New quantitative detection of pathogens in heterogeneous environmental samples

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Quantum dots and magnetic beads based genomic assay (NanoGene assay) has been developed for sensitive and inhibition resistant gene quantification to achieve in-situ bacteria monitoring in environmental samples. In this study, eaeA gene of pathogenic E. coli O157:H7 was quantified. The result demonstrated the excellent sensitivity (i.e., limit of detection: 87 gene copies for dsDNA and 890 zeptomolar for ssDNA) in the presence of nonspecific microbial populations (Kim et al., 2010; 2011a). The feasibility of the developed gene quantification for non-laboratory environment usage (in-situ use) was investigated. Therefore, DNA hybridization was achieved at ambient temperature and minimum agitation, and the analysis was completed within hours. Most importantly, the NanoGene assay demonstrated the resistance to the presence of naturally occurring inhibitors (humic acids, cations) and residual reagents (surfactants, alcohols) from DNA extraction (Kim et al., 2011b). The assay was also applied to humic acids laden soils (7 types of soils with various amount of organic matters) and successfully quantified 105 to 108 CFU of E. coli O157:H7 per gram soil (R^2 = 0.99). The results indicate that the presented NanoGene assay is suitable for further development as an in-situ bacteria monitoring method for working with heterogeneous environmental samples (Wang et al., 2013). Another aspect of the method is to transform the NanoGene assay into a portable device that can be used as a pathogenic bacteria detector in environment. The project consisted of the first inline fluidic components development and characterization as well as the first integration effort on a briefcase platform for the in-situ pathogen detection system (IPDS) (Mitchell et al., 2014). Our long term vision is to further miniaturize the briefcase platform implementation of the IPDS and to commercialize the handheld version of the IPDS.