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# Development of an immunoblot assay for carcinoembryonic antigen (CEA) in human serum using a portable UV illuminator†

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A rapid quantum dot (QD) based immunoblot assay is developed to detect CEA in human serum using a portable UV illuminator. Here, photostable QD-conjugated antibodies were utilized that bind CEA in human serum, previously blotted on a nitrocellulose membrane for point-of-care applications.

Immuno-chromatographic technologies (NCs) have driven a new era in clinical bioassay in the last decade. As an outcome of highly complementary stereochemistry, electrostatic interactions, hydrogen bonding, van der Waals forces and the integrative action of hydrophobic regions between antigens and antibodies, immunoassays have higher selectivity and sensitivity than any kind of physical and chemical analysis alone can achieve in the field of biological analysis, and thus have become critical in modern life science research.[1] A series of immunoassays have emerged in the last decade for protein detection, such as enzyme-linked immunosorbent assay,[2] fluorescence immunoassay[3] and chemiluminescence immunoassay.[4] Although they have the advantages of excellent specificity, high sensitivity and quantification, there are still some inconveniences and shortcomings. They are highly expensive, tedious, time-consuming and involve complex operation procedures. NC strips appeared in the early 1980s that serve as a detection platform which combines immuno-labeling with chromatography. It is fast, intuitive, user-friendly and inexpensive and can truly realize point-of-care applications.[5] Detection systems relying on optical signal transduction are advantageous because they are sensitive, fast and have the potential for multiplexed detection of desired analytes.

Semiconductor QDs have attracted great attention during the last decade and are regarded as promising fluorescent probes due to their unique optical properties.[6] QDs can serve as an ideal reporter for fluorescence-based assays with NC that utilize NCMs. Currently, QD-based NCs are at an early stage and few studies have reported on their potential applications. For example, a QD-based NC test is developed to detect a protein marker nitrated ceruloplasmin and for the first time quantitative analysis of protein was made based on the fluorescence intensity of QDs.[7] Such assays are imperative for developing inexpensive tools for rapid diagnosis. Therefore, development of low-cost methods needs to be investigated to prevent frequent hospital visits that are not only expensive but also time consuming. One of the greatest advantages of NC based diagnosis tools is that they do not require sophisticated equipment and long assay time, which are most desirable for point-of-care applications.

CEA is a widely used tumor marker for diagnostic and therapeutic purposes in gastrointestinal, breast and lung cancers.[8] The elevated CEA level in serum beyond the normal 2.5–5 ng $mL^{-1}$ range is an indication of possible risk of disease.[9,10] A number of microfluidics based detection methods for CEA detection have been developed but they are still not suitable for field diagnosis, *i.e.*, point-of-care diagnosis, in lower resource settings.[11] The existing methods for CEA detection still use complex detection methods and require expensive external equipment. Therefore, a cost-effective and simple method for detection of CEA with high sensitivity and specificity at the clinical level is crucial for point-of-care and early detection.

Here, we developed a one-step QD-based immunoblot assay for rapid and visual qualitative detection of CEA in human serum samples by taking advantage of the bright fluorescent QD-conjugated antibodies. We utilized a low-cost counterfeit currency detector as a hand-held UV illumination device that is available locally at a cost of ~$30. The detection principle involves (i) immunoblotting of serum containing CEA, (ii) capturing of blotted CEA with QDs-Mab conjugates on NCMs and (iii) direct visualization of results by illuminating the NCMs using a hand-held UV device (Scheme 1). The fluorescence

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intensity corresponding to the CEA concentration was rapidly assessed for the risk of cancer disease which is most desirable in point-of-care applications and the details are described in the following sections.

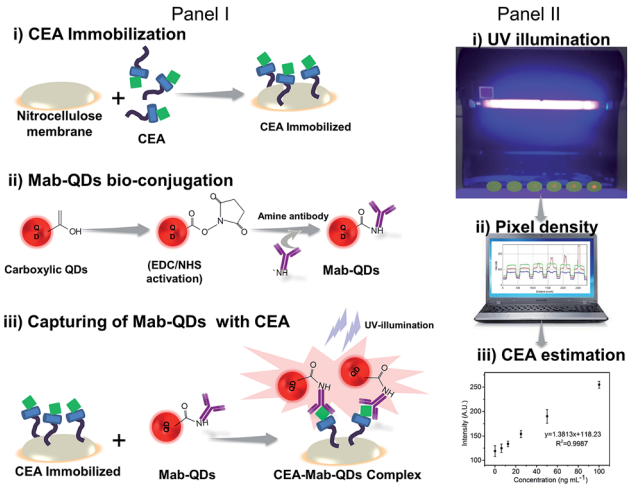
QDs were first conjugated with anti-CEA monoclonal antibodies as described in the ESI† and characterized by their emission spectra. Free QDs had an emission at 625 nm which shifted to a longer wavelength of 635 nm in QD-Mab (Fig. S1†). QDs can show effective Stokes shift in their fluorescence spectra as a result of bioconjugation due to the changes in their overall structure and spatial proximity of biomolecules.[12] However, QD-Mab antibodies retained ~95% of the original fluorescence intensity required for their visualization after excitation making the QDs conjugated CEA-antibodies qualify for the immunoblot assay. The QDs conjugated CEA-antibodies had a shelf-life of two months at 4 °C without losing their sensitivity and stability. This indicated that the QD-labeled antibodies could be stored for at least 2 months at 4 °C.

The specific affinity of QD conjugated CEA-antibodies binding with CEA on NCMs was tested using a series of QD concentrations and CEA-antibody combinations. The fluorescence intensities with respect to the concentrations of CEA-antibodies were determined by pixel densities after blotting on NCMs (Table S1†). This fluorescence intensity on NCMs was utilized to determine the concentrations of spiked CEA in the serum samples.

The developed assay was first tested for the qualitative detection of the CEA by direct visual observation after excitation with a portable UV-illuminator. Therefore, the color/fluorescence intensity of the test spots on NCMs required being high enough to visualize and distinguish the observable color/fluorescence intensity on NCMs with respect to blanks. The concentration of QDs-antibody conjugates was determined to be the key factor for the immunoblot assay. Therefore, an optimal immunoreagent concentration (QD-labeled anti-CEA antibody conjugates) was determined that exhibited a clear visible immunofluorescence on NCMs for test samples. First, a PBS/serum containing a series of CEA concentrations (6.25–100 ng mL$^{-1}$) were blotted on NCMs (2 μL per spot). A series of different concentrations of QD-labeled anti-CEA antibody were used for the range of CEA tested and the optimized concentration for the range of CEA concentrations tested was determined to be 0.25 μg mL$^{-1}$ that yielded a distinguishable color/fluorescence for visualization. The intensity of the color was found to increase with time and the optimum incubation time was found to be 10–20 min. With all the above optimal parameters, it was observed that there was a detectable difference in the intensity of color development on NCMs. A maximum intensity of color was observed in the case where there was maximum CEA present in the sample and maximum QDs-antibody conjugate bound on the NCMs. The color/fluorescence intensity with CEA concentration (6.25–100 ng mL$^{-1}$) determined the levels of available CEA molecules captured with QD-labeled anti-CEA antibody conjugates (Table S1†).

Human serum samples spiked with a series of CEA concentrations were utilized for the detection using the optimized conditions for the immunoblot assay. Similar to the standard assay for CEA detection using NCMs, a concentration dependent (6.25–100 ng mL$^{-1}$) color/fluorescence signal was clearly observed after the binding of QD-anti-CEA conjugates with CEA present in the blotted serum samples. A lower CEA concentration of 6.25 ng mL$^{-1}$ on NCM initially showed a small change in the color/fluorescence intensity as compared with the control. The minimum detectable color intensity that was visible under the UV-illumination was therefore 6.25 ng mL$^{-1}$ of CEA, which served as the lowest detectable trace amount in the serum sample (Fig. 1a). Quantitative fluorescence estimations with expensive laboratory based fluorescence detections can provide vast detection ranges but are expensive.[13] Therefore, the detection limit of the developed immunoblot assay was comparable to the laboratory based detection range of 6.25 to 100 ng mL$^{-1}$ CEA in serum samples.

The NCMs were exposed to a hand-held UV detector for the direct visualization of color in the images and the pixel intensity was determined from the developed fluorescence spot




Scheme 1 Schematic illustration of CEA detection using the QDs based immunoassay. Panel I: (i) coupling and immobilization of CEA on the NC platform, (ii) QDs labelling of antibodies using the surface chemistry of EDC/NHS, and (iii) capturing of CEA with Mab-QDs. Panel II: (i) the CEA-QDs-Mab complex was illuminated under UV-illumination using a hand-held portable device, (ii) processing the pixel density of different concentrations of CEA dots using ImageJ and (iii) CEA estimation.

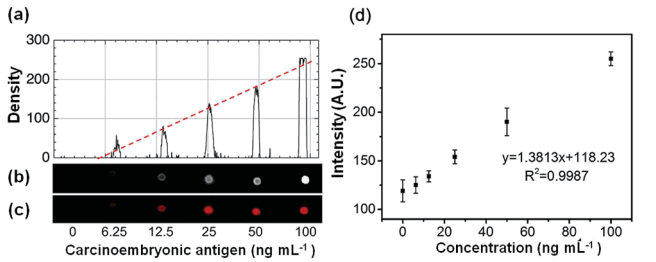



Fig. 1 Pixel density/intensity profiles of CEA-QDs-Mab complex. (a) Pixel density generated after CEA and QDs-Mab interactions showing a detection range from 6.25 to 100 ng mL$^{-1}$, (b) processed images of CEA-QDs-Mab complex on NCs, (c) raw image illuminated using a hand-held portable UV-illuminator (385 nm) and (d) fluorescence intensity plot against different CEA concentrations.

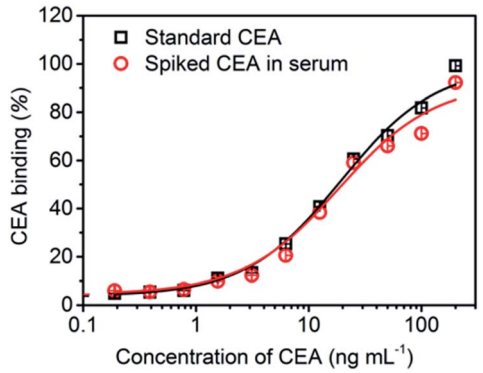



Fig. 2 Percent CEA bound on NCMs blotted with standard CEA and spiked CEA human serum at varying concentrations ($n = 3$) indicated on the $x$-axis.

(Fig. 1a–c). The intensities of each spot were plotted against CEA concentration that showed a minimum detection limit of 6.25 ng $mL^{-1}$ (Fig. 1d). The immunoblotting results were validated by the standard ELISA method using the same standard and spiked samples. Although the ELISA results exhibited a more sensitive detection range (3.12–100 ng $mL^{-1}$) it was comparable with the developed immunoblot assays (Fig. 2).

Several reported immunoassay methods on CEA detection have been documented with high sensitivity and specificity. These include colorimetry,[12] electrochemical and surface enhanced Raman scattering,[14,15] chemiluminescence, dynamic light scattering (DLS)[16] and opto-plasmonic nanosensors.[17] Although these methods provide sensitive detection limits they require sophisticated instruments. These methods not only require trained personnel to operate but also utilize expensive reagents and are not suitable for point-of-care applications. The present one-step dot blot immunoassay method overcomes all the above shortfalls through utilizing a QD-conjugated antibody fluorophore and a hand-held illuminator that is most desired especially for screening of samples near the bed-side of a patient.

## Conclusions

We have successfully developed a QD-based immunoblot assay that utilizes a simple low cost portable UV-illuminator for rapid and sensitive biomonitoring of CEA in dilute human serum. The developed immunoblot assay is designed to visually detect the elevated levels of CEA in serum for a low cost point-of-care diagnosis. Our results demonstrated that the fluorescence two-step immunoblot assay required blotting dilute serum samples followed by incubation with QDs-conjugated CEA-antibodies to rapidly and visually detect CEA using a portable UV-detector. The method developed provides preliminary information on elevated CEA levels in serum which enables determination of the potential cancer risk in suspected samples. This technique possesses several advantages including its speed, simplicity, specificity and cost effectiveness that are most desirable for point-of-care diagnosis. The method reported in this study was designed to detect the clinically significant levels of CEA that is above the normal range (2.5–5 ng $mL^{-1}$) simply by visual detection. However, more optimization is required to address even lower detection limits that allow the detection limit to fall in the normal range.

## Conflicts of interest

There are no conflicts to declare.

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