From Errors to Accuracy: Nanopore Sequencing Reliably Detects HIV Drug Resistance Variants

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BACKGROUND

Illumina next-generation sequencing (NGS) is the current benchmark for HIV drug resistance (HIVDR) monitoring. Oxford Nanopore Technologies (ONT) sequencing offers advantages in cost, portability, and long-read capability; however, earlier high error rates raised concerns regarding variant detection accuracy. Reliable detection of minority resistance variants is particularly critical, as these low-frequency mutations can contribute to treatment failure.

We therefore evaluated the performance of the latest ONT sequencing technology compared with Illumina for HIVDR detection, including minority variants.

METHODS

Twenty-one patient samples (14 plasma RNA, 7 proviral DNA) covering protease (PR), reverse transcriptase (RT), and integrase (IN) were amplified and sequenced on Illumina MiSeq and Oxford Nanopore PromethION (R10.4.1 flow cell, Kit 14 chemistry), a high-throughput Nanopore platform with greater pore density and output than the MinION, using identical PCR products (Figure 1). Resistance-associated mutations in RT and IN were classified according to the IAS–USA 2025 drug resistance mutation list. Variant calling was performed using a 2% frequency cutoff, and inter-platform concordance was assessed based on variant frequency deviation.

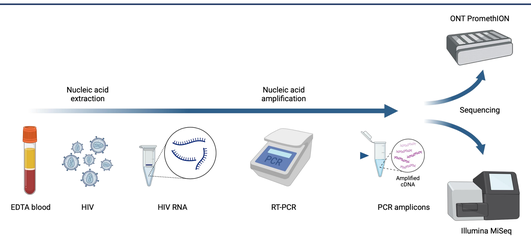


Figure 1. Workflow for HIV Target-Specific Amplification and Sequencing
EDTA-anticoagulated whole blood was subjected to nucleic acid extraction followed by HIV target-specific PCR amplification. Purified amplicons were subsequently sequenced using short-read (Illumina) and long-read (Oxford Nanopore) platforms, enabling direct comparison of the sequencing results for detection of HIV drug resistance mutations.

ADDITIONAL KEY INFORMATION

Acknowledgements

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Nanopore matches Illumina for HIV drug resistance detection - ready for routine diagnostics

RESULTS

A total of 83 HIVDR-associated mutations were identified. All 80 mutations detected by Illumina were equally detected by ONT (Table 1), demonstrating complete agreement for clinically relevant variants. The mean inter-platform deviation was −2.76%, confirming high quantitative concordance (Figure 2), with 92% of mutations showing frequency differences <5%.

Minority variants (<20%) were comparably detected by both platforms (Nanopore: n=22; Illumina: n=19; Table 2). Nanopore additionally identified three low-frequency mutations (<5%) that were below the 2% cutoff or not detected by Illumina. Discrepancies >5% (n=7) showed no systematic association with sample type, genomic region, or sequencing technology (data not shown).

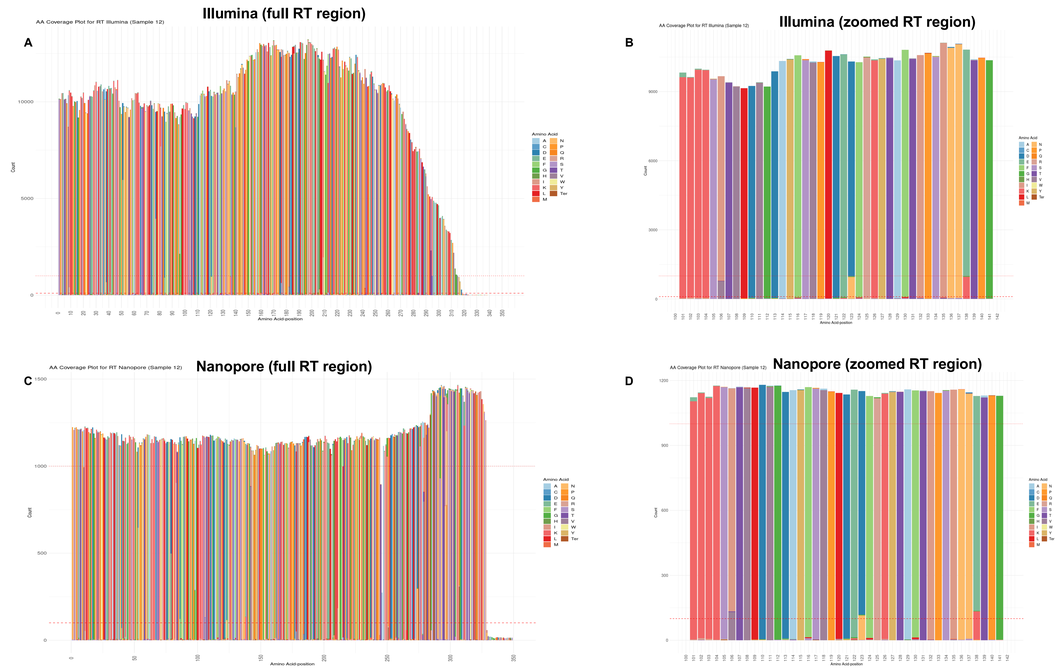


Figure 2. Comparative amino acid frequency analysis of HIV-1 reverse transcriptase (Sample 12)
Amino acid coverage and variant frequencies across the reverse transcriptase (RT) region of Sample 12 are shown for both sequencing platforms. (A) Full-length amino acid frequency plot generated from Illumina sequencing data. (B) Magnified view of a selected RT region highlighting variant distributions above and below the applied frequency threshold (dashed red line). (C) Corresponding full-length amino acid frequency plot derived from Oxford Nanopore sequencing. (D) Enlarged view of the identical region shown in panel B. Comparable amino acid frequency patterns and minor variant detection demonstrate high concordance between short-read (Illumina) and long-read (Nanopore) sequencing.

Table 1. Concordance of HIV drug resistance mutations detected by Illumina and Nanopore sequencing.
Mutation frequencies are shown as percentages. Δ indicates Nanopore − Illumina. Variant calling cutoff was 2%.

Metric	NGS platform	Value
Samples (n)		21
Total HIVDR mutations		83
Mean Δ (%)		−2,76
Mutations with |Δ| < 5%		92%
Minority HIVDR variants <20%		22
	Illumina	19
	Nanopore	22

Table 2. Minority HIV drug resistance variants detected by Illumina and Oxford Nanopore sequencing. Minority variants were defined as mutations detected at frequencies below 20% on at least one sequencing platform. Mutations present at ≥20% frequency on both platforms were excluded. Sample IDs correspond to the sample overview table. Frequencies are shown as percentages of sequencing reads. Coverages are shown as reads per position (x). Δ indicates the frequency difference calculated as Nanopore minus Illumina. Variant calling was performed using a 2% cutoff.

ID	Matrix	Subtype	Region	DRM	Illumina (%)	Nanopore (%)	Δ (%)	Illumina coverage (x)	Nanopore coverage (x)
2	Plasma	B	RT	E138A	4,37	4,39	0,02	17579	2094
3	Plasma	02_AG	RT	A98G	2,97	3,37	0,4	607	1716
3	Plasma	02_AG	RT	H221Y	15,41	15,6	0,19	519	1897
6	Plasma	A6	RT	E138K	17,86	20,95	3,09	8604	1255
10	Plasma	02_AG	RT	V90I	3,63	4,45	0,82	12247	1831
12	Plasma	B	RT	K101E	2,02	2,88	0,86	9825	1132
12	Plasma	B	RT	E138K	9,08	11,71	2,63	10826	1128
12	Plasma	B	RT	M184V	3,27	4,62	1,35	12528	1156
12	Plasma	B	RT	Y188L	2,7	3,13	0,43	12884	1176
12	Plasma	B	RT	T215F	11,94	12,6	0,66	12462	1154
12	Plasma	B	IN	E157Q	6,15	6,71	0,56	19174	4215
12	Plasma	B	IN	S230R	12,17	13,84	1,67	13480	4227
13	Plasma	B	RT	F116Y	6,55	7,19	0,64	22346	4061
13	Plasma	B	IN	S147G	5,47	5,64	0,17	20877	10579
14	Plasma	B	RT	K101R	0	4,97	4,97	6966	5478
14	Plasma	B	RT	V179I	2,05	3,12	1,07	7320	5485
15	Plasma	B	RT	E138A	7,46	8,25	0,79	5419	3506
15	Plasma	B	RT	Y181C	0	3,69	3,69	6298	3526
19	Plasma	A6	RT	M184I	18,38	21,37	2,99	3688	48941
20	Plasma	A6	RT	K70R	1,75	2,03	0,28	10900	23082
21	Plasma	B	IN	S147G	24,43	16,22	−8,21	7392	37063
21	Plasma	B	IN	G163R	3,19	5,83	2,64	7427	37468

CONCLUSIONS

The latest ONT platform provides robust and quantitatively concordant detection of HIV drug resistance mutations, including low-frequency variants. Given its strong agreement with Illumina, ONT represents a reliable and scalable alternative for routine diagnostics and surveillance, especially in resource-limited settings.

PLAIN LANGUAGE SUMMARY

We tested a newer, more portable sequencing technology to see whether it can reliably detect HIV drug resistance mutations compared with the current gold standard. The technology accurately identified drug resistance mutations, including low-level variants, suggesting it could be a reliable and more accessible option for HIV monitoring.