Centrifugation Time impacts HIV RNA quantitation using Plasma Preparation Tubes

Banda C[1], Castley A[1], Acquarola N[1], Perkov L[1], Harrison L[1], D'Orsogna L[1], John M[1,2].

[1]Department of Immunology, PathWest, Fiona Stanley Hospital, Murdoch, Western Australia, [2]Department of Clinical Immunology, Royal Perth Hospital, Western Australia.

Background:
Accurate quantitation of plasma HIV RNA or viral load is essential for monitoring treatment effectiveness in people living with HIV. K2EDTA tubes have been used widely for this assay in Australian diagnostic laboratories, however plasma preparation tubes (PPTs) have been used to enable testing from a primary tube and reduce potential pre-analytical errors. We observed an increased rate of detection of low-level viremia among those with previously undetectable HIV RNA during routine testing using PPTs, we sought to ascertain whether variations in centrifugation may account for low level HIV-RNA detection in virally suppressed individuals on effective antiretroviral therapy.

Methods:
We compared viral load results obtained from K2EDTA tubes with those obtained from PPTs during routine diagnostic testing in the PathWest laboratory from 2021 to 2022. We then evaluated the standard procedure of centrifugation for PPTs at 1200g for 20 minutes within 6 hours of sample collection, with storage at 4°C if not immediately tested, and a re-centrifugation step at 1200g for 10 minutes versus 20 minutes before testing on the COBAS 6800 system.

Results:
The initial investigation confirmed a significantly lower number of results below <40 copies/ml (cpm) associated with the use of PPTs from 2021 to 2022 (p=0.01). There were significantly more low-level viral load results (40-200 cpm) associated with PPTs compared to K2EDTA (p=0.001). No statistically significant difference was observed for samples with HIV-RNA levels >200 cpm. The evaluation of increased centrifugation time when using PPTs showed 87.5% of samples with an initially elevated viral load (40-200 cpm) result returned an undetectable result with a second re-centrifugation step at 1200g for 20 minutes.

Conclusion:
Extending re-centrifugation time improves plasma separation in PPTs and highlights the critical role of meticulous pre-analytical sample preparation in ensuring precise HIV-1 viral load quantification and reducing spurious low level HIV RNA results in a diagnostic laboratory.

Disclosure of Interest Statement:
No pharmaceutical or other external funding was used in this work.