Evaluation of a High Throughput Diagnostic System for Detection of HIV-1 in Dried Blood Spot (DBS) Samples from Infants in Mozambique

Authors & affiliations

Ilesh V. Jani1, Jennifer Sabatier2, Adolfo Vubil1, Shambavi Subbarao2, Dulce Bila1, Amina de Sousa1, Nédio Mabunda1, Albert Garcia2, Beth Skaggs3, Dennis Ellenberger2*, Artur Ramos2

1. Instituto Nacional de Saúde, Maputo, Mozambique
2. Centers for Disease Control and Prevention, Atlanta USA
3. Centers for Disease Control and Prevention, Mozambique

*Corresponding author:
Dennis L. Ellenberger, PhD
Team Lead, Molecular Monitoring Team
International Laboratory Branch
Global AIDS Program
Centers for Disease Control and Prevention
1600 Clifton Rd., MS G-19
Atlanta, GA 30333
Telephone: 404 639-1016
Email: dellenberger@cdc.gov

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Abstract

We performed a comparative analysis between Roche Amplicor HIV-1 DNA Test and CAPTAQ assay for the detection of HIV in 830 Dried Blood Spots pediatric samples collected in Mozambique. Our results demonstrated no statistical difference between these assays. The CAPTAQ assay approached nearly 100% of repeatability/accuracy. The increased throughput of testing with minimal operator interference in performing the CAPTAQ assay clearly demonstrated this method is an improvement over Roche Amplicor HIV-1 DNA Test, version 1.5.

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Early infant diagnosis (EID) and immediate initiation of antiretroviral therapy are paramount to prevent AIDS-related deaths and to evaluate the effectiveness of Prevention of Mother-to-Child Transmission (PMTCT) programs (6,8,16,17). Currently, in most resource-poor settings with high burdens of HIV infections, a significant proportion of exposed infants do not have access to timely diagnosis of HIV. While it is necessary to seek simpler diagnostic assays for decentralization of testing, it is also urgent to increase the throughput of centralized testing to meet demand.

The Roche Amplicor HIV-1 DNA Test, version 1.5 (Roche Molecular Diagnostics, Branchburg NJ) has been used by many diagnostic laboratories (13) for detection of HIV in dried blood spot (DBS) (1,10) samples. This assay has demonstrated high sensitivity and specificity (9). However, because this assay is performed manually, it has a low throughput in comparison with automated tests (3,4,5,14,15). In order to increase the testing capacity of Instituto Nacional de Saúde in Mozambique where approximately 2,900 children were tested each month for HIV infections in 2010, we evaluated the performance of Roche COBAS AmpliPrep/COBAS TaqMan (CAPTAQ) HIV-1 Qual Test (Roche Molecular Diagnostics, Branchburg NJ), which is an automated qualitative test for HIV detection in blood or DBS (12). 830 country-wide pediatric (six weeks to nine months old) DBS samples that were consecutively received and tested for HIV, by Amplicor assay in 2010 were subsequently tested using the CAPTAQ assay for comparison of results, following manufacturer’s recommendations (10,12). Results of both assays are summarized in Table 1. A total of eight samples (0.96%) provided discordant results between the
methods. All eight discordant samples were retested using the Amplicor assay and results were identical to the initial testing. Unfortunately, insufficient DBS material was available to repeat the testing with the CAPTAQ assay.

The McNemar’s test and Cohen’s Kappa statistic test were used to determine agreement between the two assays (7). Additionally, we calculated sensitivity and specificity of the CAPTAQ assay, as well as overall, positive, and negative indices of agreement, using the Amplicor assay as the gold standard method (2,11). There was no evidence of statistically significant discordance between the two assays as shown by the McNemar’s test (Table 1). Additionally, the Kappa statistic was 0.88 with a lower 95% confidence limit of 0.84. These numbers are above 0.8, which indicates good agreement between the CAPTAQ and the Amplicor assays.

Lastly, when compared to the Amplicor assay, the sensitivity and specificity of the CAPTAQ assay was 94.2% and 99.2% with lower 95% confidence bounds of 93% and 98.2%, respectively. The proportion of overall agreement was 99% with a lower bound of 98.4%.

However, due to the high number of undetected HIV-1 samples there was a tendency or bias towards the overall agreement to be high. Therefore, we also computed the proportions of agreements for HIV-1 detected and HIV-1 undetected results separately, estimating the conditional probability of both tests producing the same results (Table 2). Our data demonstrated the proportion of positive agreement is also high at 96.1% (95% CI: 93.4%, 98.8%).

Blood samples (5ml) from 20 HIV-1 infected individuals, with known viral loads, and 50 HIV-1 non-infected individuals were acquired from commercial sources (Zeptometrix, Buffalo, NY and Tennessee Blood Services, Memphis, TN, respectively) were used to determine the degree of repeatability of CAPTAQ assay. Five 100µl whole DBS blood were prepared from each donor sample. We examined the repeatability of the CAPTAQ assay along with its sensitivity and specificity by testing DBS specimens from all individuals on three separate runs following manufacturer’s recommendations. To assess repeatability of the CAPTAQ assay, first we used a generalized McNemar’s test to determine whether the proportion of detected HIV in each run was different. Second, we examined repeatability through the use of Fleiss’ Kappa statistic as described above. Results demonstrated a 99% degree of agreement in all runs (Table 4). The only false-negative result was from a HIV-infected individual with a viral load below 50
copies/ml, which is approximately the limit of detection of most molecular assays. The repeatability of the instrument across samples was high, with a Fleiss Kappa of 0.98 in each category as well as the overall rating. The lower 95% confidence bound of this statistic is 0.83, which is above the 0.81 threshold generally held to indicate strong repeatability (Table 3). Additionally, the proportion of positive agreement was 98.3% with a lower 95% CI boundary of 94.7%, indicating with reasonable confidence that the true proportion of replicates agreeing on a result demonstrating presence of HIV is between 94.7% and 100% (Table 4).

A prospective study of a cohort of HIV-exposed children would be necessary to determine the clinical sensitivity and specificity of the CAPTAQ assay. However, we can infer that these parameters are similar to the Amplicor assay based on our results and studies published elsewhere (9,12). The discordant results observed between the two methods, which constituted 1% of samples, could be attributed to the combined rates of sensitivity and specificity of both assays.

The CAPTAQ configuration used in this study was one COBAS Ampliprep instrument coupled to one COBAS TaqMan48 instrument. Method comparison, sensitivity, specificity, and repeatability parameters of the CAPTAQ assay verified by the Instituto Nacional de Saúde in Mozambique, during routine laboratory operations were satisfactory. Minor modifications in the preparation of DBS were introduced in the laboratory work flow to adapt for the CAPTAQ instrument. However, barcode scanning capability, prevention of operator-generation errors by the CAPTAQ system, and increased throughput of testing from 200 samples/technician/week to 450 samples/technician/week were advantages over the Amplicor assay. In the absence of virological point-of-care assays, automated laboratory diagnostic systems such as the CAPTAQ assay that are capable of processing large number of DBS specimens in a cost-efficient manner seem to be an appropriate solution to increase access to EID in high-demand settings.

Roche Molecular Diagnostics, Branchburg NJ, provided part of the reagents and the CAPTAQ equipment for this study as well as technical assistance. Shabnam Zavahir from Roche Molecular Diagnostics, provided logistical support and relevant suggestions facilitating the completion of this work.
References

### Table 1. Summary of comparative results of CAPTAQ and Amplicor assays.

<table>
<thead>
<tr>
<th></th>
<th>Amplicor</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>McNemar’s test p-value</th>
<th>Cohen’s Kappa (95% CI) †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV+</td>
<td>HIV-</td>
<td>total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAPTAQ</td>
<td>98</td>
<td>2</td>
<td>100</td>
<td>94.2% (93%, 99.8%)</td>
<td>99.7% (98.2%, 99.7%)</td>
</tr>
<tr>
<td>HIV+</td>
<td>100</td>
<td>100</td>
<td>0.125</td>
<td>0.29</td>
<td>0.88</td>
</tr>
<tr>
<td>HIV-</td>
<td>6</td>
<td>724</td>
<td>730</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>104</td>
<td>726</td>
<td>830</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† 95% Confidence Intervals (CI) derived from 10,000 ordinary bootstrap replicates.
HIV+ represents HIV detected.
HIV- represents HIV not detected.

### Table 2. Proportions of Overall and Specific Agreement for the CAPTAQ and Amplicor assays in detecting HIV-1 for N= 200 DBS.

<table>
<thead>
<tr>
<th>Type of Agreement</th>
<th>Proportion</th>
<th>Standard Error</th>
<th>Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Agreement (p₀) ††</td>
<td>99%</td>
<td>0.03%</td>
<td>(98.4%, 99.7%)</td>
</tr>
<tr>
<td>Positive Agreement (pₚₚ) †††</td>
<td>96.10%</td>
<td>0.14%</td>
<td>(93.4%, 98.8%)</td>
</tr>
<tr>
<td>Negative Agreement (pₚₙₙ) ††††</td>
<td>99.50%</td>
<td>0.02%</td>
<td>(99.1%, 99.8%)</td>
</tr>
</tbody>
</table>

†† p₀ = proportion of cases for which Amplicor and CAPTAQ tests agree.
††† pₚₚ = estimated probability that one test will detect HIV given that the other test will also produce the same result.
†††† pₚₙₙ = estimated probability that one test will not detect HIV given that the other test will also produce the same result.

### Table 3. Summary of repeatability of results by the CAPTAQ assay on DBS samples prepared from 20 HIV-1 infected and 50 HIV uninfected individuals.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Fleiss’ Kappa (SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV detected</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>0.98 (0.069)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HIV not detected</td>
<td>51</td>
<td>50</td>
<td>50</td>
<td>0.98 (0.069)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Overall (total count)</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>0.98 (0.069)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table 4. Proportions of Overall and Specific Agreement for the CAPTAQ assay for three replicates of DBS samples prepared from 20 HIV-1 infected and 50 HIV uninfected individuals.

<table>
<thead>
<tr>
<th>Type of Agreement</th>
<th>Proportion</th>
<th>Bootstrap Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific Agreement ps (HIV+)†</td>
<td>98.30%</td>
<td>94.70%</td>
</tr>
<tr>
<td>Specific Agreement ps (HIV-)†</td>
<td>99.30%</td>
<td>98.00%</td>
</tr>
<tr>
<td>Overall Agreement po ‡‡</td>
<td>99.10%</td>
<td>97.10%</td>
</tr>
</tbody>
</table>

† ps(j) = the proportion of agreement specific to category j is equal to the total number of agreements on category j divided by the total number of opportunities for agreement on category j, where j is either HIV + or HIV -.

‡‡ po = proportion of cases for which all three replicates of the HIV-1 Amplicor version 1.5 DNA-PCR test agree.

HIV+ represents HIV detected; HIV- represents HIV not detected