First evaluation of Next-Generation Sequencing and Sanger Sequencing for the detection of HIV1 drug resistance mutations using CE-IVD assays and Software.

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Background

Drug-resistance mutations are routinely detected using standard Sanger sequencing, which does not detect minor variants with a frequency below 20%. The impact of detecting minor variants generated by Next-Generation Sequencing (NGS) on HIV drug-resistance interpretations has not yet been studied. The objective of this study was to evaluate the performance of the CEIVD HIV-1 drug resistance assay and software using NGS platform.

Methods

A total of 69 samples and 5 negative controls were prepared, extracted (Roche MagNa Pure Compact 8; MagNA Pure Compact), amplified (ProFlex PCR System Life Technologies) for the three HIV-1 genomic targets using the CEIVD DeepChek® Assays (PR/RT and INT) and sequenced using the NGS iSeq100 (Illumina). Sequences were compared to those obtained by Sanger Sequencing using the SeqStudio system (applied biosystem). Quality control for Molecular Diagnostics (QCMD) were added to control amplification, sequencing and interpretation processes for Protease (PR), Reverse Transcriptase (RT) and Integrase (INT) regions.

Results

The limit of range detection was 1000-10^6 cp/mL. The median coverage per sample for the three amplicons (PR/RT and INT) was 13’237 reads. No interference substances were reported as no cross-reactivity occurred with the HCV and HBV spiked clinical samples. High analytical reproducibility and repeatability were evidenced by Percent Agreement being 100%. Duplicated samples in two different NGS runs were 100% homologous. NGS detected all the mutations found by Sanger sequencing and identified additional resistance variants. The score of the QCMD panel detection of drug resistance mutations (DRMs) for PR/RT and INT were 339/339 and 125/125, respectively.

Conclusions

This study is the first evaluation of the DeepChek® Assays (PR/RT and INT) and Software. A combination of NGS and DeepChek software for the interpretation of drug resistance results would help clinicians provide suitable treatments. A cut-off of 3% allowed a better characterization of the viral population by identifying additional resistance mutations and improving the drug-resistance interpretation.