Detection of Low-Frequency Drug-Resistant Variants of HIV-1 using Whole Genome Sequencing

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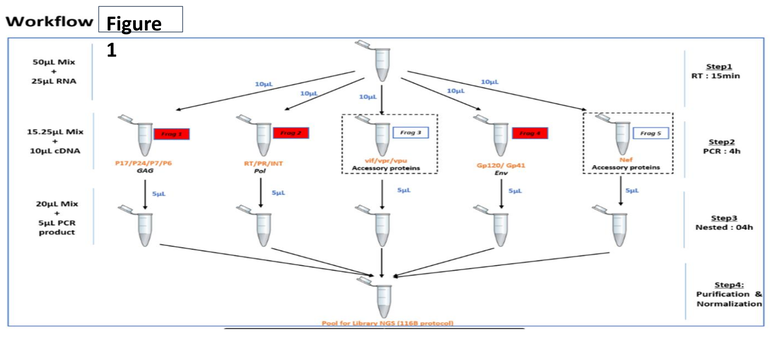
Introduction and Objectives

Highly Active Antiretroviral Therapy (HAART) has substantially improved the survival of patients with HIV-1, but successful therapy requires long-term suppression of viral replication, which may be prevented by impaired host immunity, suboptimal drug penetration in certain tissue compartments and incomplete adherence to therapy. When viral replication continues despite treatment, the high mutation rate of HIV-1 enables resistant variants to develop. Sequencing resistant variants has enabled targeted changes in treatment, which has resulted in greater reductions in viral loads than with standard care (undetectable HIV load in 32% vs 14% of patients after six months). Thus, the increasing number of drugs in development that interact with different proteins that are encoded by viral genes scattered across the genome, requires the Whole Genome Sequencing (WGS) which simultaneously captures all resistant variants and removes the need to design and optimize PCR assays for the detection of resistance to new drugs. WGS can also provide information on antigenic epitopes, virus evolution in a patient over time, and evidence of recombination between strains. To date, there has been very little direct comparison between the Sanger sequencing and WGS methods for viral genome sequencing in clinical practice. Therefore, our objective was to evaluate the results obtained from Sanger sequencing HIV-1 (*Pol*, *RT*, *INT*) genomic-regions vs. the HIV-1 WGS performance in terms of drug-resistant variants. This comparison may help in better understanding therapy response and to gain insights into the management of HAART.

Methods

A total of 10 HIV-1 positive serum samples (received at Mubarak Al Kabeer Hospital for HIV-1 genotyping) along with HIV-1 positive external control were evaluated for the standardization of the HIV-1 WGS. All samples were extracted using MagNa Pure (Roche). HIV-1 RNA was amplified using the DeepChek® Assays Whole Genome HIV-1 (ABL Diagnostics) and was sequenced using the NGS Ion Torrent S5 (ThermoFisher). Sequences were compared to those obtained by targeted NGS and Sanger Sequencing. DeepChek® HIV-1 Whole Genome software (ABL) was used for the interpretation of genotyping and drug-resistance analysis. Following the manufacturer's instructions, five different primer sets were used to amplify the following targets (*GAG*, *Pol*, *Env*, two regions corresponding for accessory proteins (vif, vpr, vpu) and (Nef). The summarized workflow is shown in **Figure 1**.

Workflow Figure 1



Accuracy, sensitivity, and reproducibility in calling consensus sequences and detecting majority drug-resistance mutations were all monitored by sequencing each sample twice in two different experimental settings (targeted NGS sequencing and WGS settings.

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Results and Discussion

Sequencing was achieved with a genome coverage and depth of >90% of the HIV genomic regions covered by >100 reads/site. Subtyping determination performed through homology testing of consensus sequence generated from all the reads mapped to HIV genomic regions and compared to an updated set of reference sequences. Duplicated samples in two different NGS runs (WGS and Targeted Sequencing) were 100% homologous. NGS detected all the mutations found by Sanger sequencing in 60% of the samples and identified additional low-frequency resistance variants which wasn't reported by Sanger sequencing in 40% of samples, as shown in **Table 1**.

Total No.	Viral Load cp/mL	HIV-1 NGS Whole Genome Results (MKH)				Drug Resistance Data Interpretation	Comparison to Targeted NGS	Comparison to Sanger Sequencing
		Subtype	RT Mutations of Interest	PR Mutations of Interest	INT Mutations of Interest			
1	12527 cp/mL	CRF15_01B	No mutation	No mutation	T66A	HR: Elvitegravir IR: Raltegravir	Match	Mismatch
2	64620 cp/mL	CRF15_01B	A98G K103N P225H	No mutation	No mutation	HR: Efavirenz HR: Nevirapine IR: Doravirine	Match	Mismatch
3	91883 cp/mL	CRF43_02G	E138A	No mutation	No mutation	IR: Rilpivirine	Match	Mismatch
4	17200 cp/mL	CRF16_A2D	T215A	No mutation	No mutation	IR: Zidovudine	Match	Mismatch
Index	HR (High Frequency Resistance), IR (Intermediate Frequency Resistance)							

Table 1. Comparison between NGS subtyping and drug resistance mutations vs. targeted and Sanger sequencing

The DeepCheck software analysis pipeline offered ease of use, scalability, data management, quality control, and limited infrastructure. The implementation of the Torrent Suite plugin for analysis and reporting simplifies and streamlines the difficult task of reviewing, interpreting, and reporting HIV-1 drug-resistance results. The plugin processes data automatically after the completion of a sequencing run and produces files that can be consumed by laboratory information systems (LIS) to minimize the amount of technical hands-on time and reduce errors, **Figure 1 (A-D)**.

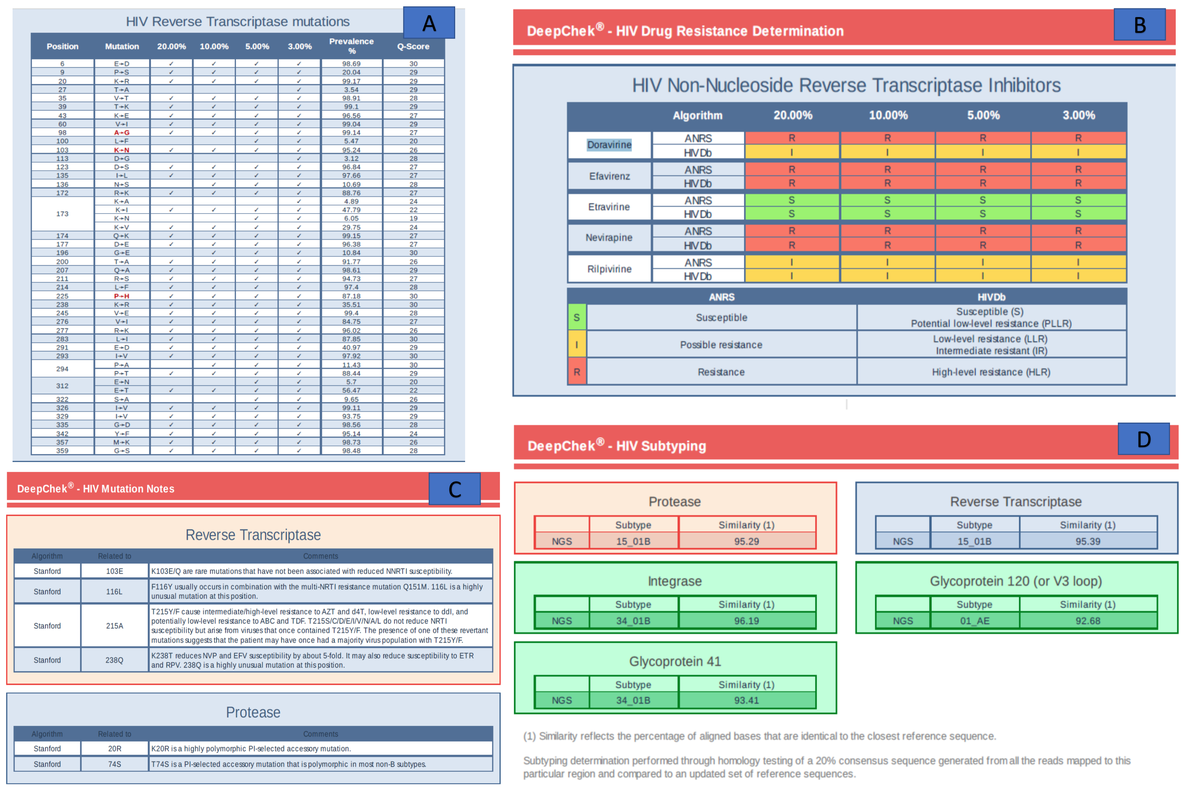

Figure 1. A shows the mutation location**, B** shows the drug-resistance frequency**, C** shows the results interpretations**, D** shows the HIV subtyping

Conclusions

1. This study is the first evaluation of the DeepChek® Whole Genome HIV-1 Assay (ABL) in Kuwait at Mubarak Al Kabeer Hospital, MOH, using the Ion Torrent S5 Sequencing Platform combined with an DeepCheck analysis software.

2. The WG NGS assay has several advantages over Sanger sequencing. Adjacent nucleotide variants within a codon that can confound Sanger sequencing can be resolved using WG NGS, eliminating ambiguous amino acid calls that can affect drug-resistance interpretation. Low frequency variants that play an important role in providing optimum treatment outcomes can be established using WGS.

References

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