Abstract  We have demonstrated a simple route for the detection of *Escherichia coli* using well plates with quantum dot (QD) fluorescent labels. The genetically engineered *E. coli* strain DH5α, containing green fluorescent protein (GFP), was used as the model system. Two approaches were employed in the detection of *E. coli*. In the first method, *E. coli* were specifically adhered onto a streptavidin-coated 96 well plate using biotin-labeled antibodies. In the second approach, *E. coli* were nonspecifically adsorbed onto the surfaces of 96 well plates without the use of antibodies. Whereas the fluorescence signal from GFP was not correlated with the number of *E. coli* cells, a linear titer dependence was found from the QD-labeled *E. coli*. The linear dependence of the antibody-immobilized *E. coli* persisted up to high (> $10^6$ cfu/mL) concentrations of *E. coli*. However, an exponential increase in the fluorescence intensity was found due to nonspecifically bound *E. coli*, and the signal intensity was much higher than that of antibody-immobilized *E. coli*.

Keywords: *Escherichia coli*, Detection, Quantum dots, Antibody, Well plate, Fluorescence

Introduction

Detection of microorganisms, such as *Escherichia coli* (*E. coli*) has been a popular quest in the field of sensor development. Some strains of *E. coli* are deadly, and *E. coli* may be used as an indicator for food, water, and environmental contamination\(^1\). The most common standard methods used to detect *E. coli* are the most probable number method (MPN) and the membrane filtration technique, both of which are labor-intensive and time-consuming\(^2\). Chromogenic enzyme-substrate tests are good substitutes for conventional methods because the detection time is much shorter and ensures high sensitivity. However, even these tests require an incubation time of 18 to 24 h for detection, which is not sufficiently short for point of care food and water screening\(^3\). Nanotechnology-related detection schemes are expected to offer high sensitivity and fast detection times, and several platforms based on nanotechnology have been recently demonstrated. For example, So *et al.* and Zelada-Guillén *et al.* reported the electronic and electrochemical detection of *E. coli* using aptamer-functionalized single-walled carbon nanotubes\(^4,5\). Sugar-coated magnetic nanoparticles\(^6\) or magnetic nanoparticles combined with bioluminescence\(^7\) proved to be effective sensors as well. Semiconductor quantum dots (QD), which are widely used for extra or intracellular imaging purposes, may substitute for organic dye molecules in fluorescence-based bacteria sensing applications. Unlike organic dye molecules, QDs offer high stability and durability in addition to a high sensitivity. Moreover, because emission spectra of QDs are narrow and may be tuned by varying the QD size, simultaneous detection of multiple analytes is possible\(^8,11\).

In the present work, we synthesized CdS shell-capped CdTe QDs and investigated the sensing characteristics of QD-based *E. coli* detection. Whereas sandwich assays using QDs have shown a linear response up to $1 \times 10^6$ cfu/mL *E. coli*, the simple nonspecific
adsorption-based method described here offers a higher signal-to-noise ratio than the sandwich assay.

**Results and Discussion**

Scheme 1 shows a schematic diagram of the experimental protocol. The first approach employed a sandwich assay in which *E. coli* were adsorbed specifically to a streptavidin-coated well plate. The second approach used nonspecific adsorption of *E. coli* onto the well plates.

**QD-based sandwich assay**

Biotinylated anti-*E. coli* antibodies were immobilized on the streptavidin-coated well plate. The concentration of antibodies in the experiment was crucial; a linear concentration dependence was observed using antibodies diluted 1/100, but a dilution of 1/1,000 did not show a concentration dependence. An *E. coli* solution (DH5α, GFP expressed) of known concentration was incubated on the well plate for an hour, and thorough washing steps were performed to prevent false positive signals. Experimental details are described in the Materials and Methods section. Two types of QD were used for this experiment, designated QD1 and QD2; QD2 (38.8%) showed a higher quantum efficiency than QD1 (18.2%). Figure 1 shows a comparison of the measured fluorescence intensity as a function of titer of *E. coli*.

As shown in Figure 1(a), both QD1 and QD2 showed a linear relationship between the logarithmic concentration of *E. coli* and the fluorescence intensity, and QD2, which had the higher quantum efficiency, showed a...