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Brief Communications

Lateral Flow Immunoassay Using Europium Chelate–Loaded Silica Nanoparticles as Labels

Xiaohu Xia,[1†] Ye Xu,[1†] Xilin Zhao,[1,2] and Qingge Li[1,3*]

[1] Molecular Diagnostics Laboratory, Department of Biomedical Sciences and the Key Laboratory of the Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Sciences, Xiamen University, Xiamen, Fujian, China; [2] Public Health Research Institute, New Jersey Medical School, Newark, NJ; [3] The Key Laboratory of Chemical Biology of Fujian, Xiamen University, Xiamen, Fujian, China; * address correspondence to this author at: Department of Biomedical Sciences, School of Life Sciences, Xiamen University, Xiamen, Fujian 361005, China. Fax +86-0592-2187363; e-mail qgli@xmu.edu.cn. † These authors contributed equally to the work.

BACKGROUND: Despite their ease of use, lateral flow immunoassays (LFIAs) often suffer from poor quantitative discrimination and low analytical sensitivity. We explored the use of a novel class of europium chelate–loaded silica nanoparticles as labels to overcome these limitations.

METHODS: Antibodies were covalently conjugated onto europium chelate–loaded silica nanoparticles with dextran as a linker. The resulting conjugates were used as labels in LFIA for detection of hepatitis B surface antigen (HBsAg). We performed quantification with a digital camera and Adobe Photoshop software. We also used 286 clinical samples to compare the proposed method with a quantitative ELISA.

RESULTS: A detection limit of 0.03 μg/L was achieved, which was 100 times lower than the colloidal gold–based LFIAs and lower than ELISA. A precise quantitative dose–response curve was obtained, and the linear measurement range was 0.05–3.13 μg/L, within which the CVs were 2.3%–10.4%. Regression analysis of LFIA on ELISA results gave: log (LFIA) = −0.14 log (ELISA) + 1.03 μg/L with $r = 0.99$ for the quantification of HBsAg in 35 positive serum samples. Complete agreement was observed for the qualitative comparison of 286 clinical samples assayed with LFIA and ELISA.

CONCLUSIONS: Europium chelate–loaded silica nanoparticle labels have great potential to improve LFIAs, making them useful not only for simple screening applications but also for more sensitive and quantitative immunoassays.

Lateral flow immunoassay (LFIA),[4] used in commercial pregnancy and drug-of-abuse tests, is a well-established and accepted point-of-care testing technique. However, LFIA has primarily been used for qualitative testing in which the analytes are present at relatively high concentrations. The major reasons for this limited use of LFIAs are the low signal intensity *(1)* and poor quantitative discrimination *(2, 3)* of the color-formation reaction based on label accumulation *(4, 5)*. Although researchers have tried to use instrumental means to generate quantitative results *(6, 7)*, the relatively low signals obtained often make such efforts meaningless *(1)*. To address this limitation, a variety of reporters have been developed, including colored particles *(8, 9)*, carbon black *(10)*, and fluorophores *(11, 12)*. However, no marked improvement in performance has been achieved, and alternative reporters remain to be developed.

We recently described a novel type of reporter *(13)* based on fluorescent silica nanoparticles covalently labeled with lanthanide chelates. These reporters have a large fluorescence emission due to multilayer chemical loading of lanthanide chelates onto the porous silica nanoparticles. Thus these reporters provide higher sensitivity in the time-resolved immunofluorometric assay of hepatitis B antigens. The apparent number of europium (Eu)(III) chelates per nanoparticle is calculated to be 6.86×10^5 *(13)*, which is much higher than commercial polystyrene nanoparticles of larger size [3.1×10^4 chelates per nanoparticle of 107 nm in diameter *(14)*]. The large Stokes shift inherent in these lanthanide-labeled nanoparticles makes it easy to distinguish specific long-wavelength emission (λ_{em} = 615 nm) signals from the background fluorescence. Because the size [mean (SD) 55 (5) nm in diameter] of this new type of nanoparticle is very close to that of the colloidal gold particles, we decided to test whether these fluorescent nanoparticles can be used as sensitive reporters for LFIA.

Details for preparation of reporters and strips, detection, and signal acquisition are provided in the Data Supplement that accompanies the online version of this article (see Supplemental Data Materials and Methods at http://www.clinchem.org/content/vol55/issue1). Our experiments used Eu (III) nanoparticles to replace colloidal gold in a commercial LFIA test strip for hepatitis B surface antigen (HBsAg). We conjugated the same monoclonal anti-HBsAg antibody used for colloidal gold conjugation in the LFIA to Eu(III):BHHCT [4,4-bis(1,1,2,2,3,3-heptafluoro-4,6-

[4] Nonstandard abbreviations: LFIA, lateral flow immunoassay; Eu, europium; HBsAg, hepatitis B surface antigen.

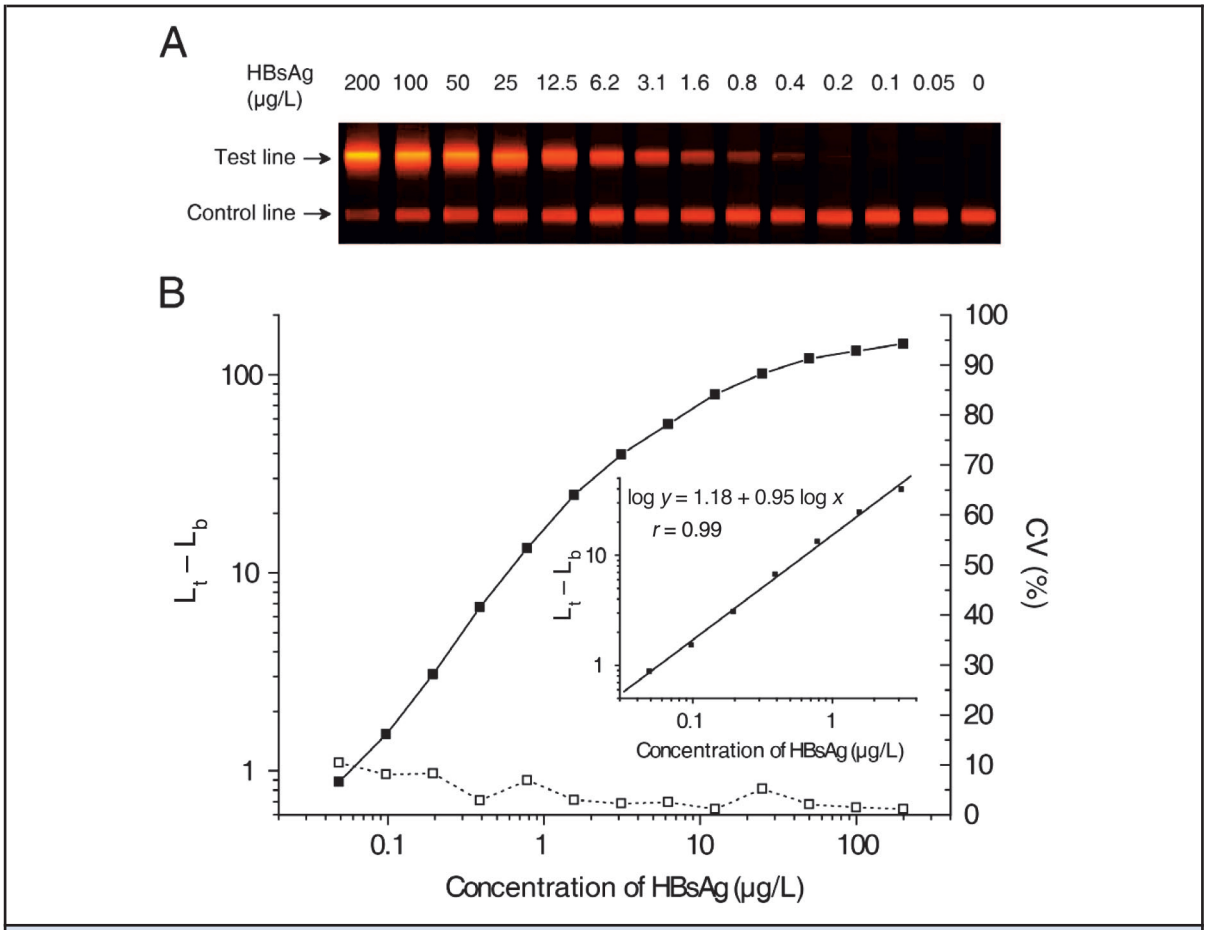

Fig. 1. Eu (III) nanoparticle-based LFIA of HBsAg.

(A), digital image of test strips (membrane area) carried out with 2-fold serially diluted HBsAg calibrator and a negative control in assay buffer. (B), Calibration curves (■) and imprecision profiles (□) of panel A. The inset illustrates the linear range region of the plot. L_t and L_b indicate the mean value of luminosity of the test line and the background, respectively.

hexanedion-6-yl) chlorosulfo-o-terphenyl]–coated silica nanoparticles via a dextran 500 linker. A series of 2-fold–diluted HBsAg calibrator solutions and a negative control were tested. After loading 80 μL of test solution on the sample pad and running at room temperature for 30 min, we visualized the results in a signal-acquisition device (online Supplemental Figure S1) equipped with an ultraviolet light source and recorded the results with a digital camera.

We observed proportional changes in fluorescence intensity of the test lines associated with the concentration of HBsAg (Fig. 1A). The control line was formed from antimouse IgG spotted onto the membrane and was used to ensure that anti-HBsAg monoclonal antibody was active and had migrated across the membrane. A red signal band from an HBsAg concentration as low as 0.2 μg/L could be seen even with visual inspection. A quantification method was developed that used a digital camera and Adobe Photoshop (Adobe Systems) software (see Supplemental Data Materials and Methods).

According to the calibration curve (Fig. 1B), the detection limit, calculated as the concentration corresponding to 3 times the SD of the background signal [3 SD method *(1)*] was 0.03 μg/L. The CVs across the entire range were <10.4% (n = 6). A linear relationship from 0.05–3.13 μg/L (r = 0.99) was observed in the calibration curve. In contrast, with the same pair of monoclonal antibodies, the colloidal gold–based LFIA had a detection limit of 3.51 μg/L, which is similar to the previously reported detection limit *(1)*. The ELISA method, with the same pair of antibodies used in the LFIA mentioned above, had a detection limit of 0.2 μg/L, as described in our previous work *(13, 15)*. We further investigated the relationship between our LFIA and ELISA. We diluted 35 HBsAg-positive serum sam-

ples with assay buffer to allow the concentration of HBsAg to fall into the linear range for each method. The concentration of each serum sample was calculated by multiplying the measured value with the dilution factor. The regression of LFIA results on ELISA results gave: log (LFIA) = −0.14 log (ELISA) + 1.03 μg/L with Syx = 0.048 μg/L, $r = 0.99$, n = 35 (see online Supplemental Figure S2).

Finally, we evaluated the qualitative response of the Eu (III) nanoparticle–based LFIA method by use of 286 clinical sera samples that had been previously identified by ELISA as positive, weakly positive, or negative. Representative results of LFIA were recorded by digital camera (see online Supplemental Figure S3). Concordant results between the 2 methods were achieved for all samples. Thus, our Eu (III) nanoparticle–based LFIA was at least as sensitive as ELISA.

Diminished fluorescence intensity in control lines was observed with strongly positive samples (Fig. 1 and online Supplemental Figs. S3 and S4). Such an observation is expected because the test line captures more labeled anti-HBsAg monoclonal antibodies along with the bound antigens in these samples than in weakly positive and negative samples, reducing the amount of anti-HBsAg monoclonal antibodies that can further migrate to the control line for capture by the goat antimouse IgG immobilized there through "sandwich-type" antigen–antibody interactions. The Eu(III) nanoparticles were basically free from background fluorescence interference owing to their unique fluorescence property (large Stokes shift, long emission wavelength) *(13, 16, 17)*. The concordance between LFIA and ELISA results indicates that fluorescence quenching did not occur in our assay. We attribute this observation to the continuous elution in LFIA, which could eliminate potential quencher from the captured labeled antibodies.

Our LFIA was rapid and easy to use and thus showed several advantages for testing large numbers of samples. We noted that with the proposed LFIA method positive sample detection was possible after only a few minutes. A follow-up, parallel experiment showed that the test enabled visualization of 0.2 μg/L HBsAg after 10 min, and the detection limit obtained at 20 min showed no difference with that at 30 min (see online Supplemental Figure S4). The hands-on time of LFIA was minimal compared with ELISA, which requires multiple incubation and washing steps.

In summary, we demonstrated that Eu (III) chelate–loaded silica nanoparticles are a promising alternative reporter for LFIA. While keeping the inherent ease of use and rapidness of LFIA, the new reporter offers improved sensitivity and quantitative discrimination.

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