V. CONCLUSION AND SUGGESTION

A. Conclusion

Based on the optimization process conducted in this study, the feasibility and accuracy of Nanopore sequencing for detecting HIV drug resistance mutations in plasma samples with low viral loads were evaluated. The following conclusions were drawn:

1. Optimization from RNA extraction to nested PCR demonstrated that Nanopore sequencing is applicable for HIV samples with viral loads below 10,000 copies/mL. Despite variations in RNA quality, most samples were successfully amplified, and DNA concentrations after purification were adequate for sequencing. These results highlight the protocol's reliability for detecting HIV drug resistance in low viral load plasma.
2. The amplification protocol using RT-PCR and nested PCR proved reliable, generating high DNA concentrations with acceptable purity despite varying sample quality. Although initial Qubit measurements showed undetectable yields, further optimization by increasing input template volume improved amplification efficiency. This confirms the protocol's robustness for processing low-viral-load HIV plasma samples.
3. After optimization during library preparation, the sequencing run achieved an average Q score of 7.78 with high coverage across most samples, showing that despite lower basecalling quality, sufficient read depth can maintain accuracy in detecting HIV resistance mutations.

B. Suggestion

Based on the findings of this study, several recommendations can improve the use of Nanopore sequencing for detecting HIV drug resistance at low viral loads. Enhancing sample preparation and purification is essential to reduce contamination and increase fragment length, which can improve sequencing accuracy and Q scores. Performing electrophoresis after each amplification step, including RT-PCR and nested PCR, is advised to verify amplicon integrity before sequencing. Alternative or supplementary purification methods should be considered alongside ExoSAP, as enzymatic

residues may interfere with end-prep reactions. Validating the workflow using clinical samples with known resistance profiles will help assess its accuracy under low viral load conditions. Finally, optimizing downstream bioinformatics pipelines to accommodate lower Q scores can improve resistance mutation detection, especially in settings with limited sample quality.

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1963