Development of a *pan*-HTLV-1 Proviral Load Assay in Australia

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Background:
While serology testing for HTLV-1 is routinely offered in Australia, there is a need for a clinical assay assessing HTLV-1 proviral load (PVL) within infected individuals. The NRL are therefore undertaking formal development work towards registering their HTLV-1 PVL assay (currently RUO) as a Class 3 Supplemental assay under their existing ISO-15189 NATA accreditation.

Methods:
The NRL HTLV-1 PVL assay is developed for use with the CFX Opus 96 (Bio-Rad) and SensiFAST Probe Kit (Bioline) using primers and probes targeting the HTLV-1 *pX* region and human *Albumin* gene. Custom clonal T-cell lines developed as Standard material were created by transfecting Jurkat cells with plasmid containing GFP and target HTLV-1 amplicon sequences (GenScript). Sequencing (Nanopore) was conducted to establish HTLV-1 target copy number and insertion site location.

Results:
Sequencing Standard material confirmed a single copy of target HTLV-1 amplicon present in an intergenic position on Chromosome 1. The PVL assay was shown to be highly quantitative and sensitive for both HTLV-1 and Albumin targets (LoD_{95} both <10 copies) over >5 logs of input Standard material. The assay also displayed low intra- and inter-assay variabilities (<10%) and preliminary validation work using clinical material showed excellent concordance with serology data. Formal clinical trials are anticipated in Q3 2023.

Conclusion:
Preliminary data for the NRL HTLV-1 PVL assay supports its further development for use in the clinical setting and assay registration is anticipated in early 2023. The use of this clinically accessible assay will allow physicians to advise patients on best practices aimed at reducing transmission of HTLV-1, further our understanding of the relationships between proviral load and disease and provide a valuable benchmark in Australia for the development of future HTLV-1 molecular assays.

Disclosure of Interest Statement:
This work is funded by the Australian Centre for HIV and Hepatitis Virology Research (ACH2). No commercial grants have been received for this study.