Qualitative HIV RNA Analysis of Dried Blood Spots for the Diagnosis of Infants

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ABSTRACT

The Gen-Probe Aptima HIV-1 RNA assay was adapted for the diagnosis of HIV infection in infants using dried blood spots. The assay was 99% sensitive (128/129) and 100% specific (162/162). This may prove useful in resource-limited settings since it precludes the need for a phlebotomist and maintenance of a cold chain.
Diagnosis of HIV-1-infected infants is essential for the evaluation of interventions for the prevention of this transmission and for identifying infants for initiation of therapy. Nucleic acid detection methods are necessary for infant diagnosis due to the presence of maternal antibodies in the babies up to 18 months after birth. 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 (2, 7). However, the limited volume of specimen (50 µl) dried on filter paper decreases the sensitivity of any assay. Current diagnostic methods that have been used on DBS include DNA PCR (3, 14), ultrasensitive p24 antigen assay (12), and viral load testing (1, 4, 8). The lower limits of detection in these RNA and DNA PCR detection methods were approximately 4000 cp/ml (1, 4, 8, 14), likely due to the small sample size on DBS.

The Gen-Probe Aptima HIV-1 RNA Qualitative Assay is extremely sensitive for the detection of HIV in plasma (5). This assay has been evaluated recently as a more sensitive screening tool for HIV+ samples from an STD clinic than typical antibody testing followed by pooled RNA testing (13). In addition, preliminary results from our laboratory suggest that plasma specimens with viral loads of at least 20 cp/ml are detectable. Therefore, we evaluated this assay for use on DBS from infants and children as a rapid and less expensive alternative to other assays for the diagnosis of HIV-1 infection in infants.

We formulated an Elution Buffer for removing the blood containing HIV from DBS (1 mM EDTA, 1 mM EGTA, 3% lithium lauryl sulfate in phosphate buffered saline). Optimization of elution resulted in the following protocol: two 6mm circles were punched from each card and placed in a 2 ml screw-top tube. The hole punch was cleaned
between cards by punching a clean Whatman 903 card twice. 525 µl of Elution Buffer was added to each tube and the specimens were rocked for two hours at room temperature. The specimens were spun down for 30 seconds at 10,000 rpm in a microcentrifuge and the filter paper was removed from the tubes using disposable wooden applicator sticks. The Aptima HIV-1 RNA Qualitative Assay was performed according to the manufacturer’s instructions using 500 µl of the eluate.

Initial experiments with DBS made from whole blood spiked with known quantities of HIV-1 indicated that our limit of detection was near 400 cp/ml, which seemed appropriate given the small sample volume represented by two punches from a single spot (approximately 10 µl plasma). However, once we began testing DBS collected from adults with low viral loads, the detection limit for the Aptima assay fell to 20-200 cp/ml (Figure 1). The lower detection limit with patient DBS, as compared to the spiked DBS may be explained by the contribution of cellular HIV RNA and possibly proviral DNA from infected cells in the blood, since all nucleic acid is included in the Aptima assay (4 cp/reaction or approximately 300 cp/ml plasma (9)). The real-time RT-PCR assay developed by Ou et al. (8) also involved isolation of total nucleic acid, which is likely why that assay was also very sensitive (lower limit approximately 8 cp/reaction or 4000 cp/ml). Cellular RNA is not measured in plasma testing, so the level of RNA may be higher in whole blood due to the infected cells.

In all, we tested 291 DBS from infants and children born to HIV-1 infected mothers (Table 1). The samples had been collected as part of prevention of mother to child transmission studies in South Africa (subtype C) (10-12); Malawi (subtype C); Tanzania (HIVNET 024, subtypes A, C, and D) (6), Vietnam (CRF 01_AE); Dominican
Republic, Haiti, and Trinidad (subtype B), and North Carolina (subtype B). The spots had been made from 50\mu L whole blood per spot on Whatman 903 cards with blood drawn by heel-stick. The samples were dried at room temperature, sealed individually in plastic bags with desiccant pouches, and stored at 4°C (Vietnam and NC infants) or room temperature prior to testing. The spots were stored in our laboratory between less than one year (NC) and 3 to 5 years (HIVNET 024) prior to testing for this study. HIV status for the samples had originally been determined by NucliSens HIV-1 QT manual viral load assay (Biomerieux, DBS from Dominican Republic, Haiti, Trinidad, Malawi, and Tanzania), DNA PCR assay (Roche Amplicor v1.5, DBS from South Africa), or an in-house HIV assay (DBS from Vietnam). All samples had been collected with appropriate Institutional Review Board approval for each country and the UNC IRB also approved the study.

The Aptima assay was very sensitive for infant diagnosis using DBS. 129 of the 291 samples had previously tested positive, and 128 of the 129 were reactive on the Aptima assay (99.2% sensitivity) (Table 1). The one false negative infant specimen had been stored for 4 years at room temperature and had the lowest VL of the HIVNET 024 samples tested (10,954 cp/ml in May 2003). We have shown in a separate study that these DBS lost an average of 1 log_{10} in VL over this length of time (Nelson et al., submitted). The 162 samples that had previously tested negative were all were non-reactive on the Aptima assay (100% specificity) (Table 1).

The results of the present study indicate that DBS can be used in the Gen-Probe Aptima HIV-1 RNA Quantitative Assay for infant diagnosis without a loss of sensitivity or specificity achieved by the currently used methods. In our laboratory, we have found
that the Aptima assay is less expensive than the NucliSens HIV-1 QT manual assay (bioMérieux, Inc.) for DBS with increased sensitivity and less labor involved, making it a good choice for infant diagnosis from DBS. In addition, this very sensitive assay would be ideal for the diagnosis of acute HIV infection via DBS.

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Figure legend

Figure 1. Aptima results (Reactive or Non-Reactive) from 39 DBS collected from adults. Viral loads were determined using the Roche Monitor v1.5 RNA assay (either standard or ultrasensitive method) on plasma from the same blood collection as was used for the DBS. Open circles designate determined viral loads, while filled triangles designate viral loads under the lower detection limit (50 cp/ml).
References


immunodeficiency virus type 1 infection in vertically exposed infants in the routine diagnostic laboratory. Clin Vaccine Immunol 14:201-3.


Table 1. Sources, subtypes, and Aptima results for infant DBS.

<table>
<thead>
<tr>
<th>Site of Collection</th>
<th>Presumed Subtypes</th>
<th>Initial testing method</th>
<th># Aptima-R(^a)/ # HIV-positive tested</th>
<th># Aptima-NR(^a)/ # HIV-negative tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Carolina</td>
<td>B</td>
<td>DNA PCR</td>
<td>2/2</td>
<td>74/74</td>
</tr>
<tr>
<td>Haiti</td>
<td>B</td>
<td>DNA PCR</td>
<td>2/2</td>
<td>0/0</td>
</tr>
<tr>
<td>Trinidad</td>
<td>B</td>
<td>DNA PCR</td>
<td>4/4</td>
<td>0/0</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>B</td>
<td>DNA PCR/ NucliSens</td>
<td>18/18</td>
<td>8/8</td>
</tr>
<tr>
<td>South Africa(^b)</td>
<td>C</td>
<td>DNA PCR</td>
<td>47/47</td>
<td>32/32</td>
</tr>
<tr>
<td>Malawi</td>
<td>C</td>
<td>NucliSens</td>
<td>26/26</td>
<td>10/10</td>
</tr>
<tr>
<td>Tanzania (HIVNET 024)(^c)</td>
<td>A, C, D</td>
<td>Roche Monitor v.1.5 (Std)</td>
<td>25/26(^d)</td>
<td>28/28</td>
</tr>
<tr>
<td>Vietnam</td>
<td>CRF01_AE</td>
<td>DNA PCR</td>
<td>4/4</td>
<td>10/10</td>
</tr>
</tbody>
</table>

\(^a\)Aptima-R denotes reactive in the Aptima assay, Aptima-NR denotes non-reactive in the Aptima assay.

\(^b\)References (10-12).

\(^c\)Reference (6).

\(^d\)False negative Aptima result was obtained with the sample in this group with the lowest VL.
Figure 1.