A NOVEL LONG-RNA HIV DRUG RESISTANCE TEST ENABLING CAPSID (LENACAPAVIR) RESISTANCE DETECTION, OPTIMISED FOR SANGER AND NANOPORE SEQUENCING

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Background:
Standard HIV-1 drug resistance testing (limit of detection: plasma viral load >2000 copies/mL) covers protease (PR), reverse transcriptase (RT), and integrase (IN) inhibitors. The introduction of the capsid inhibitor Lenacapavir in Australia highlights the need to include capsid (p24 gag) in routine resistance testing; however, no commercial assays currently sequence this region. We developed a sensitive, low-complexity HIV drug resistance (HIVDR) assay covering all major drug classes, including capsid. The assay uses Oxford Nanopore Technology (ONT) for rapid, cost-effective sequencing with minority variant detection and is adaptable to Sanger sequencing. We compared its performance with the St Vincent's Hospital Sanger method.

Methods:
We developed a nested PCR assay targeting the HIV-1 gag/pol region (>4.2 kb), including p24 gag. HIV-1 RNA was extracted from plasma. Sequencing used (i) ONT MinION Mk-1D (12 samples/run; ~1-hour runtime) and (ii) Sanger sequencing with 11 primers. Drug resistance mutations were interpreted using the Stanford HIV Drug Resistance Database and compared with the St Vincent's two-step nested PCR Sanger method targeting separate PR/RT and IN regions. A head-to-head comparison was performed on 59 plasma samples (2,240–150,000 copies/mL).

Results:
The gag/pol region was successfully amplified in all samples. Concordance between ONT and standard Sanger sequencing was ≥93% across mutation classes, with complete agreement for major and accessory mutations in RT and IN. Discordant mutations detected only by ONT occurred in 1 NRTI case (1.7%) and 4 NNRTI cases (6.7%), consistent with low-frequency variants (<20%) not detected by Sanger. The assay provided consistent capsid coverage, enabling detection of potential Lenacapavir-associated resistance.

Conclusion:

This long-read HIV-1 gag/pol HIVDR assay enables comprehensive resistance detection across PR/RT/IN and capsid. Its Sanger-compatible format supports immediate clinical implementation, while ONT provides rapid, scalable sequencing with improved minority variant detection and adaptability to emerging targets.

Disclosure of Interest Statement:
K.S., A.L., E.Y. are inventors under Australian PCT Application (PCT/AU2025/051218) covering the methodology of this study. All other authors report no conflict of interest.

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