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# Qualitative Human Immunodeficiency Virus RNA Analysis of Dried Blood Spots for Diagnosis of Infections in Infants▽

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**The Gen-Probe Aptima human immunodeficiency virus type 1 (HIV-1) RNA assay was adapted for the diagnosis of HIV infection in infants by 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 human immunodeficiency virus type 1 (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 for 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), the ultrasensitive p24 antigen assay (12), and viral load (VL) testing (1, 4, 8). The lower limits of detection in these RNA and DNA PCR detection methods were approximately 4,000 copies (cp)/ml (1, 4, 8, 14), likely due to the small sample size of 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 being a more sensitive screening tool for HIV-positive samples from a sexually transmitted disease clinic than typical antibody testing followed by pooled RNA testing (13). In addition, preliminary results from our laboratory suggest that plasma specimens with VLs of ≥20 cp/ml are detectable. Therefore, we evaluated this assay for use with 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 the removal of 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 6-mm 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. Elution buffer (525 μl) was added to each tube, and the specimens were rocked for 2 hours at room temperature. The specimens were spun down for 30 s 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 VLs, the detection limit for the Aptima assay fell to 20 to 200 cp/ml (Fig. 1). The lower detection limit with patient DBS, compared to the spiked level of 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 (a lower limit of 4 cp/reaction was achieved by Pasternak et al. [9] by using peripheral blood mononuclear cells). The real-time reverse transcription-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 (a lower limit of approximately 8 cp/reaction or 4,000 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

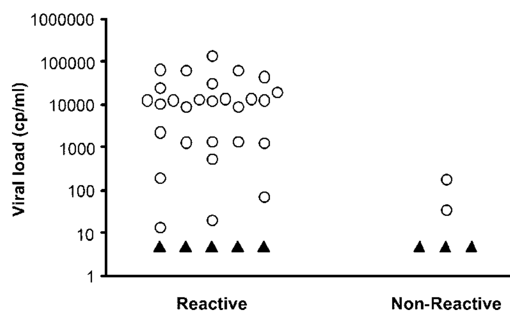


FIG. 1. Aptima results (reactive or nonreactive) from 39 DBS collected from adults. VLs were determined using the Roche Monitor v1.5 RNA assay (using either the standard or ultrasensitive method) on plasma from the same blood collection that was used for the DBS. Open circles, determined VLs; filled triangles, VLs under the lower detection limit (50 cp/ml).

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

| Site of collection | Presumed subtype(s) | Initial testing method | No. of Aptima-R samples/ no. of HIV-positive samples (%)[a] | No. of Aptima-NR samples/ no. of HIV-negative samples (%)[a] |
|---|---|---|---|---|
| North Carolina | B | DNA PCR | 2/2 | 74/74 |
| Haiti | B | DNA PCR | 2/2 | 0/0 |
| Trinidad | B | DNA PCR | 4/4 | 0/0 |
| Dominican Republic | B | DNA PCR/NucliSens | 18/18 | 8/8 |
| South Africa[b] | C | DNA PCR | 47/47 | 32/32 |
| Malawi | C | NucliSens | 26/26 | 10/10 |
| Tanzania (HIVNET 024)[c] | A, C, D | Roche Monitor v1.5 (standard) | 25/26[d] | 28/28 |
| Vietnam | CRF01_AE | DNA PCR | 4/4 | 10/10 |
| Total | | | 128/129 (99.2) | 162/162 (100) |

[a] Aptima-R, reactive in the Aptima assay; Aptima-NR, nonreactive in the Aptima assay.
[b] See references 10, 11, and 12.
[c] See reference 6.
[d] The false-negative Aptima result was obtained with the sample in this group with the lowest VL.

HIV-1-infected mothers (Table 1). The samples had been collected as part of the 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 μl 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 (for Vietnam and North Carolina infants) or room temperature prior to testing. The spots were stored in our laboratory for between $<1$ year (North Carolina) 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 VL assay (bioMérieux; 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. One hundred twenty-nine 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 (J. A. E. Nelson, A. M. Loftis, D. Kamwendo, W. W. Fawzi, T. E. Taha, R. L. Goldenberg, and S. A. Fiscus, submitted for publication). The 162 samples that had previously tested negative were all nonreactive 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 qualitative assay for infant diagnosis without the loss of sensitivity or specificity achieved by 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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