Electronic Supplementary Material (ESI) for Journal of Environmental Monitoring
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Supporting Information

Development of electrochemical based sandwich enzyme linked immuno sensor for *Cryptosporidium parvum* detection in drinking water

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Fig.S1

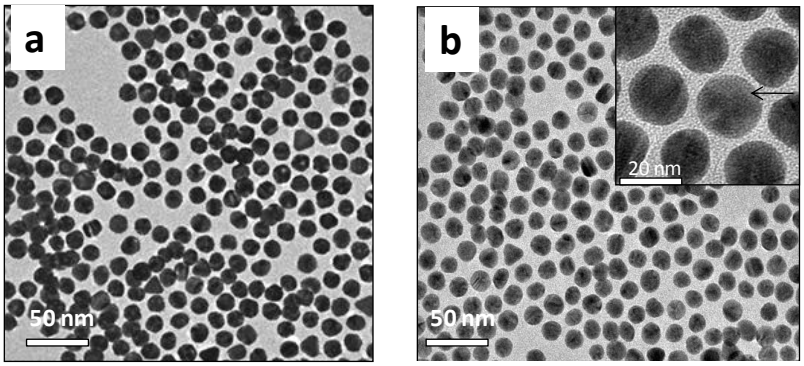

Fig. S1. HRTEM images of gold nanoparticles (A) and dual labeled (Ab and ALP) gold nanoparticles (B).

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Fig. S2

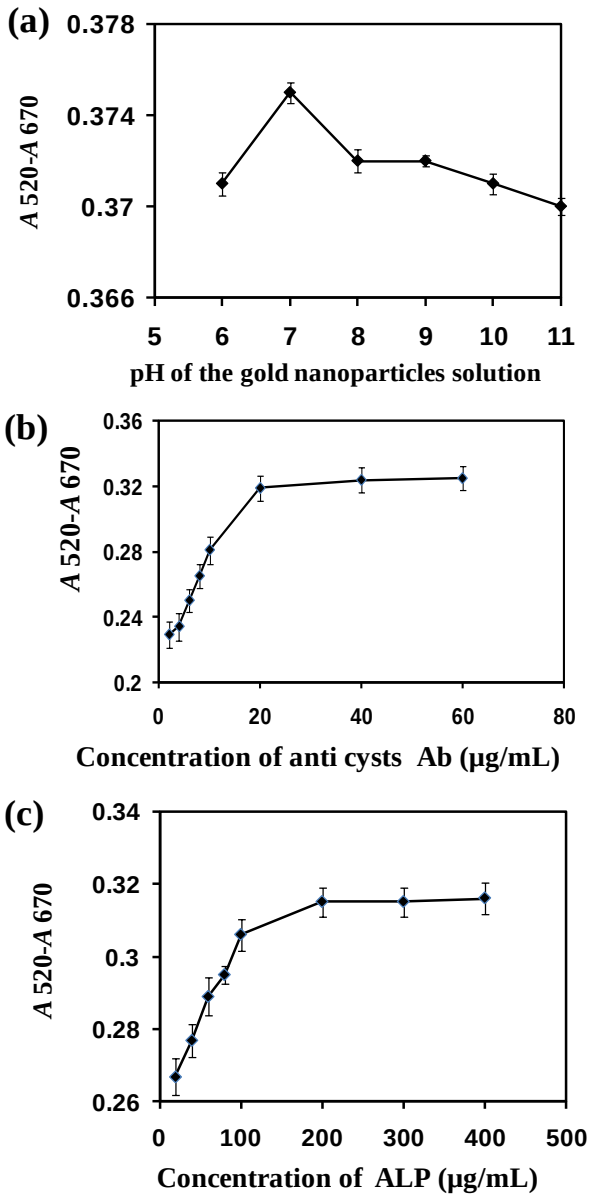

Fig. S2. Optimization of assay condition for the preparation of dual labeled gold nanoparticle probe (a) pH optimization, quantification of antibody (b) and ALP (c) concentrations needed to saturate the AuNP. The presented values are an average of triplicates ±SD (n=3).

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Fig. S3

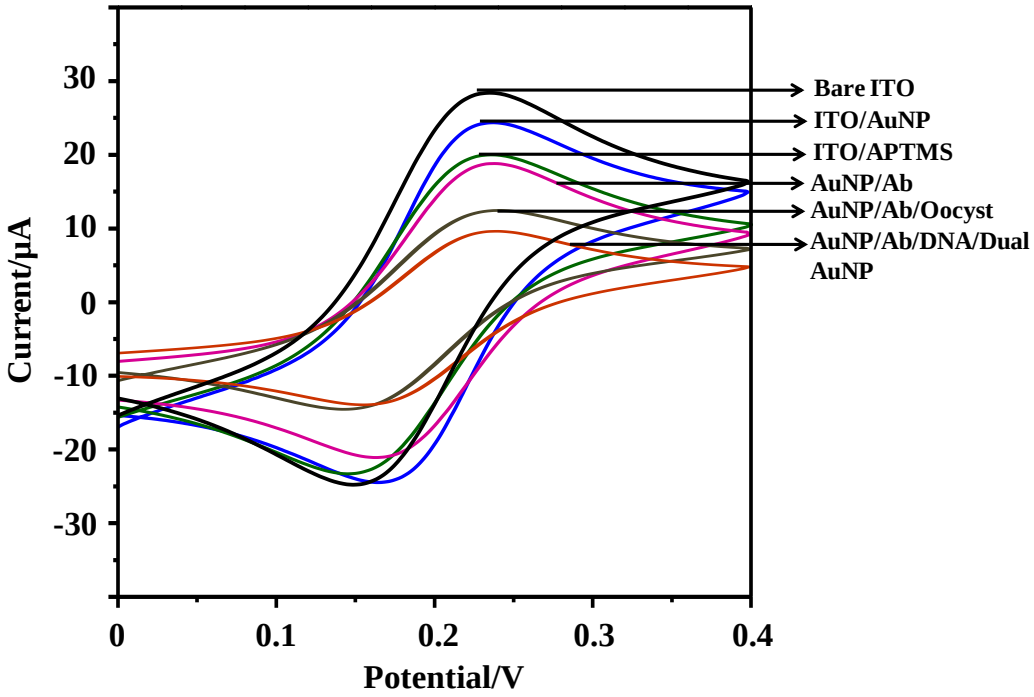

Fig.S3. Cyclic voltammetry of the ITO electrode in the presence of 2 mM Fe(CN)6 3−/Fe(CN)^{6} and 0.1 M KCl. Sequential modifications of ITO electrode–Bare ITO electrode, APTMS, AuNP immobilized ITO, antibody, oocysts immobilized (10 cells /mL) and after treated with dual labeled AuNP; Scan rate 50mVs^{-1}.

Cyclic voltammetry (CV) analysis

The sequential modification of ITO electrode such APTMS, GNP, anti–cysts Ab, oocyst and dual labeled AuNPs were characterized by cyclic voltammogram in PBS containing 2 mM $K_3[Fe(CN)_6]$. The CV responses of $Fe(CN)_6^{3-/4-}$ at bare ITO electrode, APTMS, AuNP, anti–cyst antibody, oocysts and dual labeled gold probe immobilized electrodes were shown in the Fig S3.

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The $Fe(CN)_6^{3-/4-}$ shows a reversible one–electron redox peaks in bare ITO electrode with a peak to peak separation ($\Delta E_p = E_{PA} - E_{PC}$) of 79 mV at a scan rate (v) 50mVs^{-1}. After the self assembly of APTMS on the electrode surface shows ΔE_P of 92 mV. This may due to the presence APTMS on ITO electrode, which reduces the electron transfer rate of redox couple in the PBS solution. Upon immobilization of AuNPs on APTMS/ ITO increases the surface area of the electrode and elevates the electron transfer rate of $Fe(CN)_6^{4-/3-}$, which retained closely to obtained at the bare ITO electrode. It was also explained by the amine terminated SAM was repelled the cationic $Fe(CN)_6^{4-/3-}$ redox active species, after immobilization of negatively charged gold nanoparticles the resonant electron transfer was increased. It may be fact that the electrons rapidly tunneling between one layer of nanoparticles to the next with the charging of the outer layer of nanoparticles in contact with the redox species in solution.[1] This indicates that AuNP successfully immobilized and facilitate required conduction on the electrode surface. Furthermore upon immobilization of anti–oocysts antibody the current response of the electrode was decreased. Subsequently, significant decreases in current response noticed after the immobilization of oocysts and dual labeled AuNP on the electrode surface. This noteworthy changes in the CV response of AuNP/Ab/oocysts/Dual AuNP ITO electrode denoted that an efficient electrostatic and antibody/antigen interaction occurred on the SAM modified ITO electrode surface.

1. J. B. Shein, L. M. H. Lai, P. K. Eggers, M. N. Paddon Row, and J. J. Gooding, *Langmuir.*, 2009, **25** (18), 11121–11128