Development and evaluation of a simple, filter paper-based method of plasma separation and collection to enhance HIV monitoring in resource-limited settings (RLS)

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Background

- Viral load (VL) testing is the standard method for anti-retroviral therapy (ART) monitoring
- However, currently available VL technologies are complex and expensive
- Therefore, restricted to central laboratories in RLS
- WHO recommends the use of Dried Blood Spots (DBS) in RLS (2013 WHO ART guidelines)
- However, DBS suffers from low specificity
- Over quantification due to non-plasma nucleic acids contamination from white blood cells

AIMS

- To **develop** filter paper-based method of plasma separation and collection from whole blood
- To **evaluate** prototype plasma separation device for quantification of HIV VL

<table>
<thead>
<tr>
<th>Sample type &amp; treatment</th>
<th>Failure cutoffs</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBS (n=823)</td>
<td>&gt;5000 cp/ml</td>
<td>&gt;1000 cp/ml</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPS (n=546)</td>
<td>&gt;5000 cp/ml</td>
<td>&gt;1000 cp/ml</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DBS

- Low - Specificity
- Unnecessary targeted VL testing
- Premature regimen switching

DPS

- Excellent Performance
- Plasma Vs DPS: P > 0.2
- Complex specimen processing
  - phlebotomy &
  - centrifugation

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Methodology

**RBC: Agglutination**

- Glass fiber (GF)
- GF + anti-GYPA
- RBCs captured

**WBC: Size-exclusion**

- Plasma

Membranes assembly

- A = RBC separator (Glass fiber + anti GYPA)
- B = WBC separator (nitrocellulose membrane)
- C = Plasma receiver/collector (Whatman 903)
- D = Backing plastic card

**Efficient RBCs and WBCs filtration achieved**

Prototype device

- GF + no anti-GYPA
- GF + anti-GYPA

- RBC not retained
- RBC [100% retention]
- WBC [93% retention]

94% plasma yield (∼52 µl from 100µl whole blood)

REFERRED = Filtered & dried plasma (FDP)

HIV-RNA recovery from Filtered & Dried Plasma (FDP)

- FDP: Comparable HIV RNA recovery to plasma
- DBS: Over-quantified by over 2 folds

Prospective evaluation study

- Participants: HIV patients
- Setting: Alfred HIV Clinic
- Sample size: 200 patients
- Design: Comparative evaluation of FDP Versus:
  - Plasma
  - DBS
  - FVE (Free virus elusion), Roche new method

*DBS: Co-amplifies of proviral DNA & cell-associated RNA*
DBS: Weak correlation & concordance

DBS (n=100)

FDP shows good correlation & concordance

FDP (n=191)

FVE shows good concordance

FVE (n = 191)

Performance to diagnose ART failure at 1000 copies/ml cut off

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sensitivity (CI)</th>
<th>Specificity (CI)</th>
<th>Positive predictive value (CI)</th>
<th>Negative predictive value (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBS (n=100)</td>
<td>100 (78-100)%</td>
<td>46 (35-57)%</td>
<td>27 (14-37)%</td>
<td>100 (91-100)%</td>
</tr>
<tr>
<td>FDP (n=191)</td>
<td>100 (85-100)%</td>
<td>100 (98-100)%</td>
<td>100 (85-100)%</td>
<td>100 (98-100)%</td>
</tr>
<tr>
<td>FVE (n=191)</td>
<td>87 (66-97)%</td>
<td>100 (98-100)%</td>
<td>100 (93-100)%</td>
<td>98 (95-99)%</td>
</tr>
</tbody>
</table>

Conclusion

FDP
- Effectively removes blood cell components (RBCs & WBCs)
- ~ 94% plasma yield is achieved
- Avoids proviral DNA and cellular RNA co-amplification with plasma HIV RNA
- Efficiently diagnosed ARV treatment failure at 1000 cps/ml cut-off
- Useful and simple tool for resource limited settings
- Can be used for other assays that need cell-free plasma
- Further studies in field condition using finger prick blood samples
- Further optimization for manufacturing

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