Quantitative HIV-2 DNA Droplet Digital PCR: A new tool to study HIV-2 infection dynamics and treatment response

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Introduction

- Human Immunodeficiency Virus Type 2 (HIV-2) originated in West Africa and has spread to the US, particularly NYC.
- Like HIV-1, HIV-2 can progress to AIDS.
- Since HIV-2 RNA levels in patients are generally low, a negative HIV-2 RNA result does not exclude HIV-2 infection.
- HIV-2, a retrovirus, uses reverse transcriptase to reverse transcribe RNA into double stranded DNA. Some enter the nucleus and integrates into the host's genome. This DNA could be used as a marker to improve diagnosis and monitor a patient's response to treatment.
- Droplet digital PCR (ddPCR) is a powerful tool used to achieve absolute quantification with a high level of precision and single-molecule sensitivity. It does not require a standard curve.

Objective

Develop a quantitative HIV-2 DNA droplet digital PCR (ddPCR) assay and determine if HIV-2 RNA & DNA levels are correlated in HIV-2 infected patients.

Methods

1. Extract nucleic acid from patient blood cells (BC) with Nuclisens EasyMAG
2. Prepare mastermix and make droplets
   - Bio Rad ddPCR Supermix for Probes
   - TaqMan probes (FAM for HIV-2, HEX for IC)
   - Primers for HIV-2 and Internal Control (IC)
   - Sample DNA Extract
   Use Droplet Generator to partition ddPCR reaction mix into ~15,000 nanoliter-sized droplets
3. Transfer droplets to 96-well plate and amplify DNA by PCR
4. Read fluorescence of each droplet and analyze results

Results: Blood Cells Spiked with HIV-2 Plasmids

<table>
<thead>
<tr>
<th>Strain</th>
<th>Expected Cps/90ul</th>
<th># Positive</th>
<th># Tested</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp A (A1958)</td>
<td>500</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Grp B (310319)</td>
<td>500</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>6</td>
<td>6</td>
<td>86%</td>
</tr>
</tbody>
</table>

Table 1: Blood cells spiked with HIV-2 plasmids from Group A and B viruses. Limit of detection is 125 cps/90ul for Group A and 500 cps/90ul for Group B

Results: Patient Blood Cells

**HIV-2 RNA vs HIV-2 DNA of Patient Samples**

DNA found in patient's plasma

**HIV-2 RNA Viral Load (Log IU/ml)**

y = 0.7134x + 0.1545
R² = 0.5939

**Figure 2:** HIV-2 DNA and previously quantified HIV-2 RNA levels have a positive relationship in HIV-2 infected patients. For samples with RNA values >66 International Units (IU)/ml, the R² value is 0.594 and the slope is 0.713. For 10/12 samples with undetected RNA, DNA was detected.

Conclusions

The quantitative HIV-2 DNA ddPCR assay is sensitive, linear, and reproducible. Accurate quantification of HIV-2 DNA will provide a second measure of treatment response and will improve our understanding of HIV-2 infection dynamics.

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