Evaluation of a Rapid Lateral Flow p24 Assay for the Diagnosis of HIV in Infants

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ABSTRACT

Background: Studies in the developing world have demonstrated that early intervention with anti-retroviral therapy (ART) can greatly enhance the quality of life for infants infected with HIV, and can also decrease the mortality rate among these individuals. However, the majority of HIV infected infants are in resource limited settings where several obstacles, including limited screening for HIV and the lack of a simple, affordable point of care diagnostic test, currently impede the implementation of ARTs. We have developed and evaluated a low cost, rapid ultra-sensitive lateral flow p24 antigen assay that can facilitate the diagnosis of HIV for infants in resource-limited settings.

Methods: We developed a lateral flow assay based on the use of carbon nanoparticles functionalized with monoclonal antibodies against the HIV p24 antigen as the reporter label. A simple camera setup was used to quantify the assay response. A cut-off value for the assay was established as 3 standard deviations above the mean signal of 100 HIV negative plasma samples. To disrupt the immune complexes due to maternal antibodies, a rapid heat shock pretreatment method was developed and utilized prior to performing the lateral flow assay. The performance of the assay was evaluated by testing 54 adult sero-conversion panels and 198 double blinded longitudinal infant samples obtained from the Women and Infants Transmission Study (WITS). The results were compared to previously measured RNA viral load levels to determine the sensitivity and specificity of the assay.

Results: The clinical sensitivity of the carbon lateral flow assay in detecting p24 antigen in the sero-conversion panels was equivalent to that of a commercial p24 ELISA. The heat shock pretreatment was effective in disrupting immune complexes without compromising the integrity of the p24 antigen. Upon testing the WITS samples, the carbon lateral flow assay coupled with the heat shock pretreatment achieved 90% sensitivity and 98% specificity between ages 2 and 6 months. The overall sensitivity and specificity of the assay for infants under 18 months were 81% and 98% respectively.

Conclusions: The carbon lateral flow assay for the detection of p24 shows good performance as a low cost diagnostic test for HIV infection in infants. The assay is simple to perform, requires only 25µL of plasma and can provide a result within one hour making it ideal for implementation in resource limited settings.

BACKGROUND

Studies in the developing world have demonstrated that early intervention with anti-retroviral therapy (ART) can greatly enhance the quality of life for infants infected with HIV, and can also decrease the mortality rate among these individuals[1]. However, the majority of HIV infected infants are in resource limited settings where several obstacles, including limited screening for HIV and the lack of a simple, affordable point of care diagnostic test, currently impede the implementation of ARTs. We have developed and evaluated a low cost, rapid ultra-sensitive lateral flow p24 antigen assay that can facilitate the diagnosis of HIV for infants in resource-limited settings.

Methods

Assay Procedure

The assay comprises of 4 simple user steps:

1. 25µL of plasma is aliquoted into a pre-filled tube containing 50µL of heat shock buffer.
2. The tube is heated for four minutes at 88°C, and allowed to cool passively to room temperature.
3. 39µL assay probe mix consisting of assay conjugates is then added to the sample.
4. After allowing 5 minutes for incubation, a dipstick is inserted into the tube, initiating capillary flow of the sample. After 30 minutes the test and control lines generated are read for diagnosis.

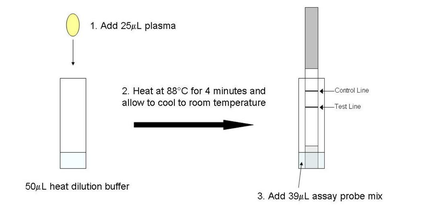

Figure 1. Schematic showing current assay procedure

Specimens

Sero-Conversion Panels: Nine well characterized sero-conversion panels (BBI 930, 940, 941, 943, 944, 945, 947, 959, and 965) were purchased and used for initial evaluation of our assay.

WITS: The Women and Infants Transmission Study (WITS) is a longitudinal study of HIV infection in pregnant women and transmission to their infants. We received 198 frozen heparin plasma samples from 49 individual infants. Blood samples were collected 1 week after birth, followed by bleeds at 1,2,4,6,9,12,15 and 18 months of age.

RESULTS

Immune-Complex Disruption

To disrupt the immune complexes due to maternal antibodies, a rapid heat shock pretreatment method was developed and utilized prior to performing the lateral flow assay. The efficiency of the pretreatment in disrupting immune complexes was determined in an artificial matrix of recombinant p24 complexed with increasing concentrations of anti-p24 antibodies. 25µL of each sample was assayed with and without heat treatment.

We found that there was complete inhibition of signal in the untreated case with as little as stoichiometric amount of competing antibody. The heat shock methodology was efficient in disrupting immune complexes even in the presence of 10,000 fold excess of competing antibodies, while protecting the integrity of the p24 antigen.

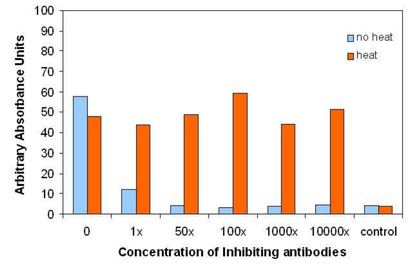

Figure 2. Assay results of 1ng/mL p24 antigen in the presence of increasing concentrations of competing anti-p24 monoclonal antibodies.

Analysis

Each specimen was run in duplicate, and scored accordingly by the camera set-up. Signal for each sample's test line was determined in Arbitrary Absorbance Units (A.A.U) and compared to a pre-determined cut-off value to generate a signal to cut off ratio or S/CO. S/CO ratios equal to or greater than 1.0 were considered HIV positive and ratios less than 1.0 were considered negative. After image acquisition and analysis, samples with discordant results were re-tested.

Sero-Conversion Panels

Nine individual adult sero-conversion panels consisting of 52 HIV positive samples and 2 HIV negative samples were tested with the carbon p24 rapid assay. The performance of the rapid assay was evaluated by comparing the results to corresponding viral load data obtained from BBI. The results of the rapid assay compared favorably with a commercial p24 ELISA assay.

Table 1. Summary of results from sero-conversion panels. The carbon p24 rapid assay performance compared favorably to a commercial p24 ELISA.

Viral Load	# of Positives	# Detected (%)	
		Carbon p24 Rapid Test	p24 ELISA[a]
0 - 1M (All)	52	34(65)	32(62)
100K - 1M	26	23(88)	24(92)
10K - 100K	12	7(58)	5(42)
1K - 10K	12	3(25)	0(0)
0 - 1 K	5	0(0)	0(0)

[a]Data provided by BBI

WITS

The performance of the carbon p24 rapid assay was evaluated by testing 198 double blinded longitudinal infant samples obtained from the WITS. The results were compared to previously measured RNA viral load levels to determine the sensitivity and specificity of the assay.

Table 2. Summary of longitudinal data from WITS

Infant Age	Total Samples	TP	TN	FP	FN	Sensitivity	Specificity
1-7 days	7	2	4	0	1	67	100
1 month	17	6	7	0	4	60	100
2 months	32	10	19	1	2	83	95
4 months	28	15	13	0	0	100	100
6 months	27	12	13	0	2	86	100
9 months	24	9	13	0	2	82	100
12 months	24	11	11	0	2	85	100
15 months	12	3	8	0	1	75	100
18 months	21	6	11	1	3	67	92
2-6 months	87	37	45	1	4	90(77,96)	98(89,99)
0-18 months	192	74	99	2	17	81(72,88)	98(93,99)

CONCLUSIONS

- The rapid assay for the detection of p24 antigen shows good performance as a low cost point of care diagnostic test for HIV infection in infants. The assay is simple to perform, requires only 25µL of plasma and can provide a result within one hour making it ideal for implementation in resource limited settings.
- Additional field studies are needed to fully evaluate the performance of the rapid test in a larger set of samples from the target population.

FUTURE DESIGN

In our future work we will be developing a point of care sample collection module and heater device. The sample collection module would enable collection and separation of plasma directly from a heel stick.

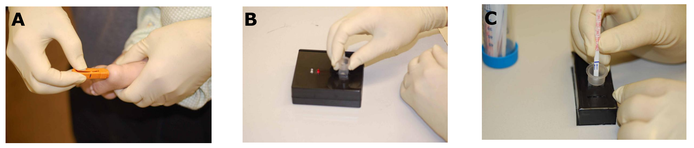

Figure 3. Illustration of future assay concept. Blood is collected from the heel stick of an infant (A), processed in a portable heater device (B) and assayed with the carbon p24 rapid assay (C).

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