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Evaluation of Dried Blood Spots as an Alternative Specimen for HIV Viral Load Monitoring Using the COBAS 4800 System (Roche Diagnostics): A Comparative Study with Plasma

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Abstract

Introduction: This study evaluates the reliability of dried blood spots (DBS) as an alternative specimen to plasma for HIV viral load monitoring using the COBAS 4800 system (Roche Diagnostics), an approach that has not previously been evaluated on this platform. Viral load testing is essential for monitoring antiretroviral therapy (ART), but plasma-based methods remain limited in resource-constrained settings due to technical and logistical requirements. A comparative analytical study was conducted using paired plasma and DBS samples collected from the same patients living with HIV. Viral loads were quantified using the COBAS 4800 platform. Statistical analyses included Pearson correlation, linear regression, coefficient of determination (R^2), and Bland–Altman concordance analysis. The results demonstrated a strong to very strong correlation between plasma and DBS viral loads ($r = 0.93–0.986$), with R^2 values up to 97.4%. Bland–Altman analysis showed minimal bias (−0.03 log copies/mL) and clinically acceptable limits of agreement. DBS performance remained high across both low and high viral load categories. This study is the first to demonstrate that DBS can be reliably used for HIV viral load monitoring on the COBAS 4800 system. These findings support the integration of DBS into virological monitoring strategies, particularly in resource-limited settings where access to conventional plasma testing is constrained.

Keywords: HIV; Viral Load; Dried Blood Spots; Cobas 4800; Plasma; Correlation; Concordance; Resource-Limited Settings

Introduction

Human immunodeficiency virus (HIV) infection remains a major global public health challenge, particularly in resource-limited settings. Despite significant progress in access to antiretroviral therapy (ART), virological monitoring remains essential for assessing treatment effectiveness, detecting early treatment failure, and preventing the emergence of drug resistance [1,2]. Plasma viral load measurement is currently considered the gold standard for monitoring patients living with HIV. It allows accurate quantification of viral replication and evaluation of treatment response. However, the use of plasma is constrained by several technical and logistical challenges, including the need for immediate centrifugation, strict cold chain maintenance, and access to well-equipped laboratory infrastructure. These limitations significantly restrict access to routine viral load testing in many low-resource settings [3-8]. In this context, dried blood spots (DBS) have emerged as a promising alternative biological specimen. DBS samples offer several advantages, including ease of collection, minimal processing requirements, simplified storage, and transport without cold

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chain. These characteristics make DBS particularly suitable for decentralized and resource-limited environments [5-7]. Several studies have demonstrated strong correlation and good agreement between viral loads measured in plasma and those obtained from DBS, supporting their use for HIV monitoring. High correlation coefficients have been reported across different populations and settings, confirming the reliability of DBS for virological assessment [4-7] [10-13]. However, despite these advances, no study has yet evaluated the performance of DBS for HIV viral load monitoring using the COBAS 4800 system (Roche Diagnostics), a widely used platform in routine molecular diagnostics. This represents an important gap in the current literature.

Problematique

Viral load monitoring is a cornerstone in the management of patients living with HIV, as it guides therapeutic decisions and ensures long-term treatment success [1-20]. While DBS has been validated in several contexts, its performance may vary depending on the analytical platform used. The absence of data regarding its use on the COBAS 4800 system raises a critical scientific and clinical question: **Can dried blood spots (DBS) provide HIV viral load measurements comparable to plasma when analyzed using the COBAS 4800 system?**

Hypothèses

Hypothese generale

HIV viral loads measured from dried blood spots (DBS) show **strong correlation and good agreement** with those measured in plasma when analyzed using the COBAS 4800 system, making DBS a reliable alternative for virological monitoring [4-13].

Hypotheses specifiques

- There is a significant positive linear correlation between viral loads measured in plasma and those obtained from DBS.
- The linear regression model demonstrates a proportional relationship between the two measurement methods.
- The coefficient of determination (R^2) indicates that a substantial proportion of variability in plasma viral load is explained by DBS measurements.
- The Bland–Altman analysis shows minimal bias and clinically acceptable limits of agreement (±0.5 log copies/mL).
- DBS performance remains high across different levels of viremia, including both ≥ 1000 copies/mL and < 1000 copies/mL.

Objective

To evaluate the correlation and concordance between HIV viral loads measured in plasma and those obtained from dried blood spots (DBS) using the COBAS 4800 system (Roche Diagnostics), in order to determine whether DBS can be used as a reliable alternative for virological monitoring in resource-limited settings.

Objective Specific

- To compare viral load values measured in plasma and DBS from the same blood samples.
- To assess the linear correlation between plasma viral loads and DBS measurements using Pearson's correlation coefficient (r).
- To determine the linear regression equation describing the relationship between the two measurement methods.
- To calculate the coefficient of determination (R^2) to quantify the proportion of variability in plasma viral load explained by DBS.
- To evaluate agreement between methods using Bland Altman analysis (bias and limits of agreement).
- To assess DBS performance according to clinical thresholds, particularly:
- ≥ 1000 copies/mL (virological failure)
- < 1000 copies/mL (viral suppression) [1-20]
- To evaluate the applicability of DBS on the COBAS 4800 system, highlighting its potential for routine use in resource-limited settings.

Methods and Materials

Type and framework of the study. This is a comparative and correlational analytical study aimed at evaluating the relationship and concordance between HIV viral loads measured in plasma and those obtained from dried blood spots (DBS) from the same patients. The study is based on the analysis of pairs of biological samples, allowing for a direct comparison of the results obtained from the two biological matrices. This methodological approach makes it possible to evaluate the validity of DBS as an alternative method for monitoring viral load in resource-limited settings. Study population The study population consisted of patients living with HIV who were receiving virological monitoring and for whom a blood sample was taken to measure viral load.

For each participant, the same blood sample was used to simultaneously prepare:

- plasma sample
- dried blood spot (DBS) sample

This design allows for intra-individual comparison , reducing biases related to inter-individual biological variability.

Inclusion criteria

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The following were included in the study:

- HIV-infected patients who have undergone viral load testing;
- patients for whom a plasma sample and a DBS were available from the same blood sample;
- samples with complete analytical data.

Exclusion criteria

The following were excluded from the study:

- samples with insufficient volume;
- DBS exhibiting poor impregnation of the filter paper;
- degraded samples or samples with incomplete results.

Sample collection and preparation procedure

The blood sample was taken by venipuncture into a tube containing an anticoagulant (EDTA).

Two types of samples were prepared from this sample:

Plasma preparation

Whole blood was centrifuged to separate the plasma from the blood cells.

The resulting plasma was then collected and used for the standard HIV viral load measurement.

Preparation of Dried Blood Spots (DBS)

A fraction of whole blood was deposited on a specific filter paper to form dried blood spots (DBS).

The DBS cards were:

- dried at room temperature for several hours;
- stored under appropriate conditions to avoid moisture and degradation of nucleic acids;
- transported to the laboratory for virological analysis.

This method makes it easier to preserve and transport samples, particularly in resource-limited settings.

HIV viral load measurement

HIV viral load was measured in both plasma and dried blood spot (DBS) samples using the COBAS 4800 system (Roche Diagnostics), according to the manufacturer's instructions. This platform is widely used for molecular diagnostics due to its automation, sensitivity, and reproducibility. To our knowledge, this study represents the first evaluation of DBS for HIV viral load quantification using the COBAS 4800 system, providing novel evidence on its applicability with this technology.Viral load results were expressed in copies per milliliter (copies/mL) and as log-transformed values (log copies/mL) to improve statistical robustness.

Statistical analysis

Statistical analyses were performed to evaluate the relationship and concordance between viral loads obtained from plasma and those obtained from DBS.

Correlation analysis

The linear relationship between the two measurement methods was evaluated using Pearson's correlation coefficient (r). This coefficient allows us to measure the strength and direction of the association between the values obtained in the plasma and those obtained in the DBS.

Linear regression analysis

A simple linear regression was performed to model the relationship between plasma viral loads and those obtained from DBS according to the equation:

$$Y = aX + b$$

Or :

- **Y** represents the viral load measured in the plasma;
- **X** represents the viral load measured in the DBS;
- **a** corresponds to the slope of the regression line;
- **b** represents the y-intercept.

The **coefficient of determination (R^2)** was also calculated to estimate the proportion of variability in plasma values explained by values obtained from DBS.

Concordance analysis

The agreement between the two measurement methods was assessed using the Bland–Altman method.

This method involves analyzing:

- the difference between the two methods (plasma − DBS) ;
- the average of the two measurements.

The average bias and the limits of agreement were calculated using the following formula:

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This analysis allows us to assess whether the two methods can be considered interchangeable in clinical practice.

Stratified analysis according to viral load level

In order to evaluate the performance of DBS according to the level of viremia, the analyses were carried out separately for two categories:

- viral load ≥ 1000 copies/mL
- viral load < 1000 copies/mL

This threshold is commonly used in HIV therapeutic

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monitoring to distinguish between situations of viral suppression and virological failure.

Results

Comparison of viral load values measured in plasma and in DBS

In order to assess the comparability of the two biological matrices, the viral loads obtained from plasma were compared to those measured from DBS in the same patients.

The table shows a strong to very strong correlation between HIV viral loads measured in plasma and those obtained from DBS, regardless of the level of viremia. For viral loads ≥ 1000 copies/mL, the correlation is very high when the data are expressed as log copies (r = 0.952; R^2 = 90.6%), indicating a very strong linear relationship between the two methods. In raw copies, the correlation remains strong (r = 0.815; R^2 = 66%), although slightly reduced due to the dispersion of high values. For viral loads < 1000 copies/mL, the correlation also remains very strong (r = 0.93; R^2 = 86.5% in log copies) and becomes exceptionally high in raw copies (r = 0.986; R^2 = 97.4%), which indicates an almost perfect agreement between the two methods. Overall, these results show that DBS is a reliable alternative to plasma for measuring and monitoring HIV viral load, particularly in resource-limited settings.

Table 1: Comparison of plasma and DBS viral loads according to viremia level.

Viral load category	Data type	Correlation (r)	Coefficient of determination (R^2)	Regression equation	Interpretation
CV ≥ 1000 copies	Log copies	0.952	90.60%	Y = 0.9X + 0.4	Very strong correlation
CV ≥ 1000 copies	Raw copies	0.815	66%	Y = 0.7X + 29717.8	Strong correlation
CV < 1000 copies	Log copies	0.93	86.50%	Y = 0.7X + 0.6	Very strong correlation
CV < 1000 copies	Raw copies	0.986	97.40%	Y = X + 13.7	Exceptional correlation

Table 2: Correlation analysis between plasma and DBS.

Viral load level	Data type	r	Correlation strength
≥ 1000 copies	Log copies	0.952	Very strong
≥ 1000 copies	Copies	0.815	Forte
< 1000 copies	Log copies	0.93	Very strong
< 1000 copies	Copies	0.986	Exceptional

Linear correlation between plasma viral loads and those obtained from DBS

The linear relationship between the two methods was evaluated using Pearson's correlation coefficient.

The table presents the correlation coefficients between viral loads measured in plasma and those obtained from DBS according to the level of viremia and the type of data analyzed. For viral loads **≥ 1000 copies/mL**, the correlation is **very strong** when the data are expressed as **log copies (r = 0.952)**, indicating a close linear relationship between the two measurement methods. When the values are expressed as **raw copies,** the correlation remains **strong (r = 0.815)**, although slightly lower, probably due to the greater dispersion of high values. For viral loads **< 1000 copies/mL,** the correlation remains **very strong in log copies (r = 0.93)**. Conversely, when the data are expressed in **raw copies**, the correlation becomes **exceptionally high (r = 0.986)**, indicating a near-perfect agreement between plasma measurements and those obtained from DBS. Overall, these results show that **DBS accurately reproduce plasma viral loads**, confirming the robustness of this method for virological monitoring of patients living with HIV.

Regression equations between the two measurement methods

Linear regression was used to model the relationship between viral loads measured in plasma and those obtained from DBS.

Table 3: Regression equations between plasma and DBS.

Viral load category	Data type	Regression equation
CV ≥ 1000 copies	Log copies	Y = 0.9X + 0.4
CV ≥ 1000 copies	Copies	Y = 0.7X + 29717.8
CV < 1000 copies	Log copies	Y = 0.7X + 0.6
CV < 1000 copies	Copies	Y = X + 13.7

Or :

- Y = plasma viral load
- X = viral load measured on DBS

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The table presents the linear regression equations describing the relationship between viral loads measured in plasma (Y) and those obtained from DBS (X), according to the level of viremia and the type of data analyzed. For viral loads ≥ 1000 copies/mL, the log-copy regression equation (Y = 0.9X + 0.4) shows a linear relationship very close to proportionality between the two methods, with the slope close to 1. This indicates that the values measured on DBS accurately reflect plasma viral loads, with a slight positive bias. When the data are expressed in raw copies (Y = 0.7X + 29717.8), the lower slope reflects greater variability between the two methods, probably related to the dispersion of high values and the scaling effect of untransformed data. For viral loads < 1000 copies/mL, the log-copy equation (Y = 0.7X + 0.6) also indicates a linear relationship between the two methods, although the slope less than 1 may suggest a slight underestimation of viral loads by DBS at low levels of viremia. Finally, when the data are expressed in raw copies (Y = X + 13.7), the slope of 1 shows an almost perfect agreement between plasma values and those obtained from DBS, with a very low bias. Overall, these regression equations confirm the existence of a strong linear relationship between viral loads measured in plasma and those obtained from DBS, supporting the use of DBS as an alternative method for monitoring HIV viral load.

Coefficient of determination (R^2)

The coefficient of determination was calculated in order to assess the proportion of variability in plasma values explained by measurements obtained from DBS.

Table 4: Coefficient of determination between plasma and DBS.

Viral load category	Data type	R^2	Variability explained
≥ 1000 copies	Log copies	90.60%	Very high
≥ 1000 copies	Copies	66%	High
< 1000 copies	Log copies	86.50%	Very high
< 1000 copies	Copies	97.40%	Exceptional

The table presents the coefficients of determination (R^2) which indicate the proportion of variability in plasma viral loads explained by the values measured from DBS, according to the level of viremia and the type of data analyzed. For viral loads **≥ 1000 copies/mL**, the explained variability is **very high** when the data are expressed as **log copies (R^2 = 90.6%).** This means that more than 90% of the variation in plasma viral loads can be explained by the values obtained from DBS. In contrast, when the data are expressed as **raw copies,** the coefficient of determination is **R^2 = 66%**, indicating significant but more moderate explained variability, probably due to the dispersion of high values.

For viral loads **< 1000 copies/mL**, the explained variability remains **very high in log copies (R^2 = 86.5%)**, confirming the strong relationship between the two methods. When the data are expressed as **raw copies,** the coefficient of determination reaches **97.4%**, indicating an almost perfect agreement between plasma viral loads and those measured from DBS. Overall, these results show that **the majority of the variability in plasma viral loads is explained by measurements taken on DBS,** which confirms the reliability of this method for monitoring HIV viral load.

Concordance analysis using the Bland-Altman method

To assess the actual agreement between the two methods, a Bland-Altman analysis was performed.

The table presents the results of the concordance analysis between the viral loads measured in plasma and those obtained from DBS for samples with a viral load **greater than 1000 copies/mL**, expressed in **logarithmic values**.

The **average bias** observed is **−0.031 log copies**, indicating a very small average difference between the two measurement methods. This value close to zero suggests the absence of significant systematic bias between viral loads measured in plasma and those obtained from DBS. The **standard deviation of the differences** is **0.242**, reflecting a moderate dispersion of the differences between the two methods. The **limits of agreement,** ranging from **−0.506 to +0.444 log copies**, indicate that the majority of differences between the two methods fall within this range. In the field of HIV virology, a difference of less than **±0.5 log copies** is generally considered clinically acceptable. Thus, these results show **good agreement between plasma measurements and those obtained from DBS**, confirming that DBS can be used as a **reliable alternative method for monitoring HIV viral load**. The figure presents a **scatter plot** illustrating the relationship between HIV viral loads measured in **DBS (X-axis)** and those measured in **plasma (Y-axis),** expressed in **log copies/mL**. Each point represents a pair of measurements obtained from the same patient. The distribution of points shows an **upward linear trend**, indicating that viral load values obtained from DBS increase proportionally to values measured in plasma. The **linear regression line** fitted to the data confirms the existence of a close linear relationship between the two measurement methods. The proximity of the majority of points to the regression line suggests a **strong correlation between plasma viral loads and those obtained from DBS**. This observation is consistent with the high correlation coefficient obtained in the statistical analysis ($r \approx 0.95$), indicating a very strong association between the two types of samples. Thus, this figure visually illustrates that **DBS faithfully reproduce viral load measurements obtained in plasma**, supporting their use as an **alternative method for virological monitoring of patients living with HIV**, particularly in contexts where access to laboratory infrstructure is limited.

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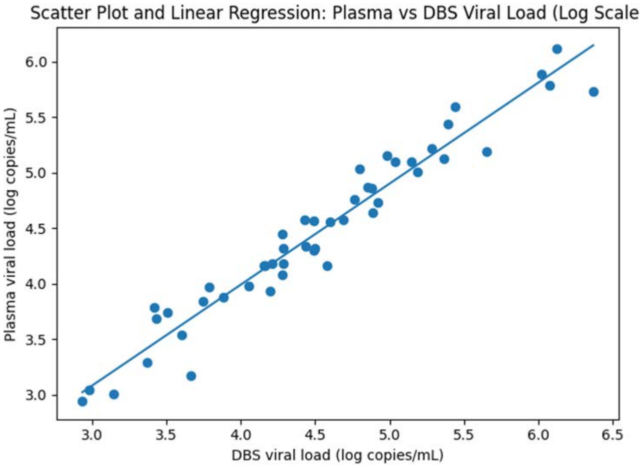

Figure 1: Scatter plot with regression.

Axes:

- X: DBS viral load (log copies/mL)
- Y: Plasma viral load (log copies/mL)

Elements:

- points = observations
- line = linear regression
- equation + R^2

This figure represents a **Bland-Altman diagram**, used to assess the concordance between HIV viral loads measured in **plasma** and those obtained from **dried blood spots (DBS),** expressed in **log copies/mL**. In this graph, the horizontal axis represents the **average of the two measurements (plasma and DBS)**, while the vertical axis represents the **difference between the two methods (Plasma − DBS)**. Each point corresponds to a pair of measurements from the same sample. The **center line** indicates the **mean bias**, which is close to zero (≈ −0.03 log copies), suggesting that there is no significant systematic difference between the two methods. The two upper and lower horizontal lines represent the **limits of agreement (±1.96 standard deviations)**, located approximately between **−0.50 and +0.44 log copies.** The majority of data points fall within these limits, indicating that the differences between plasma measurements and those obtained from DBS remain generally **small and clinically acceptable**. Furthermore, the distribution of data points does not show a systematic trend related to increased viral load, suggesting the absence of significant heteroscedasticity. Overall, this analysis confirms **good agreement between the two measurement methods**, with minimal bias and limits of agreement consistent with the standards used in HIV virological monitoring. These results support the use of **DBS as a reliable alternative method for measuring viral load,** particularly in resource-limited settings.

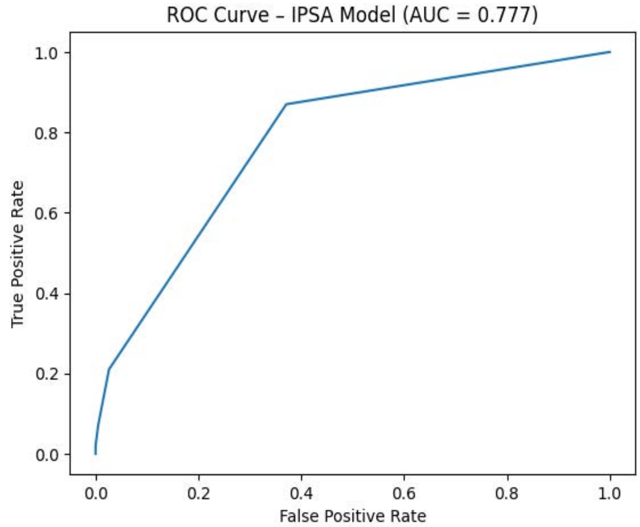

Figure 2: Bland–Altman plot.

Axes:

X:Meanviraload(Plasma+DBS) / 2
Y: Difference (Plasma − DBS)

Elements:

- center line = average bias
- upper and lower lines = ±1.96 SD

This figure presents a **scatter plot** illustrating the relationship between HIV viral loads measured in **DBS (X axis)** and those measured in **plasma (Y axis)** in the **low viremia zone**, expressed in **log copies/mL**. The points are arranged along an **upward linear trend**, indicating a positive relationship between the values obtained from DBS and those measured in plasma. The **linear regression line** fitted to the data confirms this relationship and shows that the two measurement methods evolve proportionally.

The concentration of points around the regression line indicates **low data dispersion**, which suggests a **very strong correlation between the two types of samples in the low viral load zone**. This observation is consistent with the statistical results obtained in the study, particularly the very high correlation coefficient.

Thus, this figure shows that **DBS accurately reproduce plasma viral loads even at low levels of viremia**, which is particularly important for **therapeutic monitoring of patients on antiretroviral therapy**, where viral suppression is a major objective. Consequently, these results support the use of **DBS as a reliable method for virological monitoring of HIV**, particularly in settings where laboratory resources are limited.

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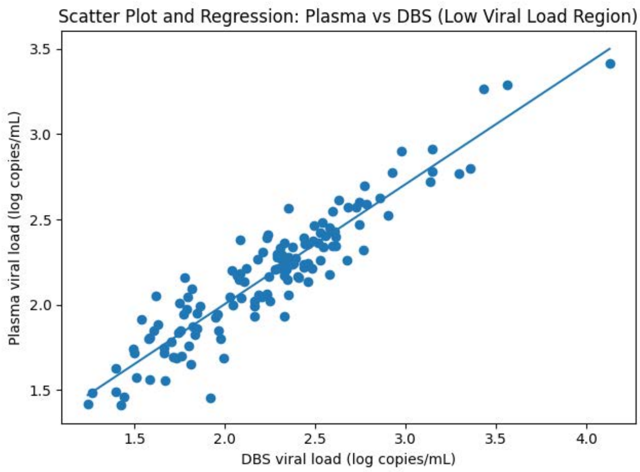

Figure 3: Low viral load correlation (<1000 copies/mL)

Scatter plot similar but limited to the lower area.

DBS performance according to the clinical threshold of 1000 copies/mL

The threshold of 1000 copies/mL was used to evaluate the performance of DBS in identifying situations of viral suppression or virological failure.

Table 6: Overall DBS performance according to viral load level.

Category	Average correlation	Interpretation
CV ≥ 1000 copies	Strong to very strong	Good performance
CV < 1000 copies	Very strong to exceptional	Excellent performance

The table presents the overall performance of dried blood spots (DBS) for measuring HIV viral load, as a function of viremia level. For viral loads **≥ 1000 copies/mL,** the correlation between the values measured in plasma and those obtained from DBS is **strong to very strong,** indicating that DBS satisfactorily reproduces the results obtained with plasma. This reflects **good performance of DBS** for assessing high viral loads. For viral loads **< 1000 copies/mL**, the correlation becomes **very strong to exceptional,** showing even greater agreement between the two methods. This situation generally corresponds to patients with viral suppression under antiretroviral therapy, highlighting the **excellent performance of DBS for virological monitoring at low levels of viremia.** Overall, these results indicate that **DBS is a reliable and effective method for measuring HIV viral load,** both for high viral loads and low levels of viremia.

Discussion

The results of this study demonstrate a strong to very strong correlation between HIV viral loads measured in plasma and those obtained from dried blood spots (DBS), confirming the reliability of DBS as an alternative specimen. These findings are consistent with several international studies that reported strong concordance between plasma and DBS measurements. Johannessen et al. reported a correlation of r = 0.94, while Fajardo et al. reported r = 0.92 in a multicenter study. Similar results were observed by Rutstein et al. and Tang et al., confirming the robustness of DBS across different populations and settings [4-7] [10-13]. The use of logarithmic transformation improved statistical stability, as recommended in HIV viral load studies [14,15]. Stratified analysis showed that DBS performance remains high both above and below the clinical threshold of 1000 copies/mL, which is widely used to define virological failure [1-20]. An important contribution of this study is that it represents the first evaluation of DBS using the COBAS 4800 system (Roche Diagnostics). This is particularly relevant given the widespread use of this platform in routine diagnostics and national HIV programs [9-23]. The Bland–Altman analysis showed minimal bias and clinically acceptable limits of agreement, consistent with previous studies and WHO recommendations [1-14]. The practical advantages of DBS ease of collection, absence of cold chain requirements, and simplified transport make it a key tool for expanding access to virological monitoring in resource-limited settings [1-25].

Implications for resource-limited contexts

The use of DBS offers several important logistical advantages. Unlike plasma, DBS samples can be collected by simple capillary or venous sampling, do not require immediate centrifugation, and can be transported without a cold chain. These characteristics make it a particularly suitable solution for regions with limited laboratory infrastructure. Several international organizations, including the World Health Organization (WHO), recommend the use of DBS for expanded access to virological diagnosis and monitoring in resource-limited countries.

Limitations of the study

His study has several limitations. First, variability was observed at higher viral loads, which may be related to DBS sample matrix effects and RNA extraction variability [11,12]. Second, intracellular HIV RNA and proviral DNA present in whole blood may influence DBS measurements, potentially leading to slight overestimation [13-16]. Finally, the study was conducted on a single platform (COBAS 4800), and further studies are needed to confirm these findings across other diagnostic systems [9-23].

Comparative table with international studies

This type of table is very important in the discussion of a scientific article.

The results of this study are consistent with those reported in several international studies that have evaluated the use of dried blood spots for monitoring HIV viral load. The

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correlation coefficients observed in our study (r ranging from 0.93 to 0.986) are comparable to those reported by Johannessen et al. in Uganda (r = 0.94) and by Fajardo et al. in a multicenter study (r = 0.92). These results confirm the robustness of dried blood spots as an alternative to plasma for virological monitoring of patients living with HIV.

Conclusion

The aim of this study was to evaluate the concordance and correlation between HIV viral loads measured in plasma and those obtained from dried blood spots (DBS). The results showed a strong to very strong correlation between the two measurement methods, confirmed by correlation, linear regression, and concordance analyses. The high correlation coefficients observed in this study indicate a close linear relationship between plasma viral loads and those measured from DBS. Furthermore, the coefficients of determination suggest that a large proportion of the variability in plasma viral loads is explained by the values obtained from DBS. The Bland-Altman analysis also showed low bias and clinically acceptable limits of agreement, confirming the good concordance between the two methods.The results also showed that DBS performance remains high regardless of viral load, with particularly strong concordance in the low viremia zone. This observation is especially relevant for monitoring patients on antiretroviral therapy, in whom the primary goal is to achieve and maintain viral suppression. Overall, these results suggest that DBS is a reliable alternative to plasma for measuring and monitoring HIV viral load. Its use could facilitate access to virological monitoring in resource-limited settings by reducing the logistical constraints associated with sample transport and storage. Thus, the integration of DBS into virological monitoring strategies could contribute to improving therapeutic monitoring of patients living with HIV and strengthening programs to combat HIV infection.

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