Evaluation of PCR detection method for *E. coli* O157 in bovine faecal samples

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Enterohaemorrhagic *E. coli* (EHEC) serotype O157:H7 is implicated in causing severe intestinal and renal illness in humans. Although various methods have been used to test for *E. coli* O157:H7 in environmental samples, a specific, sensitive, economical and rapid method still needs to be established. Various PCR assays in the literature are using primers that target single, specific genes of *E. coli* O157:H7 (Wang *et al*., 2002; Paton *et al*. 1998). These genes are encoding the protein intimin (*eae*A), galactitol 1–phosphate dehydrogenase (*gat*D), H7 flagellar antigen (*flic*H7), O157 lipopolysaccharide (*rfb*E) and ß–glucuronidase (*uid*A).

In this study a series of experiments were conducted to combine these specific *E. coli* O157:H7 primers in a single multiplex PCR assay. The sensitivity of this multiplex PCR assay in the detection of *E. coli* O157:H7 was tested using seeded faecal samples and under various DNA extraction procedures. The $Flic_{H7}$, Rfb_{O157} and Uid primers that amplify specific genes of 625 bp, 497 bp and 252 bp, respectively, in *E. coli* O157:H7 were selected for the multiplex PCR. The multiplex PCR was able to detect *E. coli* O157:H7 in pure culture and in faecal samples spiked with various dilutions of *E. coli* O157:H7 (40 to 4 x 10^8 CFU/g faeces). Additionally, the multiplex PCR assay showed higher sensitivity to detect *E. coli* O157:H7 in faecal sample once spiked and enriched for 24 hours with 3.2 CFU/g and the DNA was extracted using the bead beating method (Figure 1). However, in a separate experiment involving four naturally colonized steers, the multiplex PCR showed little sensitivity in comparison to the Immunomagnetic separation technique (IMS, Dynal). This result is difficult to explain as the numbers of *E. coli* O157 in these steers were very low (<10CFU/g faeces). Consequently, the sensitivity of this multiplex PCR still needs to be further evaluated using a larger number of naturally colonized animals. Once validated, it may be used to replace the commonly used Immunomagnetic separation technique (IMS).

Paton, A. and Paton, J. (1998). Detection and characteristics of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1 and stx2, eaeA, Enterohemorrhagic *E. coli* hlyA, rfbO111, and rfb O157. *Journal of Clinical Microbiology* 36, 598–602.

Wang, G., Clark, C.G. and Rodgers, G. (2002). Detection in *Escherichia coli* of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR. *Journal of Clinical Microbiology* 40 (10), 3613–3619.

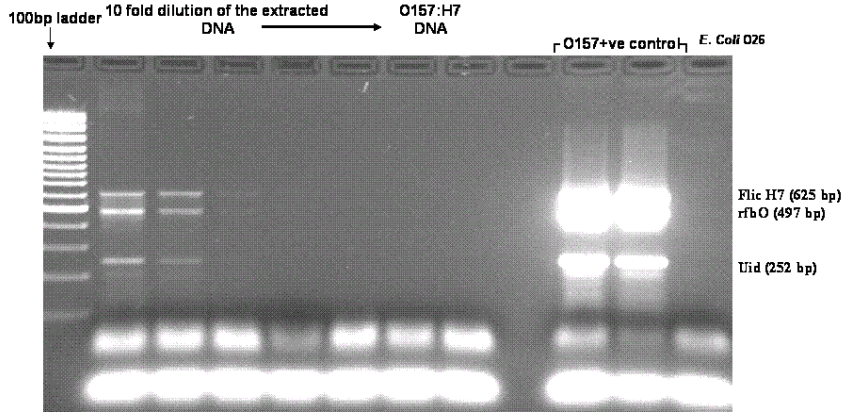

Figure 1 Detection of *E. coli* O157 in faecal sample spiked with 3.2 CFU/g and enriched over night. DNA was extracted using bead beating method.