Development of a PCR-based HIV-1 test for infants to be used at the point-of-care in resource limited settings

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Summary
DNA PCR is a recommended method for HIV diagnosis in infants but cost and complexity limit its availability in resource-limited settings. Dried Blood Spot based DNA PCR testing strategies have increased the number of infants being tested. However, the percentage of patients not being informed of the results has increased due to long turn around times. We are developing a rapid, inexpensive, point-of-care DNA PCR test for infants so that results can be delivered while the caregiver waits. DNA extraction from blood has been reduced to 2 min and combined with real-time PCR can produce results in an hour. The limit of detection of the method is 20 HIV-1 copies in 100 μL blood. We have also achieved clinical sensitivity of 99% and specificity of 100%.

Need for a POC infant testing platform
- Current testing strategy in sub-Saharan Africa is expensive and has long turn around time resulting in high lost-to-follow up rates.
- Delays initiation of treatment causing high levels of mortality

DNA Extraction by LeukoTrap takes 2-3 min
- 182 PIBMAC pellets were obtained from Women and Infants Transmission study (WITS)
- Pellets were reconstituted in leukocyte depleted HIV negative blood

DNA Extraction by LeukoTrap takes 2-3 min
- Results were compared to those obtained with viral load testing

HIV-1 can be detected from whole blood
- Stability of dried blood samples offers improved accessibility
- Blood samples can be archived
- Tests can be run later in case of high volume clinics

LeukoTrap performance is similar to QIAamp

<table>
<thead>
<tr>
<th>Concentration (copies/μL blood)</th>
<th>Total copies in reaction</th>
<th>% Samples detected by LeukoTrap</th>
<th>% Samples detected by QIAamp mini kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>400</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.4</td>
<td>40</td>
<td>100</td>
<td>75</td>
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<tr>
<td>0.2</td>
<td>20</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
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<td>Not tested</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>33.3</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

Table 1. Limit of Detection of LeukoTrap is equivalent to that of QIAamp kit

Quantifying proviral DNA in adult blood samples
- LeukoTrap was used to quantify proviral DNA in 57 adult blood samples from the ACTG clinic, Chicago, IL.
- Patients undergoing ARV therapy were tested
- 51 samples showed proviral DNA in the range of 0.05 to 11 HIV copies/μL, blood and Internal Control Ct values between 15.37 to 17.77
- 6 samples showed undetectable proviral DNA.

Pre-clinical trials in Uganda
- A research card has been designed and molded
- An in-house real-time thermal cycler is being developed in our lab.
- Objective is to perform field trials with 200 infant samples

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