SUMMARY

Human Immunodeficiency Virus (HIV) poses ongoing challenges in treatment due to its high genetic variability and the emergence of drug resistance, particularly in cases with low viral loads (LVL) below 10,000 copies/mL. Early and accurate detection of resistance mutations in such cases is essential but remains technically difficult. This study aimed to evaluate the feasibility and accuracy of Nanopore sequencing in detecting HIV drug resistance mutations in LVL plasma samples. Specifically, it sought to (1) assess Nanopore's capability in identifying resistance, (2) measure the accuracy of the RT-PCR and nested PCR amplification steps, and (3) determine whether Nanopore sequencing provides reliable results despite reduced input quality.

Ten HIV-1–positive plasma samples with viral loads below 10,000 copies/mL were collected at RSPI Sulianti Saroso using purposive sampling. Total viral RNA was extracted and reverse-transcribed, followed by nested PCR amplification targeting conserved regions. DNA purity and concentration were assessed using NanoDrop and Qubit assays. Samples meeting the input threshold were barcoded and sequenced on the GridION platform using the SQK-NBD114.24 kit. Consensus sequences were generated using Canu, Racon, and Medaka, and analyzed with the Stanford HIV Drug Resistance Database and WHO Quality Control (QC) panel to determine resistance profiles and sequencing accuracy.

The amplification protocol successfully produced sufficient cDNA concentrations in all samples, confirming its suitability for low-input conditions. Although the Nanopore sequencing output had low average Q scores (~7.78), nine out of ten samples achieved high coverage values, demonstrating that high read depth can compensate for lower basecalling accuracy. Resistance mutations were successfully identified in several samples, supporting the feasibility of this approach for resistance genotyping in low viral load cases. These findings suggest that, with proper optimization of amplification and library preparation steps, Nanopore sequencing is a promising tool for routine HIV drug resistance monitoring in resource-limited or early treatment settings.

Keywords: *HIV, low viral load, drug resistance, Nanopore sequencing, next-generation sequencing*