample using the GeneDiscCycler.
- The LOD of the GeneDiscCylcer was at 1 cfu/reaction
- Isolation of a strain is still a bottle-neck in the method. The use of IMS did not improve subsequent isolation.
- The repeatability, intra-and inter-reproducibility of the method were demonstrated

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