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Dried blood spots versus plasma samples for the quantification of HIV-1 viral load levels using the Cobas 4800 system at Kaoma District Hospital

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Abstract
HIV remains a major public health concern, especially in Zambia where access to viral load monitoring is challenging in rural areas. This study aimed to compare HIV-1 viral load levels between dried blood spots and plasma samples using the Cobas 4800 System at Kaoma District Hospital. A total of 242 matched samples were analyzed to assess performance, and a subset underwent stability testing after four weeks. Statistical analysis revealed a significant difference between the two sample types, with dried blood spots showing lower viral load values. Stability tests indicated consistent viral load levels in dried blood spots over time, while plasma showed more variability. These findings suggest that although dried blood spots offer operational advantages, they may underestimate viral loads, potentially leading to misclassification of treatment outcomes. Therefore, confirmatory testing using plasma is recommended to support accurate clinical decisions in remote settings.

Keywords: HIV viral load, dried blood spots, plasma samples, Cobas 4800, rural healthcare, Zambia

1. Introduction
HIV remains a significant global health challenge, with approximately 40 million people living with the virus by the end of 2024, two-thirds of whom are in the WHO African Region [1]. Zambia, with an HIV prevalence of 11.1%, has 1.4 million people living with HIV, and 38,000 new infections were recorded in 2023 [1, 2]. Despite advancements in antiretroviral therapy, viral load monitoring continues to be a major challenge, especially in remote areas. Effective viral load monitoring is crucial for treatment efficacy and achieving the UNAIDS 95-95-95 targets [1].

Traditional plasma-based viral load testing faces challenges related to infrastructure, storage, and transportation. As a result, dried blood spots (DBS) have emerged as a reliable alternative, offering easier collection, storage at room temperature, and reduced biohazard risk [3]. The COBAS 4800 system, a high-throughput molecular diagnostic platform, has shown strong agreement with established assays in HIV-1 viral load testing [4-6]. Several studies have demonstrated the feasibility and reliability of DBS in comparison to plasma samples. For instance, it was reported that DBS had high concordance with plasma [7]. A study in India noted 97% sensitivity and cost-effectiveness [8]. In Cameroon, minimal variability and comparable limits of detection between DBS and plasma were observed [9]. In Zambia, 100% sensitivity and specificity were demonstrated between DBS and plasma using the Hologic Panther machine platform, emphasizing the potential of DBS for wider adoption [10]. This study aims to bridge the gap in the limited studies assessing the performance of DBS on the COBAS 4800 system in Zambia. By comparing HIV-1 viral load results from DBS and plasma samples at Kaoma District Hospital, the study seeks to enhance viral load monitoring in resource-limited settings.

2. Materials and Methods
This comparative study was conducted from January 2024 to December 2024 at Kaoma District Hospital, Zambia, and involved the analysis of 484 blood samples. Convenience sampling was employed to select eligible participants who met the inclusion criteria. Blood samples were collected from individuals attending routine ART visits, regardless of their HIV genetic variant.

A trained phlebotomy nurse collected two 4 mL whole blood samples per participant in EDTA K_2 anticoagulant bottles. From one of the samples, 70 µL of blood was used to prepare dried blood spots by applying it to Whatman 903 filter paper cards. The cards were left to dry at room temperature for 16 to 18 hours before being stored with desiccants in sealed plastic bags. The remaining whole blood was centrifuged at 3000 rpm for 10 minutes to separate plasma, which was then stored at 2°C-8°C until testing.

The study included a stability assessment in which both sample types were stored for four weeks prior to testing, DBS at room temperature and plasma at -20°C to evaluate the impact of storage on test performance. Both DBS and plasma samples were analyzed using the COBAS 4800 System for HIV-1 viral load quantification. Testing was carried out by a team of qualified Biomedical Scientists in the molecular section of the laboratory at Kaoma District Hospital, following strict standard operating procedures and quality control protocols. These included barcode labeling, equipment calibration, and routine internal and external quality assessments to ensure accuracy and reliability. A double verification process was also implemented during data entry to ensure correct sample identification and prevent mismatches between DBS and plasma viral load results.

3. Results and Discussions

Comparison of HIV-1 viral load measurements in DBS and plasma samples

Descriptive Statistics of HIV-1 Viral Load Measurements

Table 1: Summarizes the distribution of viral load levels in DBS and Plasma samples

Sample Type	Observations (n)	Mean (copies/mL)	Std. Dev.	Min	Max
DBSVL	242	280.53	1481.34	1	15,700
PLASMAVL	242	23,885.51	208,717.2	1	2,510,000

Normality Testing (Shapiro-Wilk Test)

Table 2: Presents the results of Shapiro-Wilk normality test for DBSVL and PLASMAVL

Variable	W Statistic	P-Value	Normality Assumption
DBSVL	0.15967	0.0000	Not Normally Distributed
PLASMAVL	0.11718	0.0000	Not Normally Distributed

Comparison of viral load measurements (Wilcoxon Signed-Rank Test)

Table 3: Presents the results of the Wilcoxon Signed-Rank test for DBSVL and PLASMAVL

Category	Observations	Sum of Ranks	Expected Rank Sum
Positive Differences	147	18,917	14,331
Negative Differences	57	9,745	14,331
Ties (Zero Differences)	38	741	741

Wilcoxon Test Statistics

Test Statistic (z)	P-Value
4.216	0.0000

Correlation Analysis (Spearman's Rank Correlation Test)

Table 4: Presents the results for a Spearman's rank correlation test

Test Statistic	Value
Observations (n)	242
Spearman's ρ	0.4529

Stability of HIV-1 viral load in DBS over time (4 WEEKS)

Descriptive Statistics

Table 5: Summary Statistics for DBS Viral Load Measurements

Variable	N	Mean (copies/mL)	Std. Dev.	Min	Max
DVL1	92	303.09	1661.86	1	15,700
DVL2	92	274.10	1347.18	1	12,700

Normality Testing

Table 6: Shapiro-Wilk test for normality

Variable	N	W Statistic	V Statistic	Z Score	P-Value
DVL1	92	0.15851	64.820	9.212	0.00000
DVL2	92	0.17508	63.543	9.168	0.00000

Wilcoxon Signed-Rank Test

Table 7: Wilcoxon signed-rank test results

Sign	Observations	Sum of Ranks	Expected
Positive (DVL1 > DVL2)	36	1,917	2,093.5
Negative (DVL1 < DVL2)	43	2,270	2,093.5
Zero (No Change)	13	91	91
Total	92	4,278	4,278

Test Statistic	Z-Score	P-Value
Wilcoxon Signed-Rank Test	-0.688	0.4912

Spearman's Rank Correlation

Table 8: Spearman's Rank Correlation Results

Variables	N	Spearman's rho (ρ)
DVL1 vs DVL2	92	0.7145

Stability of HIV-1 viral load in plasma over time (4 Weeks)

Descriptive Statistics

Table 9: Summary Statistics for PVL1 and PVL2

Variable	Observations (n)	Mean	Standard Deviation	Minimum	Maximum
PVL1	104	28,591.19	246,578.4	1	2,510,000
PVL2	104	14,561.54	133,362.5	1	1,360,000

Normality Assessment

Table 10: Shapiro-Wilk Test for Normality

Variable	W Statistic	Z-Score	P-Value
PVL1	0.10897	9.628	< 0.0001
PVL2	0.11251	9.619	< 0.0001

Comparison of PVL1 and PVL2

Table 11: Wilcoxon Signed-Rank Test Results

Ranks	Observations	Sum of Ranks	Expected Sum
Positive (PVL1 > PVL2)	42	3,172.5	2,257
Negative (PVL1 < PVL2)	19	1,341.5	2,257
Zero (PVL1=PVL2)	43	946	946
Total	104	5,460	5,460

Test Statistic	Z-Score	P-Value
Wilcoxon Signed-Rank Test	3.083	0.0021

Correlation between PVL1 and PVL2

Table 12: Spearman's Rank Correlation Results

Variable Pair	Observations	Spearman's Rho	P-Value
PVL1 vs. PVL2	104	0.4422	< 0.0001

This study assessed the comparability and stability of HIV-1 viral load measurements between DBS and plasma samples among 242 participants. The plasma samples yielded significantly higher mean viral loads (23,885.51 copies/mL) compared to DBS samples (280.53 copies/mL), with both sample types exhibiting considerable variability. Normality testing using the Shapiro-Wilk test confirmed that the data were not normally distributed. The Wilcoxon Signed-Rank test showed a statistically significant difference between the two sample types (z=4.216, p<0.0001), while Spearman's correlation (P=0.4529, p<0.0001) revealed a moderate positive relationship indicating that while DBS tends to underestimate viral load, it still reflects overall plasma viral load trends.

When comparing test concordance, 38 participants had undetectable viral loads (Target Not Detected) in both sample types, suggesting strong agreement in identifying suppressed viremia. However, discrepancies were observed: 12 participants had detectable plasma viral load but undetectable DBS results, possibly due to DBS under-detecting low viral concentrations. Conversely, 77 had detectable DBS viral load with undetectable plasma, suggesting variations in sample characteristics or handling. The majority (115) had detectable viral load in both, supporting the overall utility of DBS. These results support findings from previous studies [10, 11] that demonstrated the feasibility of DBS for viral load monitoring in resource-limited settings, albeit with limitations in detecting low-level viremia.

In terms of sample stability, DBS demonstrated no statistically significant change in VL after four weeks of storage at room temperature (z=-0.688, P=0.4912), with a strong correlation between initial and final results (P=0.7145). This finding is consistent with previous findings [11], which reported similar long-term DBS stability. In contrast, plasma samples stored at -20°C showed a significant decline in VL over the same period (z=3.083, P=0.0021), with only moderate correlation (P=0.4422), highlighting susceptibility to degradation over time. This supports earlier work [12], which demonstrated that improper storage, particularly at elevated temperatures, leads to RNA degradation and may affect VL accuracy.

Demographic data showed that the majority of participants were aged 31-50 years, reflecting the sexually active population most affected by HIV. Female participants constituted 70.2%, likely due to greater healthcare-seeking behavior and routine HIV testing in maternal health services. While the inclusion of both sexes allows for a balanced perspective, the underrepresentation of children and adolescents may limit the generalizability of DBS performance across age groups.

Overall, the findings support DBS as a reliable alternative for VL monitoring in settings with limited access to plasma-based testing infrastructure. However, attention must be paid to its limitations at lower viral load levels. Policymakers and health programs should invest in robust pre-analytical systems, staff training, and clear guidelines on interpreting DBS results to enhance accuracy and expand access to routine VL monitoring in remote or underserved areas.

4. Conclusion

The analysis revealed significant differences in HIV-1 viral load measurements between DBS and plasma samples, with plasma consistently producing higher viral load values. Despite this difference, DBS and plasma viral loads had a moderate positive correlation. Furthermore, the stability of DBS viral load over time was evident, as indicated by minimal variation between the initial and final measurements. However, DBS demonstrated an underestimation of the viral load. This key limitation that may lead to the misclassification of patients as virally suppressed when they are not, creating a false impression of treatment success. This misrepresentation could have significant implications for patient management and public health interventions, potentially resulting in inadequate treatment adjustments and continued transmission risks.

Given these findings, there is a clear need for cautious interpretation of DBS results in clinical and programmatic settings. Policymakers should consider implementing a deliberate policy requiring confirmatory testing using plasma samples in cases where DBS indicates viral suppression, particularly for patients undergoing treatment monitoring or clinical decision-making. This approach would help ensure that patients who are not truly suppressed are accurately identified and managed accordingly. Furthermore, future efforts should focus on improving the accuracy of DBS assays, standardizing testing protocols, and training healthcare workers in proper sample collection and handling. These steps are crucial for enhancing the reliability of DBS-based viral load testing and ensuring its effective integration into routine HIV monitoring, especially in resource-limited settings.

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Conflict of Interest
Not available

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