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Detection of VTEC in foodstuffs: state of play in ISO and CEN - non-O157 VTEC

Jeppe Boel

Danish Institute for Food and Veterinary Research

**1st Annual workshop of the National
Reference Laboratories for E. coli in the EU
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CEN TC275 WG6 and ISO TC 34 SC9 have undertaken work in order to develop a method for the detection of VTEC in food, feed and faeces

Method based on PCR approach

Stefano Morabito (Italy, CRL-*E. coli*) and Jeppe Boel (Denmark) are joint project leaders.



General considerations

- ISO/CEN methods are regarded as reference methods in EU and referred to as so in EU directives
- Currently no microbiological criteria for VTEC in EU
- VTEC is included in the "zoonosis directive"
- Standardized methods facilitates the comparison of data



VTEC method development

- No clear definition of “human pathogenic VTEC”
- Target organism: VTEC (*vtx* and *eae*)
- General approach: PCR based - In accordance with general ISO standards/technical specifications developed by TAG 3
- Challenge to develop an open PCR-based method because of patents, intellectual rights etc.



Method outline

1. Sample enriched in broth
2. Purification of DNA
3. PCR screening for *vtx* + *eae* (+ other markers)
4. Isolation of VTEC from PCR positive broths
5. Characterization of isolates



Enrichment

mTSB+novobiocin in normative text
(as in O157 standard - ISO 16654:2001)

For discussion:

Concentration of novobiocin
Feacal samples: BPW+novobiocin

Preparation of broth for PCR



PCR step

Numerous Primers/probes available from the literature targeting *vtx* (several *vtx* variants)

For discussion:

- Choice of primers/probe/(RT)-PCR-chemistry
 - Requirements for validation of the PCR assay
 - Requirements for controls
 - Method should be “open”
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- Other virulence genes/genetic markers (*eae*)
 - PCR based detection of O- and H-specific genes (e.g. O157, H7, O26, O103, O111, O145 etc.)



Isolation of VTEC

- Essential to obtain isolates: Possible to test broth culture for many genes - not possible to know whether genes are present on the same bacteria
- Positive broth cultures subcultured to solid selective and indicative isolation media (TBX, other media?)
- PCR test of up to 50 individual colonies (pools)
- It can be difficult to verify screening positive enrichment broths by cultivation – how many colonies should be investigated?
- Laborious approach.....



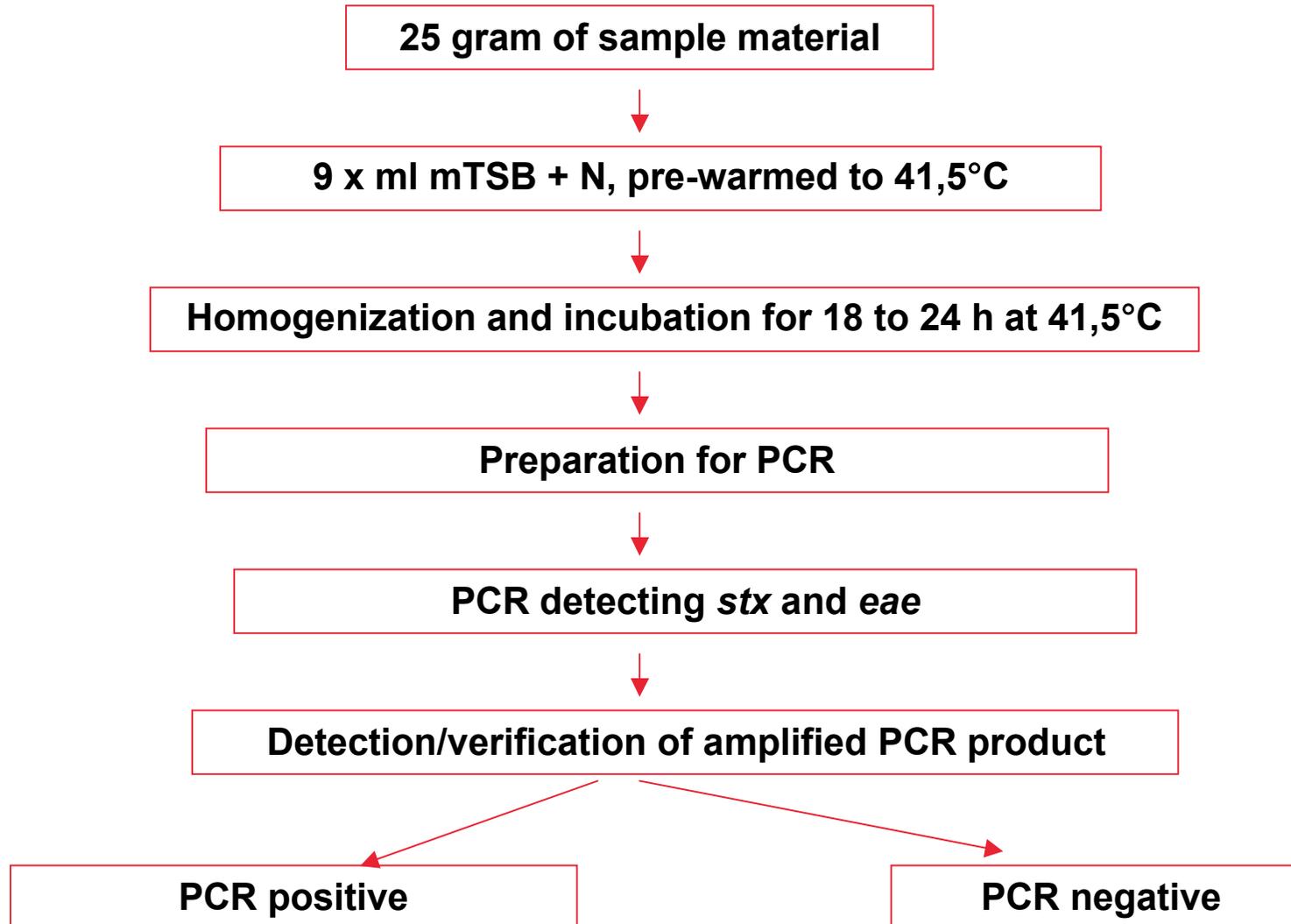
Characterization of VTEC

Routine laboratories/reference laboratories

- VT subtype
- Presence of other virulence genes
- O:H-typing (serological/DNA based)

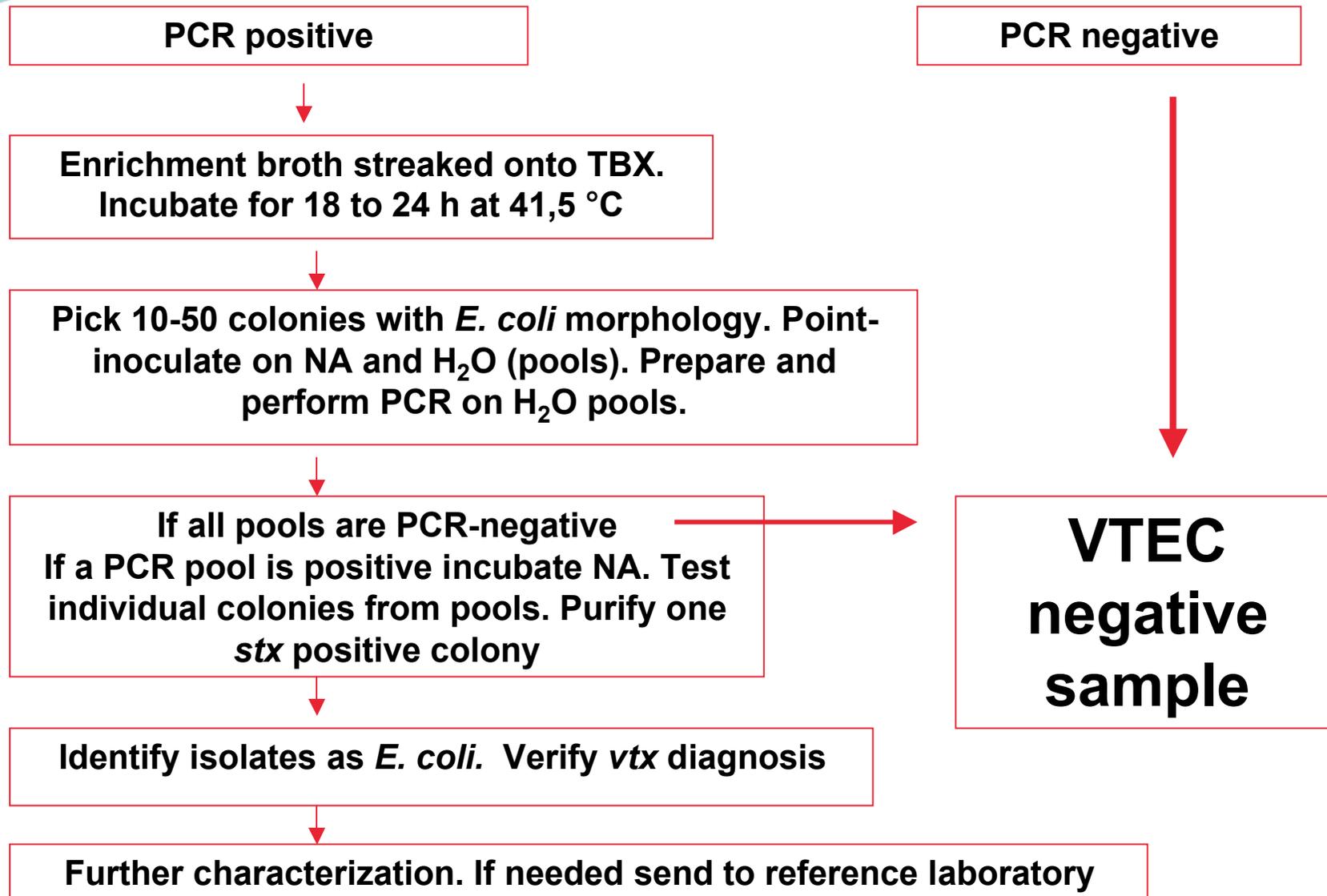


VTEC detection method (1) – PCR screening





VTEC detection method (2) isolation





Current status of the work

- There is a need for determination of the “ambition level” with regard to requirements for validation of method
- The development of a robust method will require validation work (= laboratory work) to support choice of:
 - Primers/probes (are relevant variants detected?)
 - PCR chemistry
- Method should be evaluated on different matrices
- New meeting will be scheduled (early 2007?)



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Thank you

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