Electronic Supplementary Information

Efficiently preparation of magnetic quantum dot barcodes

Gang Wang,[a†] Yuankui Leng,[a†] Heze Guo,[a] Sheng Song,[a] Zequan Jiang,[a] Xiangliang Yuan,[b] Xiebing Wang,[a] Kang Sun, *[a] Kun Sun,[b] Hongjing Dou*[a]

[†] *These two authors contributed equally.*

[a] *The State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China, E-mail: hjdou@sjtu.edu.cn (H. Dou); ksun@sjtu.edu.cn (K. Sun)*

[b] *Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200092, P. R. China P. R. China*

1. Characterization of the Nanoparticles and the MQ-Microspheres.

The morphologies of the MNPs and QDs were observed using a transmission electron microscope (TEM, JEM-2010, JEOL). The morphologies of the MQ-microspheres were observed using a scanning electron microscope (SEM, QUATNTA 250, FEI Company).

2. Characterization of the Fluorescence Properties of the QDs and the MQ-Microspheres.

The fluorescence spectra of QDs that were dispersed into a toluene solution and of the MQ-microspheres (1.5×10^7 per mL in water) were recorded using a Shimadzu RF-5301PC spectrofluorophotometer. Fluorescence images of various focalized planes of the MQ-microspheres were recorded in water using a laser scanning confocal microscope (LSM 510 META, Zeiss).

3. Investigation of on the Magnetic Properties of the MNPs and the MQ-Microspheres.

The magnetic properties of the MNPs and the MQ–microspheres were studied at 300 K by using a vibrating sample magnetometer (VSM, Model 7407, Lake Shore Cryotronics, Inc.). Saturation magnetization, coercive force and remnant magnetization measurements were obtained from the hysteresis loops.

4. Synthesis of the Magnetic Fe_3O_4 Nanoparticles.

Magnetic Fe_3O_4 nanoparticles (MNPs) were synthesized according to a method described in a previous report[1]. The product was collected *via* centrifugation and washed three times with acetone and water (v/v = 1/1). Finally, the MNPs were redispersed into toluene to form a well-dispersed MNP solution for further processing. The TEM images (shown in Fig S1a) indicated that the MNPs possessed a narrow size distribution and a high shape uniformity. The average diameter of the MNPs was ~16.7 nm. The magnetic hysteresis plot of the MNPs shown in Fig S1b indicated that they exhibited excellent magnetic properties.

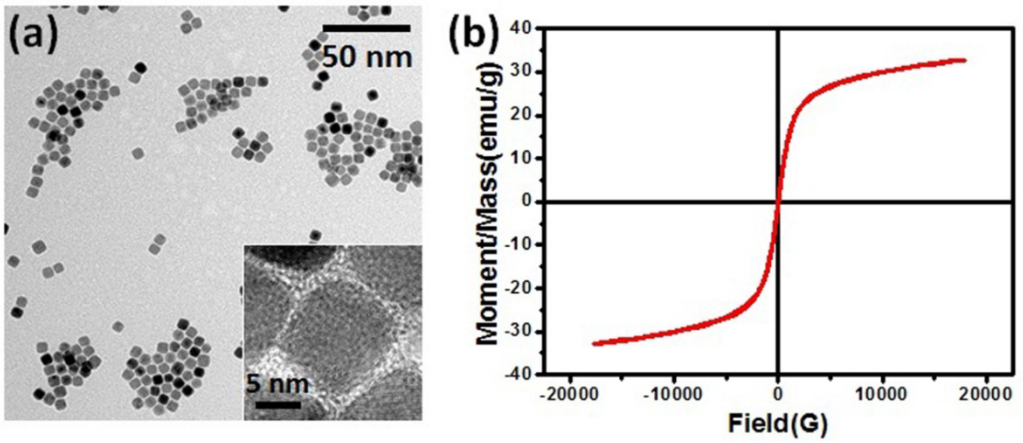

Fig. S1 (a) TEM images and (b) magnetic hysteresis plot of the MNPs.

5. Synthesis of the CdSe/CdS/ZnS and the CdTeSe/CdS/ZnS QDs.

CdSe/CdS/ZnS QDs with an emission wavelength of 520 nm were synthesized as described in a previous report[2]. Due to the nonlinear characteristics of the ternary CdSeTe QDs, it was feasible to synthesize high quality CdSeTe QDs displaying near-infrared fluorescence, which is otherwise difficult for binary II-VI QDs. Therefore, in our work, CdTeSe/CdS/ZnS QDs with an emission wavelength of 680 nm were prepared. The core CdTeSe QDs were synthesized as described in a previous report[3]. Specifically, the Cd solution was prepared by dissolving 0.156 g of CdO in a mixture of 0.4 mL oleic acid and 20 mL of liquid paraffin at 150 °C. This mixture was heated to the reaction temperature of 220 °C over a period of 10 min.

The Te and Se stock solutions were respectively prepared by dissolving tellurium and selenium powder into tri-octylphosphine (TOP). The S precursor solution was prepared by directly dissolving sulfur into liquid paraffin at 120 °C, while the Zn precursor solution was prepared by dispersing zinc stearate into liquid paraffin at room temperature. The premixed Te and Se solution was prepared from the individual stock solutions with a Se:Te molar ratio of 3:1. The amount of Cd precursor in the reaction solution was kept in excess with respect to the Se and Te precursors, and then a calculated amount of a premixed Te/Se solution was rapidly injected into the Cd solution with vigorous stirring. This solution was kept at 220 °C. After 15 min, a calculated amount of the S shell precursor solution (this amount depended on the core size) was added dropwise into the vigorously stirred core solution during a period of 5-10 min to cover the CdSeTe-core with a CdS shell. Subsequently, a calculated amount of the Zn shell precursor solution was then added into the reaction mixture, which was followed by the addition of the S shell precursor solution over a period of 5-10 min. These additions yielded a ZnS shell that surrounded the CdSeTe core and the CdS shell of the QD. After the ZnS shell had formed, the solution was cooled to 80 °C and left stirring for 1 h. Aliquots of the CdSeTe-core, CdSeTe/CdS core/shell, and CdSeTe/CdS/ZnS core/shell/shell QDs were collected at different reaction stages. Finally, these as-prepared QDs were separated from the crude solution by precipitation with methanol and redispersion into *n*-hexane for further use.

The CdSe/CdS/ZnS and CdTeSe/CdS/ZnS QDs products were purified by three times of repeated cycles of precipitation with methanol and chloroform, centrifugation, and subsequent redispersion into toluene before they were collected for further use.

From the TEM images of QDs in Fig.S2a and b, the 520 nm CdSe/CdS/ZnS QDs and 680 nm CdTeSe/CdS/ZnS QDs all possess narrow size distribution and high shape uniformity. The average diameters of the 520 nm CdSe QDs and the 680 nm CdSeTe QDs were approximately 2.8 and 5 nm, respectively. In addition, the fluorescence emission spectra of the QDs shown in Fig S2c and d were both narrow and symmetrical.

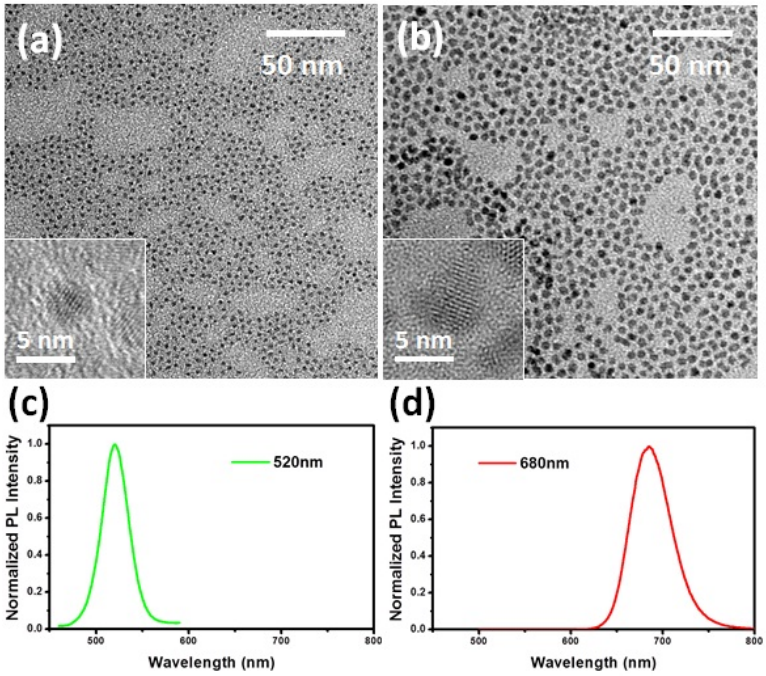

Fig. S2 Images (a) and (b) are TEM images of 520 nm CdSe/CdS/ZnS QDs and the 680 nm CdTeSe/CdS/ZnS QDs, respectively. Images (c) and (d) show the fluorescence spectra of 520 nm CdSe/CdS/ZnS QDs and 680 nm CdTeSe/CdS/ZnS QDs, respectively.

6. Fabrication of the MQ-Microspheres and the QD-Microspheres by the MESE method.

To fabricate the MQ-microspheres, a SPG membrane emulsification device (MG-20, SPG membrane/SPG Technology Co., Ltd.) was employed. Initially, two phases of an oil-in-water (O/W) emulsion were prepared individually. In order to prepare the dispersed oil phase, a designated amount of the QDs and the MNPs, along with 0.5 g of PSMA were dissolved into 8 mL of toluene. To prepare the aqueous continuous phase, the anionic stabilizer sodium dodecylsulphate (SDS) was dissolved into 200 mL of deionized water at a concentration of 0.5 wt.%. A hydrophilic SPG membrane with a 4.9 μm pore diameter was then placed in the SPG membrane emulsification device. The oil phase and the aqueous phase were stored in corresponding tanks within the device. Subsequently, the oil phase permeated through the pores

of the membrane into the continuous aqueous phase under a nitrogen pressure of 5 kPa. O/W droplets subsequently formed on the surface of the membrane and were stabilized by the SDS surfactant. With gentle magnetic stirring of the continuous phase, the stabilized oil droplets were sheared off from the surface of the membrane and suspended into the aqueous phase. After all of the dispersed oil phase was sheared off, the emulsification generation was stopped by reducing the nitrogen pressure. Meanwhile, the aqueous phase was continuously stirred to help accelerate the evaporation of toluene from the droplets at room temperature. This process was continued for 24 h to ensure the complete evaporation of the toluene from the emulsion droplets to yield the targeted MQ-microspheres. The final suspension was washed with deionized water and then ethanol and collected by centrifugation after each wash. Finally, the MQ-microspheres were freeze-dried for further use. The procedure employed for fabricating the QD-microspheres was similar to that described above, except that no MNPs were added into the dispersed oil phase.

Table S1. Protocols for the preparation of the MQ-microspheres by the MESE method.

Symbol[a, b]	Fe_3O_4 (g)	520 nm QD (mL)[c]	680 nm QD(mL)[d]
MQ1	0.005	0.8	0.0
MQ2	0.010	0.8	0.0
MQ3	0.025	0.8	0.0
MQ4	0.035	0.8	0.0
MQ5	0.010	0.4	0.0
MQ6	0.010	1.6	0.0
Q1	0.000	0.0	1.6
Q2	0.000	0.8	0.0

[a]MQ: MQ-microspheres.
[b]Q: QD encoded microspheres.
[c]: The concentration of the 520 nm QD solution was 0.2×10^{-4} M.
[d]: The concentration of the 680 nm QD solution was 0.1×10^{-4} M.

7. Morphologies of the MQ-Microspheres.

A SEM image of the morphologies of the MQ-microspheres fabricated by the MESE method is shown in Fig. S3. These inorganic/organic composite microspheres were all spherical and possessed smooth surfaces. Moreover, the microspheres were also highly monodisperse, which was also apparent from the SEM image.

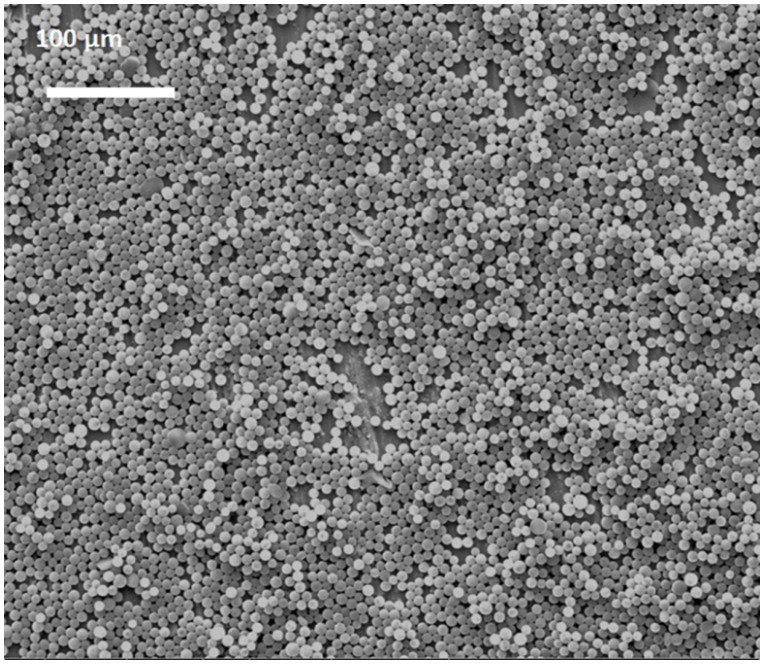

Fig. S3 SEM image of the MQ-microspheres (MQ6).

8. Surface Modification of the MQ-Microspheres

As shown in Fig. S4, a surface modification was performed in aqueous acidic condition to convert the anhydride groups on the surfaces of the MQ-microspheres into carboxyl groups *via* hydrolysis. The details of this modification were as follows: 6.25×10^8 MQ-microspheres were suspended in 15 mL of an aqueous 0.1 M HCl solution. After this mixture had been allowed to react for 24 h under magnetic stirring at 25 ºC, the MQ-microspheres were centrifuged and washed with deionized water until the pH value of the suspension became stabilized at ~6.2. Finally, these modified MQ-microspheres were freeze-dried and stored for further use.

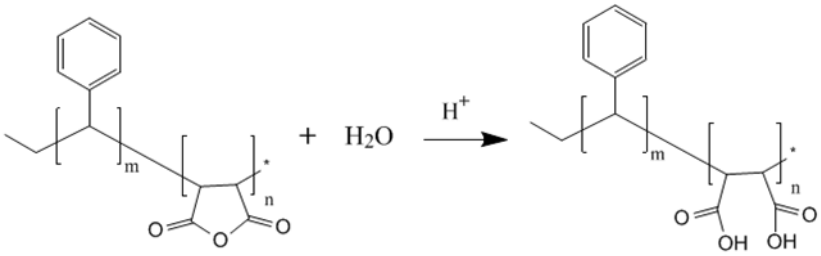

Fig. S4 Conversion of the anhydride groups on the microspheres surface into carboxyl groups *via* acid-catalyzed hydrolysis.

9. The Effect of MNPs on the QDs

Fig. S5 shows the relationship between the UV-vis spectra of MNPs and the fluorescence spectra of the 520 nm QDs that were recorded under laser excitation at 400 nm. This trend indicated that the exciting laser and emission fluorescence of the QDs were all partially absorbed

by the MNPs (which absorbed light up to 700 nm), which resulted in a decreased fluorescence intensity exhibited by the MQ-microspheres.

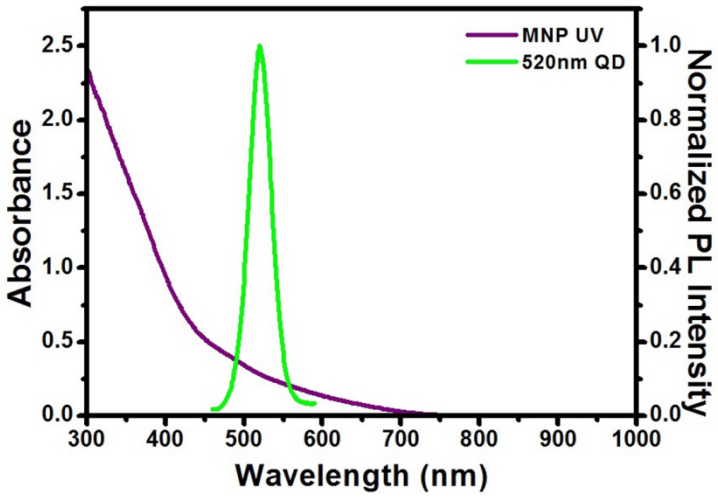

Fig. S5 The UV-vis spectrum of the MNPs and the a fluorescence emission spectrum of the 520 nm QDs.

10. Magnetic Separation and Multiplex Suspension Assays of HBV Antigens.

10.1 Flow Cytometric Analysis.

A flow cytometer (FC500, Beckman Coulter) was used in order to perform barcode library determination and suspension immunoassay detections. Microspheres were suspended in aqueous solutions and subsequently injected into the flow cytometer and were excited by a 488 nm laser. Signals from 10,000 microspheres were collected for each sample.

10.2 Reagents for Magnetic Separation and Multiplex Suspension Assays of the HBV Antigens.

The names of the reagents used to prepare the multiplex suspension assays are listed in Table S2. In addition, an ImmunoProbeTM Biotinylation Kit (Sigma-Aldrich) was employed for the preparation of the biotin-conjugated HBsAb and HBeAb antibodies, in which HBsAb (L1H00201) and HBeAb (L1H00402) were used.

Table S2. Reagents for magnetic separation and multiplex suspension assays of the HBV antigens.

Name of reagents	Suppliers
Ethylene Diamine Carbodiimide (EDC).	Sigma-Aldrich.
Sulfo-*N*-hydroxysuccinimide (S-NHS).	Sigma-Aldrich.
Hepatitis B Surface Antigen (HBsAg), L1H00301.	Shanghai Linc-Bio Science Co., Ltd.
Monoclonal Antibody to the Hepatitis B Surface Antigen (HBsAb) for probes, L1H00202.	Shanghai Linc-Bio Science Co., Ltd.
Goat Antibody to the Hepatitis B Surface Antigen (HBsAb) for detection, L1H00201.	Shanghai Linc-Bio Science Co., Ltd.
Hepatitis B e Antigen (HBeAg), L1H00501.	Shanghai Linc-Bio Science Co., Ltd.
Monoclonal Antibody to the Hepatitis B e Antigen (HBeAb) for probes, L1H00401.	Shanghai Linc-Bio Science Co., Ltd.
Monoclonal Antibody to the Hepatitis B e Antigen (HBeAb) for detection, L1H00402.	Shanghai Linc-Bio Science Co., Ltd.
Streptavidin-*R*-phycoerythrin conjugate (PE).	Sigma-Aldrich.

10.3 Preparation of the Buffers.

Seven different buffers were prepared, including phosphate buffer saline (PBS), a washing buffer, an active buffer, a blocking buffer, a storage buffer, a detection buffer and an assay buffer. The recipes for preparing these buffers are listed in Table S3.

Table S3 Recipes for preparing the buffers.

Name of buffer	Component		Volume	PH	Storage
PBS	$Na_2HPO_4 \cdot 12H_2O$	7.26 g	2000 mL	7.4	Filter was
	NaCl	16.146 g			sterilized and
	KH_2PO_4	0.48 g			stored at 4 ˚C
	KCI	0.403 g			
Washing Buffer	$Na_2HPO_4 \cdot 12H_2O$	0.7260 g	200 mL	7.4	Filter was
	NaCl	1.6150 g			sterilized and
	KH_2PO_4	0.0480 g			stored at 4 ˚C
	KCl	0.0400 g			
	Tween-20	0.100 mL			
Active Buffer	NaH_2PO_4	0.6000 g	50 mL	6.2	Filter was
	5N NaOH	0.400 mL			sterilized and
					stored at 4 ˚C
Blocking Buffer	$Na_2HPO_4 \cdot 12H_2O$	0.7260 g	200 mL	7.4	Filter was
	NaCl	1.6150 g			sterilized and
	KH_2PO_4	0.0480 g			stored at 4 ˚C
	KCl	0.0400 g			
	BSA	2.0000 g			
	Thimerosal	0.0400 g			
Storage Buffer	$Na_2HPO_4 \cdot 12H_2O$	0.7260 g	200 mL	7.4	Filter was
	NaCl	1.6150 g			sterilized and
	KH_2PO_4	0.0480 g			stored at 4 ˚C
	KCl	0.0400 g			
	BSA	0.2000 g			
	Tween-20	0.040 mL			
	Thimerosal	0.0400g			
Detection Buffer	$Na_2HPO_4 \cdot 12H_2O$	0.7260 g	200 mL	7.4	Filter was
	NaCl	1.6150 g			sterilized and
	KH_2PO_4	0.0480 g			stored at 4 ˚C
	KCl	0.0400 g			
	Tween-20	0.100 mL			
Assay Buffer	$Na_2HPO_4 \cdot 12H_2O$	0.7260 g	200 mL	7.4	Filter was
	NaCl	1.6150 g			sterilized and
	KH_2PO_4	0.0480 g			stored at 4 ˚C
	KCl	0.0400 g			
	BSA	2.0000 g			
	Thimerosal	0.0400 g			

10.4 Probe Conjugation onto the Surfaces of the MQ-Microspheres and the QD-Microspheres.

A 100 μL aqueous suspension of MQ-microspheres (1.25×10^6 /mL) was washed three times with the washing buffer. Each sample was centrifuged and subsequently resuspended into 100

μL of the active buffer. A 10 μL solution of sulfo-NHS (50 mg/mL in active buffer) and 10 μL of an 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) solution (50 mg/mL in active buffer) were then added into the sample. After this mixture was vortexed at room temperature for 20 min, it was washed three times with the washing buffer before it was centrifuged and resuspended into 500 μL of the PBS buffer. Subsequently, 10 μg of the HBV antibody probes were added into the sample and vortexed for 12 h at 10 °C. The sample was then washed three times with the washing buffer before it was resuspended into 150 μL of the blocking buffer for another 20 min to block the free carboxylates on the surfaces of the MQ-microspheres. Finally, blocked probe-coated MQ-microspheres were washed with the washing buffer and stored in the storage buffer at 4 °C. The probes were conjugated onto the surfaces of the QD-microspheres by following the same procedure as described above.

10.5 Establishment of the MFI-Ag Concentration Detection Curve for the Multiplex Suspension Assays.

100 μL samples containing both HBsAg and HBeAg HBV antigens were prepared at various concentrations. In particular, the concentrations of both the HBsAg and HBeAg antigens in the mixtures were identical, at 0, 25, 50, 250, 500 or 5000 ng/mL. A 100 μL sample at each concentration was added into a single well of a 96-well plate with ultrafiltration membranes placed at the bottom of the well, so that six wells were used. For each well, 2×10^4 HBsAb-coated 680 nm QD-microspheres (Q1) and 2×10^4 HBeAb-coated 520 nm QD-microspheres (Q2) were added into the sample simultaneously. After the samples had been vortexed for 1 h at room temperature, they were washed with the washing buffer *via* filtration in order to remove the uncaptured targets.

Subsequently, biotin-conjugated secondary antibody solutions containing 10 μg/mL of biotin-HBsAb and 10 μg/mL of biotin-HBeAb mixed in assay buffer were added into the samples (100 μL for each well). After the samples had been vortexed for another 1 h at room temperature, they were washed with the washing buffer *via* filtration to remove unreacted biotin conjugated secondary antibodies. During the next step, 100 μL of P-phycoerythrin (PE) solution (at 1 μg/mL in the detection buffer) was added into each well. After these samples were vortexed for 10 min at room temperature, they were washed with the washing buffer *via* filtration to remove any of the unreacted PE. Subsequently, the QD-microspheres in the single sample were resuspended into 100 μL of the assay buffer and injected into the flow cytometer for analysis. The median fluorescence intensity (MFI) of the reporters (detected in FL2) on the microsphere surfaces were recorded, and these values corresponded to the target concentrations of the samples.

Finally, as shown in Fig. S6, a MFI-Ag concentration detection curve was established for the multiplex suspension assay.

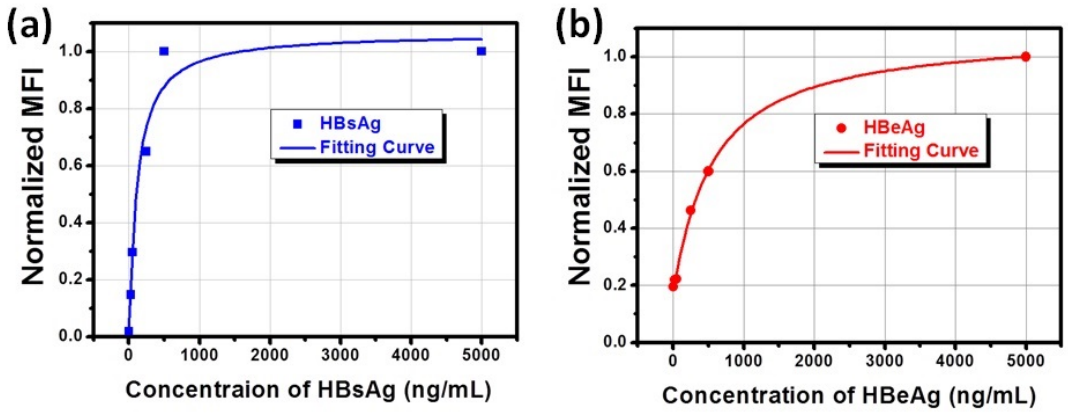

Fig. S6 Concentration detection curves corresponding to the (a) MFI-HBsAg and (b) MFI-HBeAg multiplex suspension assays.

10.6 Magnetic Separation of HBsAg.

1.25 × 10^6 HBsAb-coated 520 nm MQ-microspheres (MQ6) were added into a PBS solution containing 500 ng/mL of HBsAg and 500 ng/mL of HBeAg. After this sample had been vortexed for 12 h at 10 °C, a magnet was placed at one side of the vial and kept in place for 1 h, so that the MQ-microspheres were gradually attracted toward the magnet. Subsequently, the residual sample solution was drawn into another vial for further analysis.

10.7 Sample Preparation and Analysis of the Magnetically Separated Multiplex Suspension Assay.

2 × 10^4 HBsAb-coated 680 nm QD-microspheres (Q1) and 2 × 10^4 HBeAb-coated 520 nm QD-microspheres (Q2) were added into the magnetically separated sample simultaneously. After the sample was vortexed for 1 h at room temperature, it was washed with washing buffer *via* filtration to remove the uncaptured targets. A 100 μL biotin-conjugated secondary antibody solution containing 10 μg/mL of biotin-HBsAb and 10 μg/mL of biotin-HBeAb that was mixed with an assay buffer was added into the sample. After this sample was vortexed for another hour at room temperature, it was washed with the washing buffer *via* filtration to remove the unreacted biotin-conjugated secondary antibodies. Subsequently, 100 μL of the PE solution (1 μg/mL in the detection buffer) was added into the sample. The mixture was vortexed for 10 min at room temperature before it was washed with the washing buffer *via* filtration to remove the unreacted PE. Finally, the QD barcodes in the sample were resuspended into 100 μL of the assay buffer for flow cytometric analysis.

10.8 Calculation of the Concentration of HBsAg Remaining in the Magnetically Separated Samples.

The concentration of the HBsAg that remained after the magnetic separation was calculated according to the MFI-HBsAg concentration detection curve for the multiplex suspension assay that is shown in Fig. S6. The formula of the fitting curve is expressed by Equation S1 as shown below:

$$I_{HBsAg} = e^{\left(0.06 - \frac{101}{C_{HBsAg} + 26}\right)} \quad \text{(S1)}$$

where I_{HBsAg} corresponds to the normalized MFI of Q1 detected in FL2 and C_{HBsAg} corresponds to the concentration of HBsAg in the sample. With regard to the multiplex suspension assay of the sample that had been magnetically separated, the normalized MFI of Q1 detected in FL2 was 0.036. Therefore, based on Equation S1, the concentration of the residual HBsAg was ~3 ng/mL. Meanwhile, the normalized MFI of Q2 detected in FL2 was still greater than 1. This indicated that the magnetic separation had no effect on the concentration of HBeAg in the sample.

11. Calculation of the Physical Properties of the MQ-Microspheres and QD-Microspheres.

The average diameters of the MQ-microspheres or QD-microspheres were calculated according to Equation S2:

$$D_m = \frac{\sum_{1}^{N} D_i}{N} \quad \text{(S2)}$$

where D_m corresponds to the average diameter of the microspheres, D_i denotes the diameter of a single microsphere (as measured from the SEM images), and N denotes the number of microspheres measured in a SEM image, which was 200 in our case.

The weight of nanoparticles (QDs or MNPs) embedded within in a single microsphere was calculated according to Equation S3:

$$M_n = C_n \times \frac{4}{3} \times \pi \times (\frac{D_d}{2})^3 \quad \text{(S3)}$$

where M_n denotes the weight of the nanoparticles in single microsphere (g), C_n denotes the concentration of nanoparticles in the dispersed phase of an SPG emulsification (g/mL), and D_d corresponds to the average diameter of the droplets in emulsion prepared *via* SPG emulsification, and is expressed in cm.

The weight of a single MQ-microspheres or QD-microsphere was calculated according to Equation S4:

$$M_m = M_n + \rho_{PSMA} \times \frac{4}{3} \times \pi \times (\frac{D_{QM}}{2})^3 \quad \text{(S4)}$$

where M_m denotes the weight of a single microsphere (g), ρ_{PSMA} denotes the density of PSMA (which is 1.1 g/mL at 25 ˚C), and D_m corresponds to the average diameter of the microspheres (which was 6.6×10^{-4} cm).

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