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Optic fiber-based immunosensor for rapid and sensitive hepatitis C virus detection in serum

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Abstract: A compact and portable all-fiber evanescent wave immunosensor was developed for sensitive on-site hepatitis C virus (HCV) detection. In this system, HCV antigen was coated on the surface of the fiber probe. The evanescent wave generated at the surface of the probe excited the fluorescent molecules bound to the surface through antibody–antigen reactions and coupled back the corresponding fluorescent signal. The performance of the fiber biosensor was compared with that of conventional ELISA using serial dilutions of HCV-positive serum. The sensitivity of the fiber biosensor system was slightly better than that of ELISA, and detection only took 10 min. The fiber probes could also be reused many times with very little performance loss.

1. Introduction

Hepatitis C is a global infectious disease caused by the hepatitis C virus (HCV). World Health Organization stated that the global infection rate of HCV is about 3%. Approximately 3.15 million people are infected with HCV every year. Most infected people show no symptoms, and few patients with acute hepatitis C show symptoms lighter than those with acute hepatitis A and B. The infected patients unfortunately develop chronic infection, and a substantial proportion of patients have high probability of developing cirrhosis or even liver cancer.

Two main methods of detecting HCV, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were currently being used in HCV diagnosis.[1] Because it was quick, inexpensive, and automated, ELISA was the main method used in hospitals for HCV diagnosis. But there was still a high prevalence of false-positive results, especially among immune-compromised patients or populations without liver-related diseases,[2, 3] leading to unnecessary health-care costs and diagnosis puzzles. The PCR assay, although highly sensitive and specific, involved multiple procedural skills, specialized technical expertise, dedicated laboratory spaces,

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and it was time consuming even when simplified by automation. [4]

Therefore, developing a simple, rapid, sensitive, and specific detection method for early hepatitis C diagnosis is particularly important because it facilitates the early identification of hepatitis C infection and rational establishment of drug treatment programs. [5]

Evanescent wave fiber biosensors based on the principle of total internal reflection fluorescence had attracted much attention because of their rapid, highly specific and sensitive detection. To date, various detection targets including heavy metal ions, [6] small molecules, [7, 8] DNA, [9, 10] and proteins [11, 12] had been reported. However, few literatures involved the application in disease diagnosis, in part, because of the challenges in interference from clinical sample matrix background. [13] Recently, Konry and co-workers developed an optical fiber biosensor for the detection of hepatitis C virus. The photochemically modified fiber optics were tested as immunosensors. Antibody analyte was measured through chemiluminescence reaction.[14]

In this study, a portable evanescent wave all-fiber immunosensor for rapid and sensitive detection of Hepatitis C virus in serum had been developed. The all-fiber immunosensor utilizing a single-multi-fiber optic coupler had less optical components and no longer required optical alignment, which enhanced the efficiency of light transmission and improved the S/N ratio. This immunosensor was modified with high-affinity and highly specific HCV chimeric multi-epitope antigen to recognize the HCV antibody in serum. The real-time fluorescence signal excited by the evanescent wave displayed the dynamic process of the affinity recognition interaction between the antibody and the antigen, and thus provided reliable quantitative results for diagnosis of hepatitis C. Herein, the sensing principle and the platform configuration were introduced. The sensing time, sensitivity, specificity and reusability of the immunosensor were evaluated.

2. Experimental

2.1 Ethics Statement

The assay performed in this study did not involve human participants or human experimentation. The only human materials used were HCV serum samples collected from hepatitis C patients. Informed consent was obtained from the patients before the procedures were performed. Ethical approval for this research was obtained from the Research Ethics Committee, Academy of Military Medical Sciences, People's

Republic of China.

2.2 Materials and reagents

Bovine serum albumin (BSA) and (3-aminopropyl) triethoxysilane (APTES) were purchased from Sigma-Aldrich (Germany). Cy5.5-labeled goat polyclonal secondary antibody to human IgG was obtained from Abcam (England). Clinical HCV serum samples were collected from the Southwest Hospital of China. All solutions were prepared with ultrapure water from a Millipore Milli-Q system. All other salts and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (China), unless otherwise specified. All chemicals were of analytical reagent grade. Recombinant HCV antigen[15] was supplied by the Institute of Basic Medical Sciences, Academy of Military Medical Sciences.

2.3 Antigen preparation

In brief, the HCV antigen epitope was analyzed using BioSun software, and the fragment genes (HCVC, NS3, NS4, and NS5) were amplified by PCR. These fragments were linked together and inserted into the prokaryotic expression vector. The HCV chimeric antigen gene was expressed in *Escherichia coli* and then purified by ion-exchange chromatography.

2.4 Instrumentation

The schematic of the all-fiber evanescent wave biosensor was shown in Fig. 1. The laser beam from a 635 nm-pulse diode laser (BWT, Beijing, China) with pigtail was directly launched into the single-mode fiber of the single-multi-mode fiber coupler (Beijing Glass Research Institute, China), which reduced the optical components and eliminated the need for optical alignment.[16] The excitation light from the laser, through the fiber connector, was coupled to a fiber probe. The incident light propagated along the length of the probe via total internal reflection. The evanescent wave generated at the probe surface, interacted with the fluorescent-labeled analyte complexes bound to the surface, and excited the fluorophores. The collected fluorescence was subsequently filtered using a bandpass filter (FF01-692/40; Semrock, USA) and detected using a photodiode through the lock-in amplifier system. The probe was embedded in a glass flow cell with a flow channel having dimensions of

60 mm length and 2 mm diameter. All reagents were delivered by a flow delivery system operated with a peristaltic pump. The fluid delivery system and data processing were automatically controlled using a computer.

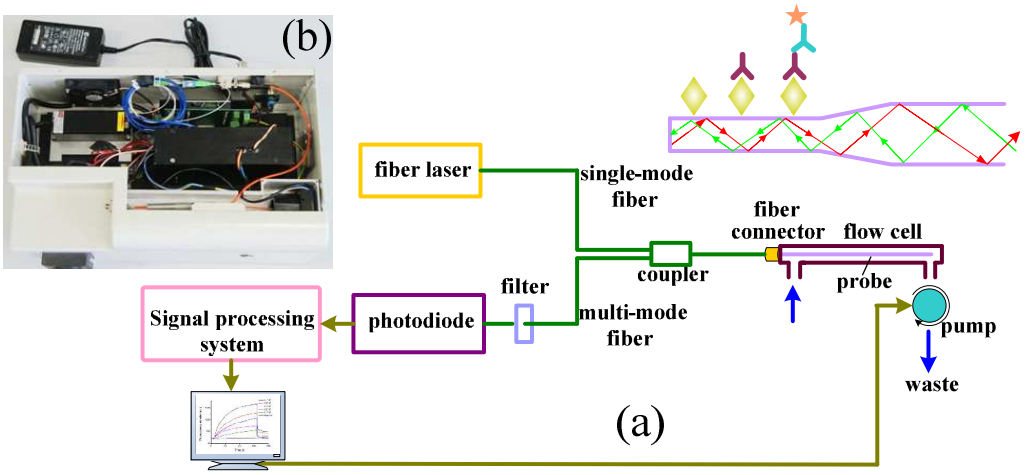

Figure 1. Schematic of the evanescent wave fiber biosensor system. (a) Principal scheme of the fiber biosensor and (b) the real-life image of the evanescent wave fiber biosensor.

2.5 Fiber probe preparation

The probes were made of a step-index silica optical fiber of 11 cm length and 600 μm diameter (Chunhui Science and Technology Industrial Co., China). The cladding was removed 6.5 cm from the distal end to form the sensing region. This region was immersed in 30% hydrofluoric acid solution and etched for about 2 h to 3 h until the diameter of the region reached approximately 225 ± 5 μm.[17–19] The lengths of the tapered section and the sensing region were about 0.3 and 6.0 cm, respectively. The combination-tapered fiber probes were sequentially immersed in H_2SO_4/30% H_2O_2 (3:1 volume ratio), 25% NH_4OH/30% H_2O_2/water (1:1:5 volume ratio), and 36% HCl/30% H_2O_2/water (1:1:5 volume ratio). Finally, the probes were sonicated with water and dried using N_2. Fig. 2 shows the modification process of the fiber probe. The cleaned fiber probes were aminated by incubating the probes in a solution of 12 mL of 95% ethanol, 12 μL of acetic acid, and 150 μL of APTES at room temperature for 30 min. The probes were then sonicated in 95% ethanol for 4 min and dried using N_2. The aminated fiber probes were incubated in 10% glutaraldehyde solution for 1 h and rinsed. The aldehydized fiber probes were incubated in

100 μg/mL solution of recombinant HCV antigen prepared in PBS, pH 7.4 at 4 ℃ overnight. The sensor was rinsed with PBST and dipped in a 2 mg/mL BSA solution for 1 h to block the nonspecific absorption sites.

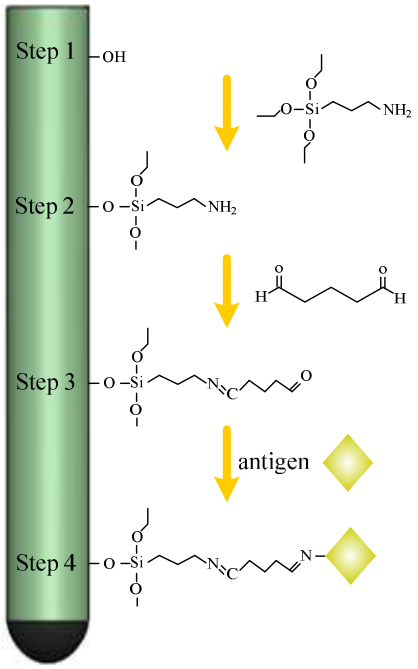

Figure 2. Schematic of fiber probe modification. The fiber probe surface was hydroxylated (Step 1) and then aminated by incubating it with APTES (Step 2). Glutaraldehyde was used to form a covalent bond (Step 3). Step 4 shows the immobilization of the recombinant HCV antigen on the aldehyde group-modified fiber probes.

2.6 Sensor immunoassay experiment

Serum samples from HCV-positive patients diluted in PBS buffer were pumped into a 200 μL flow cell at 600 μL/min flow rate. The HCV antibodies from the serum were allowed to bind to the antigen immobilized on the fiber probe for 6 min at room temperature, and the fiber probe was rinsed with PBST. The flow cell was subsequently introduced with 1 μg/mL Cy5.5-labeled human secondary antibody. The real-time fluorescence signals of Cy5.5 were measured using the all-fiber biosensor. A 0.1 M glycine–HCl solution (pH 2.0) was used to dissociate the bound HCV antibody for the regeneration of the fiber probe surface.

2.7 ELISA

Human HCV ELISA kit (Rapidbio, USA) was used to detect HCV in the serum

sample, according to the manufacturer's instructions. Diluted serum samples and HRP conjugate reagent were added in triplicate to each well of the HCV antigen-coated microelisa stripplate. The plate was incubated at 37 °C for 60 min and washed. TMB peroxidase substrate was then added and incubated at 37 °C for 15 min before adding the stop solution. The stop solution changed the color of the liquid from blue to yellow, and the optical density (OD) value of each well was measured at 450 nm wavelength using a Bio-Rad model 680 microplate reader within 15 min.

3. Results and discussions

3.1 Effect and specific detection of fiber probe modification

The effect of fiber modification was evaluated by a positive control experiment. The fiber probe coated with 100 μg/mL human IgG, as the positive control probe, was embedded in the flow cell. A 200 μL of 1 μg/mL Cy5.5-labeled secondary antibody to human IgG was pumped into the flow cell. The human IgG coated on the fiber probe specifically bound with the Cy5.5-labeled secondary antibody. The laser light from the fiber probe excited the Cy5.5 dye to produce strong fluorescence signal (shown in blue in Fig. 3), which showed that the fiber probe was successfully modified and could be used for analysis and detection.

Sera from HCV-positive and HCV-negative patients were detected using the fiber biosensor system to further assess the specificity of the fiber probes. The fiber probe coated with HCV antigen was embedded in the flow cell. The HCV-positive and HCV-negative sera were diluted 100-fold with PBS (0.01 M, pH 7.4) containing 0.1% BSA. Immunofluorescence experiments were conducted with the diluted positive and negative sera, and PBS solution was used as the blank control. The variations of the fluorescence signals of the three samples were detected in real-time. Fig. 3 shows that the fluorescence signal of the positive serum was significantly stronger than those of the negative serum and blank control, which exhibited almost no fluorescence signals. The results showed that the fiber probes had good specificity.

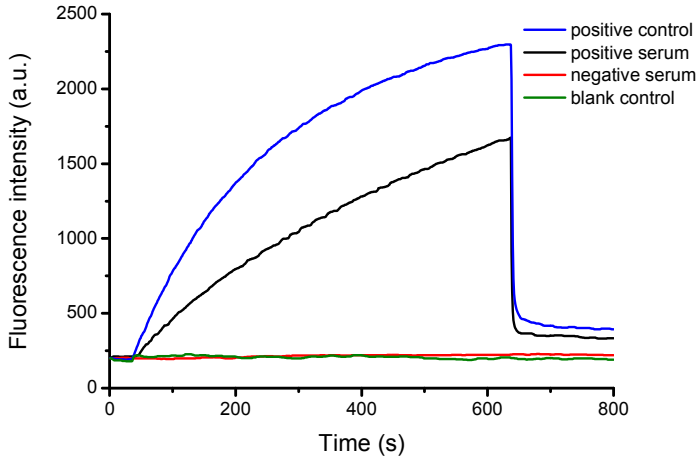

Figure 3. Performance and responses of the sensor system to various controls. The positive control was used to evaluate the effect of fiber probe modification. The positive and negative sera were the detection results of the sera from HCV-positive and HCV-negative patients, respectively. The sera were diluted 100-fold with PBS. PBS solution was used as the blank control.

3.2 Binding kinetics

The kinetics of antibody binding at the fiber probe surface was measured to determine the rate and amount of time required for the immunoassay-based fiber biosensor. HCV-positive serum (200 μL) diluted in PBS was initially pumped into the flow cell. Cy5.5-labeled secondary antibody (1 μg/mL) was then added after 6 min of incubation. Fig. 4 shows that the detected fluorescence signal exponentially increased over time as the HCV antibody bound on the fiber probe reacted with the Cy5.5-labeled secondary antibody and then gradually reached a plateau after about 10 min. The curve fitting showed that the detected fluorescence signal met the following equation:

$$I(t) = A(1 - \mathrm{e}^{-kt}) + b$$

where k is the binding rate constant, and $I(t)$ is the fluorescence intensity at time t. The calculated binding rate constant of the fiber probe surface was 1.9×10^{-3} s^{-1}, which was at least five times faster than conventional ELISA.[20] Thus, fiber biosensors completed the binding reaction between an antibody and an antigen within minutes instead of hours in conventional immunoassay.

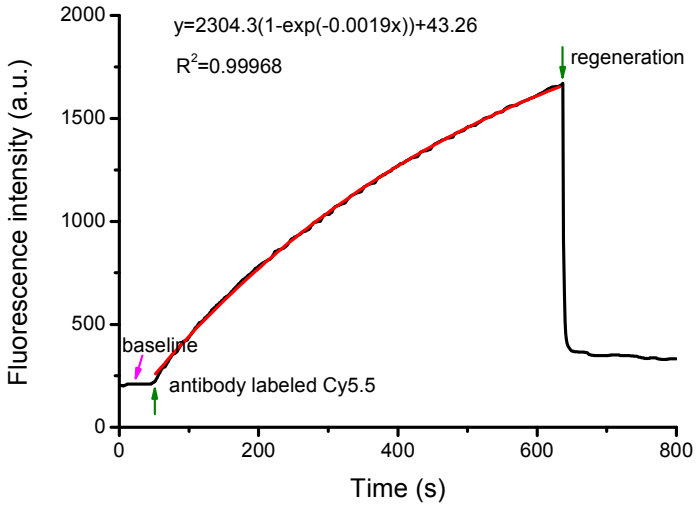

Figure 4. Binding kinetics curves of the fiber optic probe surface. The fluorescence signal was observed after short background measurement by introducing 1 μg/mL Cy5.5-labeled secondary antibody to the fiber probe surface-bound HCV antibody. Signal curve fit was applied (red line).

3.3 Fiber biosensor sensitivity

Experiments were conducted using HCV-positive serum samples of various concentrations to test the sensitivity of the fiber biosensor system. Serum from an HCV-positive patient was serially diluted 100- to 10000-fold (1:100, 1:300, 1:1000, 1:3000, and 1:10000), which was assayed using the fiber biosensor and ELISA to compare the sensitivity of the two detection methods. Fig. 5A shows that the fiber biosensor system detected the positive serum diluted 10000-fold. The detection limit of biosensors is usually defined as three times the standard deviation of the mean blank values. The experimental results showed that the fiber biosensor could theoretically detect 20000 times serum dilution. Further numerical analysis showed that the detected fluorescent signal exponentially increased with high dilution rates (Fig. 5B). Fig. 5C shows the results of ELISA. The curve fit accorded similarly to the exponential growth. Fig. 5C shows that the detection limit of ELISA was 1:10000. The comparison of the signals and detection limits of the two different detection methods indicated that the detection sensitivity of the fiber biosensor was slightly better than that of ELISA.

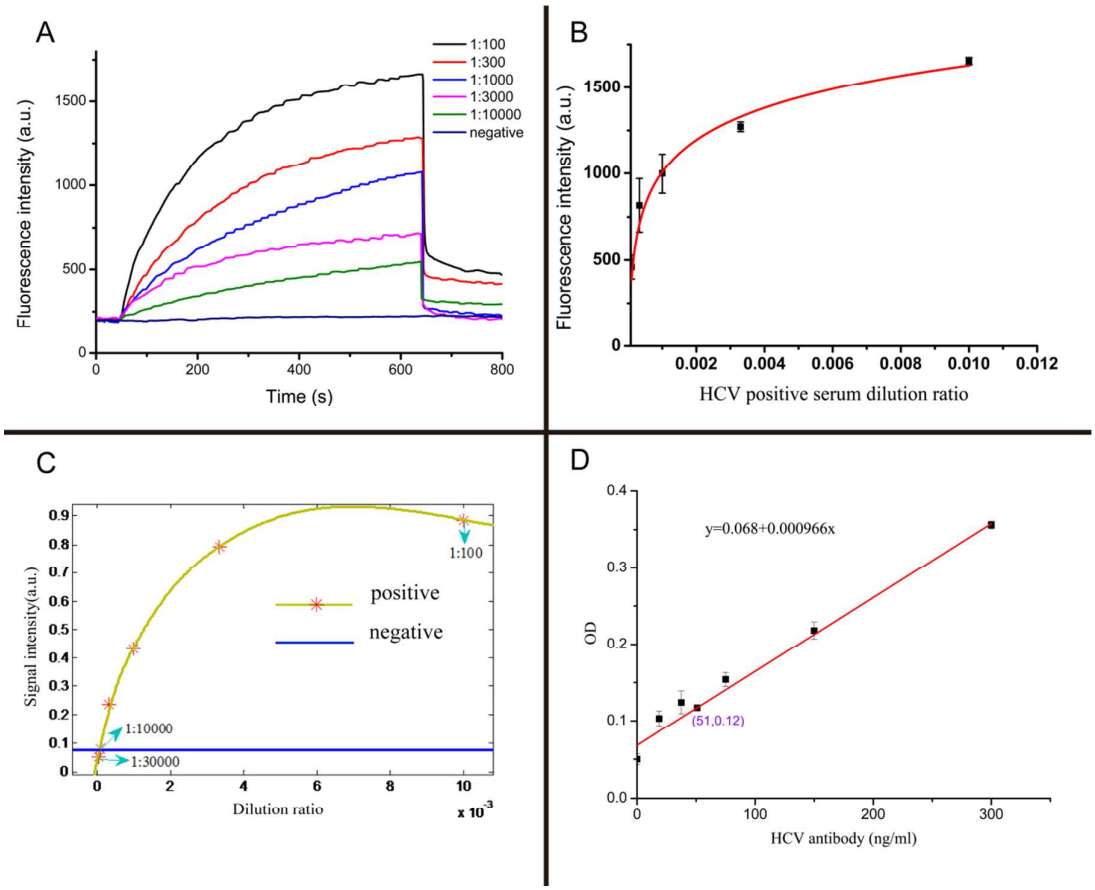

Figure 5.Comparison of fluorescence and ELISA sensitivity. (A) Sensitivity of the fiber biosensor system to serum serial dilutions from an HCV-positive patient. (B) Fluorescence signals exponentially increased with dilution rates of HCV-positive serum. (C) Sensitive detection of ELISA to serum serial dilutions. (D) Standard linear regression curve during the experiment in which the serum samples were diluted 10-fold.

The absolute concentrations of the HCV antibody in the positive serum samples were quantified to further determine the sensitivity of the fiber biosensor system. A set of calibration standards (300, 150, 75, 37.5, 18.7, and 0 ng/mL) was assayed at the same time as the samples using the ELISA kit, and a standard curve of OD versus HCV concentration was produced. The concentration of HCV antibody in the serum samples was then determined by comparing the OD of the samples to the standard curve. The standard curve showed that the concentration of HCV antibody in serum was 51 ng/mL. During the experiment, the serum samples were diluted 10-fold; thus, the original antibody concentration in the serum samples was 510 ng/mL. The serum sample was diluted 10,000-fold when comparing the fluorescence and ELISA performance. Thus, the detection limit of the fiber biosensor system is 51 pg/mL.

3.4 Fiber biosensor regeneration

The reusability of the sensing interface is an important factor for the practical implementation of biosensors. This study investigated the regeneration performance of the fiber probe surface.[21–23] The HCV antibody in the serum was initially incubated with the antigen coated on the fiber probe for 6 min during each cycle. The Cy5.5-labeled secondary antibody was then added and reacted for 10 min. SDS glycine–HCl solution (0.1 M, pH 2.0) was finally used to achieve regeneration and remove all remaining antibodies on the fiber probe surface. Fig. 6 shows that the loss of performance was less than 7% after five consecutive reactions. The regeneration cycles reached more than 10 times with larger signal loss.

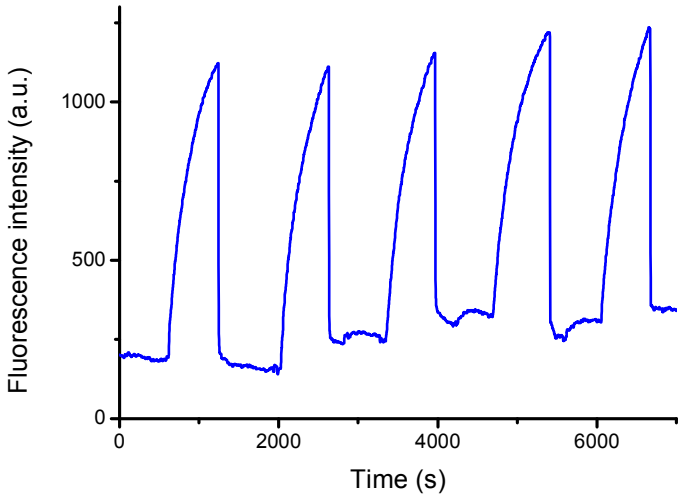

Figure 6. Regeneration detection of the fiber biosensor system. The fiber probe surface was regenerated with 0.1 M SDS glycine–HCl buffer (pH 2.0) for 5 min at each cycle.

4. Conclusions

A fiber-based immunosensor was used to develop an HCV diagnostic tool with high specificity and sensitivity. The sensitivity of the fiber-based biosensor system was comparable to that of ELISA. The fiber-based immunosensor completed a test cycle between an antibody and an antigen in over 10 min because of the fast binding kinetics of its probe surface. The regeneration technique of the optical fiber immunosensor was also investigated. The fiber optic probes could be regenerated using the optimization 0.1 M SDS glycine–HCl regeneration solution (pH 2.0) for at least five times, and the signal loss was less than 7%. The all-fiber biosensing platform presented in this study provided an important practical screening technology

that advances infectious disease diagnosis. It was easy to use, had simple, inexpensive, fast, and compact features, and could be widely applied in medicine, biology, environmental science, and other fields.

Acknowledgments

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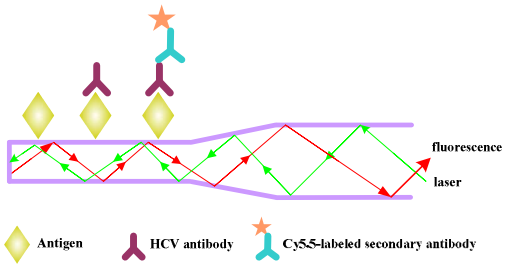

A portable optic fiber-based immunosensor is developed to achieve the rapid and sensitive hepatitis C virus detection in serum.