Evaluation of a dilution protocol using the Aptima HIV-1 RNA Quant assay on the Panther system with blood collected in EDTA-microtainer tubes

Rebecca Rossetti MS¹, Tara Smith MS², Jennifer Taussig MPH², Mariah Valentine-Graves MPH², Patrick Sullivan DVM, PhD³, Jessica Mae Ingersoll MS, MB(ASCP)⁴, Colleen S. Kraft MD, MS⁴, Jeffrey Johnson PhD¹, Silvina Masciotra MS¹

¹Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, ²Oak Ridge Institute for Science and Education, ³Department of Epidemiology, Rollins School of Public Health, Emory University, ⁴Center for AIDS Research, Emory University

Background

- FDA-approved viral load (VL) assays use only venipuncture-derived plasma collected in clinical settings
- Dried blood spots (DBS) have been evaluated as an alternative collection method; however, the limit of quantification (LOQ) of DBS VL assays is not optimal to evaluate viral failure at 2.3 log₁₀ copies/ml (log₁₀)

Methods

Example of self-fingerstick blood collection: Fill MCT to a minimum volume of 200 µl. Invert 10 times and label tube.

Viral Load Testing

Sample Processing

• Fingerstick whole blood (FSB) collected in EDTA
• Dried blood spots (DBS) have been evaluated as an alternative collection method; however, the limit of quantification (LOQ) of DBS VL assays is not optimal to evaluate viral failure at 2.3 log₁₀ copies/ml (log₁₀)
• Fingerstick whole blood (FSB) collected in an EDTA-microtainer tube (MCT) in non-clinical settings including home self-collection could be an alternative method to obtain plasma and help achieve the goals of the new initiative to increase access to HIV testing.

We evaluated the performance of a 1:7 dilution VL protocol with the Hologic Aptima HIV-1 RNA Quant assay using previously characterized plasma controls and plasma derived from FSB collected in MCTs during the Engagement sub-study¹

HIV Samples and Analysis

- Standard and 1:7 dilution VL protocols were evaluated by testing 0.7 ml of undiluted and 1:7 diluted plasma in:
  - Eight commercial controls (SeraCare and Acrometrix)
  - One control diluted to 2.95, 2.76, 2.56, 2.48 log₁₀

Results

Table 1: Results from prepared dilutions and commercial controls

<table>
<thead>
<tr>
<th>Standard Mean VL</th>
<th>1:7 Dilution VL Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>log₁₀</td>
<td>n &lt;1.47 log₁₀</td>
</tr>
<tr>
<td>2.21</td>
<td>3/3</td>
</tr>
<tr>
<td>2.48</td>
<td>7/18</td>
</tr>
<tr>
<td>2.56</td>
<td>5/15</td>
</tr>
<tr>
<td>2.76</td>
<td>0/15</td>
</tr>
<tr>
<td>2.95</td>
<td>0/10</td>
</tr>
</tbody>
</table>

The correlation between protocols was high, although the agreement was lower at low HIV-1 RNA concentrations was lower (red circle in Fig. 2)

Results Summary

- With the dilution protocol, 61% of control samples were quantified at 2.48 log₁₀ and lower VLs can be quantified
- The correlation between protocols was high, although the agreement was lower at low HIV-1 RNA concentrations
- Six samples with standard VL values ranging from 1.80 – 2.46 log₁₀ were detected <1.47 log₁₀ in the dilution VL, and one sample with standard VL 2.66 log₁₀ was TND in the dilution protocol

Conclusions

- The concordance between the standard and 1:7 dilution VL protocols was high, using both commercial and clinical HIV samples
- Although the LOQ was not close to the viral failure cutoff of 2.3 log₁₀, FSB collection in MCT offers an alternative to venipuncture plasma collection for VL testing with better sensitivity than DBS protocols
- MCT collection could facilitate surveillance for virologic suppression outside of clinical healthcare settings to optimize treatment methods and further prevent HIV transmission

References

¹Emory University Engagement sub-study: FSB specimens were collected into MCTs by trained staff from individuals participating in the Engagement study. Remnant venipuncture-derived plasma specimens from the same participants were also collected and used for the comparison.
²Photos obtained from https://ayassbioscience.com/specimen-collection-instructions/