Usefulness of *stx2* subtyping as an extension to ISO/TS 13136:2012 for detection of STEC

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Background

The work was initiated because the EU Commission was about to suggest a guidance on how to manage Shiga-toxin-producing *Escherichia coli* (STEC) findings in food, not including the most updated knowledge of *stx2* subtyping. The suggested ISO/TS 13136:2012 (ISO) method is long-lasting, and would in the first steps (which take 4-5 days), produce many false positive samples. Implementation of the guidance could hence be a practical, and economical challenge for the abattoirs and food processing industry as well, as at the retail level.

A review of the scientific literature indicated that presence of *stx2a* is the most significant disease marker. But *stx2d* has also been associated with Hemolytic uremic syndrome (HUS). *Stx2a* and *stx2c* are the most common subtypes in STEC O157, and have therefore been the main focus in our study.

Hence an extra PCR-step in the ISO method which can determine the *stx* subtypes - samples harbouring hazard genes will be identified at day one - and the further culturing and characterization of the disease properties of the bacteria, would be more targeted and faster.

Aim

Our aim is to investigate whether a nested PCR, including a step with *stx2* subtyping as an extension of the ISO method, performed better than the original ISO method. First step was to investigate the performance of primers and polymerase in regard to ability to detect *stx2* subtypes *stx2a, b, c, d, f and g)* in STEC O157.

Materials & Methods

Protocol:http://www.ssi.dk/~/media/Indhold/EN%20%20engelsk/Public%20Health/National%20Reference%20Laboratories/vtx%20detection%20%20subtyping%20protocolrev6final.ashx was used to analyze 16 *STEC* isolates O157 (8 from human and 8 from cattle), from which *stx2c* previously was isolated by Rolgaard et al. 2004

Results

Results from the 16 STEC O157 tested for the presence of *stx2a* and c subtypes are shown in figure a-c. Figure d shows control strains, as described by Scheutz et al. 2012

a

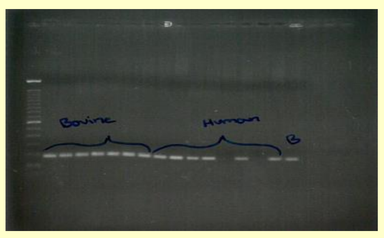

Figure a shows a gel picture of the presence of *stx2c* positive strains in the samples. The *stx2c* Bands ought to lie near 177 base pair (bp),. The gel picture clearly illustrates that 14 samples had Bands near 177 bp and two were negative (human isolates samples.)

b

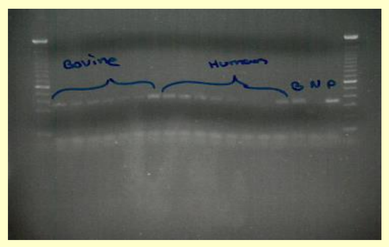

Figure b illustrates what seemed to be *stx2a*. The bands present were, however, very weak and localized around 349 bp, near the band for the positive controls. The negative control remained negative.

c

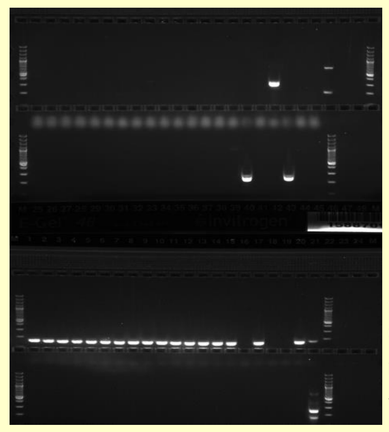

Figure c shows the results from analyses conducted at SSI. The STEC isolates were tested for *stx2a, b, c and d, but* only *stx2c* was present.

d

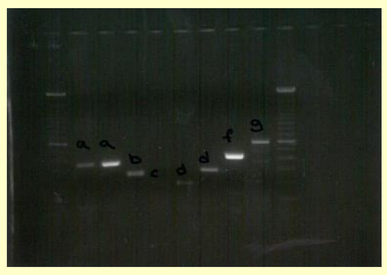

Figure d shows a gel picture of the positive controls – conducted to make sure they were stable.

Discussion

What seemed to be *stx2a* in the samples using polymerase GE Healthcare, Illustra puReTaq Ready-To-Go PCR Beads might well be *stx2c* as the position of *stx2a* and *stx2c is* very close to each other.

Because the *stx2a* bond was weak, it demanded further investigation and verification. Statens Serum Institut conducted hence an analysis using polymerase HotStarTaq, Qiagen that only gave a *stx2c* as result. This result could indicate that not all polymerase can be used. A second verification will be conducted.

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