Rapid and Specific Detection of STEC Strains O157:H7, O26 and O111 Using a Label-Free Biosensor System

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Introduction

Pathogens

- *Escherichia coli* O157:H7, a strain of enterohemorrhagic, Shiga toxin-producing *E. coli* (STEC), causes gastroenteritis, resulting in (bloody) diarrhea and sometimes acute kidney failure due to HUS (hemolytic-uremic syndrome).
- *E. coli* O26 and O111 are STEC strains, similar to O157:H7, and are emerging threats to human health.
- *Salmonella enterica* serovar Typhimurium causes gastroenteritis, resulting in diarrhea, fever and abdominal cramping.

Incidence

- Annually in the U.S. there are >100,000 cases of STEC and >1.4 million non-typhoidal *Salmonella* infections.

Problem

- Detection of foodborne pathogens is the primary means of preventing contaminated foodstuffs from entering the market.
- Traditional immunoassays are the gold standard for identifying specific strains of bacteria. However, while specific, these immunoassays are time-consuming.
- Most current biosensor applications focus on specificity or sensitivity rather than assay speed.

Materials and Methods

Antibodies

- Polyclonal antibodies against *E. coli* O157 (KPL Cat. # 01-95-90), *E. coli* O26 (KPL Cat. # 01-95-92), *E. coli* O111 (KPL Cat. # 01-95-91) and *Salmonella* species (KPL Cat. # 01-91-99) were used as capture antibodies.

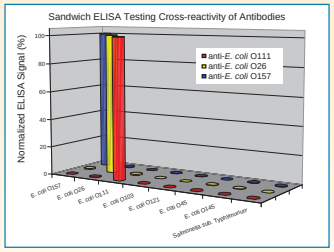

Figure 1: Antibody specificity
Sandwich ELISA showing minimal cross-reactivity between anti-*E. coli* O111, O26 and O157.

- Bacteria were serially diluted in EDTA buffer (10 mM phosphate, 150 mM NaCl, 10 mM EDTA).
- Bacteria enumeration was accomplished by plating replicates on TSB agar.
- Bacteria were disrupted by sonication and 1% Triton X-100 prior to pelleting; supernatants were assayed.
- Human serum was diluted 1:10 in EDTA buffer before use.
- Hamburger (25 g/225 mL buffer) was sonicated, pelleted and filter sterilized before use.

Aim

To develop methods using a commercially available biosensor in concert with anti-bacterial antibodies to expedite detection of foodborne pathogens.

Biosensor

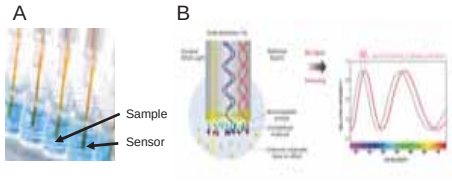

Figure 2: Biolayer Interferometry
(A) Fiber-optic biosensor tips (~1900 μm²) in solution. (B) Model depicting principle of biolayer interferometry.

- Biolayer interferometry (BLI) is a label-free immunoassay, in which the interference pattern of white light reflected from two surfaces, an internal reference layer and a layer of immobilized antigen on the tip surface, is analyzed.
- Automated dip-and-read technology permits detection of binding events within 100-300 seconds.
- The spectral shift measured by the instrument is proportional to the thickness of the antibody + antigen on the end of the probe.

Instrument

- Platform: ForteBio's Octet Red96.
- Biosensor: streptavidin functionalized.

Results – Intact Bacteria

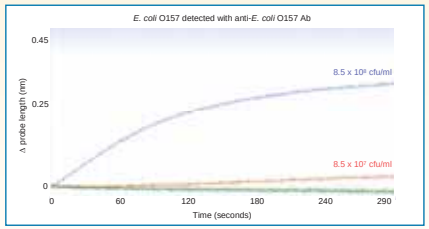

Figure 3: Detection limits for intact bacteria
Detection of serial dilutions of *E. coli* O157 with cognate biotinylated polyclonal antibodies. Rapid detection of *E. coli* O157 was obtained within 300 seconds.

Results – Disrupted Bacteria

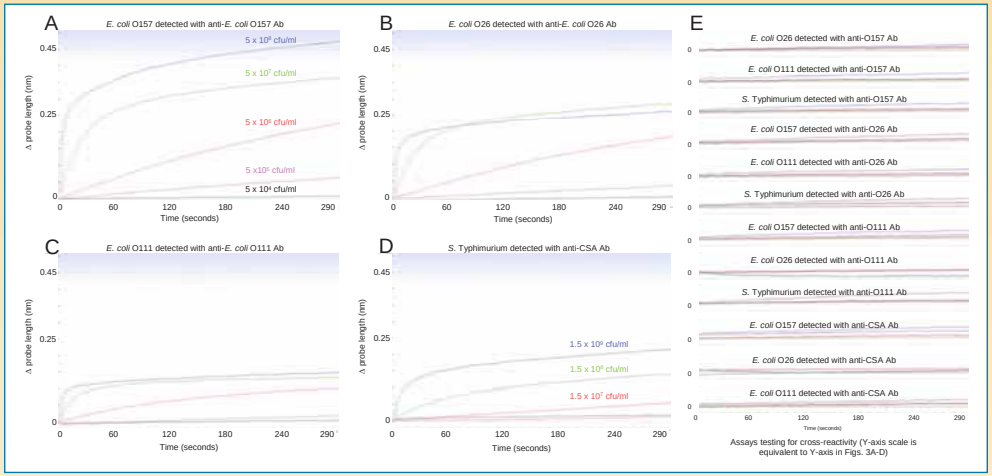

Figure 4: Detection limits for disrupted bacteria
(A-D) Detection of sonicated and detergent treated bacteria with cognate biotinylated polyclonal antibodies. (E) Spectrographic traces of antibody/bacteria interaction determining cross-reactivity. Scale on parts A-E are the same, with the limit on Fig. 3E set at 0.05 nm. For all *E. coli* strains: Blue = 5 x 10⁸ cfu/ml, Green = 5 x 10⁷ cfu/ml, Red = 5 x 10⁶ cfu/ml, Pink = 5 x10⁵ cfu/ml and Black = 5 x 10⁴ cfu/ml. For *Salmonella* Typhimurim experiments, Blue = 1.5 x 10⁸ cfu/ml, Green = 1.5 x 10⁸ cfu/ml, Red = 1.5 x 10⁷ cfu/ml, Pink = 1.5 x10⁶ cfu/ml and Black = 1.5 x 10⁵ cfu/ml

Results – Beef Extract

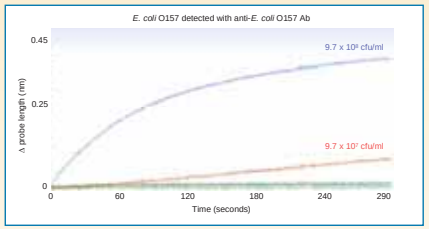

Figure 5: Detection limits of E. coli *O157* in hamburger extract
Macerated hamburger supernatant was diluted 1:10 in EDTA buffer. Detection of serial dilutions of *E. coli* O157 with cognate biotinylated polyclonal antibodies.

Results – Human Serum

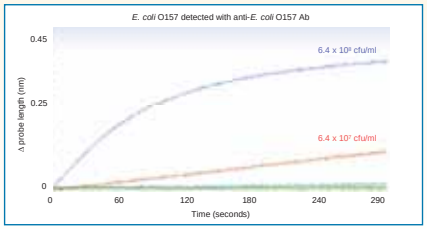

Figure 6: Detection limits of E. coli *O157* in human serum
Human serum was diluted 1:10 in EDTA buffer. Detection of serial dilutions of *E. coli* O157 with cognate biotinylated polyclonal antibodies.

Model

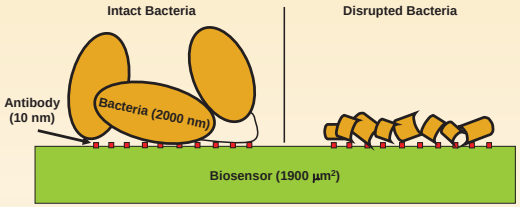

Figure 7: BLI Biosensor Model System
The BLI biosensor is dependent on efficient packing for transmission of light. Small molecules bound to antibodies form a densely packed surface and are generally straightforward to detect. Intact bacteria, however, have asymmetrical densities, and almost certainly attach with a non-uniform packing order presenting challenges to detection without processing.

Summary

- Detection of bacterial pathogens has been demonstrated in multiple matrices, for the first time, using the commercially available Octet Red96 biosensor from ForteBio.
- Biolayer interferometry, in combination with KPL's polyclonal BacTrace® antibodies, is a viable method for rapid, specific detection of various bacteria strains, including the pathogens of *E. coli* O157, *E. coli* O26, *E. coli* O111 and *Salmonella* Typhimurium.
- Using BLI, as few as 5 x 10^5 CFU/mL of bacteria can be detected in less than four hours (total assay time).
- Time consuming enrichment steps were avoided.
- Processing of the samples, in order to make a more uniform layer on the biosensor, was needed for higher levels of sensitivity.