A Lateral Flow Assay for Quantitative Detection of Amplified HIV-1 RNA

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Abstract

Although the accessibility of HIV treatment in developing nations has increased dramatically over the past decade, viral load testing to monitor the response of patients receiving therapy is often unavailable. Existing viral load technologies are often too expensive or resource-intensive for poor settings, and there is no appropriate HIV viral load test currently available at the point-of-care in low resource settings. Here, we present a lateral flow assay that employs gold nanoparticle probes and gold enhancement solution to detect amplified HIV RNA quantitatively. Preliminary results show that, when coupled with nucleic acid sequence based amplification (NASBA), this assay can detect concentrations of HIV RNA that match the clinically relevant range of viral loads found in HIV patients. The lateral flow test is inexpensive, simple and rapid to perform, and requires few resources. Our results suggest that the lateral flow assay may be integrated with amplification and sample preparation technologies to serve as an HIV viral load test for low-resource settings.

Introduction

Over two-thirds of the 33.3 million people estimated to be infected with HIV worldwide live in the developing world [1]. In response to the HIV/AIDS crisis, access to anti-retroviral therapy (ART) has increased dramatically over the past decade in low- and middle-income countries [2]. However, successful management of HIV requires that patients receiving ART be monitored routinely to assess treatment efficacy and detect treatment failure due to drug resistance. Unfortunately, current laboratory based methods to monitor ART are unaffordable, unavailable, or inappropriate for low-resource settings [3].

Rapid antibody tests are widely available in developing nations, but they cannot be used to monitor HIV progression or treatment efficacy. The standard of care to monitor ART is quantitative viral load testing based on plasma HIV RNA concentration [4]. Although CD4 count has also been used to monitor ART, recent studies suggest that it may not detect early treatment failure adequately [5]. The gold standard method for viral load testing, RT-qPCR, is unsuitable for settings where trained technicians, expensive reagents, electrically powered equipment, and dedicated laboratory space are often unavailable. Therefore, a viral load test that is appropriate for such settings is needed.

A number of commercially available viral load tests have been developed for use at the point-of-care but suffer from drawbacks that limit their widespread implementation [6–8]. Many emerging technologies that are better designed for use in developing countries may serve as improved point-of-care viral load tests. A variety of microfluidic systems have been developed to perform nucleic acid amplification on-chip [9–11]. However, microfluidic systems often require a syringe pump for operation and additional imaging equipment to acquire results. To avoid the difficulties associated with enzymatic amplification of target RNA, alternative approaches have attempted to improve the sensitivity of nucleic acid detection through signal amplification [12,13]. Other researchers have developed quantitative tests for p24 antigen [14,15], which may serve as a surrogate for HIV viral load [7]. Despite these advances, no appropriate point-of-care HIV viral load test is currently available.

Recently, paper-based devices have shown promise as point-of-care diagnostics because paper is inexpensive, portable, disposable by burning, and has the ability to wick fluids by capillarity [16]. The emergence of paper microfluidics has renewed interest in lateral flow tests, which have served as point-of-care tests for decades. For example, recent work has shown that lateral flow tests may achieve a greatly improved limit of detection (LOD) and serve as platforms for multiplexed detection [13,17,18]. Still, most examples of paper microfluidic technology are antibody tests or small molecule tests [19–22], and much work remains to be done to develop and improve paper-based nucleic acid tests.

Here we present a quantitative lateral flow test for detecting amplified HIV RNA that is appropriate for low-resource settings. Gold nanoparticles conjugated to complementary oligonucleotides are used as probes, and gold enhancement is implemented to improve the LOD. Our results indicate that this lateral flow assay may be used in conjunction with amplification to detect HIV RNA concentrations at clinically meaningful levels.
Methods

Lateral Flow Assay (LFA) Development

The LFA was designed to quantitatively detect amplified HIV-1 RNA (Fig. 1). When RNA is dispensed onto the conjugate pad of the strip, the RNA binds to complementary oligonucleotides conjugated to gold nanoparticle probes (GNPs). The target RNA – GNP complex flows down the strip via capillarity and is captured by the target capture sequence in the center of the strip. Unbound GNPs bind to the positive control sequence at the end of the strip. A wash buffer carries unbound GNPs down the strip to decrease the background, while an enhancement solution increases the size and optical absorbance of the bound GNPs. The LFA was designed so that the number of GNPs captured in the detection zone would likely be proportional to the number of RNA copies dispensed onto the strips, providing a quantitative detection modality.

Because the concentration of HIV found in patient plasma samples can be as low as a few copies per milliliter, nucleic acid amplification of genomic HIV RNA must be performed prior to detection with the LFA. The LFA was designed to detect a 142 bp NASBA product amplified from the HIV gag gene. To develop and optimize the LFA, we used a 179 bp synthetic RNA sequence containing the NASBA product sequence (Table 1). The synthetic RNA sequence was generated through in vitro transcription using the MEGAscript T7 kit (Applied Biosystems) and a plasmid containing the T7 promoter upstream of the template sequence (pIDTBlue, Integrated DNA Technologies). The in vitro transcription reaction products were purified using a RNeasy column (QIAGEN, RNeasy Cleanup Protocol) and quantified by measuring absorbance at 260 nm.

Quantitative Analysis of LFA Performance

For all experiments, the performance of the LFA was assessed quantitatively using image analysis. All LFA strips were scanned (Epson Perfection V500 Photo) and imaged using a stereo microscope (Olympus SZ61) equipped with a color camera (Zeiss, AxioCam MRc5). Images obtained with the stereo microscope were analyzed using a custom Matlab script. The green channel image was cropped to rectangular field-of-view including the captured GNPs and surrounding strip area. An intensity threshold was set by the user to make a mask, which segmented the GNP spot from the background. For images with a high noise level, the mask was drawn manually using the function ‘roispline’ (Mathworks). The intensity of each pixel was subtracted from 255 in order to invert the image data, and the signal-to-background ratio (SBR) was calculated. LFA results are expressed using the SBR.

LFA Optimization Experiments

The LFA was developed to achieve the best LOD and dynamic range possible while remaining appropriate for low resource settings. We aimed to design a test that costs less than $1, requires an assay time of less than 30 minutes, detects RNA in a volume of less than 50 μL, requires minimal instrumentation, and remains stable for months at ambient temperature. The conditions that were optimized included capture oligonucleotide sequence and concentration, buffer composition, nitrocellulose flow rate, strip width, and gold nanoparticle size (Table 2). To maximize the signal generated by the GNPs, gold and silver enhancement were explored for signal amplification. During gold and silver enhancement, metallic ions are reduced on the surface of the GNPs, increasing their size and optical extinction in order to improve the limit of detection (LOD) of the assay [17,23].

For the optimization experiments, the LFA was performed using a dilution series of in vitro transcribed target RNA for each condition to be optimized. The SBR of the detection zone was calculated for each LFA. The SBR was normalized to a maximum value of 100 for each RNA target concentration. Average normalized SBRs for each condition were used to compare conditions. The optimum conditions were defined as those that maximized the SBR, affording the best LOD and largest dynamic range.

Fabrication of LFA Strips using Optimized Parameters

For all subsequent experiments, LFA strips were fabricated using the optimized parameters. Gold nanoparticles were chosen as probes because of their large optical cross-section and stability [24]. Gold nanoparticle probes (GNPs) were made by conjugating thiolated oligonucleotides (Integrated DNA Technologies) to gold nanoparticles (Ted Pella, NanoXact tannic acid capped gold colloid). The 35 bp oligonucleotide probe sequence contained an 18-atom hexa-polyethyleneglycol internal spacer and 15 bp polyA spacer to maximize loading of oligonucleotides onto the gold nanoparticles (Table 1) [25]. The oligonucleotides (25 μL, 0.1 mM) were incubated for 30 minutes with TCEP-HCl (10 μL, 100 mM) in a total volume of 110 μL to reduce the thiols bonds. A 600 μL volume of 60 nm gold colloid at the supplied concentration was added to the reaction and incubated overnight on a rotisserie. A 3.57 μL volume of 2% SDS was added to the reaction to reach a final SDS concentration of 0.01%. After 30 minutes of incubation, five 11.57 μL volumes of 2 M NaCl were added to the solution, separated by 2-hour intervals, to reach a total NaCl concentration of 0.15 M. The following day, the GNPs were centrifuged (5000 rcf for 5 minutes) and washed four times with 1 mL of 0.15 M NaCl, 0.01% SDS. The GNPs were resuspended in GNP buffer (0.15 M NaCl, 5% BSA, 0.25% Tween, 10% sucrose) after the final wash.

LFA strips were assembled using glass fiber conjugate pads, nitrocellulose cards, and cellulose absorbent pads. Conjugate pads were cut into 1 cm by 0.5 cm rectangles from glass fiber sheets (GFPCP203000, Millipore) using a 60-watt laser cutter (Universal Laser Systems) with 3% power and 5% speed. Ten microliters of GNPs were dispensed onto each pad and dried at room temperature before strip assembly. Absorbent pads (CFSP223000, Millipore) and nitrocellulose strips with a plastic backing (Hi-Flow...
Two oligonucleotide sequences were dispensed onto the nitrocellulose strip (0.2 μL volume, 0.4 mM concentration). The target capture sequence, which is complementary to a region of the target RNA sequence, was dispensed in the center of the strip; the positive control sequence, which is complementary to the probe sequence conjugated to the GNPs, was dispensed near the end of the strip (Table 1). After the oligonucleotide solutions dried on the strip at room temperature, strips were exposed to UV light (UVP HL-2000 HybiLinker) at 125 mJ/cm² to crosslink the oligonucleotides to the nitrocellulose. Conjugate and absorbent pads were placed on the adhesive at opposite ends of the nitrocellulose strip, overlapping the nitrocellulose by approximately 2 mm. Strips were used immediately or stored in foil pouches (Clonesaver Resealable Multi Barrier Pouch, Whatman) with desiccant (FTA Card Desiccant Packet, Whatman) to prevent moisture exposure. Strips were 3 mm wide, and absorbent pads were cut into 1 cm x 0.75 cm rectangles.

| Function                                      | Name            | Sequence                                                                 |
|-----------------------------------------------|-----------------|--------------------------------------------------------------------------|
| Synthetic target sequence for LFA            | IVTX1           | 5’-GGGGATCTTGGGTGAGTGGGCGCTCTTGGAGTGTACATTCCTCCCTGTGTTTTCGCCGCTGCCCACTG-3’ |
| Probe oligonucleotide on GNPs                | GNP6B           | 5’-CAGATGGGATGAGATCTGATAGATCATTCTGCCGCCCGCACCTGACGCTGGTGGGCTGCCGGATGCA ATCCTACATTTGACGCTGGTGGGCTGCCGGATGCA ACTG-3’ |
| LFA target capture sequence                  | CPT1C           | 5’-GCCATCTAAGGAGAAGCTG-3’                                                 |
| LFA positive control sequence                | POS1            | 5’-ATGCAATCTAATCCTCCCTGGTGTGTTT-3’                                      |
| Forward primer for NASBA                    | NASBAp5         | 5’-aattctatagtacagtactagtatagggCTATGCTATGTCGTCGCCGCACTGCTGAGATGCA ATCCTACATTTGACGCTGGTGGGCTGCCGGATGCA ACTG-3’ |
| Reverse primer for NASBA                    | NASBAp6         | 5’-ATCAGAGCAGGCGACCTGACGACGCACTGCTGAGATGCA ATCCTACATTTGACGCTGGTGGGCTGCCGGATGCA ACTG-3’ |
| NASBA product sequence                      | NBPRD1          | 5’-TGCTATGCTACTTCTTTTGTTCGCTCATTGCGCTGGTGGGCTGCCGGATGCA ATCCTACATTTGACGCTGGTGGGCTGCCGGATGCA ACTG-3’ |

Detection of NASBA Products

To demonstrate that the LFA may serve as a detection platform in an HIV-1 viral load assay, NASBA was performed to generate reaction products for detection on LFA strips. A plasmid containing the HIV gag sequence (pNL43) served as the template for in vitro transcription to generate synthetic copies of HIV gag RNA (MegaScript T7 kit, Applied Biosystems). Zero, 5, 50, 500, and 5000 copies of gag RNA served as samples for NASBA reactions.

An additional control of nonspecific, genomic nucleic acid was included in some experiments to demonstrate the specificity of our assay. For this control, total nucleic acid was purified from cultured lymphoblasts (CCRF-CEM cells, American Type Culture Collection) using the QIAamp DNA Blood Mini kit (Qiagen). A total of 740 ng of purified nucleic acid served as a sample for NASBA. To further ensure specificity, we designed the LFA target capture and probe sequences to bind to the amplified sequence between the regions targeted by the NASBA primer sequences. Therefore, any sequence amplified as a result of mispriming should not be detected by the LFA.

In four experiments, NASBA products were generated and detected using the LFA. NASBA was performed using the NuclSens EasyQ Basic Kit as described by the manufacturer (bioMérieux). The KCl concentration in each reaction was 36 mM. Primers NASBAp5 and NASBAp6 as well as the NASBA product sequence are shown in Table 1. Briefly, NASBA reactions were denatured for 4 min. at 65°C, incubated for 4 min. at 41°C,
and then allowed to proceed for 90 min. at 41°C after the addition of enzyme. All incubation steps were performed using a heat block. NASBA products consisted of a 142 bp sequence and were diluted by a factor of 10, 100, or 1000 in running buffer. Twenty microliters of diluted products were dispensed onto LFA strips for detection, followed by the wash and enhancement steps as previously described. Dose response curves were made to assess detection, followed by the wash and enhancement steps as previously described. Dose response curves were made to assess detection, followed by the wash and enhancement steps as previously described. Dose response curves were made to assess detection.

**Results**

**Development and Optimization of the LFA**

The LFA was developed and optimized to provide quantitative detection of RNA for eventual use as part of an HIV-1 viral load test. The assay parameters shown in Table 2 were found to optimize performance of the LFA while maintaining conditions that are achievable in low resource settings. The parameters that most affected the LFA performance were capture oligonucleotide concentration, gold nanoparticle size, buffer composition, and temperature. Performing the assay at 37°C on a heat block eliminated any nonspecific binding of GNPs at the detection zone, which is important for avoiding false positive results. Gold and silver enhancement provided signal amplification by increasing the signal-to-background ratio of the detection zone, thereby improving the LOD. Gold enhancement increased the signal-to-background ratio (SBR) by ~25%, while silver enhancement increased the SBR by ~15%. LFA strips that underwent silver amplification resulted in a high background, while gold enhancement did not significantly affect the background. Therefore, gold enhancement was chosen for signal amplification in later experiments.

**LFA Performance and Stability**

The performance of the LFA demonstrates that the assay can serve as a quantitative detection platform. Figure 2a shows a scanned image of one set of LFA strips after the assay was performed using various concentrations of target RNA. Figure 2b shows dose response curves for two batches of LFA strips made and tested on different days. The dose response curves are based on the average SBR calculated for each concentration. For Batch 1, the dynamic range extended 2.5 orders of magnitude, from 10.5 to 13 log_{10} RNA copies. The linear region of the dynamic range extended 1.5 orders of magnitude, from 11 to 12.5 log_{10} RNA copies. The LOD, which we define as the first concentration for which the SBR is significantly different from the negative control SBR (p<0.05), was 10.5 log_{10} RNA copies. For Batch 2, the dynamic range extended 3.5 orders of magnitude, from 9.5 to 13 log_{10} RNA copies. The linear region of the dynamic range extended 2.5 orders of magnitude, from 10.5 to 13 log_{10} RNA copies. The LOD was 9.5 log_{10} RNA copies (p<0.05).

The linear range was slightly larger and the LOD was lower for Batch 2 than Batch 1. This difference in performance could be explained by batch-to-batch differences in the preparation of LFA strips, as we have observed some variability in the efficiency of the oligo loading on GNPs (data not shown). Importantly, we did not observe this variability between strips of the same batch. Therefore, a standard curve may be constructed to calibrate the LFA and to account for batch-to-batch performance variability.

After storing a single batch of LFA strips for various periods of time at room temperature and 37°C, the performance of the assay remained consistent (Fig. 3). The dose response curves for each day (Fig. 3a and 3c) show that there is no trend in assay performance variability over time. Furthermore, no significant difference was observed between the average performance of strips stored at room temperature (Fig. 3b) and at 37°C (Fig. 3d). Throughout the time course for both storage conditions, the LOD was 9.5 log_{10} RNA copies, and the linear dynamic range extended from 10.5 to 13 log_{10} RNA copies. Average SBRs for each pair of adjacent concentrations were compared using t-tests, which yielded p-values less than 0.05 for all but one pair (p = 0.07 for 12.5 and 13 log_{10} RNA copies for strips stored at 37°C). Thus, the resolution of the assay regardless of storage conditions was 0.5 log_{10} RNA copies. These results demonstrate that the assay performs consistently even after short-term storage, suggesting that

| Experimental condition | Values tested | Optimum value |
|------------------------|--------------|---------------|
| Capture oligo concentration | 0.1, 0.2, 0.4, 0.6 mM | 0.4 mM |
| Type of running buffer | 4X SSC, 4X SSC with 5% formamide, 4X SSC with 5% formamide, 1.4% Triton X-100, and 1% SDS | 4X SSC with 5% formamide |
| Ratio of detection sequence to random sequence on GNPs | 1:0, 3:1, 1:1, 1:3 | 1:0 |
| Nitrocellulose flow rate | 75, 135, 180 s/4 cm | 135 s/4 cm |
| Gold nanoparticle size | 15, 30, 60, 70, 80 nm | 50 or 60 nm |
| Gold nanoparticle orientation | 5¢ or 3¢ end of probe | 3¢ end of probe |
| Capture probe length | 16 and 20 bp | 20 bp |
| Ratio of detection probe to polyA spacer on GNPs | 1:0, 1:3, 1:1, 1:3 | 1:0 |
| Detection probe sequence | Sequence adjacent to capture probe, seq. 20 bp away from capture probe | Adjacent sequence |
| Ionic strength of wash buffer | 0.1, 0.25, 0.5, 2, 4, 10X SSC | 0.5X SSC |
| Time to incubate GNP probes and RNA target | 0, 5, 15, 30 min. | 0 min. |
| Ionic strength of running buffer | 0.5, 1, 2, 4X SSC | 4X SSC |
| Formamide concentration of wash buffer | 0, 5, 10, 20% formamide | 0% formamide |
| Lateral flow strip width | 2, 3, 4, 5 mm | 2 or 3 mm |
| Temperature | Room temp., 37°C, 42°C, 46°C | 37°C |

Table 2. Optimized experimental conditions for the lateral flow detection assay.
Detection of NASBA Products

NASBA products were detected using the LFA to demonstrate that the LFA can detect amplified target RNA. The upper limit of the clinical range for HIV-1 viral load is about 6 log_{10} copies/mL, while the LFA has an LOD of 9.5 log_{10} RNA copies in 20 μL (equivalent to ~11 log_{10} copies/mL). Therefore, NASBA was chosen to amplify HIV-1 RNA because NASBA is a well-established isothermal method that provides 10 to 12 orders of magnitude of amplification [26].

The LFA was used to detect NASBA products in four different experiments. Figure 4a shows the LFA strips for one experiment, in which NASBA products generated from 50, 500, and 5000 copies of gag RNA were detected by lateral flow strips after dilution by a factor of 10, 100, and 1000 in running buffer. A dilution factor of 100 was sufficient for the SBR to fall within the linear range of the LFA. Figure 4b demonstrates the linear relationship between the SBR and amount of template gag RNA (R^2 = 0.99). The R^2 values for all four experiments range from 0.90–0.98 with a mean value of 0.94, indicating that the LFA detects amplified RNA quantitatively (Fig. S1). However, the equations of the regression lines differ for each experiment, which suggests that the performance of NASBA varies from day to day. A standard curve should be constructed for each experiment to correct for this variation. The LOD for all four experiments was at least 50 copies, and no signal was present on LFA strips for any of the no-target controls or samples containing background nucleic acid (Fig. S1). Therefore, when combined with NASBA, the LFA may potentially report gag RNA concentrations that fall within the clinical range with adequate specificity.

Discussion

Ideally, HIV viral load is measured before a patient initiates ART and throughout the course of treatment. Typically, patients who begin to experience symptoms have plasma viral load levels exceeding 10,000 viral copies/mL [27]. After ART is initiated, patients are monitored for a significant change in viral load, which is defined as a three-fold or 0.5 log_{10} copies/mL change. After 4–8 weeks, ART is considered to be efficacious if viral load decreases by 1.0 log_{10} copies/mL [28,29]. Suppression of viral replication is considered successful if the viral load falls below 200 copies/mL [29]. Therapeutic failure due to drug resistance is characterized by a return of low-level viremia, which is defined as a viral load of at least 500–1000 copies/mL [4]. A point-of-care viral load test should achieve an LOD and resolution that accurately identifies these clinical benchmarks.

The lateral flow assay presented here achieves a resolution of 0.5 log_{10} copies/mL over a linear range that extends 2.5 orders of magnitude. When coupled with NASBA, the LFA can detect 50 copies of HIV gag RNA. We speculate that this LOD corresponds to a plasma viral load of roughly 1000 copies/mL, assuming that a plasma sample volume of 100 μL will be used and that half of viral RNA from the sample is added to the NASBA reaction. The performance of the LFA when detecting NASBA products suggests that the LFA may be sufficient to detect significant changes in viral load, suppression of viral replication, and therapeutic failure. The LFA only requires a heat block, scanner or camera, and pipette. The lateral flow assay uses a sample volume of 20 μL, requires only three steps over the course of 20 minutes, costs $0.80 per strip using commercial reagents, and performs consistently after short-term storage. By modifying the target capture, positive control, and probe sequences, the LFA may be adapted to detect other RNA targets. The LFA is capable of detecting short, amplified sequences or long, genomic sequences, although the LOD may increase with target size by an order of magnitude or more (data not shown). The LFA is suitable for low-resource settings and has the potential to be performed at the POC.

The LFA represents a different approach from other assays that may potentially serve as viral load tests. While other researchers have developed quantitative tests for p24 antigen to avoid the complications of nucleic acid tests [14,15], the LFA detects HIV
RNA, which is the traditional target for viral load tests. Like other lateral flow tests that use signal amplification to achieve appropriate sensitivity [12,13], the LFA presented here uses gold enhancement to improve performance. However, because the LFA provides detection of nucleic acids after target amplification, the LFA may be used with any amplification method that generates RNA. For example, the LFA may be used in conjunction with microfluidic systems that have been designed to perform nucleic acid amplification [9,10]. Therefore, the LFA may be integrated with new or existing amplification systems to measure HIV viral load.

Although the LFA performs well for detecting amplified HIV RNA, some of the required conditions may be difficult to achieve at the point-of-care. Currently, the LFA requires a heat block, which may be unavailable in low-resource settings because of cost and the requirement for electricity. To overcome these obstacles, the LFA may be used in conjunction with an inexpensive battery-powered heater or exothermal chemical heating unit based on engineered phase change materials [30,31]. Another drawback of the LFA is that strips are left to dry before the data are collected, which requires additional time. In order to decrease the total assay time, strips may be dried quickly by heating or imaged while wet. In this study strips were imaged with a microscope, which often is not available in the field. To eliminate the necessity of expensive imaging equipment, strips may be imaged with a digital camera, point-of-care optical reader, or cell phone [32,33]. Alternatively, the color of the detection zone may be compared to a color scale to completely eliminate the need for imaging equipment. Finally, the LFA requires multiple pipetting steps and a dilution series of standards to achieve quantitative results; other work has shown that multi-step assays may be integrated in a paper microfluidic device to simplify the procedure and avoid the need for a pipette.

**Figure 3. Performance of the lateral flow assay after storage.** To assess the effects of storage on LFA performance, lateral flow strips were fabricated on the same day, placed in foil pouches with desiccant, and stored at room temperature or 37 °C. The lateral flow assay was performed and analyzed on the day of strip creation and 1, 3, 7, 14, 21, and 28 days after strip creation. The signal-to-background ratio (SBR) for each log_{10} copy number is shown for strips performed on different days. The negative control SBR is shown for comparison. The regression line and equation were calculated for the average SBRs over the linear range of the assay, from 10.5 to 13 log_{10} RNA copies. (A) Dose response curves at each time point and (B) average dose response curve for strips stored at room temperature. (C) Dose response curves at each time point and (D) average dose response curve for strips stored at 37 °C.

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Despite current limitations, the LFA may be modified or used in conjunction with other available technology to serve as an appropriate point-of-care test. Further work would be required to integrate the LFA with sample preparation and nucleic acid amplification in a format that is suitable for low resource settings.

We have described a quantitative LFA that detects amplified HIV RNA by using gold nanoparticle probes and gold enhancement. The assay has the potential to serve as part of a point-of-care test in low-resource settings. Because this assay serves as a detection platform, the LFA may be adapted to quantify RNA targets for other diseases as well. The LFA may be integrated with amplification and sample preparation to comprise an HIV viral load test for low-resource settings. Point-of-care tests for viral load measurement in low resource settings have the potential to allow proper monitoring of HIV patients receiving ART, improving the management of HIV in the developing world.

Supporting Information

Figure S1 Detection of NASBA products in three additional experiments (A–C). To the left, scanned images of LFA strips are shown at the appropriate dilution for the SBRs to fall within the linear range of the assay. The total number of gag RNA copies added to each NASBA reaction are shown next to each image. Note that although the contrast was adjusted in the figure, raw images were used for signal-to-background calculations. (TIF)

Author Contributions

Conceived and designed the experiments: BR VL RRK. Performed the experiments: BR VL. Analyzed the data: BR. Wrote the paper: BR. Overall project design and management: EM RRK. Revised the manuscript: VL EM RRK.

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