Background: The aim of this study was to evaluate the diagnostic performance of the Vela Sentosa next-generation sequencing system in conjunction with the Sentosa SQ HIV Genotyping Assay for sequencing and genotyping HIV-1 samples.

Materials and methods

Plasma samples were extracted and template prepared on the Sentosa SX instrument. Reverse transcriptase (RT), protease (PR) and integrase genes were sequenced on the Sentosa SX-301 Sequencer (PSM Ion Torrent). Sequence analyses and lists of mutations were provided by the Vela software. HIV-1 specimens of various subtypes harboring resistance-associated mutations were used to validate all analyses. Methods were compared using 46 clinical samples (mean viral load: 6.15 log copies/mL) that had been characterized by direct sequencing and on two NGS platforms, 454 GS-Junior Roche or MiSeq Illumina.

Results – Analytical performance

The analytical sensitivity for detecting major resistance mutations in HIV-1 subtype B samples was 200 copies/mL. It was 500 copies/mL for CRF02-AG specimens (Table 1). Minor variants were detected with a sensitivity of 5% at 100,000 copies/mL (Figure 1). The sequences of reference HIV-1 strains (A, B, C, D, F, G) were concordant with those obtained by direct sequencing (Table 2).

Table 1. Analytical sensitivity for subtype B and CRF02-AG

<table>
<thead>
<tr>
<th>HIV-1 subtype</th>
<th>Viral load cp/mL</th>
<th>VELA sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>200</td>
<td>95%</td>
</tr>
<tr>
<td>CRF02-AG</td>
<td>500</td>
<td>95%</td>
</tr>
</tbody>
</table>

Results – Methods comparison

The Vela DX system detected 100/103 mutations identified by direct sequencing (concordance 97%). Vela DX identified 3/17 mutations, accounting for 1-20% of the quasi-species identified by MiSeq and 50/50 mutations >20% (23 clinical samples) (Table 4). Vela DX also identified 3/17 mutations, accounting for 1-20% of the quasi-species identified by the 454 GS-Junior and 6164 of the >10% mutations (43 clinical samples) (Table 5). The Vela DX and 454 GS-Junior quantified 55 mutations (Spearman correlation ρ=0.688; p=0.0001; mean difference: 2.3% by Bland Altman plot). The Vela DX and MiSeq quantified 53 mutations (Spearman correlation ρ=0.696; p=0.0026; mean difference: 1.1%) (Figure 2).

Figure 1. Sensitivity for detecting minor variants. One sample with major resistance mutation (RT E138A) was mixed with 454 GS-Junior to obtain proportions of 75%, 20%, 5%, 1% and 0% of mutation load. Each mixture was tested in triplicate.

Conclusions

- The Vela DX and HIV Genotyping Assay accurately identified HIV-1 genotypic resistance mutations.
- Nucleic acid extraction, PCR amplification, library preparation and bio-informatic analysis are all automated.
- Vela DX identified the same resistance-associated mutations as those found by direct sequencing.
- The three NGS platforms, Vela DX, 454 GS-Junior and MiSeq, all detected variants accounting for more than 20% of the quasi-species.