Supporting Information

Simple and Sensitive Detection of HBsAg by Using a Quantum Dots Nanobeads Based Dot-Blot Immunoassay

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Antigen-down enzyme-linked immunosorbent assay (ELISA)

An antigen-down ELISA test was also used to detect HBsAg. Polystyrene microtiter plate wells (Corning, USA) were coated with 100 μL HBsAg in carbonate buffer (50 mM, pH 9.6) at 4 °C for 18 h. The excess antigen solution was discarded, the plates were washed three times with washing buffer (PBS containing 0.05% Tween-20, PBST) and the excess binding sites were blocked with 1% (w/v) BSA in PBST for 2 h at 37 °C. After blocking, 100 μL of the corresponding anti-HBsAg antibody diluents (1% BSA in TBST) was loaded into the wells, and the plates were then incubated for 1 h at 37 °C. After three washes, 100 μL of horseradish peroxidase (HRP)-labeled goat-anti-mouse secondary antibody dilutes (1% BSA in TBST) were loaded into wells and incubate for another 1 h at 37 °C. The microplates were washed again, and 100 μL of substrate solution (3,3',5,5'-tetramethylbenzidine/H_2O_2) was added. After 10 min, the reaction was stopped by adding 50 μL of 1 M H_2SO_4. The absorbance was measured at 450 nm in an ELISA microplate reader (Kehua ST-360, Shanghai, China). The average absorbance was calculated from triplicate wells.

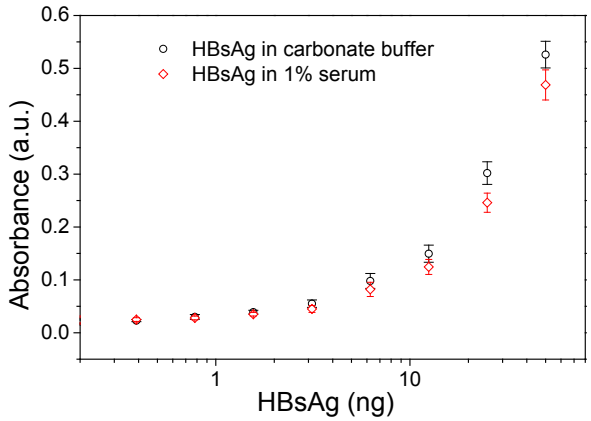


Figure S1. Detection of HBsAg in carbonate buffer and 1% human serum with ELISA test.

Calculation of the number of QDs encapsulated per nanobeads

Number of QDs in each nanobead is mainly dependent on the size of nanobeads and the ratio of QDs to PSMA, with the assumption that QDs were totally encapsulated into nanobeads and evenly dispersed in the matrix polymer. The average number of QDs in each QDNB (N_{sQDNB}) was

calculated from the follow equation:

$$N_{sQDNB} = \frac{N_{QD}}{V_{QD}+V_{PSMA}} V_{sQDNB}$$

Here, N_{QD} is the total number of QDs added in nanobeads, V_{QD} is the total volume of QDs, V_{PSMA} is the total volume of PSMA polymer, and V_{sQDNB} is the volume of single QDNB. The results were shown in Table 1.

Table S1. Optical properties of oil soluble QDs, water soluble QDs and the prepared QDNBs.

Name	Absorption Peak (nm)	PL Peak (nm)	PL FWHM (nm)	QYs[a] (%)	DLS Size (nm)
Oil QDs	557.7	582	30.4	41.2	7.5 ± 0.5
Water QDs	558.5	582	31.8	27.3	14.5 ± 1.5
QDNBs	558.2	582	32.9	15.9	118.4 ± 13.4

[a] quantum yields (QYs) of QDBNs were measured by dissolving QDNBs in chloroform and compared with standard dyes (Rhodamine 6G).

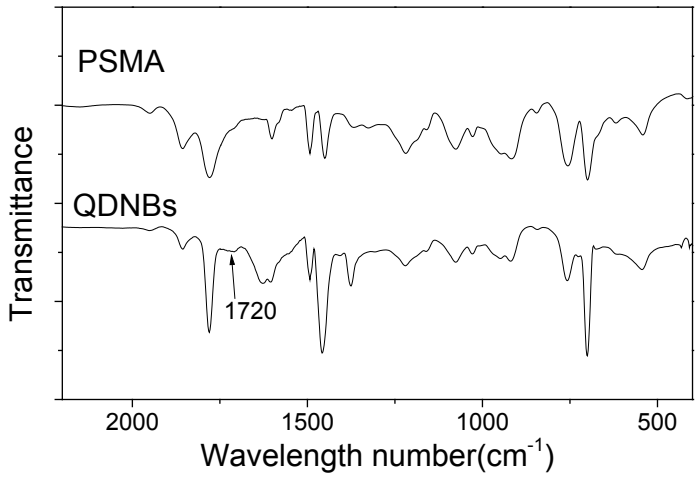


Figure S2 FTIR spectra of PSMA polymer and QDNBs.

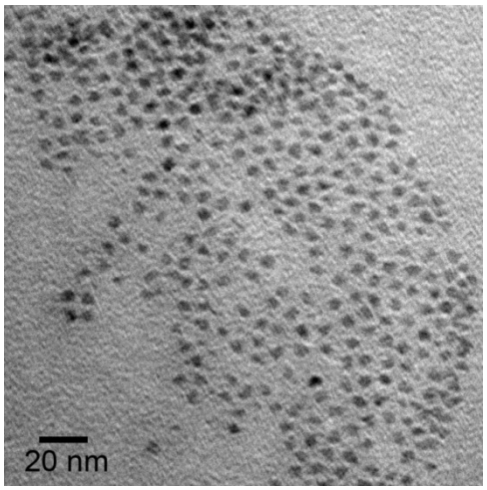
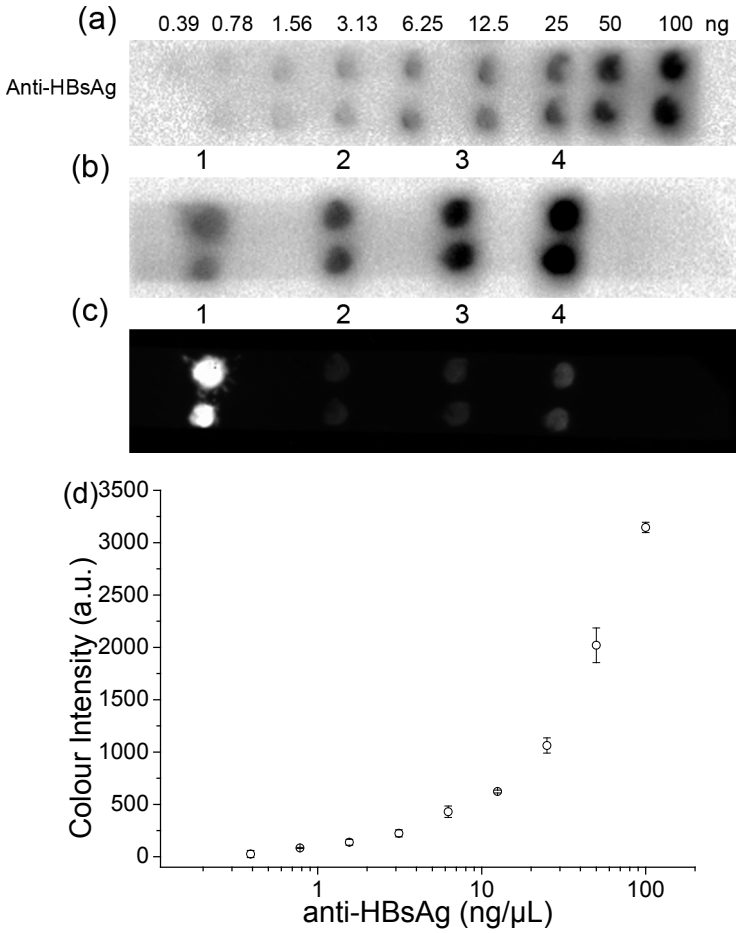


Figure S3 TEM image of oil soluble CdSe/ZnSe/ZnS QDs (average size: 5.7± 0.5 nm).

Determination of the number of antibodies attached per nanoparticle

The number of antibodies conjugated per nanoparticle (N) was calculated according to following equation: $N=C_{ab}/C_{np}$. Concentration of nanoparticles (C_{np}) was determined by measuring the absorption intensity of nanoparticles at the first absorption peak, and compared with a standard curve of nanoparticle solutions of known concentration. Concentration of IgG (C_{ab}) in antibody conjugates was measured by a chemiluminescence dot-blot immunoassay. In this approach, a known volume of conjugates spotted on PVDF membrane and incubated with HRP-labeled secondary antibody. IgG dots on PVDF membrane detected using chemiluminescence generated by SuperSignal West Femtochemiluminescent substrate (Thermo Scientific, USA) acquired with a CCD camera (Bio-Rad ChemiDoc, USA), and quantified using Image Lab software (Bio-Rad). A standard curve constructed by spotting a known anti-HBsAg concentration range on PVDF membrane was used for comparison in each experiment.



Sample	1	2	3	4
Nanoparticles	QD	QDNBs	QDNBs	QDNBs
C_{ab} (μM)	0.32	0.43	0.73	1.37
C_{np} (μM)	0.71	0.012	0.019	0.023
N	0.45	35.8	38.4	59.6

Figure S4. Membrane chemifluorescent immunoassay of concentration known anti-HBsAg antibody (a) and antibody conjugates (b), and corresponding fluorescent image (c) of antibody conjugates under UV light illumination. (d) Chemifluorescence intensity of spots against pure antibody concentration.

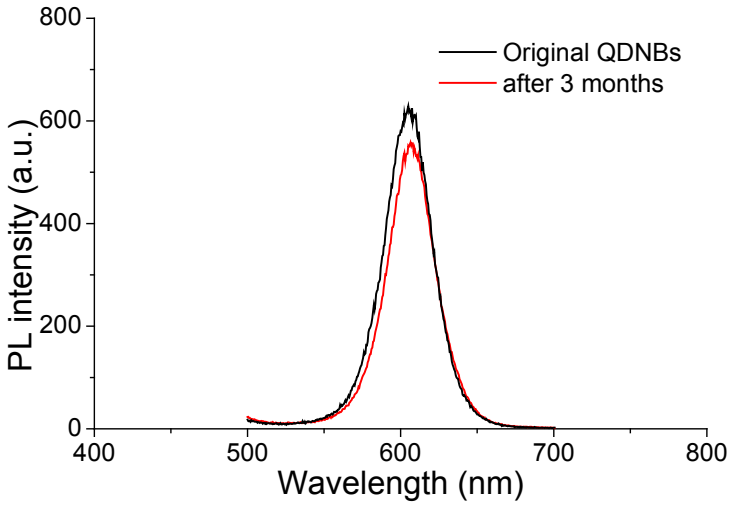


Figure S5 Fluorescent stability of QDNBs dispersed in water.

As shown in Figure S6, stability of as-prepared QDNBs was investigated by dispersing in different pH buffer. Fluorescence intensity of QDNBs is relatively stable in pH 4-11, this result is similar to previous reports. [1, 2]

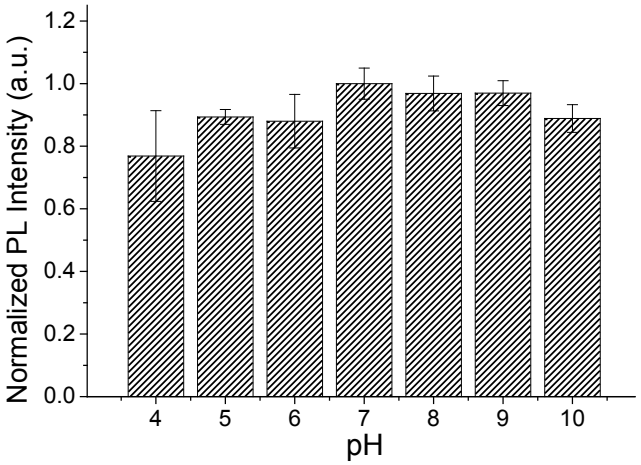


Figure S6 stability of QDNBs dispersed in different pH buffer.

Commercial ELISA kit assay

Sino-FDA approved chemiluminescent ELISA kit was provided by Beijing Chemclin Biotech and the instruction given with the kit was exactly followed. All solutions used were provided with the kit. Briefly, ELISA plates coated with capture antibody were warmed to room temperature. Then, 50 μL of serum and a series of standard samples were added to each well, and mixed with 50 μL of HRP labeled HBsAg antibody. A plastic plate sealer was applied and the plate was incubated in a dry

incubator at 37 °C for 1 hour. After the incubation, the plate was washed 5 times using supplied washing buffer, with 10 second intervals between washes. 100 μL of fresh prepared luminol-hydrogen peroxide solution was added into each well and incubated in dark for 5 min at room temperature. After this, the chemiluminescence intensity was immediately measured using a plate reader. Results for the commercial ELISA kits are shown in Figure S7.

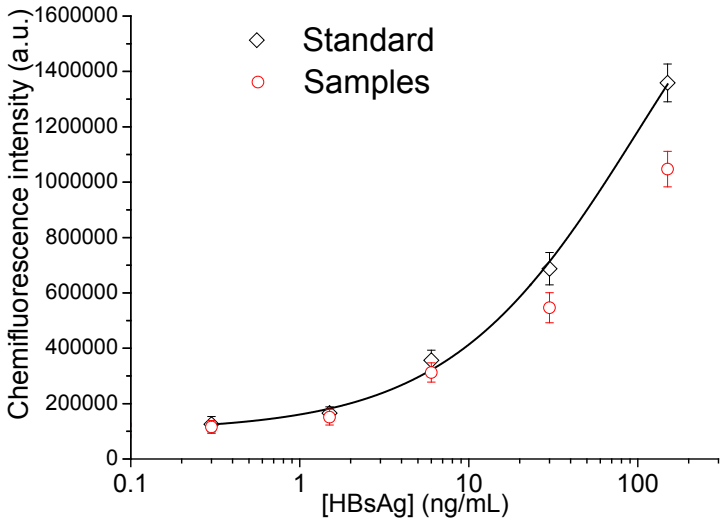


Figure S7 The test results of SFDA approved HBsAg ELISA kit.

References

[1] Zhang P, He Y, Ruan Z, Chen FF, Yang J. Fabrication of quantum dots-encoded microbeads with a simple capillary fluidic device and their application for biomolecule detection. J Colloid Interf Sci. 2012; 385: 8-14.

[2] Zhou C, Yuan H, Shen H, Guo Y, Li X, Liu D, Xu L, Ma L, Li L-S. Synthesis of size-tunable photoluminescent aqueous CdSe/ZnS microspheres via a phase transfer method with amphiphilic oligomer and their application for detection of HCG antigen. J Mater Chem. 2011; 21: 7393-400.