Electronic Supplementary Information

(Manuscript Number: AY-ART-06-2013-041062)

Manuscript title : Detection of pathogenic Salmonella with nano-biosensor

Conjugation of antibodies to QDs

QD labeling was performed by 3 different methods as shown in Fig. S1 (ESI). Method 1 used stepwise immune-binding reactions similar to that described by Gazouli.²⁴ Cell separation, binding of biotinylated anti-*Salmonella* antibodies, and streptavidin-functionalized QD labeling were performed sequentially. In method 2, biotinylated anti-*Salmonella* antibodies were conjugated to streptavidin-functionalized QDs in parallel with a cell separation step. Biotinylated anti-*Salmonella* antibodies (100 μ L) were incubated with 100 μ L streptavidin-functionalized QDs at room temperature for 30 min with an RKVSD mixer (IKA, Wilmington, NC, USA) at 10 rpm. Method 3 used the Qdot antibody conjugation kit with which antibodies were coupled directly to a QD surface. QD-antibody conjugation was performed according to the manufacturer's instructions.

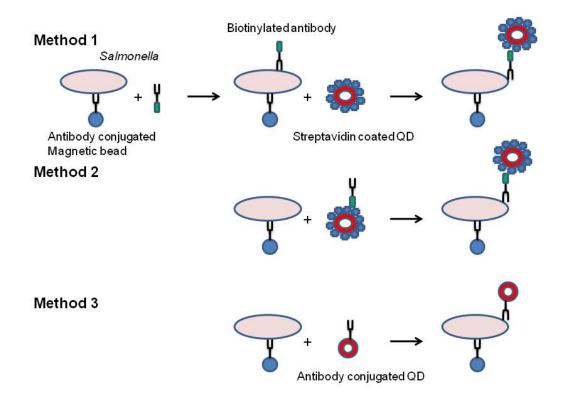
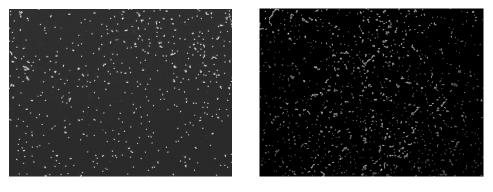


Fig. S1 Schematics of three different quantum dot conjugation methods.

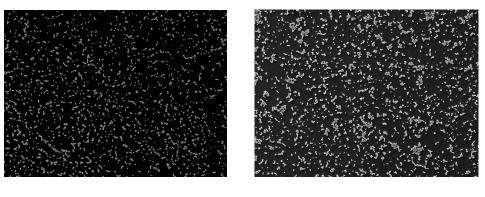
Verification of the detection procedure

To validate the immunomagnetic separation, a solution containing anti-*Salmonella* antibody functionalized magnetic beads were applied to the fixed cells and incubated for 30 min at room temperature. The slide was rinsed with PBS and distilled water, then air-dried and examined by scanning electron microscopy. As shown in Fig. S2, the number of attached magnetic beads increased with increasing numbers of cells fixed to the glass slide. SEM images ($200\times$) were taken with a JEOL JSM-5500 operated at 20 kV, at a working distance of 17 mm.



(a)

(b)



(c)

(d)

Fig. S2 SEM images (200×) of the magnetic beads attached onto the *S. typhimurium* cells covered slide glasses: (a) 10^5 CFU/mL; (b) 10^6 CFU/mL; (c) 10^7 CFU/mL; (d) 10^8 CFU/mL.

Calibration of the custom built fluorometer

Before the detection experiments, the custom-built fluorometer was calibrated with the QDs. The results showed that the fluorometer has good linearity and enough dynamic range to measure fluorescence signals of QDs over a concentration range of 0.2 pM to 2 nM (Fig. S3). The standard curve showed linear relationships between the concentrations (C) and fluorescence intensity (FI) of QDs in solution. The regression model can be expressed as: FI = 433617C + 945.42 with R² = 0.9998.

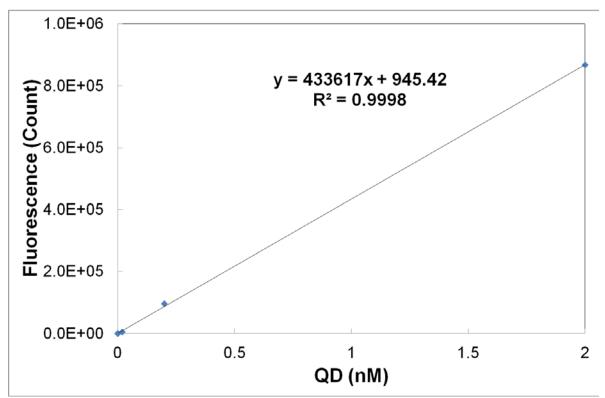


Fig. S3 The calibration curve of fluorescence intensity measured with the custom built fluorometer and its corresponding QD concentration.