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Use of Dried Blood Spots and the Gen-Probe Aptima HIV-1 RNA Qualitative Assay for the Diagnosis of Infants and Detection of Acute Infection from Pooled Samples

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Introduction

The Gen-Probe Aptima assay is an extremely sensitive, qualitative HIV RNA assay. Preliminary results from our laboratory suggest that plasma specimens with viral loads of at least 20 cp/ml are detectable. Dried blood spots (DBS) are an easy way to collect and ship specimens for diagnostic testing of HIV and preclude the necessity of a phlebotomist and maintenance of the cold chain. However, the limited volume of specimen dried on filter paper decreases the sensitivity of any assay. If the Aptima assay could be adapted for use with DBS, the increased sensitivity of the assay should improve diagnosis. There are two potential applications for a highly sensitive qualitative HIV RNA assay – diagnosis of perinatal HIV infection in infants and young children and the diagnosis of acute infection in high risk individuals or vaccine recipients. We developed a cost-effective, sensitive method for diagnosing acute HIV infection and infant using DBS and the Gen-Probe Aptima HIV-1 RNA Qualitative Assay.

Methods

DBS were prepared by placing 50ul whole blood on Whatman 903 paper and air drying overnight. The DBS were then placed in individual ziplock bags with a dessicant sachet and stored at room temperature. A PBS/ detergent buffer was developed to elute the blood, including HIV-1 virions, from DBS by rocking 1-2 hr at ambient temperature. Two punches were used from each spot. 500-750ul of the eluates were put into the Gen-Probe Aptima HIV-1 Assay. The limit of detection of the assay was tested using DBS made with spiked whole blood, DBS from HIV-1-infected and uninfected adults (n=36 and n=10, respectively), and DBS from infants (n=222). Optimal pooling strategies using a single punch per spot were determined and then used to assess the ability of the pooled DBS assay to detect HIV infection in adults and infants.

Results

Table 1. Aptima assay results for DBS from patients with viral loads <200 cp/ml

Viral load	APTIMA result
<50	Positive
<50	Positive
<50	Positive
<50	Positive
<50	Positive
14	Positive
21	Positive
72	Positive
<50	Negative
36	Negative
191	Negative

Results

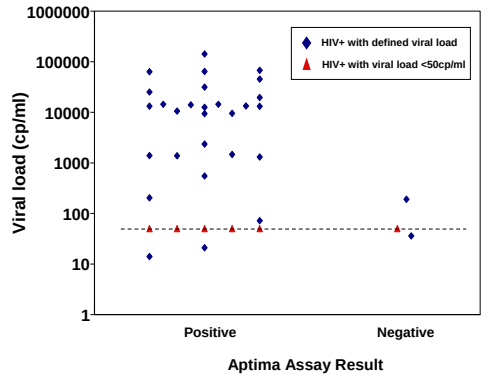
Using spiked whole blood, the limit of detection with DBS was ~400 cp/ml. However, when DBS from HIV-1-infected patients were tested, the assay proved to be more sensitive (Figure 1). The additional sensitivity is likely due to viral RNA and DNA in the infected cells of the blood that are absent from spiked whole blood or plasma.

51/52 specimens from infected infants (subtypes A, B, C, and D) with viral loads >1000 cp/ml were positive. The one false negative infant specimen was from a DBS that had been stored for 4 years at room temperature (98% sensitivity).

26/27 specimens from infected adults (subtype B) with detectable viral loads in the Roche HIV RNA assay (viral loads >50 cp/ml) were positive in the assay (96% sensitivity). In addition, 7 of 9 infected adults with viral loads <50 cp/ml were detected in the assay (Table 1).

180 specimens from uninfected adults (n=10) and infants (n=170) were negative (100% specificity).

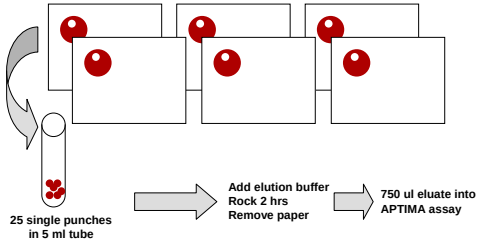
Figure 1. Aptima assay results vs. viral load for 36 adult DBS



Results

Pooling of up to 50 DBS with a single positive punch was reactive in the assay if the positive punch had a viral load of at least 10,000 cp/ml. Greater sensitivity was achieved with a smaller pool size: when 25 single punches were co-eluted (Figure 2), the lower limit of detection of the single positive punch was approximately 1000 cp/ml.

Figure 2. Pooling strategy for DBS and APTIMA assay.



Conclusions

The Gen-Probe Aptima HIV-1 RNA Qualitative Assay was successfully adapted to work with DBS. Sensitivity in both infected infants and adults was 96 to 98% and specificity was 100%. Up to 25 DBS could be pooled and tested with a limit of sensitivity ~ 1000 cp/ml. This assay could be used as a sensitive, specific, cost-effective way to determine acute infection in populations such as infants, vaccine recipients, blood donors, pregnant women, or STD clients.

This work was supported by IMPAACT and the UNC CFAR.