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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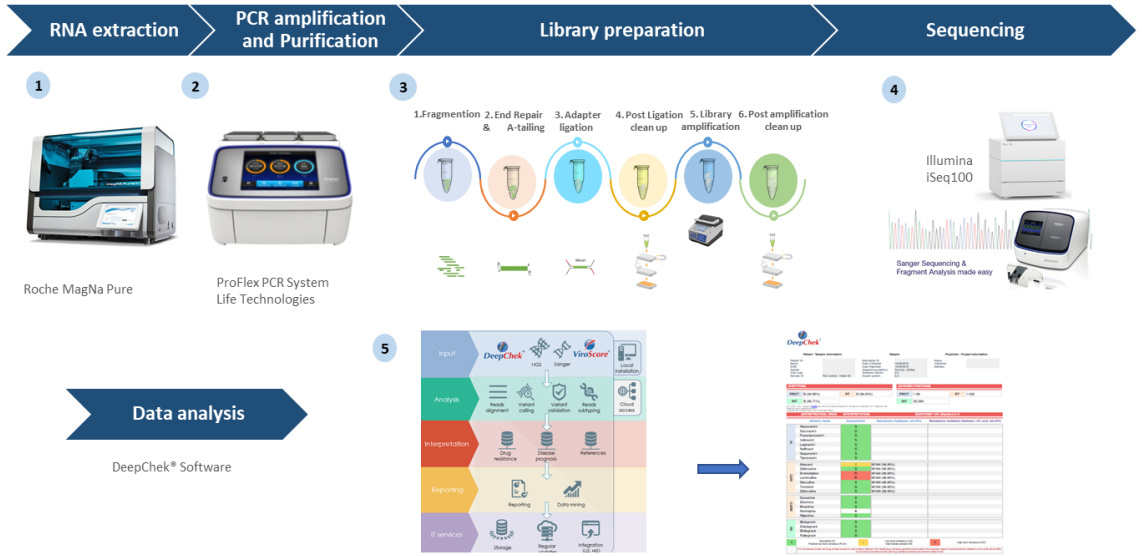
Background

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.

Aim

The objective of this study was to evaluate the performance of the CEIVD HIV-1 drug resistance assay and software using NGS platform

Methodology



Results

- The limit of range detection was 1000-106 cp/mL.
- 100% homologous.
- All mutations detected by NGS
- Additional resistance variants identification
- QCMD Score panel detection form PR/RT and INT were 339/339 and 125/125, respectively

NB. of samples	**301**
Nb. of sites (median number of samples per study)	27 (8)
Nb. of controls / EQA samples (positive/negative/EQA)	33 (15/6/12)
Nb. of viral loads available	215
Nb. of viral loads >= 1000 cp/ml	186
Median viral load (cp/mL)	26915
Nb. of subtypes available	252
% of subtypes B / non-B	63% / 37%
Nb. of PR/RT or of PR/RT/INT DeepChek® Assay ran	91 / 210
Nb. of samples with viral load >=1000 cp/ml and subtype B	149

	Amplification Successful *	Median viral load (cp/mL)	Inter-Quartile range viral load (cp/mL)	Min viral load (cp/mL)
PR/RT + INT	159	26'915	6'998 - 100'000	104

* (excluding controls and unspecified viral loads)

	Amplification Successful**	Subtype B (%)	Non-B subtype # (%)
PR/RT + INT	196	58%	42%

** (excluding negative controls and unspecified subtypes)
# Main non-B subtypes are C, 02_AG, A1, D (44% of non-B)

DeepChek® Assay	Downstream sequencing instrument used with DeepChek® Assay	Device 2 used for agreement concordance	Nb. of samples tested	Concordance (%)
PR/RT + INT	Illumina MiSeq	Abbott® Dx - ViroSeq® HIV-1 Genotyping PR/RT + INT (Sanger)	23	100%
PR/RT	Illumina MiSeq	LDT (German laboratory) PR/RT (Sanger)	12	92%*
PR/RT + INT	Illumina MiSeq	Vela Dx - Sentosa® HIV-1 Genotyping PR/RT/INT (NGS)	18	100%

* Only 1 sample was not amplified by DeepChek Assay but it was not done a second time. The small number of samples have an impact on the reported figures.

Conclusion

- Next-Generation Sequencing (NGS)
- Variants detection
- Reproducibility and repeatability
- HIV drug-resistance interpretations

IVD
Qualitative in vitro diagnostics

For use with downstream sequencing instruments

CE

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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.