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A Combined Immunological-PCR Biosensor for Detection of Water-Borne *E. coli* O157:H7

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Enterohemorrhagic *E. coli* (e.g., *E. coli* O157:H7) has emerged as a serious problem in developed countries. Symptoms include bloody diarrhea and kidney failure, which can be fatal. Enterohemorrhagic *E. coli* strains may be candidates for bioterrorism agents because of their virulence and the very small infectious dose. Epidemiological data suggests that consumption of relatively few cells (ca. 10) can result in infection. Traditional methods for detection of *E. coli* O157:H7, which rely on enrichment, plating on selective media, and identification via biochemical/serological testing, are time consuming and labor intensive. Recently, faster immunological- and PCR-based methods have been developed. However, the limit of detection for both methodologies is approximately 100 cells/mL, which is inadequate. Consequently, these methods must be combined with concentration or enrichment prior to detection. In addition, neither immunological or PCR assays alone are definitive for enterohemorrhagic *E. coli*. We describe here a biosensor which combines immunological and PCR techniques. Anti-O157 antibodies attached to the inner surface of capillary tubes allow for cell capture from a flowing stream of water (i.e., concentration). Subsequently, tubes are incubated with a second antibody conjugated with Cy-5 (sandwich assay), allowing for detection via the Integrating Waveguide Biosensor (Ligler *et al.*, *Anal. Chem.* 74:713-719, 2002). Alternatively, capillary tubes can be filled with enrichment medium and incubated, resulting in growth of the captured viable cells within the tube. Tubes can then be analyzed via the Integrating Waveguide Biosensor, or cells lysed in the tube followed by real-time PCR analysis. Data will be presented demonstrating each of the assay components. Experiments are in progress to optimize each component and to integrate the components into a single system.

Key words: *E. coli* O157:H7, integrated waveguide biosensor, real-time PCR