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Magnetic Field Assisted Preconcentration of Biomolecules for Lateral Flow Assaying

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Abstract

Lateral flow assays (LFA) have been extensively explored for rapid and cost effective point-of-care diagnostics. Typically, LFA responses are influenced by the complexity of sample matrices containing analogues or molecules that may potentially yield non-specific responses, for instance, when assaying for biomarkers in blood, serum and plasma. Therefore, isolation of analytes of interest from sample matrices would significantly improve the LFA responses. Herein, we report a magnetic field assisted preconcentration approach for extraction and assay of proteins in an LFA format. Cardiac marker, Troponin (cTnICT complex), is utilized as a model system for validation of the proposed approach. Magnetic fields of different strengths are evaluated for retaining cTnICT on an LFA membrane utilizing magnetic beads conjugated with anti-Troponin I (anti-TnI) antibodies. The sample matrix components subsequently flows through to the absorbent pad via a hydrophilic passivation layer that is protecting the capturing anti-Troponin C (anti-TnC) antibodies in the test zone. Isolated TnI-magnetic bead complexes are then released to flow downstream along the LFA strip to the test zone upon removal of magnetic field and the passivation layer. The capture of cTnICT magnetic bead complexes at the test zone produces a characteristic brownish band, enabling concentration dependant visual detection of cTnICT. Experimental results indicate that the assay yields pM level sensitivity within 15 min using very low sample volumes (<100 μ L), and with possibilities for signal enhancement. The reported assay offers a promising avenue for sensitive detection of target analytes in complex matrices at clinically relevant concentration levels without requiring tedious sample pre-treatment protocols.

Keywords: Lateral Flow Assay, Protein Extraction, Magnetic Separation, Magnetic Field Assisted Preconcentration, Troponin Assay

1. Introduction

Point-of-care (POC) assaying of biomolecules in complex matrices has attracted significant research interest over the past few decades. One of the most promising POC assay format is the LFA, consisting of membranes with dried reagents that are activated by application of a fluid sample. LFA typically yield qualitative optical responses with a potential for quantification in conjunction with image processing devices.[1-3] LFAs are cost effective, easy to fabricate and generally do not require sophisticated instrumentation for read out.[4, 5] However, in clinical applications, LFA has been limited by two main challenges; sensitivity and specificity, owing to the interferences associated with the complexity of sample matrices such as whole blood[6-8], serum[9, 10] and plasma[11]. Therefore, efforts have been devoted to explore methodologies such as sample extraction/preconcentration, antifouling surface treatment, use of reporter molecules with improved optical properties and efficient read out strategies in order to overcome the limitations of LFA.

Typical LFA utilizes gold nanoparticles (AuNP) as optical reporters.[12-14] Although AuNPs possess advantages such as good biocompatibility, high surface-to-volume ratio and high intensity signal readouts[14, 15], their colloidal stability often causes an inhibitory effect on the performance of LFAs[16, 17]. Alternative reporters including composites such as gold-silver[18], gold-iron[19] and gold-graphene oxide[20] therefore have been investigated for sensitive detection of analytes. However, synthesis of these composite materials is cumbersome and often yield a broad distribution of particles with non-homogenous surface areas for conjugation, which significantly influences the performance of the assay. Polystyrene-magnetic beads have been reported for detection of several biomolecules such as proteins and nucleic acids.[21-23] These beads are homogenous, stable and bear functional groups that can be modified and effectively conjugated to recognition molecules, such as antibodies and aptamers. Magnetic beads provide an additional advantage of spatial control with application of an external magnetic field, enabling efficient biomolecule extraction from a complex matrix.[23] Studies involving magnetic separation of analyte molecules have shown significant enhancement in sensitivity and specificity of such assays.[24-26] Most of these assays follow a layout wherein the antibody conjugated magnetic beads are added to a matrix consisting of the analyte and incubated for a certain period of time for antigen-antibody binding, followed by extraction using an external magnetic field. The separated beads are then re-suspended in a buffer solution (PBS, DI water, etc.) for assaying. Use of magnetic beads as reporters in LFA devices has been studied previously. However such assays require an external magnetic assay reader in most cases to detect trace signals.[26-29] This approach, although effective, involves external instrumentation and tedious extraction protocols such as several intermediate

centrifugation and pipette-based transfers of reagents, which may result in artefacts influencing the assay reproducibility.

Herein, we propose a facile extraction and assaying approach using polystyrene magnetic beads (MB) under a magnetic field for preconcentration of biomolecule and for subsequent concentration-dependent visual detection of biomolecules. The proposed LFA consists of a backing pad incorporated with a nitrocellulose (NC) membrane and a detachable magnet for preconcentration of target biomolecules. The sample containing the target analyte is added to a vial containing the target specific antibody-conjugated magnetic beads. As illustrated in Fig 1, the magnetic field of the detachable magnet is utilized to retain the magnetic beads on the LFA, enabling preconcentration of the bound analytes. The magnet is then detached from LFA to release the captured magnetic beads once the matrix solution has flown across the test zone on the NC membrane into the absorbent pad. A hydrophilic passivation layer has been evaluated for channelizing the sample matrix solution, and for protecting the test zone from non-specific adsorption of molecules present in complex sample matrices.

Cardiac Troponin I-C-T complex (cTnICT), a biomarker for contractile regulation of cardiac and skeletal muscles, is used as a model system for validation of the proposed approach[30]. In healthy humans, cTnI levels in blood are in the range of 20-30 pg/ml, whereas it increases rapidly after a heart attack[31, 32], reaching a peak of 190-200 ng/ml after 11 h [32, 33], thus serving as an important marker for diagnosis of acute myocardial infarction (AMI). Currently most LFA devices for colorimetric detection of Troponin mainly rely on tedious sample pre-treatment protocols to achieve the required detection limits, resulting in a long turnaround time for assaying, thereby preventing early diagnosis of AMI. The proposed assay demonstrates a facile approach to isolate target proteins on a LFA membrane for sensitive and specific evaluation of cTnICT concentrations within a time span of around 15 min. The visual detection limit (VDL) of ~ 1 ng/ml achieved by the proposed assay is clinically relevant and comparable to existing LFAs for cTnICT assaying.[34-36] Moreover, an approach involving protein A conjugated magnetic beads has been evaluated for further enhancing the VDL. The simplicity of the proposed approach facilitates point of care assaying of analytes in complex matrices. Another advantage offered by the proposed methodology is the generic approach of the assay, which could be translated for detection of other biomarkers in complex matrices.

2. Materials and Methods

2.1 Reagents, Membranes and Apparatus

PBS, BSA (Lyophilized), sodium azide and tween 20 were purchased from Sigma-Aldrich. Troponin I-C-T complex (cTnICT, 100 µg/ml), anti-TnI and anti-TnC antibodies were purchased from Hytest Inc. Commercially available superparamagnetic carboxylated

polystyrene beads (200nm) purchased from Chemicell were used in this study. EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride) and sulfo-NHS (*N*-Hydroxysuccinimide) were purchased from Sigma-Aldrich. PMB of size 1 μm was procured from Thermo Scientific. NC membrane cards (Hi-Flow Plus 75), glass fibre membranes and cellulose absorbent pads were obtained from Merck-Millipore. Images of strips were captured by Sony Alpha a7R III and processed in Sony Imaging Edge Edit RAW software. A permanent neodymium-iron-boron magnet (LifeSep 96F) purchased from Sigma Aldrich was used for the assay. The magnetic strength was measured using an ESCO Tesla/Gauss meter (EA703G-11).

2.2 Preparation of Antibody Conjugated Magnetic Beads

EDC/NHS protocol[37] was adopted and optimised to conjugate anti-TnI antibodies to carboxylated magnetic beads. A 200 μl solution of magnetic beads (concentration 2.5 mg/ml) was prepared in 100 mM MES buffer (pH 5). The beads were washed twice with MES buffer prior activation with EDC (400 mM) and NHS (300 mM) for 30 min. The excess of EDC and NHS was then removed by separating the beads using a magnetic separator followed by washing them twice with PBS. The beads were then re-suspended in 250 μl of anti-TnI antibody solution (100 $\mu\text{g}/\text{ml}$) and incubated for 2 h under gentle shaking. They were then separated and washed twice with PBS to remove the unbound antibodies followed by incubation in 1% BSA in PBS for 30 min in order to block any free and active carboxyl groups on the bead surface. The modified beads were eventually stored in a storage buffer consisting of PBS, 0.05% tween-20, 0.1% sodium azide and 1% BSA until use.

2.3 Preparation of Test Strips

The test strip consisted of a NC membrane (2.5 cm x 3 mm) and a cellulose absorbent pad. A test line consisting of 3 μl of anti-TnC antibody (1 mg/ml) was immobilised onto the NC membrane over a width of 3 mm. NC membranes were blocked with 1% BSA in PBS and incubated for 1 h at 37°C. Post incubation, they were washed with PBS and dried. NC membranes were then assembled with a cellulose absorbent pad (2 cm x 1 cm) with an overlap of 2 mm. The proposed layout does not require a control line as the bulk MB aggregates at the interface between the NC membrane and cellulose absorbent. The assembled strips were stored in dry conditions at 4°C before use.

2.4 Preparation of Samples for LFA

cTnICT was assayed in a concentration range from 0 to 1 $\mu\text{g}/\text{ml}$. Two types of samples were prepared; first in PBS and the second in 10x diluted plasma. Standard serial dilution procedures were adopted to prepare the various concentrations of cTnICT. In case of plasma dilutions, the analyte with highest concentration was spiked in 10x diluted plasma and subsequently serially diluted in 10x diluted plasma to prepare lower concentrations.

The anti-TnI antibody conjugated magnetic bead solution was diluted to a concentration of 10^9 beads/ml. 100 μ l of this solution was incubated with 100 μ l of the sample matrix containing cTnICT in varying concentrations for 10 min. 100 μ l of this mixture was then applied onto LFA strips for assaying.

2.5 Assay Layout

Fig 1: (a) Magnetic beads conjugated with anti-TnI antibodies are added to the sample matrix containing the analyte. (b) Use of magnetic field for isolation of analyte from the sample matrix and a hydrophilic passivation layer (glass fibre) to protect the test line (c) Removal of passivation layer and magnetic field followed by release and capture of beads along the test line immobilized with anti-TnC antibodies.

As illustrated in Fig 1, the assay layout consists of an LFA strip, a detachable magnet and a passivation layer. Typically, 100 μ l of sample and anti-TnI conjugated magnetic beads (MB-Ab) mixture is dropped on to the strip for assaying. The magnetic field induced by the detachable magnet retains the MB-Ab complexes containing the analyte, if any, on the LFA membrane. The sample matrix components are subsequently channelized via a hydrophilic passivation layer to reach the absorbent pad. The magnet and passivation layer are then removed followed by addition of 100 μ l of PBS as the assay buffer. The captured MB-Ab then flows along the NC membrane and are specifically captured by the anti-TnI antibodies immobilized on the test line in the presence of analyte, yielding a visible band due to accumulation of magnetic beads on the test line. Images of the bands were then captured after 15 min for analysis.

2.6 Signal Enhancement

Protein-A is known to bind to antibodies and therefore a signal enhancement strategy based on protein-A magnetic bead (PMB) complexes has been evaluated. A 50 times diluted stock solution (as purchased) was used for signal enhancement. Once the assay was complete, 5 μ l of diluted PMB solution was dropped on the test line. After an incubation period of 10 min, 50 μ l of PBS was used to rinse off the excess of PMB solution. Tests were carried out using strips corresponding to 1 ng/ml and 0.1 ng/ml cTnICT concentrations, with the use of passivation layer, in both PBS and plasma.

2.7 Analysis of Assay Readout

The images were analysed using ImageJ software. Ten different regions of interest (10 x 10 pixels) of the test line bands were selected and analysed to acquire the calibration curves. The relative luminance values were obtained by calculating the changes in luminance of the images from that of the control luminance value (absence of cTnICT). The relative luminance values were further normalized with respect to the control luminance value and plotted for analysis.

3. Results and Discussions

3.1 Principle of Assay

Herein, an LFA layout incorporating a magnetic field assisted sample preconcentration step is proposed to isolate analyte molecules from complex matrices. Carboxylated polystyrene magnetic beads conjugated with anti-TnI monoclonal antibody were utilized to specifically capture cTnICT. The amount of antibody conjugated to the beads was quantified by a bicinchoninic acid assay (BCA) and found to be around 25 μ g/mg of beads (Fig S1,

Supplementary Information). The MB-Ab complexes were used to capture cTnICT from the matrix solution in a vial (Fig 1a). An incubation time of 10 min was provided to ensure efficient binding between cTnICT and anti-TnI Ab to form MB-Ab-cTnICT complexes. It should be emphasized that the number of conjugated antibodies available for binding were maintained in excess to the typical cTnICT levels in clinical samples, in order to ensure that no free cTnICT remains in the sample solution.

The solution containing the MB-Ab-cTnICT complexes, when transferred to the LFA strip, starts flowing along the NC membrane. However, the magnetic field in the sample injection area induced by a magnet retains the MB-Ab-cTnICT complex, enabling isolation of the bound cTnICT from the matrix on the LFA membrane. A glass fiber membrane was evaluated as a passivation layer to channelize the matrix solution and to protect the test line comprising of pre-immobilized anti-TnC antibodies from non-specific adsorption of the sample matrix components. The passivation layer shown in Fig 1b and c, channelizes the flow of matrix solution, owing to its superior hydrophilicity as compared to that of the NC membrane, thereby minimizing exposure of the sample matrix to the test line. To release the MB-Ab-cTnICT complexes, the magnet was detached, and an assay buffer was added on the strip to facilitate the flow. The passivation layer was simultaneously peeled off to reveal the test line for capturing the MB-Ab-cTnICT complexes using immobilized anti-TnC antibodies. A characteristic brownish band was observed on the test line for samples containing cTnICT and the intensity of the band correlated with cTnICT concentrations. Qualitative analysis was then carried out by visual observation of the band intensity, followed by a quantitative analysis using ImageJ for ascertaining the visual estimation of cTnICT concentrations.

3.2 Effect of Magnetic Field on the Flow of Magnetic Beads

Magnetic fields of different strengths were applied on the LFA strip to evaluate the retaining efficiency of the magnetic beads. The strength of the applied magnetic field was controlled by adjusting the distance between the strip and the magnet (Fig 2a) and the magnetic field strength was measured using a Gauss meter. As illustrated in Fig 2b, a dark brownish coloration of NC membrane was observed near the sample injection area in the presence of a magnetic field, which is due to capture and

Fig 2: (a) Schematic NC strip before flowing MB-Ab complexes and the approach adopted for controlling the magnetic field strength. (b)-(e) Photographs of NC strips showing effect of varying magnetic field strength on the flow of MB-Ab complexes.

accumulation of magnetic beads. However, the beads could not be released upon removal of magnet, which is attributed to extensive penetration of the beads into the NC membrane owing to the magnetic attraction. For instance, magnetic field with strengths of 200 mT (strip placed directly on the magnet) (Fig 2b) and 100 mT (corresponding to 3 mm distance between magnet and the strip) (Fig 2c) lead to significant adhesion of beads to the NC membrane. In this case, a lesser number of beads flow through to reach the test line leading to formation of band with low intensity. On the other hand, if the magnetic field is too weak, almost all the beads flow through due to insufficient retaining of magnetic beads in sample injection area, inhibiting isolation of the analyte from the sample matrix. This was visualized on the strip where a 20 mT field strength was applied, that corresponded to a distance of 9 mm between the strip and magnet (Fig 2e). A distance of 6 mm between the strip and the magnet yielded an optimized magnetic field of ~ 40 mT (Fig 2d) for retaining and release of beads in the presence and absence of magnetic field, respectively. At this optimized magnetic field, the magnetic beads are retained near the surface and when assisted by a liquid medium they flow through towards the test line, without significant adhesion onto the NC membrane, upon removal of magnetic field.

3.3 Assay Performance

3.3.1 Assay in Buffer and 10x Plasma without Passivation Layer

Figure 3a-3b show the test bands obtained for different concentration of cTnICT in buffer and in 10x plasma, without the use of the passivation layer, respectively. A sample volume of 100 μ l was necessary for obtaining a distinct band. Once the sample matrix solution reaches the absorbent pad, another 100 μ l of PBS was added as a rinsing buffer to release the MB complexes from the sample injection area (magnet removed). The signal read out was observed at a fixed time frame of 15 min after adding PBS. A test band was visually observed for assay in PBS, at a concentration ≥ 1 ng/ml, which was considered as the VDL, without involving any instrumentation for analysis. In the absence of cTnICT, no band was observed at the test line, showing minimal non-specific adsorption of the MB-Ab complexes (without bound cTnICT) on the test line.

For the assay in 10x plasma, experimental parameters such as the sample and rinsing volumes were the same as that of assay in buffer. Visual observation for bands at cTnICT concentrations between 0 and 1000 ng/ml, illustrates that the LFA responses in 10x plasma correlate to that of responses obtained using the buffer solution. Figure 3b shows a dark band at 10x plasma spike with 1000 ng/ml of cTnICT. As the cTnICT concentration decreases, the bands become less observable and below 100 ng/ml, the band could not be observed by naked eye, indicating a VDL of 100 ng/ml for assay in 10x diluted plasma samples.

3.3.2 Influence of Passivation Layer

The 100 ng/ml VDL obtained in plasma is significantly higher than the 1 ng/ml VDL obtained in PBS could be attributed to interferences present in the matrix solution. Interferences such as dissolved proteins, clotting factors, electrolytes, etc., in plasma could inhibit the binding of MB-Ab-cTnICT complexes to the anti-TnC antibodies deposited on test line. This inhibitory effect can be reduced significantly if the interaction of the matrix solution with the test line antibodies is minimized. Thus, the concept of a hydrophilic passivation layer was incorporated with the proposed assay to channelize the flow of matrix solution over the test line. Glass fiber membrane was used as a passivation layer in this study. The hydrophilic nature of the membrane enables channelization of flow of sample matrix, thus protecting the capturing antibodies in the test line. Fig 3c and 3d illustrate the LFA responses in buffer and 10x plasma, respectively, with the passivation layer. It is evident that the band for 1 ng/ml cTnICT in buffer is more distinct in the presence of a passivation layer compared to the band obtained without the passivation layer (Fig 3a). The influence of passivation layer is best visualized for the assay in 10x diluted plasma (Fig 3d). A band is visually observed for 10 ng/ml, unlike the responses without the passivation layer (Fig 3b), where a band was visually observed only up to 100 ng/ml.

3.3.3 Calibration Curves in Buffer and 10x Plasma

Calibration curve between 0 and 1000 ng/ml was plotted for all the assay configurations (Fig 3a to 3d) using the normalized relative luminance values obtained via image analysis using ImageJ software. The luminance value is a weighted average of the red, blue and green components of an image and it is inversely proportional to the intensity of the band.[38] Thus, the band at 1000 ng/ml being the darkest in coloration, yield the lowest luminance and highest relative luminance, whereas, band that are difficult to visualize with naked eye (for instance, 1 ng/ml), yield a higher luminance value and a lower relative luminance. As observed from Fig 3e and 3f, concentration dependent normalized relative luminance responses are obtained for cTnICT, which correlate well with the visual observations for all the assay configurations, illustrating that quantification of cTnICT concentrations is feasible by the proposed methodology. The effect of the passivation layer in buffer and 10x plasma also can be seen in the normalized relative luminance read outs. For all test concentrations, the normalized relative luminance value increases upon incorporation of passivation layer as compared to the read outs without the passivation layer (Fig 3e and 3f). This observation suggests that interferences from plasma, that affect the binding ability of MB-Ab-cTnICT complexes to the antibodies deposited on the test line, are significantly reduced by employing a glass fiber passivation layer.

Fig 3: Photographs of NC membranes showing the test bands for assay in buffer (a) and 10x plasma (b) without and with passivation (buffer, (c)) and (plasma, (d)), respectively (e). Quantification of color intensities via measurement of normalized relative luminance of test bands for assay in buffer (e) and 10x plasma (f). Here, “Control” refers to absence of cTnICT in sample matrix.

3.3.4 Benchmarking against Reported cTnICT Colorimetric LFA

As illustrated in Table 1, other colorimetric LFA assays have been reported with better sensitivities by employing optical and enzymatic enhancement protocols. For example, signal enhancement approaches using a secondary Au nanoparticle have been reported to yield good sensitivities[35, 39]. Apart from nanoparticles, enzymatic assays using HRP have also been reported to be sensitive.[40] Although these methods are sensitive, they require either cumbersome particle synthesis or tedious LFA fabrication protocols [36, 38] with elaborate signal enhancement approaches. [39] The proposed methodology of using magnetic beads as optical reporters is facile and cost effective as it requires neither signal enhancement nor sample pre-treatment to reach clinically relevant concentration ranges in complex matrices such as plasma. In addition, it should be noted that the proposed methodology demonstrates assaying

in plasma (Table 1), which is a more complicated matrix than serum that is utilized in most of the existing approaches with better LODs.

Table 1: Performance characteristics of colorimetric LFAs for Cardiac Troponin

Matrix	Label	Assay Time	LOD	Pros	Cons
Serum	Dual AuNP	10 min	0.01 ng/ml [39]	Sensitive Demonstrated efficacy in clinical samples	Elaborate synthesis and LFA fabrication protocols Requirement of additional reagents for signal amplification
	Dual AuNP	15 min	1 ng/L [35]	Sensitive Multiplexed detection	Elaborate synthesis and LFA fabrication protocols
	AuNP	20 min	0.24 ng/ml [41]	Sensitive	Complex device fabrication
	HRP	20 min	0.027 ng/ml [40]	Sensitive	Requirement of reagents for signal amplification
	MB	15 min	0.01 ng/ml [27]	Sensitive	Requires Magnetic Assay Reader for signal readout
Plasma	MB	15 min	10 ng/ml [This work]	Facile fabrication Cost effective, minimal reagents required	Further optimisations needed to improve sensitivity
Plasma (with signal enhancement)		25 min	1 ng/ml [This work]	Sensitive Facile fabrication	Requirement of additional reagents for signal amplification

3.3.5 Signal Enhancement Approach

A signal enhancement approach based on PMB was explored to enhance the sensitivity of the assay. Protein-A binds to the detection antibodies (anti-TnI) captured on the test line, thus, localizing them on the test line. As observed from Fig 4a, the bands obtained upon assaying appear darker after treatment with PMB complexes. In case of assay in PBS, a band is observed at a cTnICT concentration of 0.1 ng/ml, implying that the VDL could be improved up to 10-fold. In case of assay in plasma, the band corresponding to 1 ng/ml is visible compared to lower concentrations, thus improving the VDL to 1 ng/ml in plasma. These results correlate with the normalized relative luminance plot, which shows an increase in normalized relative luminance values at all concentrations except for control and 0.1 ng/ml of cTnICT in plasma (Fig. 4b),

indicating that PMB complexes does not adsorb on test line nor bind to the anti-TnC antibodies immobilized along the test line.

Fig 4: (a) Comparison of strips before and after signal enhancement, in PBS and 10x plasma. (b) Normalized relative luminance plot before and after signal enhancement. Here, “Control” corresponds to absence of cTnICT in sample matrix.

3.3.6 Limitations and Scope for Improvement

Although the proposed approach enables assaying at clinically relevant levels, further optimizations are required to achieve a lower VDL in plasma samples, given than 10x diluted plasma has been utilized in this proof of concept study. The addition of a signal enhancement step is feasible, but this approach influences the complexity, leading to an increased overall cost and turnaround time for assaying. This may also limit its applicability for point of care (POC) assaying. Hence, there is a need for making the assay more competitive, without compromising the assay performance. Exploration of composite materials as reporters is a feasible approach for sensitivity enhancement, which constitutes our current work. The uniformity and intensity of bands can be improved by immobilization of the test line antibodies using a LFA dispenser. Uniformity in test line, optimization of the MB complex-protein concentrations and binding protocols as well as novel composite materials can provide an avenue for further improving the detection limits of the proposed assay. Furthermore, the detection limits can be improved by several orders of magnitude with the use of an external magnetic reader. However, such an approach increases the complexity for POC assaying.

4. Conclusions

In this study, an approach to incorporate extraction and assaying of a model target protein (cTnICT) on an LFA strip is demonstrated. The layout comprised of an external magnet that assisted in capturing of anti-TnC antibody conjugated magnetic bead complexes. The effect of varying magnetic fields on the capture and release of these bead complexes on the strip was studied and optimized. The activity of the capturing antibodies in the test zone was preserved by the use of a hydrophilic passivation layer that protected the test zone by channelizing the flow of sample matrix. The layout was tested against cTnICT spiked in PBS as well as 10x diluted plasma. The VDLs achieved were 1 ng/ml and 10 ng/ml in buffer and 10x plasma, respectively before signal enhancement, which meets the requirement for clinically relevant assaying of cTnICT. We demonstrated a 10-fold improvement of VDL to 0.1 ng/ml and 1 ng/ml in buffer and 10x plasma, respectively, after signal enhancement by subsequent exposure of Protein A modified magnetic beads to the test line. The proposed methodology could serve as a facile and appealing alternative to the current LFA schemes and as an approach towards the development of an LFA device that does not require tedious sample pre-treatment protocols. Furthermore, the generic approach of the proposed methodology allows translation to detect several other relevant biomolecules of interest.

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