A sensitive HIV-1 DNA quantitative PCR assay for measuring the residual HIV-1 reservoir

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BACKGROUND

Sensitive and feasible methods for measuring HIV-1 reservoirs are critical to evaluate interventions for HIV-1 eradication. Though total HIV-1 DNA measurements overestimate latent reservoir size, recent studies suggest that HIV DNA reflects the size of replication-competent virus and is predictive of disease progression, response to ART, and viral rebound. Thus, HIV DNA is a valuable biomarker for HIV reservoirs (1,2).

AIM

Evaluate the performance — sensitivity, linearity, precision, accuracy and specificity — of the UltraQ HIV-1 DNA quantitative assay.

MATERIALS & METHODS

The UltraQ HIV-1 DNA quantitative assay:
- A multiplex PCR measuring the HIV-1 long-terminal repeat region and human beta-actin gene.
- Recombinant DNA, serving as quantitative standards, included to report HIV DNA copies/106 cells.
- In this study, Qiagen QiaAmp DNA blood kit and QuantStudio 5 were used to isolate DNA and perform PCR, respectively.

Sample list:
- Varied amounts of DNA extracted from 8E5/LAV cells, a single proviral DNA copy/cell, were spiked to ~1.67 µg human PBMC DNA for each PCR reaction to evaluate assay's limit of detection, linearity and precision.
- Assay specificity included viral stocks of the M group subtypes A to G and O groups, HIV-2 (ROD, EHO), HCV, HBV and Plasmidofalpum strain 3D7.
- Peripheral blood of viral suppressed patients (plasma RNA < 40 copies/mL) were tested in duplicate.

Statistical analysis:
- Microsoft Excel was used to calculate mean, SD, %CV and correlation.
- R statistical software 3.1 was used to conduct the probit analysis.

RESULTS

Limit of Detection (LOD) Probit analysis: LOD = 4 (95% CI, 2-8) copies HIV-1 DNA/reaction

Peripheral blood viral-load suppressed patients

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>HIV DNA copies/million cells</th>
<th>%CV</th>
<th>Strain</th>
<th>Measured copies/mL</th>
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<tr>
<td>0001</td>
<td>281±36</td>
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<td>G</td>
<td>7.00E6</td>
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<tr>
<td>0002</td>
<td>223±34</td>
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<td>G</td>
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<td>G</td>
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</tbody>
</table>

The assay gave undetected results for HIV-2 (ROD, EHO), HCV, HBV and Plasmidofalpum.

CONCLUSIONS

Key features of the UltraQ HIV-1 DNA quantitative assay
- The assay’s LOD is 4 copies (95% CI, 2-8) HIV-1 DNA/reaction in the presence of human genome equivalent to 200,000 cells.
- It potentially quantifies HIV-1 DNA in PBMC from patients infected with HIV-1 strains of M and N groups.
- Quantification of HIV-1 DNA in U1 cells ranging from 30 to 7290 per million cells – corresponding to 1.78 to 4.16 log10 HIV-1 DNA copies/million cells, respectively – is highly correlated with nominal values provided by QVA. SD is less than 0.22 log10 copies/million cells.
- The UltraQ assay has potential ability to estimate HIV-1 DNA reservoir size and assess the efficiency of HIV-1 therapeutic strategies.


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