Validation of the Gen-Probe Aptima Qualitative HIV-1 RNA for Diagnosis of Human Immunodeficiency Virus Infection in Infants

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Abstract

The qualitative Roche HIV-1 DNA Amplicor assay has been used for the past 20 years to diagnose HIV infection in infants and young children, but is being phased out; hence, alternative assays must be found. The Gen-Probe Aptima qualitative HIV-1 RNA assay is currently the only FDA cleared HIV-1 nucleic acid assay approved for diagnosis, but data on the use of this assay with infant plasma are limited. We assessed Aptima’s performance using control material for reproducibility and limit of detection and 394 plasma samples (0.2 to 0.5 ml) from HIV exposed infected and uninfected infants and children for analytical sensitivity and specificity. Assays to assess within run repeatability and between run reproducibility indicated that the 10,000 (5 of 5), 200 (5 of 5), 100 (16 of 16), 50 (12 of 12), and 25 (20 of 20) HIV-1 RNA copies/ml controls were always positive and negatives were always negative (20 of 20). The limit of detection was 14 cp/ml as determined by probit analysis. The analytic sensitivity of the assay was 99.5% (189/190; 95% confidence interval (CI): 97.1-99.9%) and specificity was 99.5% (199/200; 95% CI: 97.2-99.9%). These results suggest that the assay is suitable for early infant diagnosis of HIV-1.
The Joint United Nations Program on HIV/AIDS (UNAIDS) estimates that children less than 15 years of age accounted for roughly 13% of new HIV infections in 2011 (1). Additionally, a meta-analysis concluded that with no intervention about 35% of African HIV infected children die before their first birthday and more than 50% die by the time they are 2 years old (2). However, data from the Children with HIV Early Antiretroviral Therapy (CHER) trial demonstrated that early, as opposed to delayed, initiation of antiretrovirals (ARVs) in young infants significantly reduced mortality (3), and data from several clinical trials have indicated that infected infants exposed to prophylactic ARVs often develop resistance to the drugs which may limit future therapeutic drug choices (4-6). Thus, early identification of HIV infection in infants is important so that ARV prophylaxis can be stopped to reduce the development of resistance to the ARVs and therapeutic doses of ARVs can be initiated as quickly as possible. Early diagnosis of HIV infection using serologic testing of antibodies, however, is hindered by the presence of maternal antibodies that cross the placenta during gestation. Consequently, early infant diagnostics must test for either viral antigens or nucleic acids.

For many years the Roche Amplicor HIV-1 DNA assay, version 1.5, has been the mainstay and gold standard for early infant diagnosis (EID), having been validated and used extensively in many countries, and recommended by the World Health Organization, as well as the US Center for Disease Control and Prevention, for EID programs using both whole blood pellets and dried blood spots (DBS) (7). However, Roche plans to discontinue this assay in the next few years, so alternative assays must be found (8). Although there are several alternatives including the Roche TaqMan and Abbott RealTime quantitative HIV-1 RNA and qualitative HIV-1 total nucleic acid assays, the Gen-Probe Aptima HIV-1 RNA qualitative assay is the only nucleic acid assay currently approved by the FDA for HIV diagnosis using serum or plasma (9). Although its utility in EID using DBS (10-12) and whole blood (11) has been reported, data on the use of this assay using infant plasma are limited. The NY State Department of Health evaluated Aptima for infant diagnosis, but only tested plasma from 28 HIV exposed uninfected
babies, and 68 HIV infected infants (Sullivan TJ, Miller TT, Warren B, Parker MM. Presented at the 3rd HIV Diagnostics Conference, Orlando, FL, March 24-26, 2010). An additional 48 sera from HIV-exposed, uninfected infants were tested and found to be non-reactive in the Aptima assay (13).

Materials and Methods

Samples - The limit of detection, within run repeatability and between run reproducibility were assessed using control material obtained from the Virus Quality Assurance Program (VQA, Rush University Medical Center, Chicago, IL) (14) diluted in BaseMatrix (SeraCare, Milford, MA). Repeatability was assessed by diluting the VQA HIV-1 RNA 200 cp/ml control material to final concentrations of 100 and 25 HIV-1 RNA copies/ml in and testing in duplicate in 4 separate runs by two technologists. Reproducibility was determined by testing three positive VQA controls (10,000, 200, and 50 cp/ml) and a negative control in singlet over 5 days using 2 different kit lots. To assess the limit of detection, we made additional dilutions to 0.4 cp/ml that were tested in 4 separate runs by two technologists using 3 kit lots. Fewer samples were tested at the highest concentrations (n = 5-8 replicates) compared to lower concentrations that were tested more frequently (n = 10-20 replicates). There were a few invalid results that were not included in the analysis. BaseMatrix was used as HIV RNA negative control material for all these experiments.

Analytic sensitivity was assessed using 114 plasma samples obtained from HIV infected infants aged 3 to 12 months (median 7 months) and 79 plasma samples from older children (13 months – 18 years; median 9.3 years) who had been enrolled in Pediatric AIDS Clinical Trials Group Study 152 (15). Specificity was evaluated using plasma samples from 201 HIV-exposed uninfected infants enrolled in International Maternal Pediatric Adolescent AIDS Clinical Trials Study P1025 (16). Infection status for these infants had been determined previously either by HIV culture (17) or HIV DNA PCR testing (Roche Amplicor, Roche Molecular Systems, Branchburg, NJ) in laboratories successfully participating...
in the VQA proficiency testing program (17, 18). Testing personnel were blinded to the previously
determined infection status of each specimen.

Assay - The Gen-Probe Aptima qualitative HIV RNA assay (Hologic Gen-Probe, Inc, San Diego, CA)
was performed following the manufacturer’s package insert (9) using 0.5 ml of plasma, when available.

In 42 cases, less than 0.5 ml was tested (0.4ml – 19 times; 0.35 ml – 10 times; 0.3 ml – 8 times; 0.25
ml – 4 times and 0.2 ml – once). Assay software was used to compare the relative light units reported
by a luminometer to run-specific cut-offs.

Analysis - Analyses were conducted with Stata SE, version 13.0. The 95% limit of detection was
assessed using probit analysis incorporating the base 10 logarithm of the HIV-1 RNA cp/ml. Sensitivity
and specificity were calculated using standard methods, considering HIV culture and HIV DNA PCR
with Roche Amplicor as the reference standard. Ninety-five percent confidence intervals (95% CI) were
calculated for sensitivity, specificity, and repeatability using the Wilson method (19).

Results

Assays to assess within run repeatability and between run reproducibility indicated that the 10,000 (5 of
5), 200 (5 of 5), 100 (16 of 16), 50 (12 of 12) and 25 (20 of 20) HIV-1 RNA copies/ml controls were
always positive and the negatives were always negative (20 of 20) (Tables 1 and 2). Experiments to
assess the limit of detection indicated that the assay could detect 25 HIV RNA cp/ml 100% of the time
and 6-12.5 cp/ml 75-90% of the time. Detection dropped off sharply after that (Table 2). In probit
analysis, the 95% limit of detection was 14.1 cp/ml.

Of the 394 clinical samples tested, only 4 were invalid (1.0%); these were excluded from data analysis.
Analytic sensitivity was 99.5% (189/190; 95% CI: 97.1-99.9%) (Table 3), despite limited sample volume
for 42 infants. Specificity was 99.5% (199/200; 95% CI: 97.2-99.9%). Signal to cut-off (S/CO) ratios for reactive samples ranged from 2.7-38.3, with >96% (182/189) of reactive samples with S/CO ratios greater than 19.0 (median S/CO ratio for all reactive specimens was 22.0). There was no indication that specimens from infected babies where less than 0.5 ml of plasma was used had lower S/CO ratios since 11.5% of specimens (17/148) with full volumes had S/CO ratios < 20.0 compared to 11.9% of specimens (5/42) with < 0.5 ml. The single false negative had a S/CO ratio of 0.30. The single false positive result had a S/CO ratio of only 2.68 and would have been repeated under the laboratory’s standard operating procedures had there been sufficient plasma. Signal to cut-off ratios for non-reactive specimens ranged from 0.06-0.55, with > 90% (181/200) < 0.20.

Discussion
The Aptima assay appears extremely sensitive with a lower limit of detection of 14.1 cp/ml. Kerr et al. (10) found that DBS from 5 of 8 adults with HIV RNAs < 50 cp/ml were detected in the Aptima assay. Similarly, Stevens et al. (11) performed dilution experiments with DBS and found that the Aptima assay generated reactive results that were 1-3 log units more sensitive than the Roche Amplicor assay. Lilian et al. reported that at birth, 2 weeks, and 4 weeks, Aptima detected 76%, 64%, and 96% of infected children compared to 68%, 64%, and 88% by the Roche Amplicor assay using DBS (12). Furthermore, Aptima detected HIV infection in the sera of 6 children an average of 28 days earlier (range 18-65 days) compared to HIV DNA testing (Sullivan TJ, Miller TT, Warren B, Parker MM. Presented at the 3rd HIV Diagnostics Conference, Orlando, FL, March 24-26, 2010). This increased sensitivity may be important as more infants are exposed to combination ARVs for prevention of mother to child transmission (20, 21).

Previous work in our laboratory suggested that S/CO ratios between 1.0-5.0 were suspect and should be repeated (unpublished observations). Only three of the 394 clinical specimens had S/O ratios that fell in this range (2.68, 4.08, and 4.17) and none of these had sufficient volume to repeat. Stevens, et al...
(11) also reported improved specificity when DBS with S/CO ratios less than 10 were repeated. Analytic specificity in HIV exposed, uninfected children < 18 months of age has been consistently high ranging from 99.4-100% (10-12).

A major limitation of this study was that none of the specimens from infected children were obtained before the first 6 weeks of life when sensitivity for HIV diagnosis is typically lower (22). With increased uptake of more potent ARVs for the prevention of mother to child transmission, the proportion of intrauterine transmission compared to intrapartum transmission has increased and thus more infected babies are detected at birth rather than at 6 weeks (23). Another limitation is that the infected infants had minimal exposure to ARVs, and then only to zidovudine and not to combination prophylaxis ARVs that are commonly in use today (20, 21). Some have argued that detection of HIV DNA or total nucleic acid may be more sensitive than HIV RNA detection when infants are exposed to prophylactic or therapeutic levels of ARVs (20, 21), although a recent study does not confirm that hypothesis (24).

A further limitation is that in most cases the recommended 500 uL of plasma was tested, but it is often difficult to obtain a full 500 uL of plasma from newborn infants. Our limited data on samples with less than the full 0.5 ml of plasma and the exquisite sensitivity of the assay (14 cp/ml limit of detection), suggest that smaller volumes of plasma could be used if appropriately validated. We did not have access to fresh whole blood from infected infants since perinatal HIV infection is now rare in North Carolina (S. Fiscus, unpublished data). In addition, the Aptima package insert states that whole blood should not be frozen prior to testing. However, Stevens, et al (11) diluted whole blood in reverse osmosis water prior to transport to the laboratory where it was apparently immediately loaded onto the Tigris instrument for analysis. This option would only be feasible in a high throughput laboratory.

Since sufficient volumes of whole blood and plasma may be difficult to obtain, especially in neonates, DBS may offer an attractive alternative. Our previous data (10) and those of others (11, 12),
demonstrate that DBS yield extremely sensitive and specific results and have been recommended for use in low resource countries with other HIV-1 nucleic acid assays as a means to provide greater accessibility to testing (7, 8, 25). The Aptima assay is currently being used in New York State to identify HIV infection in infants (26) and should be considered as an alternative to the Roche Amplicor HIV-1 DNA assay for infant diagnosis.
Acknowledgments:

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References:


Table 1. Within-run and within-laboratory repeatability of the Aptima HIV-1 RNA assay.

<table>
<thead>
<tr>
<th>Concentration of control HIV-1 RNA</th>
<th>Number Reactive/ Number Tested (%)</th>
<th>Concentration of control HIV-1 RNA</th>
<th>Number Reactive/ Number Tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 cp/ml</td>
<td>8/8 (100%; 95% CI: 67.6-100%)</td>
<td>10,000 cp/ml</td>
<td>5/5 (100%; 95% CI: 56.6-100%)</td>
</tr>
<tr>
<td>25 cp/ml</td>
<td>8/8 (100%; 95% CI: 67.6-100%)</td>
<td>200 cp/ml</td>
<td>5/5 (100%; 95% CI: 56.6-100%)</td>
</tr>
<tr>
<td>BaseMatrix Negative Control</td>
<td>8/8 (100%; 95% CI: 67.6-100%)</td>
<td>50 cp/ml</td>
<td>5/5 (100%; 95% CI: 56.6-100%)</td>
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Table 2. Limit of Detection of the Aptima HIV-1 RNA Qualitative Assay.

<table>
<thead>
<tr>
<th>Concentration of control HIV-1 RNA</th>
<th>Number Reactive/ Number Tested (%; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 cp/ml</td>
<td>8/8 (100%; 67.5-100%)</td>
</tr>
<tr>
<td>50 cp/ml</td>
<td>7/7 (100%; 64.6-100%)</td>
</tr>
<tr>
<td>25 cp/ml</td>
<td>12/12 (100%; 75.8-100%)</td>
</tr>
<tr>
<td>12.5 cp/ml</td>
<td>11/12 (91.6%; 64.6-98.5%)</td>
</tr>
<tr>
<td>6.1 cp/ml</td>
<td>9/12 (75%; 46.8-91.1%)</td>
</tr>
<tr>
<td>3.1 cp/ml</td>
<td>5/10 (50%; 23.7-76.3%)</td>
</tr>
<tr>
<td>1.6 cp/ml</td>
<td>5/10 (50%; 23.7-76.3%)</td>
</tr>
<tr>
<td>&lt; 1 cp/ml</td>
<td>0/12 (0%; 0, 24.2%)</td>
</tr>
<tr>
<td>Negative control</td>
<td>0/12 (0 %; 0, 24.2%)</td>
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</table>
Table 3. Sensitivity and specificity of the Aptima HIV RNA Qualitative Assay

<table>
<thead>
<tr>
<th></th>
<th>Positive Culture or DNA PCR</th>
<th>Negative Culture or DNA PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Aptima</td>
<td>189</td>
<td>1</td>
</tr>
<tr>
<td>Negative Aptima</td>
<td>1</td>
<td>199</td>
</tr>
</tbody>
</table>