

Reaching the Third 90: Dried Blood Spot Specimens for HIV-1 Viral Load Determination Using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0 in Swaziland

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Background

In 2015, HIV viral load (VL) monitoring was adopted as a standard of care for monitoring anti-retroviral therapy (ART) in Swaziland. The National Molecular Reference Laboratory (NMRL) is currently using plasma as a gold standard. However, processing and storage of plasma is technically demanding, requires cold chain and may incur additional transportation costs. To scale-up routine VL monitoring, the Swaziland Health Laboratory Services (SHLS) is looking into the possibility of using dried blood spots (DBS) for VL testing. The objectives of this study include 1) to verify if VL test results using DBS prepared from venous blood can produce comparable results to plasma, and 2) to verify if VL results from finger prick DBS can produce comparable results to plasma.

Methods

A cross sectional study was conducted between December 2015 and April 2016 at Mbabane Government Hospital. Venous blood was collected into a 5 ml ethylenediaminetetraacetic acid (EDTA) tube from HIV infected patients. A finger prick DBS specimen was also collected by directly spotting on Munktell TFN cards. Both finger prick DBS and the venous blood samples were transported to the NMRL. Corresponding venous DBS specimens were prepared before the whole-blood specimen was centrifuged to separate plasma (Fig 1). The DBS cards were processed using the Free Virus Elution protocol within 14 days. Viral load in plasma and eluted DBS samples was processed using COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0.

Results

Paired plasma and DBS (both venous and finger prick specimens) from 207 patients (160 pre-ART and 47 ART) were tested. Plasma VL was target-not-detected (TND) in 31 patients, below limit of detection (<20 copies/ml) in 5 patients and detectable (≥ 20 copies/ml) in 171 patients. VL from venous DBS was detectable in 112 samples, below limit of detection (<400 copies/ml) in 31, and non-detectable in 48 patients. VL from finger prick DBS was non-detectable in 56 patients, below limit of detection in 26 patients, and detectable in 125 patients. Linear regression analysis for paired plasma and venous DBS VL showed strong correlation ($R^2=0.88$). There was also a strong linear correlation ($R^2=0.84$) between paired plasma and finger prick VL (Fig 2 and 3). Similarly, VL results from both venous and finger prick DBS showed strong correlation ($R^2=0.87$) and were almost in perfect agreement ($\kappa=0.87$, $P=0.000$). Using a VL of 1000 copies/ml as the threshold for virological failure, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) was calculated to be 81.6%, 100%, 100% and 60.7% for venous DBS and 76.7%, 100%, 100% and 57.1% for finger prick DBS respectively. On average, the HIV-1 RNA levels were higher in plasma than venous DBS with a mean difference (\pm standard deviation [SD]) of $0.57 \pm 0.25 \log_{10}$ copies/ml. Similarly, plasma HIV-1 RNA levels were higher than those detected in finger prick DBS with a mean difference (\pm SD) of $0.79 \pm 0.46 \log_{10}$ copies/ml. All results from both venous and finger prick DBS were within 1.96 SDs (2.3 to 6.5 \log_{10} copies/ml) of plasma viral load levels. Five (4.5%) venous and twenty (18.7%) finger prick DBS samples yielded $>1 \log_{10}$ copies/ml difference between plasma HIV-1 RNA levels.

Conclusion

In the study population, the VL results from V-DBS and FP-DBS were found to be comparable to plasma. This indicates that DBS can be used as an alternate sample of choice to monitor the virological failure of patients on ART programme. Introduction of DBS as an alternative specimen will expedite the rollout of routine VL testing services to include infants and access to patients from hard to reach health facilities with minimal regular sample transportation.

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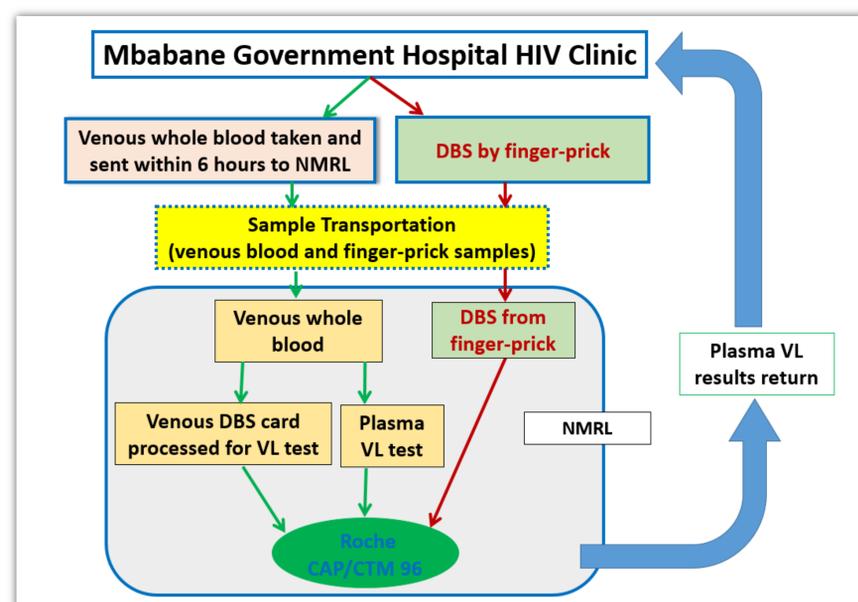


Fig 1: Flow of sample collection and result return

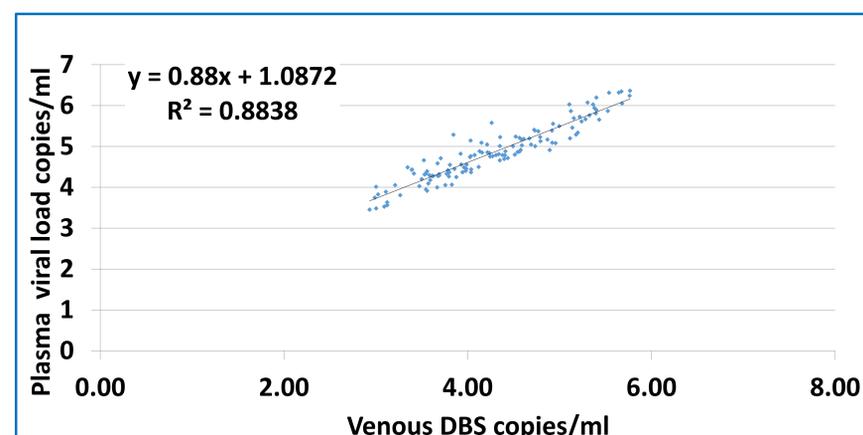


Fig 2: Log Linearity of Plasma and venous DBs viral load (copies/ml)

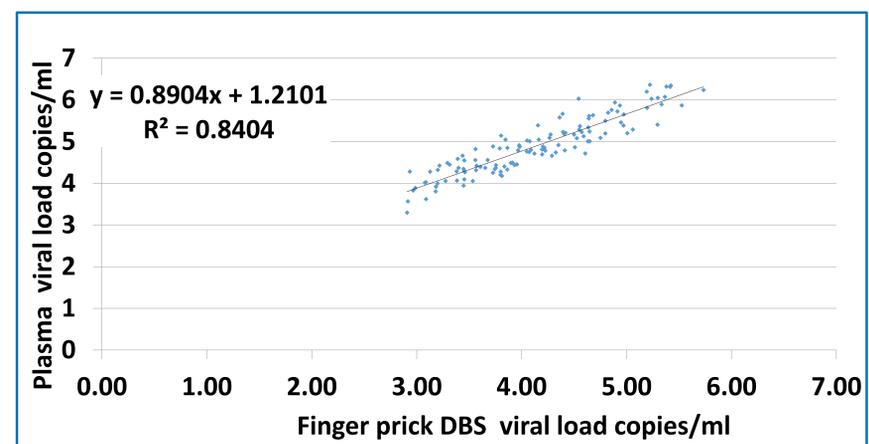


Fig 3: Log Linearity of Plasma and Finger prick DBs viral load (copies/ml)