Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supplementary material

A novel Time-resolved fluorescent lateral flow immunoassay for quantitative detection of the Trauma Brain Injury biomarker-Glial fibrillary acidic protein.

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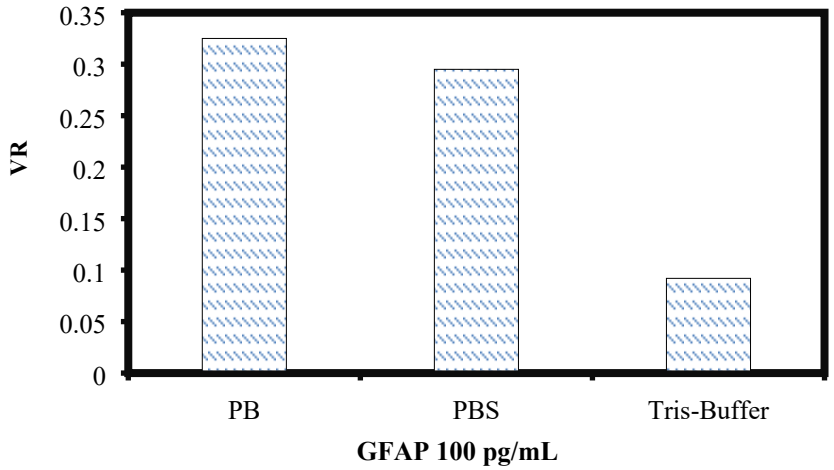
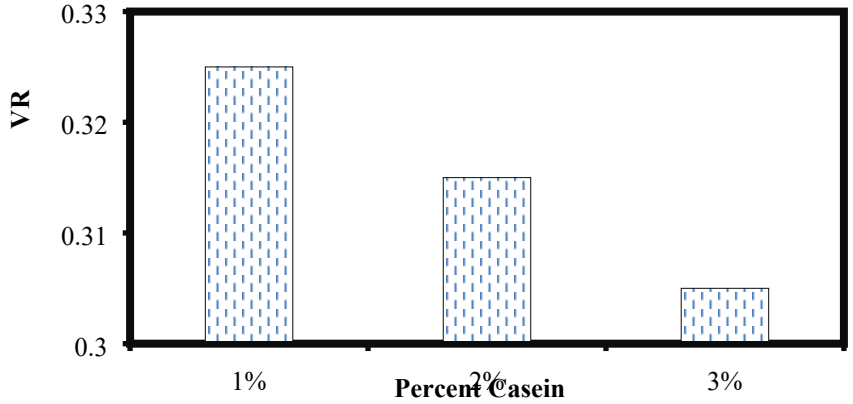
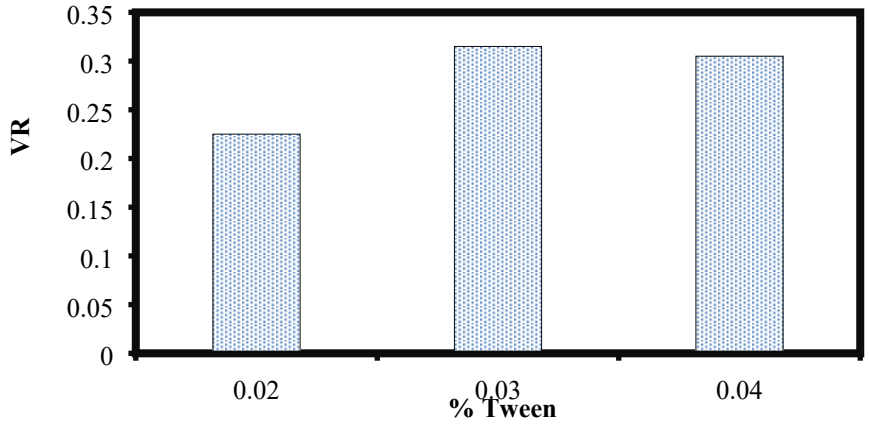
Optimization of the LFIA parameters

Components of running buffer mainly influence the flow of the CM-EU-D_{AB} conjugates, the binding efficiency of antigen-antibody immunoreaction and the level of nonspecific adsorption, the effect of running buffers on the Volume Ratio (V_R) of the assay were studied in the first place. The different buffer (Fig S1a) percent casein (S1b) percent Tween-20 (S1c) and EDTA (S1d) were tested. Sample solutions containing 100 pg/mL of GFAP were prepared by diluting the GFAP stocking solution with the running buffers. It can be seen from Fig (S1a) as the running buffer matrix is very crucial for immunoassay, as it is interconnected with all the lateral flow immunoassay components such as analyte, detection and capture antibodies, and fluorochrome. A good buffer will stabilize all these molecules to improve the performance and sensitivity of the lateral flow immunoassay. Fig (S1b) Along with that the buffer also reduces non-specific binding sites on the membrane, and Casein is the commonly used blocking reagent in the immunoassays. We also compared these buffers with the immunoassay buffers PBS and Tris. For GFAP detection, significantly higher V_R at 100 pg/mL concentrations were obtained with the PB buffer compared to the other buffers ($p < 0.05$) Fig (S1b). With the addition of casein into the running buffer, the V_R increased significantly due to a better movement of the conjugates on the membrane. Fig (S1c) Tween is a nonionic surfactant that is often used in the lateral flow assays to improve the performance of the LFIA. The best V_R was obtained with the running buffer containing 0.03% Tween. Fig (S1d) presents the effect of EDTA concentration on the V_R. It is worth mentioning that the influence of EDTA concentration on the performance of LFIA shows a sharp peak. One can see the V_R increased significantly with the increase of the EDTA amount from 0 to 0.8 mM. The addition of EDTA in the running buffer improved the mobility of CM-EU-D_{AB} on the nitrocellulose membrane and reduced the background signal. Further increase of the EDTA resulted in the decrease of the V_R of LFIA. High EDTA concentration would affect the immunoreactions between the antibody on the CM-

EU surface and GFAP. Therefore, phosphate buffer + 1% Casein + 0.03% Tween + 0.6 mM EDTA was employed as the running buffer in the following assays.







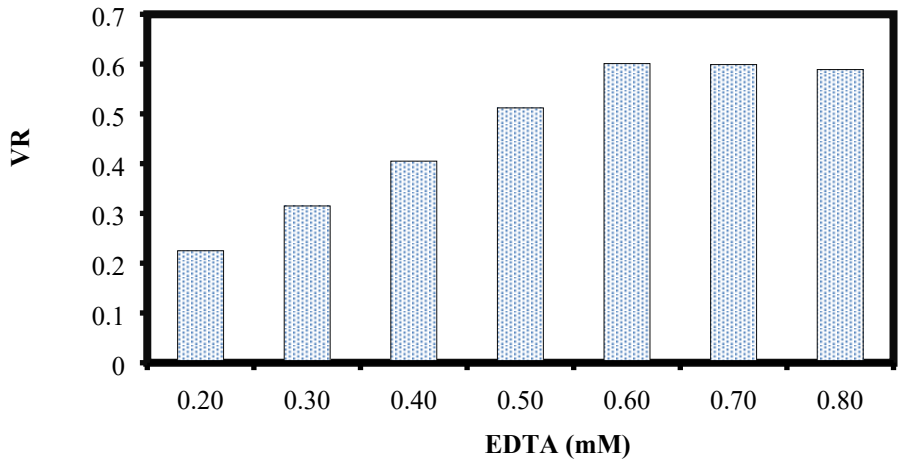


Fig S1: (a) Effect of Buffer (b) Casein Percent (c) Tween Percent and (d) EDTA amount in running buffer on the V_R of the assay. Buffer volume 85µl; assay time: 25 min.

The detection antibody amount (D_{AB}) attached to the CM-EU, the capture antibody amount (C_{AB}) dispensed on the test zone and the CM-EU-D_{AB} conjugate amount had significant impacts on the V_R of the test and control line, the detection efficiency, and sensitivity of the assay. As shown in Fig S2a the V_R raised when the D_{AB} amount increased from 0.15 ng to 0.5 ng in the conjugation process, further increment to 0.5 ng had no contribution to the V_R because the carboxyl groups on the CM-EU surface had been used up. Fig S2b presents the effect of the C_{AB} amount on the V_R of the assay. The amount of C_{AB} on the test line was determined by dispensing different concentrations of the C_{AB}. The V_R was the highest for the cycle of 1.0 mg/ml C_{AB}. A further increase would not increase the V_R ratio because the C_{AB} amount is saturated at the test line.

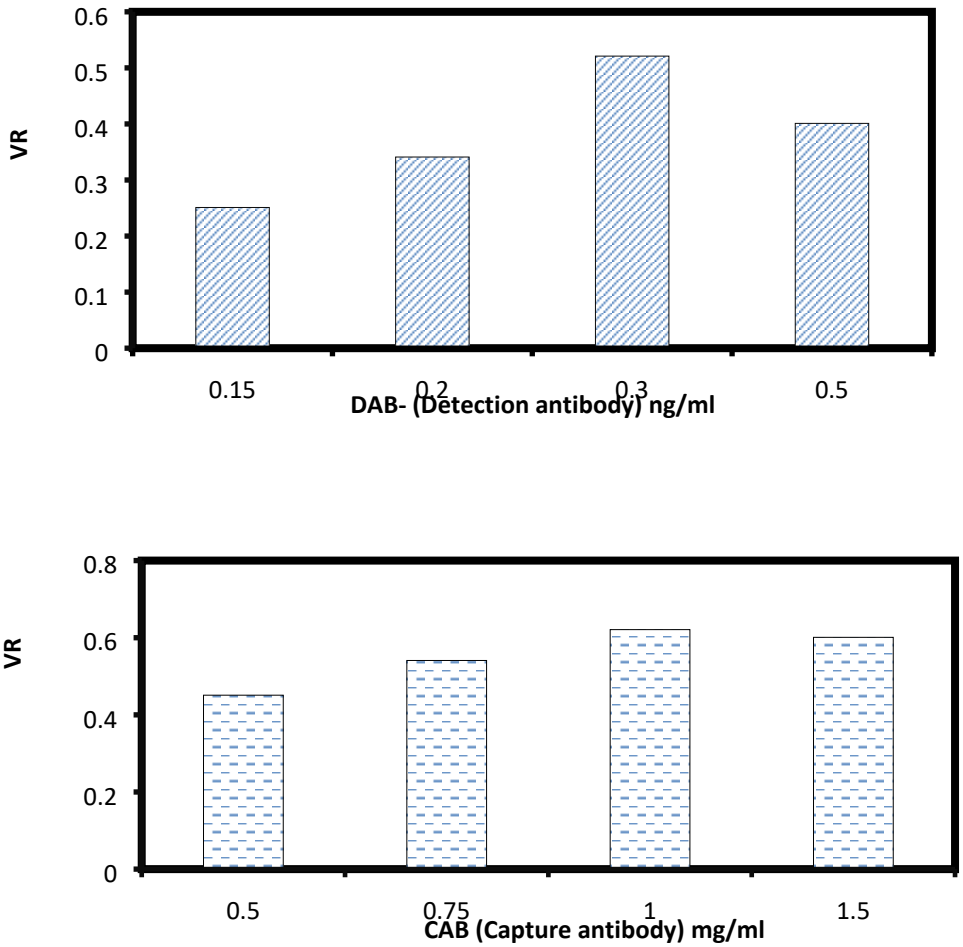


Fig S2 The effect of the (a) amount of capture antibody on the V_R ratio of the assay (b) amount of detection antibody in the conjugation pad. Spiked GFAP concentration: 100 pg/mL; assay time: 25 min. Each data point represents the average value obtained from three different measurements

Fig S3 Reproducibility of detecting GFPA in spiked in a blood sample

The reproducibility of the assay was examined by testing six times of the spiked blood samples 100 pg/mL of GFAP. Reproducible signals were obtained with a relative standard deviation (RSD) of 5.8 % (Fig**.** S3).

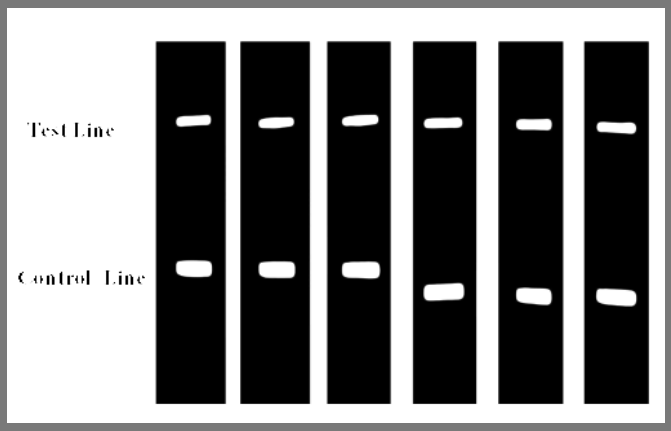
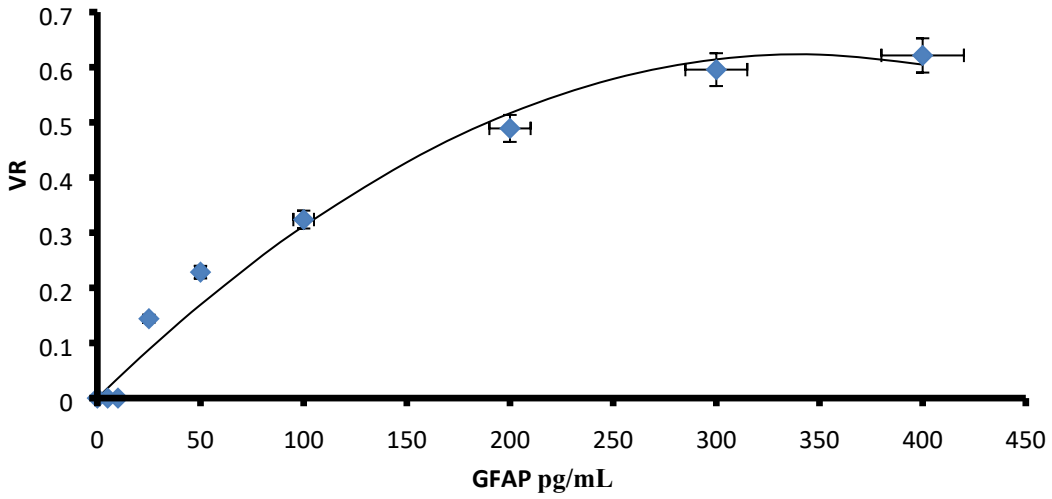


Fig S3. The LFIA pictures illustrates the reproducibility assay in spiked in a blood sample

Analytical validation and detection limit

A standard curve was obtained based on the measurement of six serial conference standards (0, 10, 50, 100, 200, 300, and 400 pg/mL). The standard curve was carried out by plotting the V_R (y) against the GFAP concentration (x). Under those optimized conditions, we obtained a reasonable calibration curve for the proposed assay (Fig S4a). The equation of the regression curve was $5E\text{-}06x^2 + 0.0033x + 0.0295$, n = 10, (r = 0. 9892). The analytical sensitivity of the proposed assay was 5 pg/mL, defined as the mean plus two SD (n = 20) of the zero standards. These results showed the excellent performance of the developed CM-EU test strips



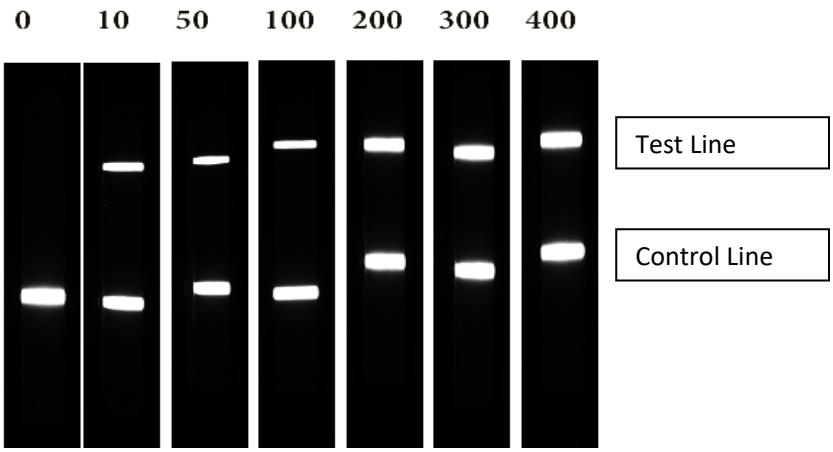
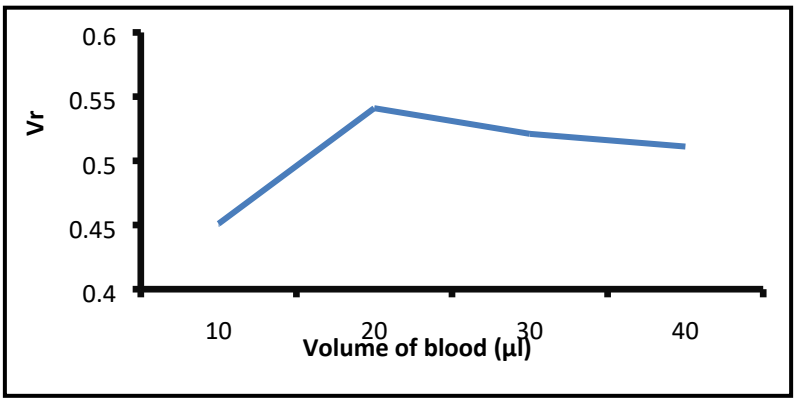


Fig S4a. The calibration plot of the LFIA for GFAP, which was constructed by plotting the V_R × various concentrations of the GFAP standard solutions. Data represent the average of three measurements. Fig S4b. The LFIA strip picture for the corresponding concentration.

Optimization of the assay parameters in the spiked blood.

The volume of the blood sample and incubation time was optimized. The performance of the Immunoassay could be influenced by the volume of blood. It was found that the best V_R was obtained with mixing of 15µl of blood with 85µl running buffer (Fig.S5a). The incubation time of CM-EU-D_{AB} in the blood also influences the V_R of the assay. It was found that the highest V_R was observed after 10 min of the incubation time (Fig.S5b). Consequently, 15µl blood sample, 10 min incubation time were used in the analysis of GFAP in blood.



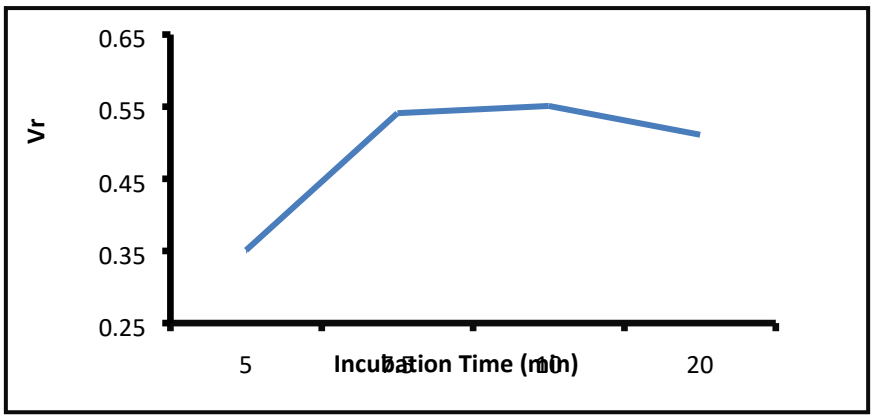


Fig S5. The volume and incubation time was optimized. (Fig S5a). The performance of the assay could be affected by the volume of blood, which brought substantial interference for the assay. It was found that the best V_R was obtained with mixing of 15 µl of blood with 85 µl running buffer (Fig S5b). The incubation time of GFAP in the blood also affected the V_R of the assay. It was found that the intensity of the test band reached a plateau at the 10 min incubation time