Comparison of Sanger and Next Generation Sequencing for Detection of HIV Protease Inhibitor Mutations

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Introduction

South African national guidelines recommend HIV drug resistance (HIVDR) testing for all HIV-infected patients failing a protease inhibitor (PI) based regimen[1]. Patients with PI Genotypic Susceptibility scores (GSS) >15 are eligible for 3rd line treatment. Detection of drug resistance mutations (DRMs) using Sanger sequencing remains the gold standard and is currently used for testing purposes. Implementation of next generation sequencing (NGS) considered an alternative approach in order to improve sensitivity, reduce cost and increase sample throughput. The aims of this study were to compare Sanger and Illumina NGS methods for their ability to detect PI drug resistance mutations among patients failing a PI regimen.

Materials and Methods

- Patients failing a PI regimen for at least one year were referred from across South Africa.
- Whole blood specimens were tested for HIV drug resistance at the HIV genotyping Unit at Charlotte Maxeke Johannesburg Academic Hospital
- 162 specimens with PI mutations by Sanger sequencing were selected for this study.

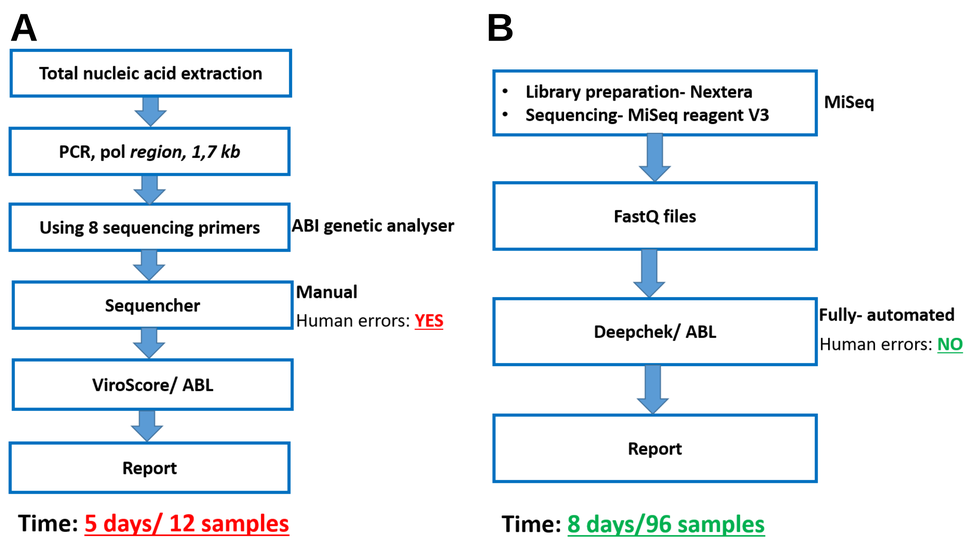

Figure 1: Sequencing workflow. (a) Sanger (b) NGS/ MiSeq

- A consensus sequence was generated for each specimen using 15% cut-off to compare with Sanger sequences.
- Validation criteria of the two methods was done using phylogenetic analysis and pairwise analysis. Pairwise distance at the nucleotides level expected to be >99% while at the amino acids level >98%.

Results

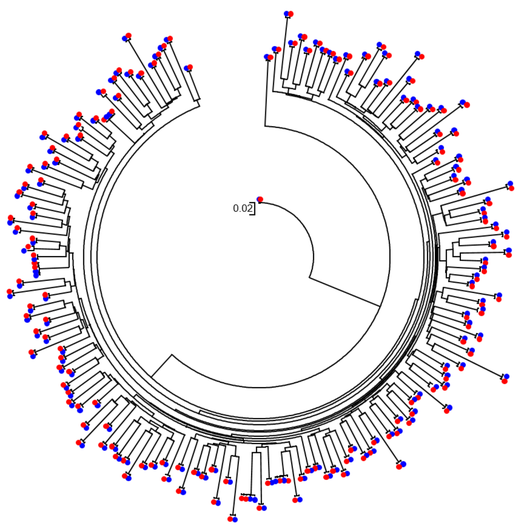

Figure 2: Neighbor-joining phylogenetic tree showing clustering of samples sequenced by Sanger and NGS. Sanger sequences are represented in **Red dots,** NGS sequences are in **Blue.**

Validation- Pairwise analysis

Pairwise analysis was done to confirm the sequences similarity between NGS and Sanger. At the nucleotide level sequences pairwise distance were >99%, while at the amino acids level >98%.

Resistance scores analysis

Using a ≥15% cut-off for generating a consensus sequence, 155/162 (95.7%) of samples showed similar resistance scores between Sanger and NGS, while 7 (4.3%) showed discordance (Table 1A). When using 5-15% cut-off, an additional 13 samples (8%) showed discordance (Table 1B).

Table 1: Discordance between resistance scores, using (A) a 15% cut-off for generating consensuses FastQ files of NGS and (B) using a 5-15% cut-off

A

Sample ID		PR Major	PR Accessory	Atazanavir/r	Lopinavir/r	Darunavir/r
IF01391949	NGS	I54V,V82A	None	40	55	0
	Sanger	**M46MI**,I54V,V82A		60	75	0
LD01326634	NGS	I54V,V82A	**L10FI**,L23I	40	60	5
	Sanger		L23I	40	55	0
LD01494526	NGS	M46I V82A	L10F,L23I	35	55	5
	Sanger	M46I,**I54IV**,V82A		60	80	5
NJ00216656	NGS	**M46I**,I54V,L76V,V82A	None	60	115	20
	Sanger	I54V,L76V,V82A	**M46KR**	40	85	20
PE00759577	NGS	M46I,I54V,I84IV	**L10F**	85	60	20
	Sanger		Unknown	85	55	15
PM00399744	NGS	I54V,V82A	**L10F,L33LF**	45	65	10
	Sanger		Unknown	40	55	0
TD01607974	NGS	G48GA,I54V,V82A	**L24LI**	60	75	0
	Sanger		None	50	65	0

B

Sample ID		PR Major	PR Accessory	Atazanavir/r	Lopinavir/r	Darunavir/r
FA00462664	NGS	M46I,**I50IV**,I54V,L76V,V82A	Q58E	60	145	40
	Sanger	M46I, I54V,L76V,V82A		60	115	20
ID00331994	NGS	V32VI,I47A,**I84IV**,L90LM	K20KRT	110	135	55
	Sanger	V32I,I47A, L90LM		50	95	30
ID00351416	NGS	**I54IV**,V82VA	L10LF	40	60	5
	Sanger	V82A		15	35	5
IF01214993	NGS	I47IV,I54IV	K20KRT	30	30	10
	Sanger		None	25	30	10
IJ00990056	NGS	M46I,L76V,V82VCFG,I84V	L10F,L23I,L33LF	100	130	60
	Sanger	M46I,L76V,V82CG,I84V		100	115	45
IK01642894	NGS	V32I,I47A,**V82VA**	L10F	35	115	35
	Sanger	V32I,I47A		20	85	35
NJ00235428	NGS	M46I,I54V,L76V,V82A	**L10F,L33LF**,Q58QE	65	125	30
	Sanger		Q58E	60	115	20
PQ00127065	NGS	M46MIL,I54V, V82A	L24LI	70	85	0
	Sanger	M46I,I54V,L76V,V82A	L10F,L33F	65	125	30
QD00803826	NGS	M46I,I54V,V82A	L10F,K20RT,**L33F**	70	85	10
	Sanger		L10F,K20T	65	80	5
QD00841461	NGS	M46I,I54V,**L76LV**,V82A	L10F,L24LI,K43T	70	130	25
	Sanger	M46I,I54V, V82A		70	90	5
QD00867516	NGS	**V32VI,M46M**I,I47IV	K20KT,F53FL	55	45	30
	Sanger	I47IV		25	15	10
RA00537874	NGS	M46I,I54V,V82A	L10F,L24LI,T74PS	80	95	10
	Sanger		L10F,L24I	70	90	5
TD01888072	NGS	**M46MI**,I54V,L76LV,V82A	L10F,**K20KRT**,L33LF	70	125	30
	Sanger	I54V,L76LV,V82A	L10F, L33LF	45	95	30

High level resistance
Intermediate resistance
Low level resistance
Potential Low level resistance
Susceptible

Conclusion

- Detection of DRMs using MiSeq using Sanger sequencing showed high concordance (95.7%).
- The difference in the phenotypic interpretation of resistance was due to presence of discrepancies in mutations detected (4.3%).
- The discrepancies had minor impact on clinical interpretation as all GSS >15.
- The use of NGS for HIVDR testing is therefore reliable and allows for large sample numbers to be tested in a more efficient workflow and potentially be more cost-effective.

References

1. Meintjes G, Moorhouse MA, Carmona S, et al. Adult antiretroviral therapy guidelines 2017. S Afr J HIV Med. 2017;18(1), a776.

Acknowledgments

We would like to thank Arshad Ismail, Mushal Allam and Senzo Mtshali from the Sequencing Core Facility, National Institute for Communicable Diseases, South Africa