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Rapid screening of beef carcass swabs for enterohaemorrhagic E. coli (EHEC) by real time PCR

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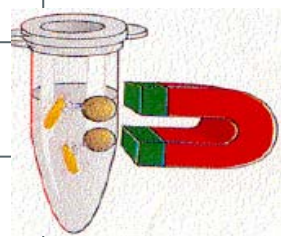
Introduction

- *E. coli* O157:H7 - haemolytic uremic syndrome (HUS) and bloody diarrhoea
- other serogroups (O26, O103, O111, and O145) become more important
- detection and isolation methods in food are available for *E. coli* O157:H7 strains (ISO 16654:2001)

Detection of *E. coli* O157:H7 (ISO 16654:2001)

Selective enrichment	Modified tryptone soy broth + novobiocin (mTSB+n) (6h and 24h at 41.5°C)
Immunomagnetic separation and concentration (IMS)	
Isolation	50 µl on CT-SMAC (24h at 37°C) 50 µl on 2nd medium of choice
Confirmation	Biochemical identification Agglutination with *E. coli* O157 antisera



Detection other VTEC O26, O103, O111, and O145

- No standard protocol

- differential medium for the isolation of non-O157 shigatoxigenic *E. coli* strains (Possé *et al*., 2008)

  → laborious and time consuming

- Alternative: multiplex real time PCR (Pall Gene systems, France)

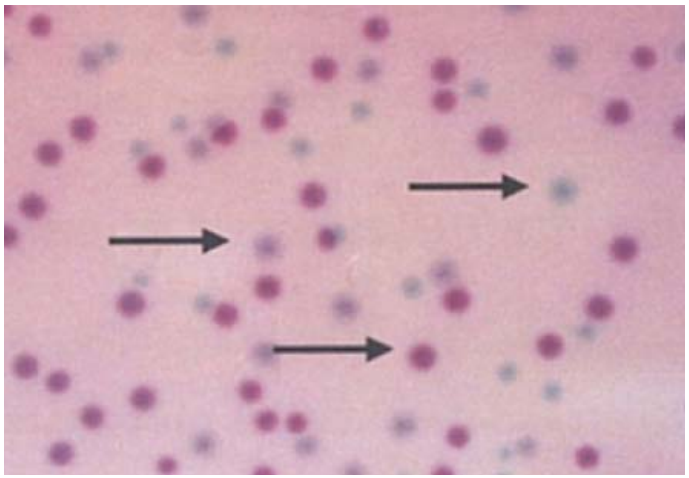
→ presence of the virulence factors (stx1/2, eae, ehx)

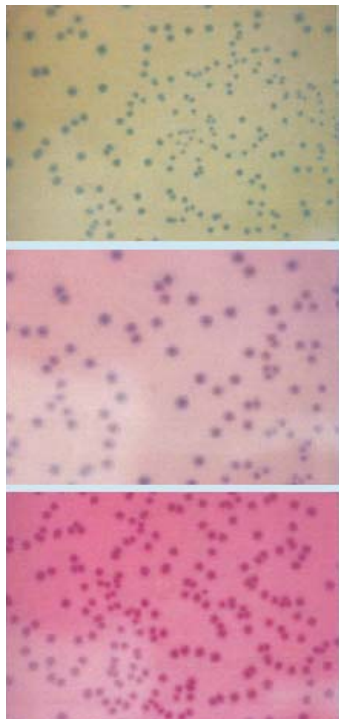
→ second screening: serotype specific gene amplification

→ If both positive: Isolation of the strain

The new selective media for STEC

(Possé et al. Letters Microbiol 2008; 282:124-31)





O145

O111 en O103

O26

Study beef carcass swabs

- 45 beef carcass swabs
- *E. coli* O157:H7 Vidas Eco/Vidas ICE (Biomerieux)
- Other EHEC: Gene Disc Cycler (Pall Gene systems)

DNA extraction

STEP I

Sample (25 g) + enrichment broth mTSB+n

enrichment (42°C for 6 and 24h)

Extraction of DNA

method 1

50 µl of enrichment in lysisbuffer (Gene Systems)

10 min waterbath at 90°C

method 2

1 ml enrichment broth

Centrifuge 5 min at 500g

Take 900 µl of the supernatant and transfer to lysis buffer

Centrifuge 5 min at 10 000g

Discard supernatant and dissolve pellet in 200 µl dilution buffer

10 min waterbath at 90°C

Enrichment in CT Mac Conkey 17-19h 37°C

Detection of E. coli O157 by VIDAS ECO

If + isolation and confirmation of *E. coli* O157

STEP II

molecular detection of the virulence genes, O157 and other *E. coli* serotypes

First screening presence of the virulence genes

MULTIPLEX PCR
stx 1 and stx 2
eae
Ehx
Internal control for inhibition

Second screening presence of different serotype specific genes

MULTIPLEX PCR
O145
O26
O111
O103
H7
Internal control for inhibition

Results

- 8 out of 45 samples EHEC
  - 6 O145 EHEC: stx1, stx2, eae, ehx
  - 1 O26 EHEC: stx1, ehx, eae
  - 1 O103 EHEC: stx1, ehx, eae
- 9 % inhibition using method 2 DNA extraction
- 24/45 no pathogenic combination

Conclusions

- GeneDisc system useful method first screening
- necessary to confirm PCR results
  isolate corresponding strain
- How?
  - Serotype specific IMS
  - Serotype specific enrichment
  - Or serotype differential isolation media (Possé et al. 2008)

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Thank you for your attention!

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