Validation and implementation of a full-length *pol* RT-PCR and Next Generation Sequencing pipeline for routine HIV drug resistance genotyping.

Authors:
Castley A[1], Crisafio A[1], Raut A[1], Chin, A[1], Rao J[1], Gizzarelli L[1], D'Orsogna L[1], John M[1,2].

[1]PathWest, Department of Immunology Fiona Stanley Hospital, Perth WA,
[2]Department of Immunology Royal Perth Hospital, Perth WA.

Background:
Bulk sanger sequencing has been the standard method for HIV drug resistance genotyping however this method has limitations in throughput and rate of detection of minor quasispecies that may mediate drug resistance. We therefore developed a full-length HIV-1 *pol* PCR next generation sequencing assay used to detect HIV variants, including those at low frequency, and validated the method against the standard sanger sequencing-based method.

Methods:
A complete HIV-1 full length *pol* PCR, next generation sequencing (NGS) and reporting pipeline has been designed, validated and implemented. This includes nested RT-PCR, NGS Ion Torrent S5 sequencing, CLC genomic workbench for sequence analysis at 10% variant frequency threshold and the use of Stanford HIV database for determining HIV drug resistant mutations and diverse HIV-1 subtypes present in Western Australia.

Results:
The results show, successful amplification of more samples using the HIV-1 FL *pol*-RT-PCR method when compared to the routine partial *pol* HIV-1 RT-PCR method (95.3% vs 88.3%), including success at amplifying samples with low level viremia(<10,000cpm).
Next generation sequencing was also successful, with the overall total length of target region covered for all samples being 99.7%, the specificity of the mapped reads (MR) over the target region being 99.99%, the average MR was 312447 with a MR average length for all runs of 313 and the percent MR after trim was 92.5%.
High concordance of diverse HIV-1 subtypes was achieved (97.5%). Importanly, the results from the HIV FL-*pol* PCR/NGS evaluation showed, 95.0%, 97.5% and 100% of the DRM were concordant to the current routine HIV PCR/Sanger sequencing results for protease, reverse transcriptase and integrase respectively.

Conclusion:
This newly developed in-house HIV-1 FL *pol* PCR/NGS assay is highly concordant with the standard Sanger based method in detection of genotypic drug resistance and may increase the efficiency of HIV genotyping in a diagnostic laboratory.

Disclosure of Interest Statement:
Nothing to disclose