An automated microfluidic chemiluminescence immunoassay platform for quantitative detection of biomarkers

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Abstract

A rapid, sensitive and quantitative biomarker detection platform is of great importance to the small clinic or point-of-care (POC) diagnosis. In this work, we realize that an automated diagnostic platform mainly includes two components: (1) an instrument that can complete all steps of the chemiluminescence immunoassay automatically and (2) an integrated microfluidic chip which is disposable and harmless. In the instrument, we adopt vacuum suction cups which are driven by linear motor to realize a simple, effective and convenient control. The method of acridine esterification chemiluminescence is adopted to achieve a quantitative detection, and a photomultiplier tube is used to detect photons from acridine ester producing in alkaline conditions. We use the laser cutting machine and hot press machine to accomplish the product of microfluidic chips. The automated microfluidics-based system is demonstrated by implementation of a chemiluminescence immunoassay for quantitative detection of ferritin. We observe a linear relationship between CL intensity and the concentration of ferritin from 5.1 to 1300 ng mL⁻¹ and the limit of detection (LoD) is 2.55 ng mL⁻¹. At the same time, we also used the automated microfluidics-based system to test clinical serum samples. The whole process of chemiluminescence experiment can complete within 45 min. We realize that this lab-on-a-chip chemiluminescence immunoassay platform with features of automation and quantitation provides a promising strategy for POC diagnosis.

Keywords Chemiluminescence · Microfluidic chip · Acridine ester · Ferritin

1 Introduction

The lab-on-a-chip (LoC) platforms have made significant progress in diagnosis in the past years, due to its inherent advantages such as reduced consumption of reagents, highly integrated operation of the experiment, rapid detection of

multiple biomarkers (Gervais et al. 2011; Gorkin et al. 2010; Nahavandi et al. 2014; Ng et al. 2010). There are some LoC platforms which have been applied for a variety of biomarkers including proteins, (Fan et al. 2008; Lee et al. 2013; Li et al. 2013; Liu et al. 2009, 2011; McRae et al. 2016; Nie et al. 2014) nucleic acids, (Cai et al. 2014; Chen et al. 2016, 2017; Fang et al. 2011; Gan et al. 2014; Liu et al. 2017; Zhang et al. 2011; Zhuang et al. 2016) and cells (Hyun et al. 2015; Shen et al. 2014) in blood or urine with the characteristics of the high sensitivity and high throughput, but few of them can be of the marketization.

The main challenges we face in LoC platforms are how to transport the fluids in microchannels precisely (Li et al. 2014; Song and Shum 2012) and design an automated instrument which can sensitively detect the biochemical signal inside the reaction reservoir and provide an quantitative result. Now most drive using peristaltic pump make the vent hose directly connect with the chip (or related links of pillar on chip), which makes the chip production process more difficult and the experiment operation more complicated. Centrifugal forces propel liquids radially outwards, which is considered a major

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limitation of centrifugal platforms, as the radial path provided is limited by the radius of the centrifugal disks (Zehnle et al. 2012). Meanwhile, driven by the centrifugal force, the chip on the instrument can't stop until the completion of the experiment, which can't realize a new coming sample detection at anytime. So a simple and flexible reagent drive mode is very important.

The immunological detection is a method that mainly uses the specific reaction of the antigen and antibody to detect trace substances such as proteins, hormones etc. Meanwhile, the detection signal is amplified due to exploiting of isotope, enzyme and chemical luminescence material etc. Chemiluminescence immunoassay method is a new method on the basis of radiation immunity analysis method and enzyme-linked immunoassay, which is not radioactive and of no carcinogenic substances, no pollution to environment and human. With the features of simple operation, high sensitivity, and stable results, it is of great significance in the early diagnosis of many diseases.

Now microfluidic platforms using chemiluminescence method for the quantitative detection of biomarkers, most are using enzymatic glowing methods (Marzocchi et al. 2008; Zhao et al. 2012) by looking at the depth of the color reaction to reflect the biomarker concentration, then read out through the instrument, and obtain a relative quantitative result; the detection of this approach having a longer reaction time and easy to detect by a CCD camera, but the depth of the color reaction is not easy to distinguish when the concentration difference is not obvious, thus appearing the insufficient sensitivity and not absolute quantification. Zirath H etc. (Zirath et al. 2016) present a compact diagnostic platform for a rapid and sensitive detection of plasma biomarkers. With a novel system for dispersion and manipulation of the magnetic particles in combination with chemiluminescence detection, the sensitivity of the immunoassay is improved and enables a rapid assay in a microfluidic format. Using the microfluidic technology realized the blending of reagents, the scatter of magnetic beads, incubation, target capture and detection in the chamber of the chip. The system adopts injection pump to drive and chip wrapped by antibodies, which belongs to the design of no typical centrifugal chip. Czilwik G etc. (Czilwik et al. 2015) have developed an automated magnetic chemiluminescent immunoassay (MCIA) on a mobile analyser for rapid POC determination of CRP. The MCIA is fully automated after the initial loading of sample and immunoreagents at the inlet ports. The automated protocol involves the transportation of magnetic capture microparticles between adjacent reaction compartments using a set of stationary magnets, amicrofluidic polymer disposable and a specific centrifugal protocol.

In this study, we report an automated microfluidic chemiluminescence immunoassay platform for quantitative detection of ferritin sensitively. Using a flexible and reliable suction

method to drive the liquid flow, overcoming the drawbacks of the previous gas drive to connect many tubes on the chip. This makes the installation of our instrument easier, and also provides external reference for some adopting the method of centrifugal to flow driver such as lab-on-a-disc platforms. When the centrifugal force is not enough to drive fluid flow, the drive mode of vacuum suction cups adopted in our study can be used in the lab-on-a-disc platforms to realize flexibly control of fluid flow. The method of acridine esterification chemiluminescence is adopted. By detecting photon signal from acridine ester in alkaline conditions, the device is able to achieve a quantitative detection. The photon detection range is able to be from 0 - millions, so it can make greatly improvement for the sensitivity of the detection. The main components of the microfluidic device by the laser cutting machine and hot press machine which allow for low-cost production of chips. The related reagents first are pre-loaded to the liquid storage chamber of chip, and then we use the vacuum suction cups as a driver interface which can be flexible to connect and interrupt with our chip, and only need to fit the corresponding holes on the chip to realize driving reagents, which makes the drive more flexible and easier. The reagents are released orderly by a flexible vacuum suction cups that generates power by pneumatic pump and the microchannels is hydrophobic. After sample introduction, the chip is placed into a customized instrument, and the release of reagents allows for the automated operation of the chemiluminescence immunoassay. The chemiluminescence signals are acquired and processed inside the instrument. After the reagent is loaded into the chip and placed in the machine, quantitative results can be obtained within 45 min. The proposed LoC platform shows advantages in terms of quantitation, sensitivity, low cost, portability and intuitive use, and it might be promising in diagnostic fields.

2 Materials and methods

2.1 Materials and reagents

Purified specific mouse monoclonal antibody of Ferritin (Ferritin-Ab₁, 4.5 mg·mL⁻¹) are pre-coated on the surface of magnetic particles (Package ratio, magnetic particles: Ferritin-Ab₁ = 1 mg:10 μL), another purified specific mouse monoclonal antibody of Ferritin (Ferritin-Ab₂, 4.4 mg·mL⁻¹) are marked with the acridinium ester (Mark ratio, acridinium ester: Ferritin-Ab₂ = 15 mol:1 mol), ferritin standard, pre-trigger solutions (PTS) is an acidic solution containing 1% (w/v) hydrogen peroxide, trigger solutions (TS) is an Alkaline solution containing sodium hydroxide, sample dilutions are phosphate buffer containing bovine serum, concentrated wash buffer is phosphate buffer containing 0.05% (w/v) Tween 20, all of these are from Xiamen innoDX. (Xiamen, China). Magnetic particles (Magnosphere MS300) are tagged with carboxyl group,

obtained from JSR Life Sciences. (Japan) and the diameter of the magnetic beads is 300 nm. Acridinium ester is purchased from Suzhou Chemsolarism Chemical Co., Ltd. (Suzhou, China). The clinical samples are from the First Affiliated Hospital of Xiamen University. Caris200 is used from Xiamen UMIC. (Xiamen, China), which is used to detect clinical serum protein as a standard control. The water used in the experiments is deionized using a water purification system (Millipore, USA). All experiments are performed in compliance with the hospital guidelines (Independent ethics committee approval was obtained from the Ethics Committee of the National Institute of Diagnostics and Vaccine Development in Infectious Diseases (NIDVD)).

2.2 Design of the microfluidic chip

The single-use microfluidic chip is designed by SolidWorks® 2015 3D CAD software (Concord, MA, U.S.A.). The microfluidic chip (75 mm wide, 79 mm long, and 5 mm high) is composed of three layers: the top layer (1 mm thick) with eight air holes, a pressure port and an open hole; the middle layer (2 mm thick) with embedded microchannels, reagent reservoirs (seven reagent reservoirs and one sample reservoir, and the volume of each reservoir is 150 μL), a reaction reservoir and a waste reservoir (there is a filter paper to guide the waste liquid), and the bottom substrate layer. To avoid reagent contamination and unwanted waste, we design relatively small air holes (1.5 mm in diameter) above the open reservoirs (3 mm wide, 25 mm long, and 2 mm high) for loading reagents. Each reservoir is connected to an individual connection microchannel which is connected to the reaction reservoir (different reagent reservoir is not the same as the distance to the reaction reservoir). The microchannel is 0.5 mm wide, and 2 mm high (all the microchannel is hydrophobic). The microchannels gather in reaction reservoir. The reaction reservoir is a u-shape which is 10 mm in diameter of the semicircle and 2 mm high with a volume about 300 μL . The upper part of the reaction reservoir has an air hole which is 1.5 mm in diameter to facilitate the reagent to enter. The reaction reservoir is connected to the waste reservoir through an s-shaped pipe which is 0.3 mm wide, 2 mm high and 22 mm long. In order to save the chip space and increase the volume of waste reservoir, we design it as shown in the Fig. 1a below. A microchannel at the upper right of the waste reservoir connects to a pressure port (a through-hole 1.5 mm in diameter). The total volume of the waste reservoir is about 2000 μL (left part of the waste reservoir fills with filter paper in order to ensure that waste liquid is able to enter it, which makes full use of the volume of waste reservoir. Otherwise it will be difficult to get into the left part of the waste reservoir, because the surface tension hinder waste liquid into the left chamber.). The third layer (2 mm thick) is a bottom substrate layer. The microfluidic is depicted in Fig. 1(a) and (b).

Fig. 1 Design and assembly of an integrated microfluidic chip for chemiluminescence immunoassay. **(a)** Schematic of the top layer with different holes, the middle layer with embedded microchannels and reservoirs, and the bottom silicone substrate layer before assembly **(b)** Photograph of the assembled microfluidic chip

2.3 Fabrication and assembly of the microfluidic chip

Because the chip on the size we designed is mm level, it is convenient for industrial production later and reduces our processing difficulty. The material we use is PMMA, which is a kind of environmental protected, cheap material, and shall not affect the reaction between different reagents. Injection molding is a reliable way to manufacture the layers with a high production volume, but the complex shapes and high aspect ratios of microstructures may lead to the difficulty of demolding of layers. So we take the laser cutting combination of hot-pressing to produce chips. (1) The back of the 1 mm and 2 mm PMMA board are pasted with double-sided adhesive. (2) We use a laser cutting machine cutting different shapes of microchannels in different layers (1 mm or 2 mm PMMA board) of the chip (cutting a piece of chip will be in about two minutes) and remove the cutting part. (3) We pull off the other side of the double-sided adhesive and we put the same size of the chip alignment and against of each layer, put them in hot-pressing machine to bond (the above board and bottom board of hot pressing machine set up at 70 °C and pressure is 0.4 MPa). The process of hot pressing is about half an hour, we can get 12 chips (three layers) per hour.

3 Instrumentation

The portable instrument is approximately $48 \times 37 \times 24$ cm (length $\times$ width $\times$ depth, 7 kg in weight) (Fig. 2(b)), with two main functions: (1) all the necessary steps of chemiluminescence immunoassay experiment including the release of the reagent, blending of reagent and magnetic beads, and automated fluid transportation in microchannels and (2) capture and analysis of chemiluminescence signals in microfluidic chips. The design of the automated instrument for chip-based chemiluminescence immunoassay is shown using SolidWorks® 2015 3D CAD software (Concord, MA, U.S.A.). The main components of the instrument include a PMT Assembly, linear motors, vacuum suction cups (different specifications of the vacuum suction cups can cooperate with different diameter of holes) (Fig. 2(b)), silicone tube, magnet, circuit board, lens, peristaltic pump, and mechanical slider (Fig. 2(a)). The PMT Assembly is purchased from Hamamatsu Co., Ltd. (Beijing, China). The convex lens used

to converge the chemiluminescence signals from the microfluidic chip to the PMT Assembly are purchased from Sai Electronics Co., Ltd. (Beijing, China). The magnet is obtained from Crazy Magnet Co., Ltd. (Beijing, China). The peristaltic pump is obtained from Baodingdichuang Technology Co., Ltd. (Baoding, Hebei, China). A customized printed circuitboard (PCB) for hardware module automation is designed using Altium Designer (Altium, Australia) and manufactured by Chuanglijia Co., Ltd. (Shenzhen, China). The protective enclosure and structural parts are designed using SolidWorks® 2015 3D CAD software (Concord, MA, U.S.A.) and machined by Shengjiafu Metal product Co., Ltd. (Xiamen, China). The linear motor is purchased from Shanghai zexin trade Electrical equipment (Shanghai, China). The vacuum suction cups is purchased from Hongyuan machine hand accessories Co., Ltd. (Suzhou, China). The mechanical slider is purchased from Oubang Transmission Co., Ltd. (Jiangsu, China). The silicone tube is purchased from Kamoer Co., Ltd. (Shanghai, China).

Fig. 2 (a) Design and photograph of the portable and automated instrument for chip-based chemiluminescence immunoassay. (b) The main components of the instrument include a photomultiplier tube, vacuum suction cup, circuit board, peristaltic pump, and negative pressure connector

4 Results and discussion

4.1 Operation and actuation of fluid flow

To realize automated fluid manipulation in microfluidic chips, we customize a set of simple, easy-to-control, and effective Valve actuator. The main components of the valve actuator include two vacuum suction cups (8 mm in diameter), a

silicone tube (inner diameter is 3 mm, outer diameter is 4 mm), and a peristaltic pump. The vacuum suction cups are connected to the peristaltic pump through silicone tube, so the vacuum suction cups are able to blow air to provide a positive or negative pressure. The flow rate inside the channel linearly depends on the applied negative pressure, which is generated by altering the rotation speed of the peristaltic pump connected to the outlet pressure port. The inner and outer diameters of

Fig. 3 The automatical experiment of reagents reaction in the chip **(a)** Positive pressure opening of the second reservoir drive ferritin sample and magnetic beads pre-coated with Ferritin-Ab1 crossing the microchannel together from the reservoir into the reaction chamber. **(b)** Peristaltic pump provides negative pressure to blow the hole and realize the reagent from reaction reservoir to waste liquid reservoir. **(c)** Peristaltic pump provides positive pressure to blow the hole and the mixture in the reaction chamber gets bubbles

the peristaltic pump runner herein are 22 mm and 24 mm, respectively. The speed of the peristaltic pump is 35 r/min. The silicone tube is placed between the runner of the peristaltic pump and the pressing device of the silicone tube, and when the runner rotates, the silicone tube is squeezed to form a positive and negative pressure; Vacuum suction cups are driven by linear motor on the sliding platform forward or backward; When you need the liquid from the reagent reservoirs get into the reaction reservoir, the vacuum suction cup moves forward until fit closely with chip and stop immediately, the peristaltic pump provides a positive pressure to make the liquid down into the reaction reservoir until the reagent completely released, then the linear motor drives vacuum suction cup restoring to the original position by backward movement. The bottom of the linear motor is a sliding platform, thus vacuum suction cup can also move around by the platform sliding around. So when the release of the liquid from the reagent reservoir to the reaction reservoir finished, moving to the next reservoir to release reagent in turn until finishing the release of all related reagents.

This methods is simple, reliable and easy to realize, and will not cause the relative interference between different reagents. We can ensure reagent into the reaction chamber in the equal initial velocity under the condition of providing the consistent gas pressure.

4.2 Automated microfluidic chemiluminescence immunoassay

The reliability of the automated microfluidic device is demonstrated by measuring biomarker of ferritin by direct sandwich immunoassay. Ferritin is a kind of soluble protein, normal serum contains a small amount of ferritin. The concentration of ferritin in human serum ranges from 15 to 200 $\mu\text{g/L}$ in men and from 12 to 150 $\mu\text{g/L}$ in women. Serum iron level decreases during pregnancy, and elevates during chronic liver damage and liver cancer. An automated platform developed for ferritin detection with high sensitivity has great significance in clinical diagnostics.

To enable the automated detection of ferritin, we first assemble the microfluidic device and manually load different reagents into the inlet reservoirs using a syringe. For ferritin detection, the direct sandwich immunoassay is adapted. 120 μL of wash buffer, 25 μL of ferritin sample and 50 μL magnetic beads, 120 μL of wash buffer, 100 μL of Pre-trigger solutions (PTS), 100 μL of Trigger solutions (TS), 120 μL of wash buffer, 100 μL of acridinium ester, and 120 μL of wash buffer, are loaded into inlet reservoirs 1 to 8, respectively. After preparing the microfluidic chip, we slide the chip into the instrument and initiate the detection of ferritin. The instrument automates the chemiluminescence immunoassay as follows (Fig. 3): (1) The instrument initializes itself and the vacuum suction cup driven by linear motor moves forward until

fit closely with chip and stop immediately. The positive pressure connector connecting the outlet pressure port on the microfluidic chip to the peristaltic pump. The operation of the peristaltic pump provides a positive pressure of 3.5 kpa; (2) The positive pressure opens the second reservoir then drive ferritin sample (25 μL) and magnetic beads (50 μL) pre-coated with Ferritin-Ab₁ together across the microchannel from the reservoir into the reaction chamber, incubating for 15 min. (3) Sliding platform drives vacuum suction cup moving to the hole of waste liquid chamber, then vacuum suction cup driven by linear motor moves forward to the hole closely and begins providing negative pressure. Cylindrical magnet just arrived in the reaction chamber at the same time and magnetic beads in the reaction chamber are attracted, which assure the reagent completely get into the waste liquid chamber after incubation and magnetic beads are completely preserved in the reaction chamber. (4) Positive pressure opening of the first and third reservoir drives wash buffer (each 120 μL) crossing the microchannel together from the reservoir into the reaction chamber, and sliding platform drives vacuum suction cup2 moving to the hole of waste liquid chamber, then vacuum suction cup2 driven by linear motor moves forward to the hole closely. Peristaltic pump provides positive pressure to blow to the hole and bubbles generated in the reaction chamber (about 10s). As bubbles produced from the bottom of the reaction chamber and constantly rise toward liquid level, the bubbles are bursting. So as to achieve the purpose of the magnetic beads and wash buffer fully blending. Finally still moving liquid of reaction to waste liquid chamber leaving magnetic beads. (5) Positive pressure opening of the seventh reservoir drive acridinium ester marked Ferritin-Ab₂ crossing the microchannel from the reservoir into the reaction chamber, and blending through the way of blowing bubbles in shock, and then incubation for 10 min, finally still moving liquid of reaction to waste liquid chamber leaving magnetic beads. (6) Open the sixth and eighth reservoirs and wash the microchannel and reaction chamber again two times. The washing steps have the same parameters of the two washing rounds as mentioned before. Only magnetic beads left in the reaction chamber finally; (7) Positive pressure opening of the fourth and fifth reservoir orderly drives pre-trigger solution (PTS 100 μL) and trigger solution (TS 100 μL) crossing the s-shape microchannel from the reservoir into the reaction chamber, then they react in reaction chamber. Since the chip is placed vertically, when the liquid is flowing toward the reaction chamber, the liquid naturally flows downward under the action of gravity, and does not enter other parallel sampling microchannels; At the same time, a vent hole is left above the reaction chamber. When the liquid enters, the gas in the reaction chamber is discharged from the vent hole, thereby avoiding the influence on other liquid storage chambers. The generated photon signal is captured by the photomultiplier tube inside the instrument. Photon acquisition time can be

Fig. 4 The state of the magnetic beads in different reaction stages (a) The magnetic beads are mixed by the bubbles (b) The magnetic beads are adsorbed and fixed in the chamber by the magnets

set through the PC software (i.e. all the photon number is obtained during the set period time). The parameters used in this paper: gate time (300 ms), settling time (10 ms), acquisition time (60s), pulse pair resolution time (1 ns). The reaction process produces photon about 3 s, so we set 300 ms as the acquisition time and accumulates about ten points value as the amount of photons generated in the reaction. In order to better reflect the state of the magnetic beads in different reaction stages, we photographed the mixing state of the magnetic beads and the aggregation state of the magnetic beads when the magnets were adsorbed under an inverted microscope (Fig. 4). In the figure, it is only the local part of the reaction chamber. It can be seen that the magnetic beads can be well mixed by the oscillation when the bubbles are broken, and since the diameter of the magnetic beads is only 300 nm, it will be in a suspended state after being mixed, and will not sink to the bottom. Under the adsorption of the magnet, the magnetic beads exhibit a stable aggregation state, so that the magnetic beads are not taken away when the reagent is transferred from the reaction chamber to the waste liquid chamber. The total photon after background subtraction is used to calculate the concentration of ferritin. The part of experimental

reaction principle likes (Fig. 5). The whole microfluidic chemiluminescence immunoassay is completed within 45 min in an automated manner. The used microfluidic chip is disposed, and all reagents are kept within the waste reservoir or inside the inlet reservoirs of the microfluidic chip. We should note that the preparation of the microfluidic device requires manual assembly of the chips and loading of different reagents. However, the chemiluminescence immunoassay inside the integrated microfluidic device is automatically performed using a customized instrument.

4.3 The performance of automated detection of ferritin

We next characterize the performance of the microfluidic chemiluminescence immunoassay for ferritin detection in terms of linear detection range, reproducibility, and limit of detection (LOD). The optimized concentrations of purified specific Mouse monoclonal antibody of Ferritin-Ab₁ pre-coated on the surface of magnetic particles (Package ratio, magnetic particles: Ferritin-Ab₁ = 1 mg:10 μ L) is 4.5 mg mL⁻¹, another Purified specific Mouse monoclonal antibody of Ferritin-Ab₂ marked on the acridinium ester (Mark ratio, acridinium ester: Ferritin- Ab₂ = 15 mol:1 mol) is 4.4 mg·mL⁻¹, respectively. We use the microfluidic device to detect ferritin with different concentrations. In the study, the amount of photon statistical graph is obtained by PC software, and we add and cumulate the points in the area of the peak in the curve in order to get the amount of photon approximately, and subtract the background value, getting the approximately total amount of photons (Fig. 6). We first used the same experimental reagents to test using traditional methods, which proved that the reagents and samples were no problem (Fig. 7(a)). And then we use the automated microfluidics-based system to detect ferritin. we observe a linear relationship between CL intensity and the concentration of ferritin from 5.1 to 1300 ng·mL⁻¹

Fig. 5 Schematic of microfluidic chemiluminescence immunoassay for ferritin detection. Workflow of CRP detection (a) The magnetic particles pre-coated with Ferritin-Ab1 and ferritin are in incubation for 15 min (b) The combination is washed by wash buffer (each 120 μ L) for two times. (c) Acridinium ester marked Ferritin-Ab2 from the reservoir into the reaction

chamber, blending with the combination, and then incubation for 10 min. (d) The combination is washed by wash buffer (each 120 μ L) for two times again. (e) PTS and TS are introduced into reaction reservoir and generate the CL signal

Fig. 6 the amount of photon statistical graph is obtained by PC software

(Fig. 7(b)) and the LoD is $2.55 \text{ ng}\cdot\text{mL}^{-1}$. To demonstrate the use of our device in clinical serum samples, serum ferritin was also tested in this paper. We first used the instrument named Caris200 from UMIC to test the concentration of 8 clinical samples, and then tested with our microfluidic chemiluminescence platform and made a correlation analysis between the

two. The results are illustrated in Fig. 7(c). It can be seen that our device also has a good sensitivity and reliability in the detection of serum samples. These results indicate that the automated microfluidic chemiluminescence immunoassay have good reproducibility and high sensitivity for ferritin detection.

Fig. 7 (a) The linear relationship between CL intensity and the concentration of ferritin by the traditional method. (b) The linear relationship between CL intensity and the concentration of ferritin from 5.1 to $1300 \text{ ng}\cdot\text{mL}^{-1}$ by the automated microfluidics-based system. (c) Comparison of detection of ferritin in serum samples by the automated microfluidic chemiluminescence immunoassay and the instrument named Caris200 from UMIC

5 Conclusion

In this work, we developed an automated and portable microfluidic chemiluminescence immunoassay platform composed of a microfluidic chip and an instrument for quantitative detection of ferritin in serum samples. The system can also be extended to the detection of other clinical targets. The disposable, self-contained, microfluidic chips are manufactured by laser cutting machine and hot press machine with a high production volume and will be manufactured by injection molding. The automated detection of biomarkers inside the microfluidic chips shows good reproducibility and high sensitivity. The limitation of the platform might be the difficulty in long-term storage of the integrated microfluidic chips with pre-patterned antibodies/antigens as well as pre-loaded reagents. New storage methods incorporated within the microfluidic devices could be attempted in future work. This microfluidics-based platform might be viable for POCT of a variety of biomarkers in clinical applications.

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