

Evaluation of the Concordance of the Results of the Dried Plasma Spot and Liquid Plasma for the Determination of the Viral Load of the HIV in Kinshasa: Preliminary Study

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Abstract

Objective: This study aims to compare the determination of the Viral Load of HIV using plasma samples and Dried Plasma Spots (DPS) in the context of Biological Monitoring in Kinshasa.

Methods: An experimental study was conducted to determine patients' Viral Load (VL) on liquid plasma and DPS. It was performed at the Molecular Biology Laboratory of the UNIKIN Faculty of Medicine. The size of the population was 48 ARV-naïve patients in a reasoned manner in the various selected centers. Five milliliters of blood were collected in a tube with EDTA anticoagulant from the vein of the elbow crease. The collected blood was centrifuged at 1000 g for 10 minutes to obtain the plasma. The blotting paper was prepared from 140 µl of plasma. After extraction of the RNA, the VLs were carried out on different algorithms for DPS and liquid plasma.

Results: Forty-eight (48) plasma samples and DPS were analyzed simultaneously for the determination of the VL. All samples were successfully extracted and amplified. At 60 Cycles, the results reveal 100% amplification for liquid plasma and DPS. The median values of VLs were respectively 4.68 log₁₀RNA copies/ml on plasma and 4.52 log₁₀RNA copies/ml on DPS. The correlation between the 2 methods was strong and the coefficient R² was of 0.9452 (p<0.001). The approved limits for both samples are between -1.20 and 0.80 log₁₀ copies/ml and the 30% confidence interval is -0.2 to 0.2.

Conclusion: The results found for the viral load on DPS and liquid plasma had shown that the results were consistent and correlated.

Keywords: Viral load; Dried Plasma Spot; HIV; PCR; Kinshasa

1. INTRODUCTION

According to the Organization of United Nations against HIV and AIDS (UN AIDS), the number of new HIV infections for 2013 was closed to 2.1 million people, more than thirty years after the start of the epidemic [1]. Despite substantial funding provided, the response against HIV, especially in sub-Saharan Africa, has given mixed results [1].

Globally, on December 1st, the 2015 UNAIDS report made subject of 36.9 millions of People Living with the Human Immunodeficiency Virus (PLHIV) in 2014 and nearly 2 million new HIV infection [2].

The UNAIDS has launched a new challenge to eradicate the HIV epidemic by 2030, with

intermediate targets, called "90-90-90" for 2020 [1]. To reach the 3 x 90%, patients with known Viral Load (VL) should reach viral suppression even in Low to Median Income Countries (LMIC). Over 70% of this population lives in rural areas in these countries without basic infrastructure with difficulties in moving samples to the reference laboratories found in urban areas. Thus the need of introducing Dried Plasma Spot (DPS) in large scale for VL detection in these LMIC. Hence, the aim of this study was to evaluate the correlation between DPS and Liquid Plasma samples in our environment.

2. METHODS

The present study was an experimental study carried out with different monitoring and follow

-up centers for PLHIV randomly chosen in Kinshasa, Democratic Republic of Congo (DRC). The inclusion of patients was from November 15th, 2016 to May 15th, 2017.

2.1. Criteria for Inclusion

Patients included in this study were HIV positive patients, naive of Antiretroviral Treatment (ART), over 18 years at the time diagnosis, and positive according to the national recommendations [3] and consenting to participate.

2.2. Variables of Interest

The values of the VL count on fluid Plasma and on DPS were the main variables of interest in the present study.

2.2.1. Blood Sampling and Rna Extraction

Five milliliters of blood were collected in a tube with anticoagulant EDTA from the vein in the elbow crease. One milliliter of plasma was transferred to a labeled micro-tube and 140 µl of this plasma was deposited on the blotting paper (DPS). The DPS were dried at room temperature for at least 2 hours before being sealed in the plastic bags.

The RNA was extracted from 140µl of plasma and 10µl of the internal extraction control (Diagenode RNA-050.vsl) using the QIA amp RNA Mini Kit from QIAGEN® for RNA extraction [4]. The extracted samples were stored at -20° C in the Molecular Biology Laboratory of the Faculty of Medicine (UNIKIN).

2.2.2. Quantitative Real-Time PCR for Viral Load

Real-Time Quantitative Multiplex PCR (q PCR) was performed to determine the amount of HIV pro-viral RNA in the samples according to previously described protocols [5-7]. The targeted viral region for amplification is part of the Long Terminal Repeat (LTR) which is a constant and conserved region of genome of HIV type 1 [5, 8]. The calibration curve has been plotted with standard controls. The standards used to graph are previously quantized plasmas 10² to 10⁷ viral particles per increment of a logarithm of 10 (Acro Metrix Control Panel # 94-2013). The curves were made on liquid plasma and on DPS separately. The acceptable coefficient for the curve was between -3.40 and -3.10 according to the Applied Bio

systems (ABS) 7500Fast Real-Time PCR System thermal cycler [8], and between -3.60 and - 2.90 according to the viral quantification protocol by Bio centric [9].

Primers and probes used for the amplifications, as well as PCR cycles are presented in the table 1 and 2.

Table1. *Primers and probes for amplification Real Time HIV*

Primers	Sequences
HIV1MGForward	GCCTCAATAAAGCTTGCCTTGA 5'-3'
HIV1MGReverse	GGCGCCACTGCTAGAGATTTT 5'-3'
HIV1MGProbe FAM	5'-AAGTAGTGTGTGCCCGTCTGT TRTKTGACT-3' BHQ1

Table2. *PCR/ Taq Man One Step cycle on ABI® 7500 Fast*

Temperature (° C)	Time (min : dry)	Step	Amplification
50	30 :00	Inverse Transcription	50/60 *
95	10 :00	Activation of the Enzyme	
95	0 : 15	Denaturation per cycle	
60	1 : 00	Hybridation by cycle	

* *Liquid plasma samples were all amplified at 50 cycles while samples on DPS were all amplified at 60 cycles.*

Table3. *Results Viral Charge PCR Plasma Vs DPS (log₁₀ copies of RNA / ml)*

Values	Liquid plasma	DPS*
Maximum CV (log ₁₀)	7.95	7.70
Minimal CV(log ₁₀)	2.38	1.98
Median CV(log ₁₀)	4.68	4.52
Standard deviation	1.25	1.32

* *at 60 cycles of amplification*

2.2.3. Data Processing and Analysis

The data collected were entered on Windows Excel version 2010. The statistical analysis of the results was done on SPSS version 17.0 for Windows. The Pearson Correlation test was used to evaluate the correlations between the different data. The significant value (p) retained for the probability was p<0.01 for the study. The results were expressed in median [Extreme value]. The sensitivity and specificity has been calculated in relation to the values of plasma VL. The concordance liquid plasma VL

and DPS was determined by the Bland Altman Test.

2.2.4. Ethical Consideration

Participation in this study was voluntary and all subjects had previously signed a consent form. The confidentiality of patients had been respected in accordance with the standards of Good Clinical Practice (GCP). The collection of blood samples was done by the technical team of the different centers selected for the study.

3. RESULTS

3.1. Determination of Viral Load

Forty-eight (48) plasma and DPS samples were analyzed simultaneously for Viral Load (VL) determination. All these samples were successfully amplified according to the protocol. At 50 amplification cycles, there was 100% of amplification for the liquid plasma samples and 33% of amplification for the DPS samples. At 60 cycles of amplification, there was 100% of amplification for both assays.

3.2. Results of Samples Made on Plasma

The median value of VL on liquid plasma was of 4.68 \log_{10} RNA copies/ml with extreme values of 2.38 and 7.95 \log_{10} RNA copies of RNA/ml.

3.3. Results of Samples Made on DPS

The median value of VL on DPS was of 4.52 \log_{10} RNA copies/ml with extreme values of 1.98 and 7.70 \log_{10} RNA copies/ml at the 60-cycle amplification.

3.4. Correlation and Concordance of VL Liquid and DPS

The results of VL were comparable on the 48 amplified samples on DPS and liquid plasma. The degree of association between the two methods was high and the coefficient R^2 was 0.9452 ($p < 0.001$). The merge of 0.5 \log_{10} was used to evaluate the differences between the results of the two values. The dispersion of the values was constant for the 2 methods used; it was included between -1.13 and 0.57 \log_{10} copies / ml.

3.5. Accreditation Limits

To calculate the accreditation limits for this study, it was necessary to calculate (i) the average of the differences ($d = -0.2$); (ii) the Standard Deviation (SD) of differences ($sdd = 0.5$); and

(iii) the lower and upper limit ($d \pm 2 \times sdd$) which was equal to the interval between -1.20 and 0.80.

3.6. Confidence Interval

To assess the degree of confidence the limits of agreement calculated confidence intervals around the graph Bland-Altman. With 30% of confidence interval, the values were included in the interval between -0.2 and 0.2.

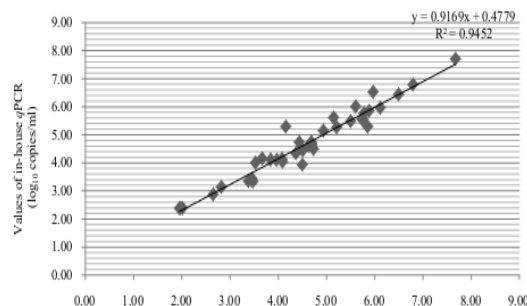


Figure1. Correlation Coefficient between VLs on liquid plasma and on DPS

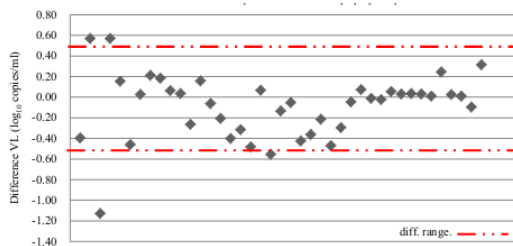


Figure2. Confidence interval between VLs on liquid plasma and on DPS

4. DISCUSSION

The aim of this study was to evaluate the correlation between blotting paper (DPS) and liquid plasma or the determination of HIV Viral Load (VL) in our environment, in order of contributing to the management, monitoring and follow up of People Living with HIV/AIDS (PLHIV).

The study involved 48 positive samples collected in different centers of the city of Kinshasa. The samples were aliquoted to give 48 liquid samples and 48 DPS. The results showed amplification of all samples in 100% of the corresponding using 60 cycle's amplification assays. At 50 cycles, only liquid plasma samples amplified at 100% and 33% of DPS samples amplified. Only high VL samples did amplify on DPS at 50 cycles. These data are consistent with those reported in our environment [12, 13].

The Spearman coefficient (R^2) found was 0.9452 for both types of samples (liquid plasma

and DPS) which were a good and strong correlation coefficient.

These data confirm the literature on the amplifications of DPS and DBS [9, 12, 13]. Different research groups reported an amplification detection limit in the range of 3.0 log₁₀ and 3.33 log₁₀ RNA copies/ml with the use of blotting paper [9, 12, and 13]. With 20% of the samples having a VL lower than 3.0 log₁₀, the results obtained after amplification in this study met the standards and confirmed the literature [9, 12, and 13].

The margin of ± 0.5 log₁₀ RNA copies/ml was used to evaluate the differences between the results of the two techniques [9]. The dispersion of values was constant for both techniques; it varied in the range.

These data showed a concordance of the results of the viral loads made on the samples on plasma and on DPS, with a mean of the differences of -0.2. This corroborates the literature data reported by Kouassi of Burkina Faso who found a difference average of 0.17 [14].

The approval limits for this work range from -1.2 to 0.8; they are in the range of -2 to 2 which determines the degree of confidence of the method and when the limits of approval are between the interval -2 to 2, the measure is said to be concordant [10]. The confidence interval in this study was in the interval of -0.42 to 0.21 for 95%; and from 0.2 to 0.2. This shows that the degree of associativity between the two techniques is very significant [10].

5. CONCLUSION

The results of this study on the comparison of Viral Load on liquid plasma and DPS, as an alternative method for monitoring PLHIV in the environments where resources are limited, gave a strong and good correlation using the Spearman correlation.

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