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Simultaneous Multiplex Whole Genome Sequencing of HIV-1 and Influenza A/B viruses Using Nanopore Technology

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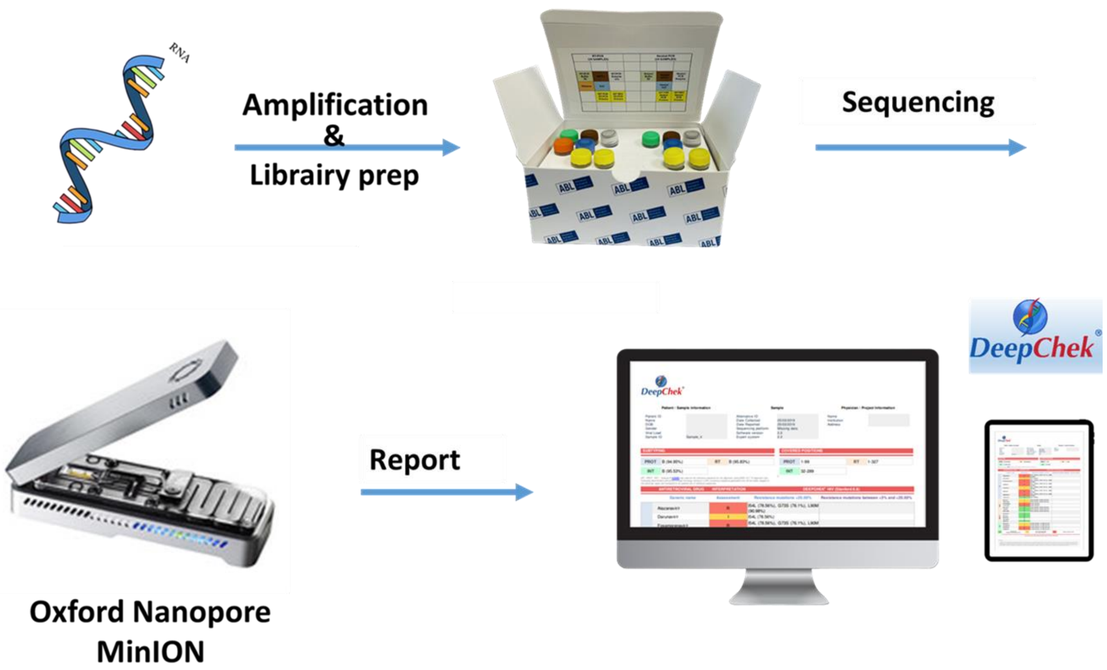
Introduction

The Human Immunodeficiency Virus-1 (HIV-1) and the Influenza Virus are RNA viruses responsible for serious illness, causing hundreds of thousands of deaths worldwide each year. The development of high-performance surveillance tools, such as whole genome sequencing, has made it possible to make significant advances in the knowledge of these pathogens with a view to controlling and/or eradicating them in the medium and long term. In this work, we show that simultaneous sequencing of the whole genomes of these two viruses using Nanopore technology offered an excellent alternative to expensive technologies.

Methods

Twelve samples from PCR-positive patients (5 for HIV-1 and 7 for influenza) were prepared with the QIAamp Mini (Qiagen) kit. OneStep RT-PCR was performed with the ABL Diagnostics DeepChek® Whole Genome HIV-1 and DeepChek® Whole Genome Influenza A/B kits, respectively. Whole genome sequencing with the MinION Nanopore device was performed after multiplexing the 12 samples included in this study. To evaluate the accuracy of Nanopore sequencing, a set of sequences was also subjected to Illumina iSeq100 sequencing. DeepChek® (ABL) software was used to assemble genomes, detect mutations, and build phylogenetic trees.



Results

The sequences generated by the MinION and Illumina technologies were comparatively similar and consistent with the reference genomes of the HIV-1 and Influenza A/B viruses analyzed here. Coverage of the sequenced genomes was complete for all 12 targeted samples, namely, the five HIV-1 samples (subtypes: two B, two C and one 02AG) and the seven influenza samples (two A/H1N1, two A/H3N2 and three B/Victoria). The identified subtype and mutations (>20%) found in Illumina and Nanopore were similar.

Results of multiplex Whole Genome Sequencing
Minion Oxford Nanopore vs Illumina

Nanopore QCMD4 HIV DR

	ANTIRETROVIRAL DRUG	INTERPRETATION	DEEPCHEK® HIV (HIVDb 9.4)
	Generic name	Assessment	Resistance mutations sanger
PI	Atazanavir/r	R	M46I, I54V, V82A
	Darunavir/r	S	
	Fosamprenavir/r	I	M46I, I54V, V82A
	Indinavir/r	R	M46I, I54V, V82A
	Lopinavir/r	R	M46I, I54V, V82A
	Nelfinavir	R	M46I, I54V, V82A
	Saquinavir/r	R	M46I, I54V, V82A
	Tipranavir/r	I	M46I, I54V
NRTI	Abacavir	I	M184V
	Didanosine	S	M184V
	Emtricitabine	R	M184V
	Lamivudine	R	M184V
	Stavudine	S	M184V
	Tenofovir	S	M184V
	Zidovudine	S	M184V
NNRTI	Doravirine	S	
	Efavirenz	S	
	Etravirine	S	
	Nevirapine	S	
	Rilpivirine	S	
INI	Bictegravir	S	
	Cabotegravir	S	
	Dolutegravir	S	
	Elvitegravir	S	
	Raltegravir	S	

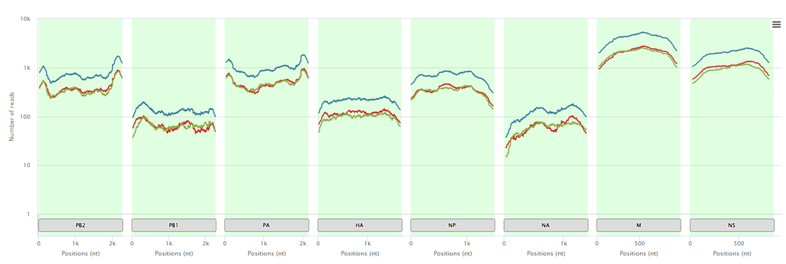
S Susceptible (S) Potential low-level resistance (PLLR) | I Low-level resistance (LLR) Intermediate resistant (IR) | R High-level resistance (HLR)

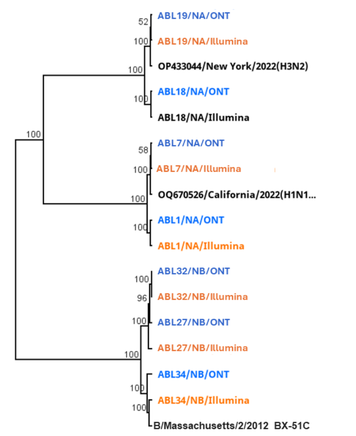
iSeq100 QCMD4 HIV DR

	ANTIRETROVIRAL DRUG	ANALYSIS	DEEPCHEK® HIV (HIVDb 9.4)	
	Generic name	Assessment	Resistance mutations >20.00%	Resistance mutations between >3% and <20.00%
PI	Atazanavir/r	R	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Darunavir/r	S		
	Fosamprenavir/r	I	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Indinavir/r	R	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Lopinavir/r	R	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Nelfinavir	R	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Saquinavir/r	R	M46I (94.97%), I54V (99.65%), V82A (99.6%)	
	Tipranavir/r	I	M46I (94.97%), I54V (99.65%)	
NRTI	Abacavir	I	M184V (99.68%)	
	Didanosine	S	M184V (99.68%)	
	Emtricitabine	R	M184V (99.68%)	
	Lamivudine	R	M184V (99.68%)	
	Stavudine	S	M184V (99.68%)	
	Tenofovir	S	M184V (99.68%)	
	Zidovudine	S	M184V (99.68%)	
NNRTI	Doravirine	S		
	Efavirenz	S		
	Etravirine	S		
	Nevirapine	S		
	Rilpivirine	S		
INI	Bictegravir	S		
	Cabotegravir	S		
	Dolutegravir	S		
	Elvitegravir	S		
	Raltegravir	S		

S Susceptible (S) Potential low-level resistance (PLLR) | I Low-level resistance (LLR) Intermediate resistant (IR) | R High-level resistance (HLR)

Influenza A/B virus	Seg. 1_PB2 2,4 kb	Seg. 2_PB1 2,4 kb	Seg. 3_PA 2,2 kb	Seg. 4_HA 1,8 kb	Seg. 5_NP 1,6 kb	Seg. 6_NA 1,5 kb	Seg. 7_M 1,0 kb	Seg. 8_NEP 0,9 kb
Illumina	✓	✓	✓	✓	✓	✓	✓	✓
ONT	✓	✓	✓	✓	✓	✓	✓	✓





Conclusions

Nanopore technology, which enables multiplex sequencing of complete genomes of dangerous viruses such as HIV-1 and Influenza A/B, offers a real alternative to complex and expensive sequencing methods, especially for developing countries.

In addition to being specific and compact, Nanopore technology is easy to use. This will inevitably have an impact on the monitoring of antiviral resistance as well as on epidemiological and clinical studies soon.