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A rapid test strip for diagnosing glycosylated hemoglobin (HbA1c) based on fluorescent affinity immunochromatography

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Abstract

In this research, we developed a rapid and easy to operate point-of-care testing (POCT) strip based on fluorescent affinity immunochromatography to quantitatively determine HbA1c concentrations in whole blood. This assay based on a sandwich method performed on test strips effectively utilized the principle of affinity chromatography column which was commonly used in the detection of HbA1c, and the technology of traditional fluorescence immunochromatographic test strips (FICTS) were combined. In our test strips, the test line of traditional FICTS was transformed into the region of affinity chromatography, improving the linearity and reducing the interference of the precursor of HbA1c and hemoglobin variants. The test strips could quantitatively detect HbA1c in a wide range (3%-13.8%) with excellent linearity (R^2>0.99) and the assay accuracy were demonstrated by comparing with high performance liquid chromatography (HPLC) (R^2>0.95). The simple, rapid, effective and quantitative strips will provide a novel method for detection of HbA1c in clinical.

Keywords: Fluorescent affinity immunochromatography, HbA1c, Rapid test strips, Affinity chromatography, Lateral flow assay

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Introduction

Diabetics is a global public health problem, characterized by hyperglycaemia. People with type II dibetes have many complications such as high blood pressure, cerebral infarction and nephrotic syndrome[1-4]. In China, the prevalence rate of adult with diabetes is 10.9% , and the morbidity is as high as 5.9% under the age of 40. The number of patients is increasing, and the diabetes has a trend of attacking young adults[5]. In the 1990s, Britain and the United States did research on a large scale for diabetes by clinical pathology and physiology and the scientists found that the decrease of diabetic complications was related to the lower percentage of glycosylated hemoglobin to total hemoglobin (Hb)[6]. Glycosylated hemoglobin can accurately reflect the long-term blood glucose control of diabetic and is an important indicator for the condition of diabetes[7-8]. Glycosylated hemoglobin helps people understand diabetes complications and guide patients to control blood glucose[9-11]. In 2010, the American Diabetes Association (ADA) suggested that all diabetics should make at least two regular glycosylated hemoglobin tests every year[18].

HbA1c is a stable compound of glucose and hemoglobin. In vivo, once the hemoglobin is in contact with the glucose, the non-enzymatic glycosylation could occur, and the degree of glycosylation depends on the time and concentration of glucose. An unstable schiff base (the HbA1c precursor) is formed at initial stage. The HbA1c precursor goes through by the Amadori rearrangement to form HbA1c. The formation process of the ketone structure is irreversible, slowly and throughout the erythrocyte life span (120 days)[12-13]. It is worth mentioning that total glycosylated hemoglobin and HbA1c represent different meanings[12]. HbA1c is defined as a stable compound of n-terminal valine residues on Hb combined with glucose, and glycosylation sites on Hb forming glycosylated hemoglobin are more than that one. However, the ratio of HbA1c to total glycosylated hemoglobin is similar (70%) in different people and HbA1c is a effective long-term indicator of blood glucose level in diabetics[12].

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In recent years, ADA and the International Federation of Clinical Chemistry (IFCC) have been working together to find a consistent, standardized, and parametrical method to detect the glycosylated hemoglobin in the whole blood[14-17]. At present, the proportion of HbA1c to Hb is used internationally as the HbA1c results. In 2010, ADA indicated that 6.5% was the cutoff value for HbA1c at the guideline for diabetes diagnosis[18]. In the process of diabetes diagnosis, relying on the results of the fasting glucose tests to monitor diabetics is not enough. Though the cost of HbA1c tests is higher, HbA1c tests have certain advantages over the fasting glucose tests in terms of the prevention of complications. Therefore, the glycosylated hemoglobin and blood glucose for joint detection will be of great importance in the diagnosis and treatment for diabetes [19].

At present, HbA1c is clinically detected through HPLC. This method is charactered by high sensitivity and accuracy and its test results are presented in the form of percentage. But it still has some shortcomings, on the one hand the supports from large-scale instrument and professional experiment operation are required, on the other hand the cost of equipment maintenance is high. Therefore, it's difficult to promote HPLC method in community hospitals and families[20]. Affinity chromatography is also widely used in the detection of glycosylated hemoglobin (not only HbA1c). This test is not affected by such as HbF, HbS, HbC, formylation Hb and acetylated Hb because of the presence of boric acid group in column[21]. These hemoglobin variants have a great influence on many measurements due to the similar surface charge of variants with HbA1c, for example, the ion exchange method[22-23]. The precursor of HbA1c cannot interfere the measured value of glycosylated hemoglobin in affinity column, as a result the precursor does not need to be removed from samples[21]. However, the affinity column is difficult to operate and takes a long time to get results. Traditional FICTS for HbA1c is simple and meets the requirements of POCT. But the cost is higher because of the scarcity of excellent antibodies for HbA1c and the precursor could affect the test results[24]. Therefore, it is

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necessary to find an ideal, fast, low-cost and convenient analysis method to achieve rapid and accurate detection of HbA1c[25].

In the current study, combining the principle of affinity chromatography, boric acid affinity area was built on the nitrocellulose membrane (NC membrane) of the lateral flow immunoassay[26]. Like many FICTS, quantitative fluorescent signal on test line was the test result and fluorescent signal from control line guaranteed the validity of the test strips, establishing a POCT strip based on sandwich assay. Compared with a new hemoglobin A1c analyzer from Biohermes, Biohermes's instrument can only measure HbA1c in semi-quantitative detection according to the color of hemoglobin, but results obtained from our HbA1c test strips based on fluorescent affinity immunochromatography were more accurate and reliable[27]. We prepared a stable fluorescent microspheres (FMS) for immunoassay by swelling and covalent coupling methods to embed fluorescent molecules into carboxyl polystyrene microspheres and to crosslink Hb antibodies with FMS. The affinity probe BSA-APBA was constructed by covalently coupling bovine serum albumin (BSA) with aminophenylboronic acid (APBA), which was immobilized on the NC membrane to capture the glycosylated hemoglobin. Compared with traditional FICTS, our HbA1c test strips based on fluorescent affinity immunochromatography ensured accuracy and the effectiveness to differentiate negative or positive results (below or above 6.5% of HbA1c), at same time linear correlation was improved. In addition, the fluorescenct affinity immunochromatography rapid diagnostic strips had good repeatability with the clinical results (60 samples) from HPLC. In a sense, our HbA1c test strips based on fluorescent affinity immunochromatography combined the advantages of affinity chromatography and fluorescent immunochromatography with high accuracy and high sensitivity to detect HbA1c. And the test procedures of our strips was simple, rapid and suitable for whole blood, without the complicated pretreatment process and professional requirements for operators.

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Experimental

Materials and Instruments

The fluorescent molecular (Cy5) was purchased from AAT Bioquest, Inc. Carboxyl polystyrene microspheres (10%, w/v) were obtained from JSR Life Sciences. Dimethyl sulfoxide (DMSO), 4-morpholineethanesulfonic acid (MES), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), TritonX-100, Tween-20, the goat-anti-mouse polyclonal antibody and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Sigma-Aldrich Co., LLC. BSA was acquired from Shanghai Yeasen Biotechnology Co., Ltd. The monoclonal antibody for hemoglobin was supplied by Suzhou Lansion Biotechnology Co., Ltd. Monoclonal antibodies for HbA10 (Hb6) and HbA1c (75C9) were purchased from HyTest Ltd. Nitrocellulose membrane was supplied by Millipore. Conjugate pad, absorbent pad, polyvinyl chloride (PVC) board and a complete set of equipment for preparation of strips were obtained from Shanghai Kinbio Tech.Co.,Ltd. APBA was supplied by Aladdin. Human serum albumin (HSA) was purchased from WuHan AmyJet Scientific Inc. The water used in the experiment was pure water.

FMS were characterized with a particle size analyzer (Malvern Instruments Ltd.). The size distributions and surface morphology were observed by scanning electron microscope (SEM) from ZEISS. Fluorescent signals from solid phase was read by fluorescent strips reader Nanoeasy 1700 (NE 1700) (Nanjing Nanoeast Biotech Co.,Ltd.).

Buffer solutions

Buffer solutions used in the study were activating buffer (15mM MES, pH5.5), binding buffer for labeling antibodies on FMS (20mM carbonate buffer, pH9.0), blocking buffer (2% BSA(w/v), pH 8.3), washing and saving buffer for FMS (5mM K_2CO_3 (w/v), 0.375% glycine (w/v), 0.5% casein (w/v), 0.01% sodium azide (w/v)), saving and binding buffer for affinity probe (20mM borate saline buffer, pH 9.0, the hemolytic agent (1.925% ammonium acetate

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(w/v), 0.01% sodium azide (w/v), pH 8.6 using ammonium hydroxide), antibody dilution buffer (20mM phosphate buffer, 0.9%NaCl, pH7.4) and sample pad treatment buffer[28] (100 mmol L^{-1} $Na_2B_4O_7.10H_2O$, 1% PVP (w/v), 0.2% casein (w/v)). All solutions were freshly prepared before use.

Preparation of FMS for immunoassay

FMS were prepared as previously reported with modifications using swelling method[29]. Cy5 (10mg/mL, DMSO) at 13.5μL was added into 486.5μL of DMSO. Carboxyl polystyrene microspheres (10%, w/v) at 100μL were diluted in ultra-pure grade water at 5mL. Then the mixture of Cy5 and DMSO was added into microspheres. The solution was vibrated at 220rpm at 25°C for 5h in glassware. The mixture was centrifuged at 20000g at 25°C for 15min three times. The precipitation was suspended in ultra-pure grade water at 1mL (1% FMS).

After FMS were prepared, we prepared two kinds of microspheres for the traditional FICTS and fluorescenct affinity immunochromatographic test strips with the same way[30]. FMS (1%) at 60μL were centrifuged at 20000g at 25°C for 10min. The precipitation was suspended in 200μL of activating buffer. An EDC/NHS solution including 0.8mg EDC and 0.8mg Sulfo-NHS dissolved in 100μL of activating buffer was added into FMS which was incubated at 37°C for 30min. The mixture was centrifuged at 20000g at 25°C for 10min and the precipitation was suspended in 200μL of binding buffer for labeling antibodies on FMS. The antibody (the monoclonal antibody for HbA10 or the monoclonal antibody for Hb) at 120μg in antibody dilution buffer was added into the latex which was incubated at 37°C for 2 hours. The mixture was centrifuged at 20000g at 25°C for 10min. The precipitation was suspended in 200μL of blocking buffer and incubated at 37°C for an hour. Finally the mixture was centrifuged at 20000g at 25°C for 10min three times and the precipitation was suspended in 200μL of saving buffer (0.3% FMS). The illustration of making FMS was shown in Fig. 1a.

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Construction of affinity probe BSA-APBA

2.68mg BSA was dissolved in 300μL of activating buffer. An EDC/NHS solution including 2mg EDC and 2mg Sulfo-NHS dissolved in 100μL of activating buffer was added into the BSA solution which was incubated at 37 ° C for 30min. The mixture was collected with 30K ultrafiltration tube by centrifugation at 4000g for 10min and the activated BSA solution was added into 160μL of binding buffer. 2.4mg APBA dissolved in 240μL of saving buffer for affinity probe was added into the activated BSA solution which was incubated at 37 ° C for 2 hours. The solution was collected with 30K ultrafiltration tube by centrifugation at 4000g for 10min three times and the obtained affinity probe was dispersed in 400μL of saving buffer (6.7mg/mL BSA).

Whole blood samples

Whole blood samples from a total of 75 patients were kindly provided by Zhongda Hospital Southeast University (Nanjing, Jiangsu, China), the Second Affiliated Hospital of Nanjing Medical University (Nanjing, Jiangsu, China), and Nanjing Drum Tower Hospital (Nanjing, Jiangsu, China). Samples with the test results from HPLC included 25 negative samples (below 6.5% of HbA1c) and 50 positive samples (above 6.5% of HbA1c). All samples were stored at −80°C until use. The study was approved by the ethics committees of three hospitals.

Preparation of test strips

The test strips consists of four parts as shown in Fig. 2a, including the sample pad, NC membrane, absorbent pad and PVC board. The sample pad was treated with sample pad treatment buffer[28]. In traditional FICTS, FMS (3mg/mL) with the antibody for HbA10 were dispensed on the front-end of NC membrane at a rate of 1.2μL/cm using a Kinbio XYZ HM3030 dispenser. The antibody for HbA1c (75C9) and the goat-anti-mouse polyclonal antibody diluted to 3mg/mL and 0.8mg/mL by antibody dilution buffer were respectively dispensed on NC membrane like Fig. 2b at a rate of 0.8μL/cm as the test line and

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control line.

In our test strips, the affinity probe of BSA-APBA (6.7mg/mL BSA) was dispensed on NC membrane like Fig. 2c at a rate of 5μL/cm as the test line. The goat-anti-mouse polyclonal antibody diluted to 0.8mg/mL was also dispensed on NC membrane. The distance between test line and control line was near 3.7mm.

Fluorescence lateral flow assay procedure

In traditional FICTS, 3μL of the whole blood samples was added to 997μL of the hemolytic agent at first and the mixture was incubated at 37°C for 10min. After that, 100μL of as-obtained mixture containing Hb and HbA1c was loaded onto the sample pad. During the sample flow, the analytes were trapped by the HbA10 antibody on FMS to form the composites which were subsequently bound to the HbA1c antibody on the test line. Finally, the fluorescent signals were obtained by fluorescent strips reader NE 1700 after a 10min reaction.

In our test strips, 3μL of the whole blood samples was firstly added to 992μL of the hemolytic agent at 37°C for 10min. Because only 3μL of whole blood as the sample was tested in dilution, the affinity probe on the NC membrane was enough to capture glucose and HbA1c.

Next, 5μL of FMS (3mg/mL) with the Hb antibody was added into the mixture at 37°C for 10min in order to form the composites of FMS-Hb antibody-analytes (Hb and glycosylated hemoglobin). The obtained samples of 100μL were added to the sample pads. During the sample flow, the composites were trapped by BSA-APBA on the test line. After a 10min reaction the fluorescent signals were also recorded by reader NE 1700. In the developed method, FMS labeled by the Hb antibody could capture Hb including HbA1c in the proportion of HbA1c/Hb, therefore, the fluorescent signals on the test line represents the percentage results of HbA1c.

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Results and Discussion

Principle of methods

FMS containing Cy5 dye were firstly prepared by swelling and adsorption method to act as signal amplification label. As shown in Fig. 1c, FMS have uniform and spherical morphology with an average size of 200nm. Dynamic Light Scattering (DLS) measurement indicated a hydrodynamic size of 209 nm with polydispersive index of 0.008 (Fig. 1b). The fluorescence spectra of FMS was shown in Fig. 1d with the fluorescent peak at 671nm which was consistent with Cy5. The stability of FMS was shown in Fig. 1e. The hydrodynamic size and dispersivity of FMS was stable during the six months' storage time.

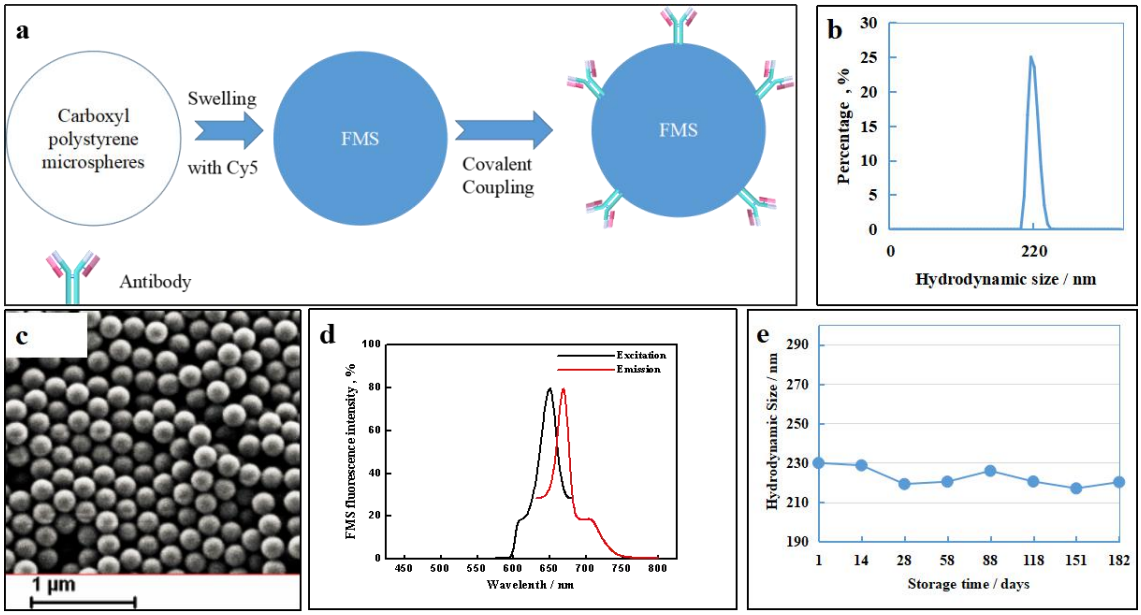

Fig. 1 a, The illustration of making FMS for immunoassay; b, The hydrodynamic size distribution by number; c, SEM image; d, Excitation and emission spectra of FMS; e, The stability of FMS.

The characteristics of affinity probe BSA-APBA was described in Supporting Information (Fig. S1 and Table S1). The data demonstrated the successful preparation of the affinity probe.

The traditional FICTS were illustrated in Fig. 2b using double antibody sandwich method. After adding the hemolytic sample, Hb and HbA1c were firstly trapped in the certain proportion by

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the HbA10 antibody coupled FMS to form the composites, which were subsequently immobilized by the HbA1c antibody on the test line to form a sandwich structure. The residual FMS-antibody conjugates were captured by the goat-anti-mouse polyclonal antibody on the control line and excess FMS and solutions were absorbed at last with the aid of the capillarity of the absorbent pad. After completion of the reaction, the test line and the control line were detected by reader NE 1700 (Fig. 2d). The test line reflected the concentration of HbA1c. The signal value of test line increased with increasing the concentration of HbA1c. The control line reflected the validity of the test strip. It is worth mentioning that the fluorescent signal value (data) of reader NE 1700 was processed by the initial fluorescent intensity (readable) by a standardized formula.

The principle of our strips based on fluorescent affinity immunochromatography for HbA1c was slightly different from the traditional FICTS, which was also based on the sandwich method, as shown in Fig. 2c. After hemolysis of the whole blood sample, FMS coupled with the Hb antibody were incubated with the sample for 10min to ensure sufficient reaction. After adding as-obtained mixture sample, the FMS-Hb antibody-HbA1c composites were captured in the affinity chromatography area because of the ability of boric acid to capure polyhydroxyl groups, which replaced the test line in traditional FICTS. The fluorescent signals obtained by reader NE 1700 at the test line were the results of all subsequent validation experiments.

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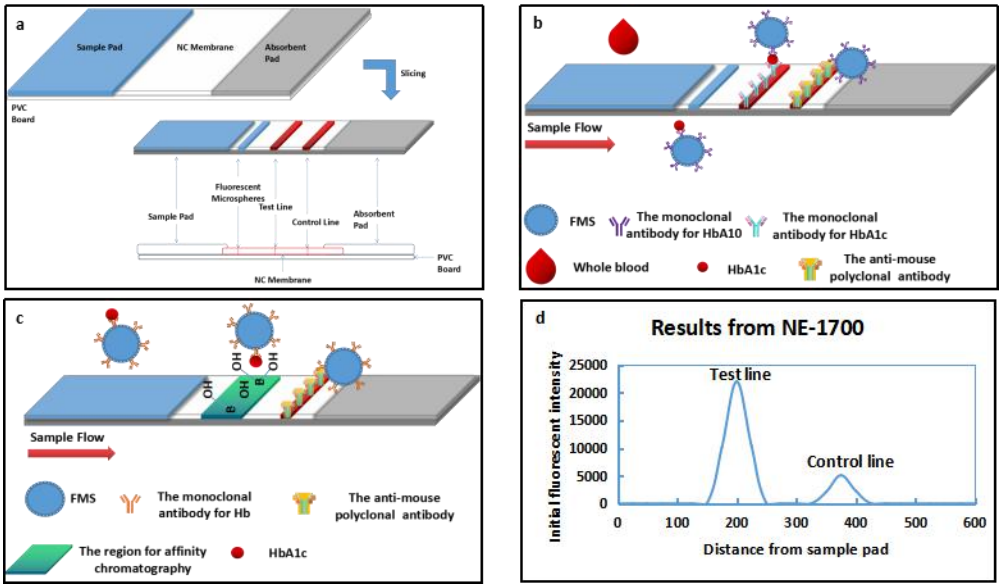

Fig. 2 Schematic illustration of different lateral flow assays based on FMS; a, Components and assembly of lateral flow test strips; b, Schematic illustration of traditional FICTS; c, Schematic illustration of test strips for HbA1c based on fluorescent affinity immunochromatography; d, The initial fluorescent intensity results were detected by reader NE 1700.

Linearity and Precision

The standard curves for traditional FICTS and our fluorescent affinity immunochromatography test strips were constructed based on the measurement of a series of different concentrations of HbA1c (4.7%, 7.4%, 9.8%, 10.8% and 13.8%) from clinical, which were accurately detected by HPLC before we used. The readout curves of the strip based on fluorescent affinity immunochromatography on the reader NE 1700 are shown in Fig. 3a.

After recording the fluorescent intensities, we obtained two standard curves by plotting the fluorescent signals (y) against the concentration of HbA1c (x) as represented by two equations: $y = 10849x - 57.166$, $R^2 = 0.9639$ (traditional FICTS); $y = 3810.6x - 25.15$, $R^2 = 0.9961$ (strips based on fluorescent affinity immunochromatography), and linearity was displayed as shown in Fig. 3b.

In the strips based on fluorescent affinity immunochromatography, the coefficients of variation

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(CVs) were less than 15% based on five duplicated measurements at each concentration (4.7%, 7.4%, 9.8% and 13.8%) as shown in Fig. 3c. The batch-to-batch variation described in Supporting Information was under 16% (Table S2).

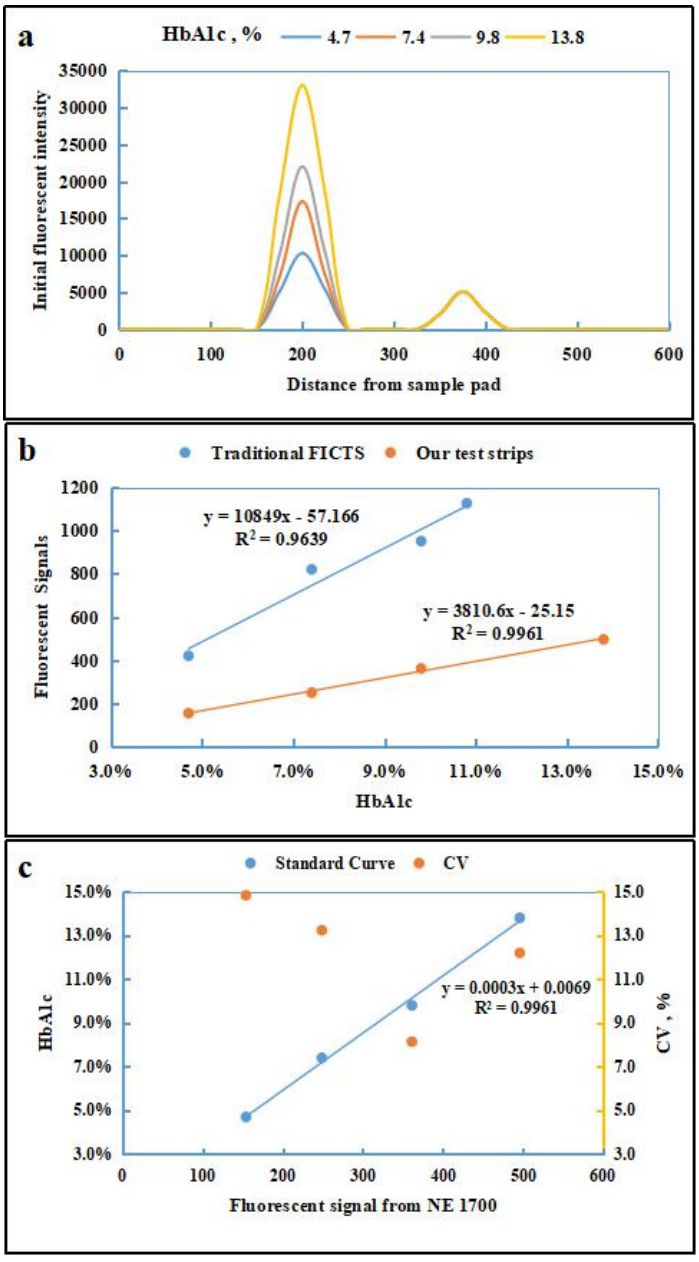

Fig. 3 Linear relationship and CVs measurement; a, The readout curve for strips based on fluorescent affinity immunochromatography at concentration of HbA1c from 4.7% to 13.8%; b, Linearity of two assays; c, The standard curve and CVs for strips based on fluorescent affinity immunochromatography.

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Discrimination

The effective discrimination of samples below or above 6.5% was the key to the rapid test strips for HbA1c. To define the effectiveness of our fluorescent affinity immunochromatography test strips, we analyzed the whole blood samples including 25 negative results (near 4.7%) and 25 positive results (near 7.4%). In addition, 25 serum samples were taken as control group. The test results of negative samples were all negative (25/25), and positive samples were mostly positive (23/25) bounded by the signal value of 200 in the strips based on fluorescent affinity immunochromatography (Fig. 4).

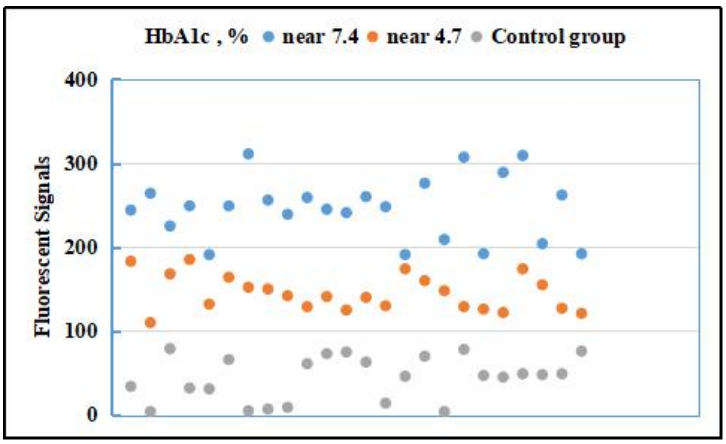

Fig. 4 Discrimination of our fluorescent affinity immunochromatography test strips

HbA1c measurements in whole blood samples from clinical (HPLC)

In addition, for comparison between methods, 60 HbA1c samples were analyzed using both the test strips based on fluorescent affinity immunochromatography and HPLC on clinical. As shown in Fig. 5, a good level of agreement between the two methods was observed. The equation was $y = 1.0245x - 0.0031$, $R^2 = 0.9516$, n=60, where y represented the concentration of HbA1c obtained by the test strips and x was the concentration obtained by HPLC. Results from the test strips based on fluorescent affinity immunochromatography had good consistency with the clinical results (60 samples) from HPLC. The results suggested that the test strips based on

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fluorescent affinity immunochromatography were effective for the quantitative determination of HbA1c in whole blood samples.

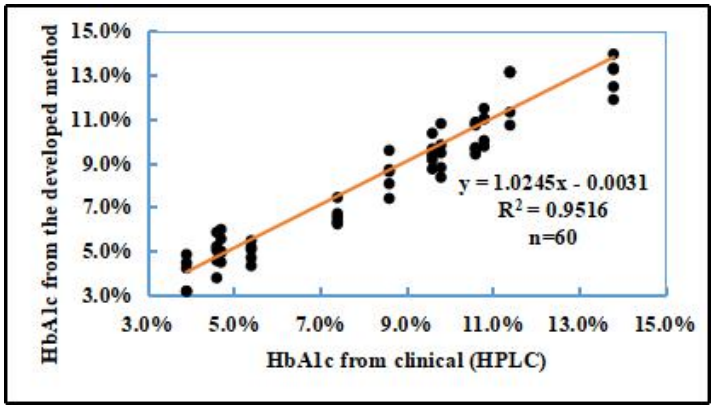

Fig. 5 Comparison of different methods for measuring the concentration of HbA1c using the developed method and HPLC.

Cross-reactivity and interference experiments

We verified the effects of several types of reagents including proteins (BSA, HSA), surfactants (Triton x-100, Tween-20) and glucose on the strips based on fluorescent affinity immunochromatography (Fig. 6). On the one hand, we needed to know whether there would be false positive, so the sample (0.1% solution of the above regents) was measured on the strips with negative results (the average fluorescent signals of ordinary zero samples were 45). On the other hand, we wondered whether there is a false negative, so 13.8% of HbA1c in the hemolytic agent containing the above reagent (0.1%) were tested (the average fluorescent signals of 13.8% of HbA1c were 496). As a result, we found that surfactants (Triton x-100, Tween-20) would slightly decrease the fluorescent signal value, because surfactants would wash off the BSA-APBA from the NC membrane. At the same time, glucose significantly reduced the value of fluorescent signals, because excess glucose competed with HbA1c in affinity chromatography.

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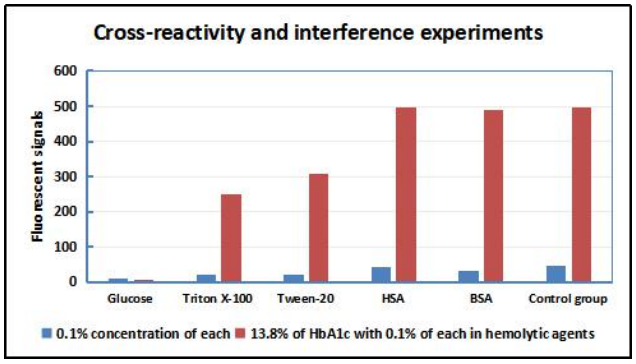

Fig. 6 Cross-reactivity and interference experiments on the strips based on fluorescent affinity immunochromatography

HbA1c is an effective indicator for clinical diagnosis of diabetes[7-8]. It is also a "gold standard" for long-term assessment of diabetics[9-11]. However, there are only a few methods to quantitatively analyze HbA1c level in whole blood with plenty of limitations such as requiring expensive instrumentation, complexity of the procedures, and long operation times[20-21]. These methods cannot meet the current requirements for the detection of HbA1c.

Traditional FICTS based on the double antibody sandwich method effectively optimize the processing steps, shorten the testing time and meet the requirements of the POCT. However, test results with narrow linear range (Fig. 3b) will be affected by the precursor of HbA1c [22-24]. It is also notable that high quality antibodies for HbA1c are rare and expensive.

In the past decade, affinity chromatography has been used to detect glycosylated hemoglobin in clinical departments. Although its procedures are complex and the time for obtaining results is long, its results are accurate in accordance with clinical requirements[31-32]. In this paper, we design a test strip for rapidly diagnosing glycosylated hemoglobin (HbA1c) based on fluorescent affinity immunochromatography, which can be used to quantitatively detect HbA1c, and the strips only need 3μL of the whole blood sample. This kind of strips based on the sandwich method combine the advantages of affinity chromatography and fluorescent immunochromatography, and ensure the operating time within 30min which meets the

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requirements of POCT. Compared with the color results of hemoglobin in affinity chromatography, the strips can improve accuracy and reduce interference by quantitative fluorescent signals. Compared with traditional FICTS, the strips have larger linear range (3%-13.8%) and good linear correlation (Fig. 3b), exclude the impact of precursor and save the cost of the antibodies for HbA1c. Moreover, the strips ensure a good accuracy (CV<15%, Fig. 3c) and the discrimination (Fig. 4) on the cutoff value (6.5%) of HbA1c. In comparison with clinical samples measured by HPLC, good consistency is still demonstrated on the strips (Fig. 5).

Conclusions

In summary, we have successfully constructed a test strip for HbA1c based on fluorescent affinity immunochromatography using the affinity probe to replace the monoclonal antibody for HbA1c on the test line in traditional FICTS. The developed method could improve the linear correlation compared with traditional FICTS and rapidly provide qualitative results of HbA1c. The assay is convenient, rapid (only 30 min) and low in cost. The method which is consistent with clinical (HPLC) test results has excellent linearity, accuracy, and good discrimination of positive and negative samples. This kind of strips has its unique advantages and good performance on detecting HbA1c, which can provide a new method for the clinical detection of HbA1c.

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Supporting Information

APBA had a strong absorption peak between 260nm and 300nm due to the presence of benzene ring and the presence of the chromophore (amino). APBA was faint yellow and the absorption of APBA in the visible band increased gradually from 700nm to 400nm. The standard curve of APBA was represented by the equation: y=8.5443x+0.0508, in which y represented the absorption peak at 287nm and x represented the concentration of APBA (mg/mL). The affinity probe BSA-APBA was characterized by UV-Vis absorption spectrum as shown in Fig. S1. In the visible band, BSA-APBA (6.7mg/mL BSA) had absorption, while the absorption value of BSA with the same concentration was near 0. The BSA-APBA was faint yellow, while BSA (6.7mg/mL) was colorless under visual observation. The conjugating rate of BSA on APBA was calculated by analyzing the percolate in the last procedure of construction of affinity probe (Table S1). The conjugating ratio was 10.12%, that is, 1mg BSA could be coupled with 0.036 mg APBA .

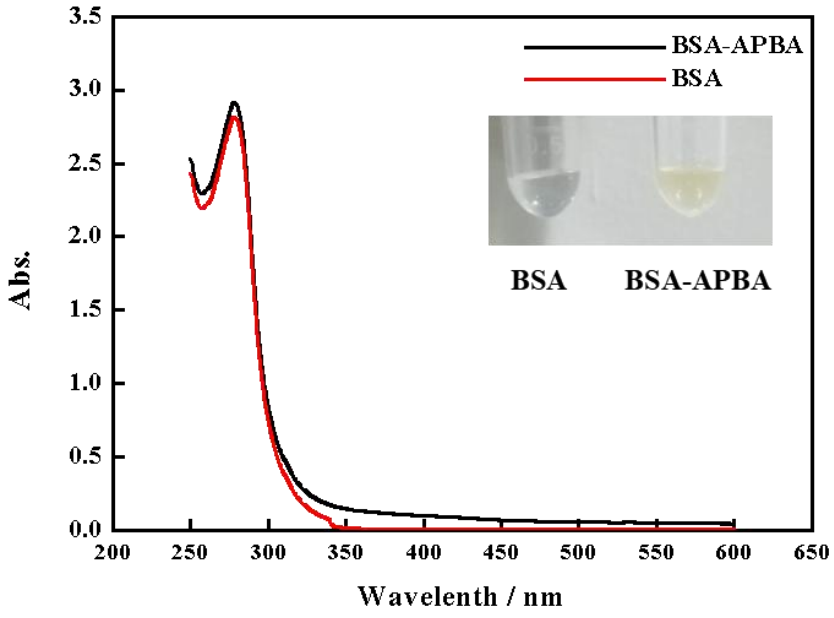

Fig. S1 UV-Vis absorption spectrum of affinity probe BSA-APBA and BSA

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Table S1 The conjugating rate of APBA on BSA

	The absorption at 287nm	APBA in percolate / mg
The first percolate	2.35	1.77
The second percolate	0.51	0.35
The third percolate	0.10	0.04
Conjugating rate	=(2.40-1.77-0.35-0.04)/2.40=10.12%	

Three HbA1c samples in different concentration (4.7%, 7.4% and 9.8%) were tested under five duplicated measurements by three different batches strips. As shown in Table S2, the batch-to-batch variation was under 16%.

Table S2 The batch-to-batch variation of the developed method

Batches	4.7%	7.4%	9.8%
1	183	244	332
	110	264	398
	168	225	302
	185	249	386
	132	191	389
2	153	229	377
	152	220	302
	145	218	318
	133	258	361
	175	230	329
3	140	288	372
	140	266	365

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	135	259	386
	116	280	350
	154	213	330
Batch-to-batch variation , %	16.0	12.0	10.0

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Figure Captions

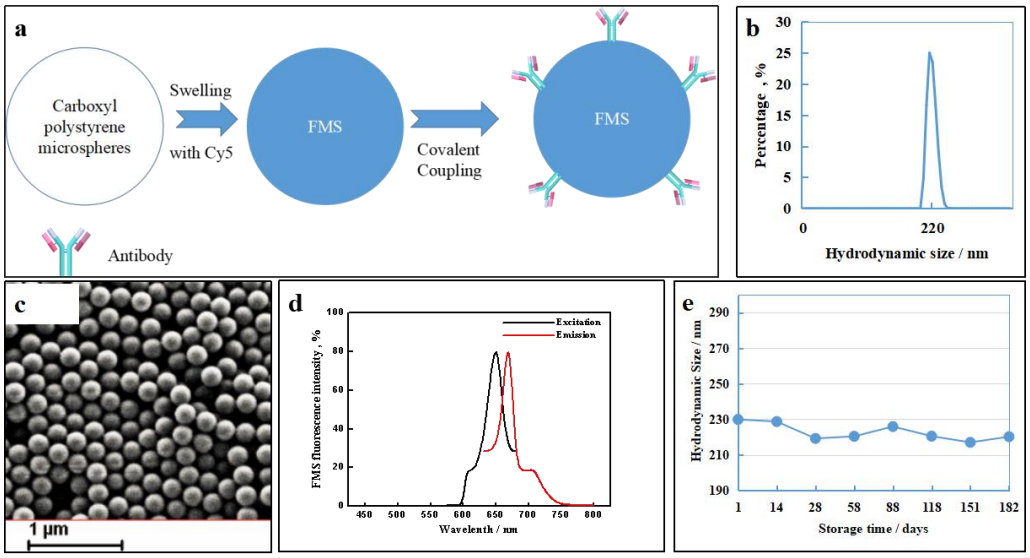

Fig. 1 a, The illustration of making FMS; b, The hydrodynamic size distribution by number; c, SEM image; d, Excitation and emission spectra of FMS; e, The stability of FMS.

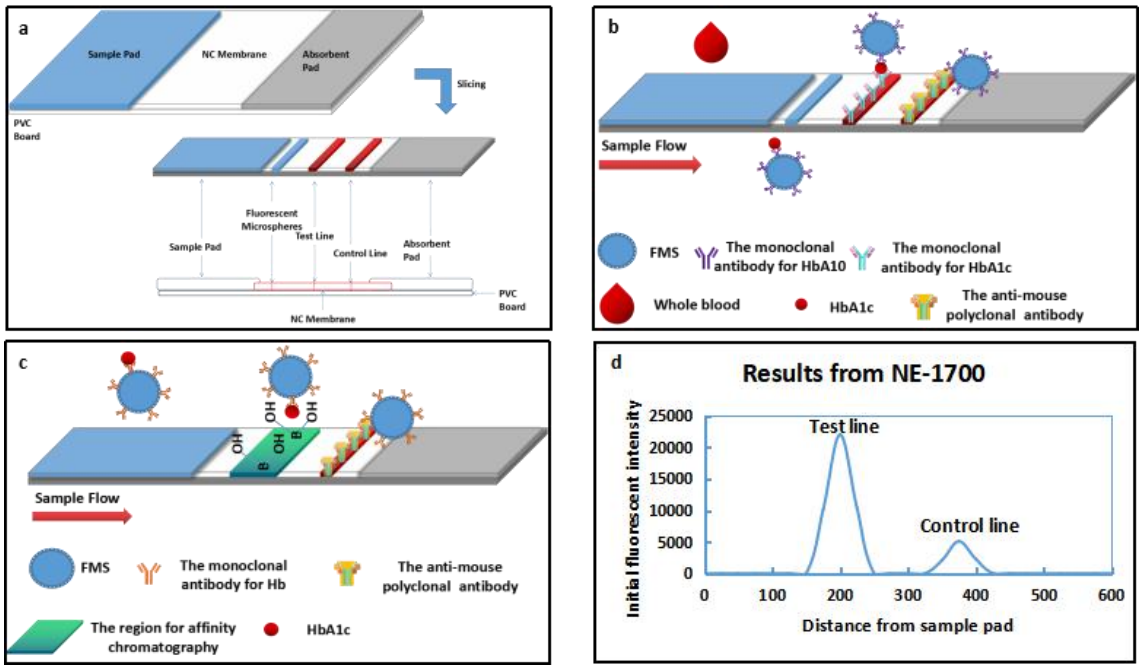

Fig. 2 Schematic illustration of different lateral flow assays based on FMS; a, Components and assembly of lateral flow test strips; b, Schematic illustration of traditional FICTS; c, Schematic illustration of test strips for HbA1c based on fluorescent affinity immunochromatography; d, The initial fluorescent intensity results were detected by reader NE 1700.

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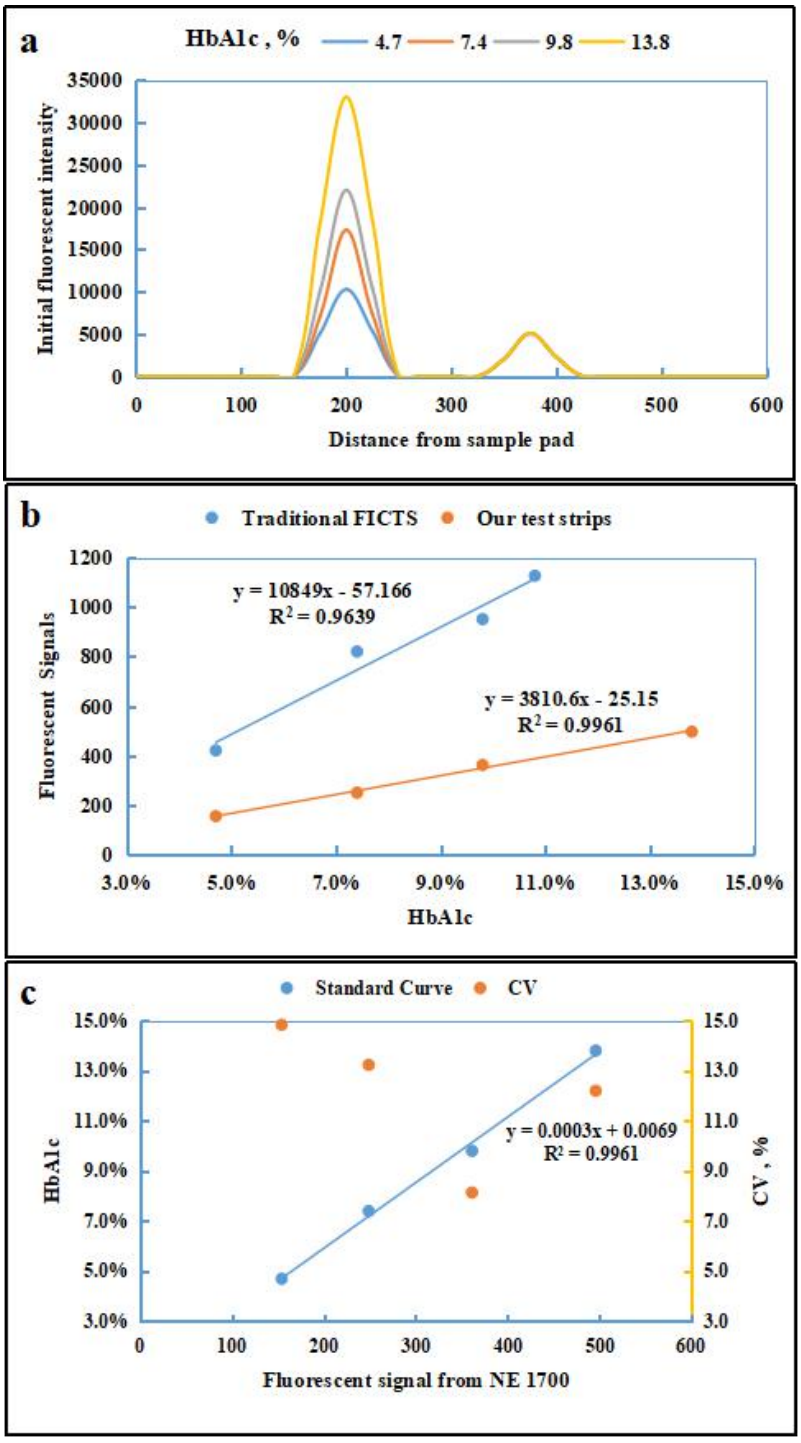

Fig. 3 Linear relationship and CVs measurement; a, The readout curve for strips based on fluorescent affinity immunochromatography at concentration of HbA1c from 4.7% to 13.8%; b, Linearity of two assays; c, The standard curve and CVs for strips based on fluorescent affinity immunochromatography.

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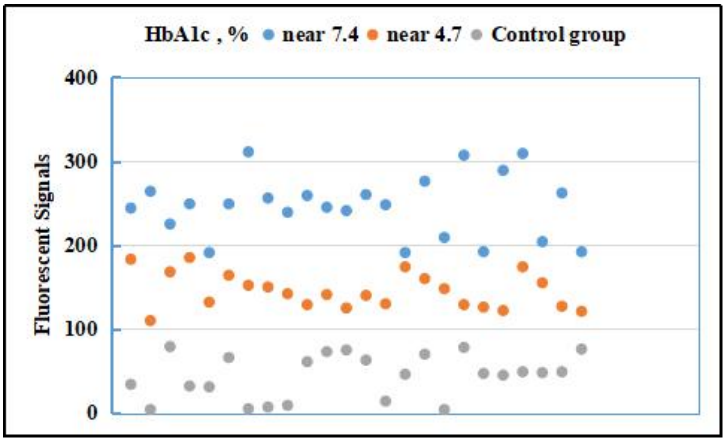

Fig. 4 Discrimination of our fluorescent affinity immunochromatography test strips

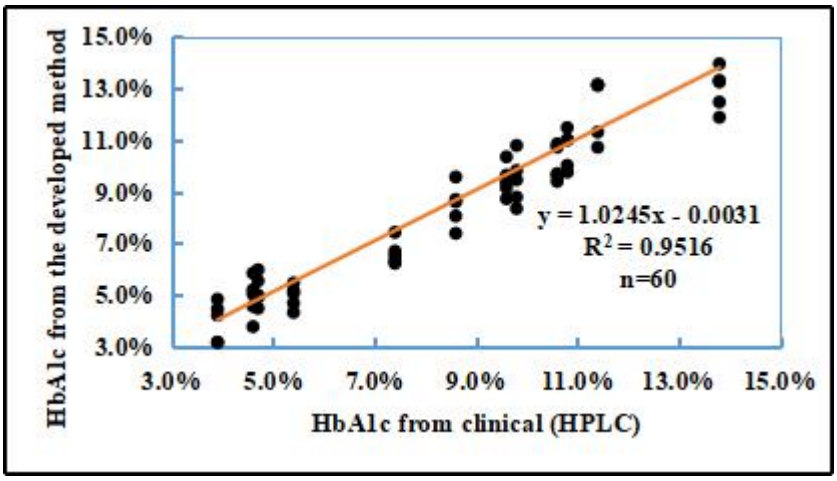

Fig. 5 Comparison of different methods for measuring the concentration of HbA1c using the developed method and HPLC.

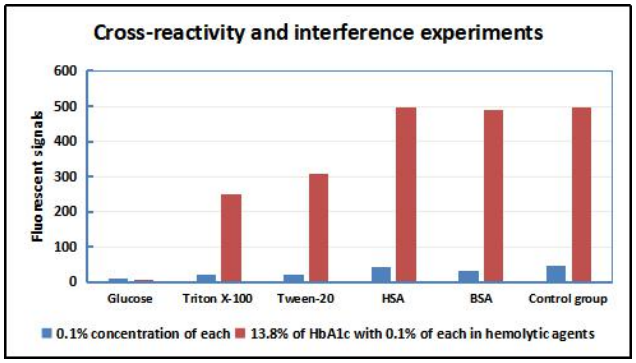

Fig. 6 Cross-reactivity and interference experiments on the strips based on fluorescent affinity immunochromatography

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