Development of a microchip Europium nanoparticle immunoassay for sensitive point-of-care HIV detection

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Abstract
Rapid, sensitive and specific diagnostic assays play an indispensable role in determination of HIV infection stages and evaluation of efficacy of antiretroviral therapy. Recently, our laboratory developed a sensitive Europium nanoparticle-based microtiter-plate immunoassay capable of detecting target analytes at subpicogram per milliliter levels without the use of catalytic enzymes and signal amplification processes. Encouraged by its sensitivity and simplicity, we continued to miniaturize this assay to a microchip platform for the purpose of converting the benchtop assay technique to a point-of-care test. It was found that detection capability of the microchip platform could be readily improved using Europium nanoparticle probes. We were able to routinely detect 5 pg/mL (4.6 attomoles) of HIV-1 p24 antigen at a signal-to-blank ratio of 1.5, a sensitivity level reasonably close to that of microtiter-plate Europium nanoparticle assay. Meanwhile, use of the microchip platform effectively reduced sample/reagent consumption 4.5 fold and shortened total assay time 2 fold in comparison with microtiter plate assays. Complex matrix substance in plasma negatively affected the microchip assays and the effects could be minimized by diluting the samples before loading. With further improvements in sensitivity, reproducibility, usability, assay process simplification, and incorporation of portable time-resolved fluorescence reader, Europium nanoparticle immunoassay technology could be adapted to meet the challenges of point-of-care diagnosis of HIV or other health-threatening pathogens at bedside or in resource-limited settings.

1. Introduction
As reported by UNAIDS, there were 34.2 million people living with HIV and 2.5 million incident cases globally, while 1.7 million people died from AIDS-related diseases. In HIV-infected population, 68% was in sub-Saharan Africa. 91% of Children under 15 years old carrying HIV virus were also living in this area. Despite the striking facts of the pandemic, however, it is very encouraging that with help of persistent global efforts against HIV/AIDS, the essential treatment becomes more accessible to HIV-infected individuals residing in developing countries and resource-limited regions, resulting in remarkable declines in both incidence and death rates ([http://www.unaids.org/en/resources/presscentre/factsheets/]).

In typical ARTs, patients who are diagnosed HIV positive need to take antiretroviral drugs regularly to suppress the viral loads in their life spans. This life-long treatment scenario is similar to those for chronic diseases (Colvin, 2011; Wainberg and Jeang, 2008), and its practice requires the combined use of HIV diagnostic techniques and sufficient supply of highly effective antiretroviral (ARV) drugs. A sensitive, specific and reliable HIV diagnosis technique plays an equally important role as ARV drugs in this strategy (Wong and Hewlett, 2010). Through continuous disease monitoring using HIV diagnosis technique, clinicians are able to determine the most appropriate timing of therapy to stop the progression of HIV disease in infected individuals and thus reduce their infectiousness to healthy populations. Enzyme-linked immunosorbent assay (ELISA) and nucleic acid test (NAT) are two major assay techniques widely employed for HIV/AIDS diagnosis in hospitals and laboratories (Iweala, 2004; Wong and Hewlett, 2010). ELISA is commonly used for screening purposes. Its newest version, 4th generation HIV combo immunoassay, detects both HIV IgM/IgG antibodies and HIV capsid protein p24 antigen (detection limit: 4–10 pg/mL (Speers et al., 2005)) and thus enabling the detection of early HIV infection. NAT is by far the most sensitive and accurate
HIV diagnosis platform (detection limit: 30–60 copies/mL (Busch et al., 2005)). It serves in early diagnosis of HIV infection in infants, monitoring infection progress, blood donor screening and confirmatory tests. A laboratory HIV diagnosis algorithm combining the combo immunoassay and NAT was recommended recently (http://www.cdc.gov/hiv/testing/lab/guidelines/index.html). In the initial step, the combo immunoassay is used to screen for acute HIV-1 infection and for established HIV-1/2 infections. Positive specimens will receive further inspection using HIV antibody immunoassay to differentiate HIV-1 infection from HIV-2 infection. Specimens positive on the combo immunoassay and negative on the antibody assay should be tested with NAT to confirm the infection. Unfortunately, application of both ELISA and NAT techniques requires abundant resources. The high cost, long assay time, complex instrumentation, requirement for well-maintained environment and highly trained professionals for testing limits access of these technologies to the less-developed world. As UNAIDS data indicate, most HIV-infected populations in need of ART are located in low- or middle-income countries and regions. Therefore, one must balance multiple factors in selecting a proper HIV diagnosis technique for use in these areas. Besides accuracy and reliability, a suitable HIV diagnosis technique for resource-limited settings (RLS) should also have attributes including low cost, short turn-around time, low reagent/energy consumption and simplicity. Portability and disposability are also desirable (Allain and Lee, 2005; Wong and Hewlett, 2010).

The single-use lateral flow assay (LFA)/immunochromatographic strip system is a widely accepted rapid point-of-care (POC) diagnostic platform in RLS (Desai et al., 2011; Posthum-Trumpie et al., 2009). The core of a LFA system for HIV detection is a filter paper or porous membrane strip carrying detection zone immobilized with anti-HIV antibodies. Presence of HIV antibodies in samples will initiate specific capture events in the zone and visualization of the capture is implemented by infusing gold nanoparticles (AuNPs) conjugated with anti-IgG/IgM antibodies through the detection zone. No reader is necessary to detect the immuno-sandwiches formed since the color of AuNPs is visible to the naked eyes (Wong and Hewlett, 2010). Unfortunately, most LFA diagnosis platforms, including the recently developed rapid POC HIV antigen/antibody combo test, suffer from relatively low analytical sensitivity or high detection limit, an adversary factor that often compromises their performances in detection of early/acute HIV infections (Desai et al., 2011; Gordon and Michiel, 2008). LFA platform alone is incapable of accurate quantitative analysis, large-scale multiplex pathogen detection and performing multiplex assays, thus further limiting their usage in diagnosis of HIV co-infections and identification of different HIV strains. Several solutions aiming at overcoming the problems haunting the current LFA systems have been proposed, which include using more sensitive bioconjugated probes, introducing flow control to core strip, integrating functional components such as detectors to LFA system, and developing a new generation of POC tools based on lab-on-a-chip/microchip (LoC) concept (Chin et al., 2012; Corstjens et al., 2007; Gordon and Michiel, 2008).

LoC is one subset of microelectromechanical system (MEMS) with emphasis on chemical/biological processes (Reyes et al., 2002). Shortly after its introduction in early 1990s the research on LoC rapidly attracted great attention and ignited widespread enthusiasm in broad areas such as chemistry, biology, medicine, material science, bioengineering etc. (Manz et al., 1990; Reyes et al., 2002). The root of this excitement resides in the fascinating benefits LoC can offer. With downsizing of geometric dimension, diffusion distance shortens, surface-to-volume ratio increases and heat capacity reduces, leading to faster mass/heat transfer and thus higher reaction rate. Dimension shrinkage also brings extra advantages such as lower sample/reagent/energy consumption, lower waste generation and smaller instrument footprint. In addition, multiple functional modalities or same assay units can be miniaturized and packaged in one cartridge to perform complex assays or high-throughput parallel analysis, respectively. Recognizing the tremendous impacts and unique opportunities LoC will bring to global health, investigators from both academia and industry have been working diligently to develop novel LoC diagnostic devices and transform proof-of-concept devices to practical POC tools (Chin et al., 2012; Desai et al., 2011; Yager et al., 2006). To mention a few of such efforts, Sia’s group developed an immunoassay based microfluidic POC cartridge to simultaneously detect HIV and Syphilis and field testing in Rwanda has validated its effectiveness in RLS (Chin et al., 2011). Kim et al. (2012) introduced an immunofluorescence microchip system for rapid detection of Mycobacterium tuberculosis complex sputum. While Cheng et al. employed a cell isolation microfluidic device to separate CD4+ T cells from 10 μL of blood and count the cell number through either microscopy and impedance sensing (Cheng et al., 2009). Soh’s group reported a disposable microfluidic chip for sample-to-answer genetic analysis of H1N1 virus, (Ferguson et al., 2011). Lutz’s group demonstrated a fully automated microfluidic centrifugal disc system capable of detecting antibiotic resistance gene mecA of Staphylococcus aureus using isothermal recombinase polymerase amplification, (Lutz et al., 2010) and Lin’s group developed a highly sensitive microchip colorimetric assay for detecting a biomarker of Alzheimer’s disease using rolling circle amplification and G-quadruplex DNAzyme (Lin et al., 2014). Some LoC devices are sufficiently mature and have been launched into market. Typical FDA approved examples include Abbott’s i-STAT™ blood analyzer, Alere’s Pima™ CD4 counter, Focus (Quest) Diagnostics’ 3M™ Integrated Cycler, and IQuum’s Liat™ rapid NAT analyzer.

Most core assays for pathogen detection on LoC POC systems can be categorized into immunoassays and NATs, and the detection mechanisms are either label-free or requiring sensing probes. Label-free detection approaches are ideal for simple, rapid and multiplex pathogen diagnosis (Emaminejad et al., 2012; Stern et al., 2010; Vasan et al., 2013; Yoon and Kim, 2012). However, their accuracy, reliability, reproducibility and cost-effectiveness are yet to be examined in “real-world” applications. To date, detection based on visible, fluorescent, luminescent or electrochemically active molecules sensing probes is still prevalent in most diagnostic assays. With rapid progress of nanotechnology, many nanomaterials possessing excellent physical and chemical properties have been discovered and introduced for high sensitivity detection, including quantum dots, gold/silver nanoparticle (NPs), carbon nanoparticles and carbon nanotubes. The Europium (III) chelate–dyed nanoparticles (Eu NPs) used in this work are synthesized composite nanospheres with an average diameter of 107 nm. Each Eu NP has a carboxylated polystyrene shell to encapsulate more than 104 high-quantum-yield Eu (III) ions chelated by β-diketones (Harma et al., 2001). These lanthanide chelates have relatively high photostability and resistance to self-quenching effect, (therefore, Eu NPs can emit stable and extremely high-intensity fluorescence under proper excitation (Ukonah et al., 2007). Additionally, the functionalized polymer shell provides great amount of chemical handles for bioconjugation and a protective barrier to prevent other strong chelating ligands in solution (e.g., EDTA) from destructing the highly fluorescent Eu (III) chelates. A large 300 nm Stokes shift exists in Eu NPs, allowing convenient separation of excitation from Eu NP emission wavelengths in measurements. When time-resolved fluorometry (TRF) is used in measurement, rapidly decayed background fluorescence from matrixes can be temporally decoupled from long-lifetime Eu NP fluorescence. All these features facilitate assays incorporating Eu NPs as probes to achieve high sensitivity. Harma et al. reached a
2.2. Preparation of bioconjugated Europium (III) nanoparticles

EDC/sulfo-NHS chemistry was used to covalently link primary amines of streptavidin to the carboxylic groups on the surface of Eu NPs. Briefly, Eu NPs were first added to 10 mM phosphate buffer (pH 7.0) with 10 mM EDC and incubated for 10 min, followed by activation with 10 mM sulfo-NHS in the same buffer for 20 min. After a buffer exchange with 10 mM carbonate buffer (pH 9.0), 2 mg/mL streptavidin in the carbonate buffer was added to the activated Eu NPs and the reaction was allowed to proceed for 2 h. The remaining active groups on EuNP surface were blocked with 10 mM hydroxylamine for 30 min. Bioconjugated Eu NPs were washed 5 times with 10 mM glycine buffer (pH 8.5) and stored at 4 °C. Buffer exchange and EuNP wash were implemented using NanoSep® centrifugal ultrafiltration devices with a molecular weight cutoff of 300 kDa (Pall Life Sciences, Ann Arbor, MI, USA). All reactions were performed at room temperature.

2.3. µENIA of p24 antigen and HIV antibodies

Capture antibody or antigen was dissolved in a PBS (pH 7.2) solution and loaded to a freshly prepared microchip with a pipette gun. The device was incubated at 4 °C for at least 24 h and the capture antibody or antigen coating solution was replaced with PBST solution for blocking non-specific adsorption sites on the microreactor and microchannel walls. PBST solution was evacuated after incubation for 40 min at room temperature or 20 min at 37 °C.

In step 1 of HIV-1 p24 antigen and HIV antibody assays, sample solutions were loaded to PBST-blocked microchips and the devices were kept at 37 °C for 15 min (antigen assay) or 30 min (antibody assay). In step 2, biotinylated secondary antibody solutions were filled in the devices following a wash step and the on-chip reactions were allowed to proceed at 37 °C for 30 min. In step 3, EuSA solution was loaded to the microchips after a second wash and the biotin-streptavidin coupling reactions were incubated again at 37 °C for 15 min. The devices were subjected to a final wash and then placed on a modified rack for time-resolved fluorescence signal reading in a Perkin-Elmer 1420 Victor®3 multi-label counter. Fluid pumping in the microchip assays was achieved manually using a rubber pipette filler (Thermo Fisher Scientific) connected to the devices through a section of PTFE tubing (1/32" ID x 1/16" OD, Cole-Plamer, Vernon Hills, IL, USA).

3. Results and discussion

3.1. Fabrication of testing microchip

A large number of materials are available for microdevice fabrication. We conducted a series of screening experiments to identify the most appropriate substrate materials based on costs, fabrication easiness and optical properties. We finally chose PDMS as µENIA device substrate in our initial tests (Microfabrication process can be found in Supplementary Material).

Current PDMS testing microchip contains 12 independent microreactors and each one has a total volume of 22 μL. We initially employed a microreactor format having a 10-cm-long double spiral microchannel and a total volume of 0.9 μL in µENIA. Unfortunately, the microchips were unable to reliably detect sub-20 pg/mL level of target analytes in p24 antigen assay, possibly because the small microreactor did not have enough volume to contain a sufficient number of immunoreaction events detectable by the reader in one sample loading. The sensitivity issue associated with this type of microchip may be solved by adopting a more sensitive yet costly reader and loading more samples via multiple manual injections or a precise pump to increase immunoreactivity in the devices. However, these approaches are apparently not favored in POC settings since they require complex instruments, intensive human intervention and long assay time.
We finally modified the microreactor to a serpentine channel capable of accepting relatively large volume of sample in one loading. Our μENIA tests showed that this modification simplified sample/reagent loading and effectively enhanced assay sensitivity.

3.2. Preparation of microchip platform for ENIA

The first step to implement ENIA on LoC platform is to immobilize highly specific capture proteins on PDMS microchannel surface. Two major categories of approaches, chemical bonding and physical adsorption, are available for this purpose (Kim and Herr, 2013; Lin et al., 2014; Liu et al., 2011; Schramm et al., 1993; Zhou et al., 2010). The chemical approaches provide strong and durable biomolecule anchoring leading to improved assay performances, however, multiple steps are usually required in most methods and stringent quality controls are essential to achieve high immobilization density as well as uniform coverage. As a comparison, immobilization based on direct physical adsorption is a simpler and more reproducible method. Because of its convenience and reasonable assay results, we adopted the approach in this evaluation work to coat capture species to our disposable, single-use microdevices.

PBS (pH 7.2) and sodium bicarbonate (pH 9.4) buffers are commonly used in antibody coating and we were able to coat antibodies to ELISA microtiter plates in both buffers. However, we found that no signals were detected in μENIA tests if sodium bicarbonate buffer was used as antibody coating buffer, whereas good results were repeatedly obtained when the coating buffer was replaced with PBS. We suspect that the basic condition of sodium bicarbonate buffer rendered the capture antibodies negatively charged, which dramatically reduced their physical interaction with highly hydrophobic PDMS surface and thus total number of capture antibodies remaining on microreactor surface.

Following studies on immobilization of capture species, we evaluated the blocking effect of 1% BSA, 1% BSA/0.1% P-123 mixture and PBST, a commercial ELISA plate blocker containing a small surfactant Tween 20 and a proprietary blocking protein. However, our assay results suggest that actually all the blockers can effectively depress non-specific adsorption of EuSA on microreactor surface in μENIA. However, we finally chose PBST simply because air bubbles could form and become trapped in microchips treated with BSA blockers more easily, which negatively affected reproducible sample/reagent loading and increased assay variation.

3.3. Optimization of μENIA performances

Because of its particularly important role in early detection of HIV infection prior to seroconversion and the commercial availability of pure protein standards, we adopted the HIV p24 antigen assay to study μENIA performances. Before assays, we optimized the concentration of EuSA by measuring signal-to-blank (S/B) ratio of 50 pg/ml of p24 in μENIA using serial dilutions of EuSA (Fig. 1(A)). We found that signals from both μENIA reaction and blanks increased nonlinearly with EuSA concentration. The highest S/B ratio appeared at 1 x 10^9 particles/ml. However, when the EuSA concentration was higher than this value, the signal intensity of the blank increased more rapidly than that of the μENIA signal, indicating that non-specific adsorption of the EuSA to PDMS surface increased. We used the optimal EuSA concentration in all on-chip p24 antigen assays and Fig. 1(B) shows a typical result of μENIA of p24 antigen. 5 pg/ml (0.21 pM) of p24 can be reproducibly detected at an S/B ratio of 1.5 on the test microchips (limit of detection: 3.7 pg/ml or 0.15 pM), with a linear dynamic range extending from 0 pg/ml to 500 pg/ml. However, the signal is indistinguishable from the blank signal when p24 concentration dropped below 1 pg/ml. The signal variation in the same device was below 15% (relative standard deviation, RSD) and up to 25% (RSD) for different batches of devices.

To investigate the impact of various factors and optimize the performances of μENIA, we carried out a series of experiments based on the microchip HIV p24 antigen assay. We first evaluated the impact of coating concentration of capture antibody on the final μENIA results. As indicated in Fig. 2, only weak responses were detected from chips coated with low concentrations of the capture antibody (2-5 μg/ml). When the antibody concentration was increased to 10 μg/ml, strong signals were recorded in the μENIA. However, the calibration line deviated from linearity at 200 pg/ml point. Further increase of capture antibody concentration to 20 μg/ml extended the linear dynamic range to 500 pg/ml, while both μENIA signal intensity and analytical sensitivity (slope of calibration line) remained unchanged below 200 pg/ml as compared to those obtained from devices coated with 10 μg/ml of capture antibody. Since we were more interested in the detection of low pg/ml levels of p24 in this work and no significant μENIA improvements were obtained in this concentration range when higher coating concentrations were used in device preparation, in most cases, we chose solutions with an antibody concentrate of 10 μg/ml to coat microchips for the p24 antigen assay.

In regard to the optimization of reaction time, we varied the time of a particular step while other assay conditions remained the same in μENIA. The optimal time was then identified according to the highest analytical sensitivity of the assays (Fig. 3). To our surprise, the figure of merit did not monotonically increase with reaction time in a 100-min long time frame as found in parallel ENIA tests on NUNE microtiter plates. Instead, it reached a constant number within a relatively short period of time. In step
antigen assay. We coated the devices with p24 antigen, used following the same procedures for the microchip HIV-1 p24 exposed PDMS surface.

The result implies that strong non-specific adsorption of bio-matrix species occurred on PDMS surfaces and their intense competition for EuSA with p24 immunocomplexes greatly interfered with μENIA. To alleviate impact of the plasma matrix effect, we diluted the spiked plasma in PBST before loading it to chip. The data shown in Fig. 5(A) indicate when the dilution level reaches 10 the μENIA result of plasma is equivalent to that of typical clean samples, while the performance of μENIA in the testing of a 5-time diluted plasma is 85% of that in a clean sample assay in terms of analytical sensitivity. Plasma matrix effects become significant at dilution factors lower than 5 and the performance drops considerably. Additionally, the lowest detectable p24 concentration at an S/B ratio of 1.5 increased from 5 to over 10 pg/mL when plasma samples were diluted less than 5 times. We therefore conclude that a dilution factor of 5 is a reasonable balance point between enhancing detection sensitivity and reducing matrix effects and may be adopted in future μENIA of p24 antigen in real clinical plasma samples using the current microchip platform.

Following the on-chip p24 antigen assay, we evaluated the feasibility of applying μENIA to qualitative HIV antibody detection in plasma samples. Again, a specific dilution is necessary in analysis of these samples and we used a dilution factor of 100 in the assays to avoid matrix effects while reducing sample consumption. Nine samples were randomly chosen from a pool of HIV positive plasma specimens (proved by ELISA assay and microtiter plate ENIA) and loaded to p24-coated microchip platform after anti-p24 monoclonal antibody as a standard sample, and chose biotinylated rabbit anti-mouse IgG as the secondary/detection antibody. In a typical microchip antibody assay (Fig. 4), 25.0 pg/mL (0.17 pM) of antibody can be detected at an S/B ratio of 1.9 and the limit of detection was estimated to be 10.6 pg/mL (0.07 pM). The detection sensitivity of on-chip antibody assay is actually equivalent to that of microchip p24 antigen assay in terms of molar concentration.

3.4. μENIA in complex bio-matrix

To evaluate its potential in real-sample assays, we first challenged the microchip platform with p24-spiked plasma samples. Our initial results indicate that without any pretreatment, the complex bio-matrix species of plasma greatly distorted μENIA results. In these tests we observed that the background signal intensity surged at least 4-time higher than that of clean samples, and the responses from p24 serial dilutions in plasma significantly deviated from a line in the concentration range from 0–200 pg/mL. The results imply that strong non-specific adsorption of bio-matrix species occurred on PDMS surfaces and their intense competition for EuSA with p24 immunocomplexes greatly interfered with μENIA. To alleviate impact of the plasma matrix effect, we diluted the spiked plasma in PBST before loading it to chip. The data shown in Fig. 5(A) indicate when the dilution level reaches 10 the μENIA result of plasma is equivalent to that of typical clean samples, while the performance of μENIA in the testing of a 5-time diluted plasma is 85% of that in a clean sample assay in terms of analytical sensitivity. Plasma matrix effects become significant at dilution factors lower than 5 and the performance drops considerably. Additionally, the lowest detectable p24 concentration at an S/B ratio of 1.5 increased from 5 to over 10 pg/mL when plasma samples were diluted less than 5 times. We therefore conclude that a dilution factor of 5 is a reasonable balance point between enhancing detection sensitivity and reducing matrix effects and may be adopted in future μENIA of p24 antigen in real clinical plasma samples using the current microchip platform.

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being diluted in PBST doped with 0.55% TritonX-100. Plasmas of two healthy persons were subjected to the same treatment as that for other HIV positive plasmas and injected into the microchips as negative controls. The μENIA result (Fig. 5(B)) indicates all HIV patient plasmas show strong positive responses, with average signal-to-negative ratios (S/N) ranging from 5 (plasma 9) to a maximum of 61 (plasma 7). This qualitative result suggests that the microchip platform has a potential to be applied to detection of HIV antibodies from real patient samples. Furthermore, one may combine the HIV p24 antigen assay together with HIV antibody assay on current microchip platform for simultaneous detection of both HIV antibodies and antigen, an essential feature most of commercial 4th generation HIV combo assays have for early detection of HIV infection.

3.5. Future developments

Several improvements are required before validation with clinical samples and field tests. First, it would be beneficial to further improve the sensitivity of the PDMS microchip. Proper surface treatments as well as good blocking strategies could help to achieve this goal by increasing the density of immobilized bio-active capture species and depressing non-specific adsorption. Although many surface treatment and blocking methods are available for these purposes (Zhou et al., 2010), there is still a compelling need to identify simple, robust, uniform and inexpensive ones with negligible effects on physical properties of substrates and microfabrication process. Second, to mimic POC assay practice in RLS settings, we only used a pipette gun and a rubber pipette filler in μENIA to manually manipulate samples and reagents in the devices. The operation unfortunately incurred much inconvenience and fatigue to operator, and caused assay variance as well. Integration of micropumps and microvalves to the core microchip would be of great help to improve usability of future μENIA platform. Integration of sample cleanup/processing modules adds additional functionalities to the microdevice and thus enhances its capability to handle real clinical samples in POC settings. μENIA shown in this work follows a time-consuming, standard multi-step sequential assay process. We are currently working on reduce the assay steps to one with the use of EuNP-labeled detection antibodies. This assay process improvement would hopefully shorten the total assay time without sacrificing assay sensitivity. Relative large variation in μENIA assay results is another issue worthy of more efforts. We believe improvements in coating uniformity of capture species, consistency of sample/reagent loading and microchannel/microreactor geometry design would partially remedy the problem. As pointed out by Harma et al. (2001) and Ukonaho et al. (2007) a more fundamental source of Eu-NP assay variation may come from EuSA aggregates. These aggregates have broad size distribution and large steric hindrance that contribute to their relative low specific binding tendency and high non-specific binding possibility. Collecting non-aggregated EuSA using size-based purification/separation methods for the assay could reduce μENIA assay variation. Using conditions that discourage nanoparticle aggregation in assays would also help for this purpose. Finally, the reader we are currently using is a benchtop TRF fluorometer for research purposes. An ideal TRF reader suitable for POC applications would have several features including high sensitivity, compact size, simplicity, low power consumption, and ability to upload diagnostic results through wireless networks to center laboratories, to list a few. Such a detector is crucial to implementation of μENIA at the bedside or in remote regions.

4. Conclusions

We successfully implemented ENIA on a PDMS microchip platform in this work. Using bioconjugated Europium nanoparticle as probes, sensitivity of microchip immunoassay can be effectively enhanced without the employment of labile catalytic enzymes and help of complex signal amplification process. We reached a reasonably low detection level of 5 pg/mL in HIV–1 p24 antigen assay using μENIA. Meanwhile, we achieved a 4.5-fold reduction in sample/reagent consumption and a 2-fold reduction in assay time compared to conventional microtiter plate assay (Supplementary Material). We demonstrated both HIV antigen and antibody assays in plasma specimens using μENIA and found that without other sample processing processes, dilution was necessary to overcome interference from the complex bio-matrix. Further efforts are needed to improve sensitivity, usability and assay reproducibility. Optimization of the assay to a rapid format and identification of a portable TRF reader suitable for the platform are essential to apply μENIA to POC HIV diagnosis in RLS settings.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2014.04.057.

References