



Correlation between Sequencing Results from Liquid Plasma and Dried Plasma Spot (DPS) for Determination of HIV Type 1 Non-B Subtypes

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Abstract

Background: The blotting paper is an alternative to the collection of blood in the tubes for analysis, especially in the field of Human Immunodeficiency Virus infection. This technique allows to easily send the collected samples to specialized laboratories while limiting the stresses of storage and transport.

Objective: The objective of this study was to compare the results of sequencing performed on liquid plasma and Dried Plasma Spot (DPS) for the variants of HIV-1 non-B. **Methodology:** Fifty subjects diagnosed positive for HIV Type 1 using the Rapid Screening Tests voluntarily participated in this study.

Two hundred microliters of plasma are deposited on blotting paper Whatman 903 and 500 µl in a micro tube. RNA was extracted from 140 µl of plasma fluid and from a piece of DPS of 5 mm of diameter using the QIAamp RNA Mini Kit QIAGEN®. After extraction, the Viral Load (VL) was performed on each sample of liquid plasma. A Reverse Transcription PCR and Nested PCR were used to amplify the regions of interest on the Protease and Reverse Transcriptase for subsequent sequencing. **Results:** Protease and Reverse Transcriptase were amplified and sequenced respectively for 44 (88%) and 48 (96%) with the liquid plasma samples and 40 (80%) and 45 (90%) with the DPS. The results of Viral Loads were in the range of 2.5 log₁₀ and 6.5 log₁₀. The results of sequencing are comparable for plasma samples and DPS. The correlation coefficient (R²) between the two methods is good (R² = 0.903, p < 0.001). **Conclusion:** Liquid Plasma and Dried Plasma Spot give highly correlated results for sequencing strains of HIV type 1 non-B.

Subject Areas

HIV

Keywords

Sequencing, Liquid Plasma, Dried Plasma Spot, HIV-1 Non-B, Kinshasa

1. Introduction

Human Immunodeficiency Virus (HIV) is classified into 2 types (HIV-1 and HIV-2) which each one comprises different groups and subtypes [1]. Worldwide, the subtype C of HIV-1 group M dominates the epidemiology with nearly 70% of cases [2] [3]. Meanwhile, in sub-Saharan Africa, molecular epidemiology is dominated by subtypes A, C, G and various recombinant forms [1] [2] [3].

Used for more than 50 years, the blotted paper or Dried Blood Spot (DBS) is an alternative to the collection of blood in the tubes for analysis, especially in the field of HIV infection [4]. The DBS technique allows to easily send collected samples to specialized laboratories while limiting constraints due to storage and transport conditions. DBS can be crafted from any medium (blood, plasma, serum) in compliance with Good Laboratory standards and even routed by a local postal service [5].

To optimize the performance of the RNA extraction, the Dried Plasma Spot (DPS) was developed as an alternative to DBS [5] [6] [7] [8] [9]. With this variant, whole blood collected with anticoagulant is previously centrifuged and only the plasma is then deposited on the support; this allows to limit the presence of DNA and other contaminants in a sample where should be detected only viral RNA [5] [6] [7] [8] [9]. This technique is often used for the amplification of RNA for genotyping and Viral Load (VL) in resource-limited settings [5] [6] [7] [8] [9].

The objective of this study was to compare the results of sequencing performed on Liquid Plasma and DPS for HIV-1 non-B variants.

2. Methodology

2.1. Study Population

Fifty (50) subjects diagnosed positive for HIV Type 1 using the Rapid Screening Tests (RDTs) voluntarily participated in this study after signing consent. They were recruited from different centers in Kinshasa. The sampling was done from August 1st, 2013 to February 28th, 2014. HIV positive patients were confirmed in the Laboratory of Molecular Biology of the University of Kinshasa using a PCR technique previously described [10].

2.2. Blood Samples

Five milliliters (5 ml) 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 at room temperature to obtain a clear separation into 3 phases (plasma, buffy coat and Cullot). A milliliter of plasma (supernatant) was transferred into a pre-labeled micro centrifuge tube. These samples were mixed to ensure homogeneity and then aliquoted into two tubes (A and B) of 500 μ l each. Two hundred microliters (200 μ l) of plasma from tube B was deposited on blotting paper Whatman 903 (DPS). The tube A and the DPS were sent to the AIDS Reference Laboratory (ARL) of the University Hospital of the University of Liège (CHU-ULg) in Belgium for sequencing.

2.3. RNA Extraction, Amplification and Sequencing

RNA was extracted ARL-CHU Liege from 140 μ l of liquid plasma and a piece of DPS of 5 mm of diameter using RNA Mini Kit QIAamp[®] Kit (QIAGEN) [11]. The DPS samples were incubated for 2 hours at 70°C to maximize the extraction results. RNA extracted samples were stored at -80°C until use.

After extraction, the Viral Load (VL) was determined on each sample of liquid plasma using an in-house Quantitative PCR assay previously described for quantification of RNA [12].

A Reverse Transcription PCR (RT-PCR) and Nested PCR were used for amplifying the regions of interest on the Protease and Reverse Transcriptase (RT) for subsequent sequencing in conditions and temperature cycles previously described [13]. These PCR assay were used under the same conditions as described in the previous study [13].

The fragments obtained after amplification (Protease 500 base pairs and TR 800 bp) were sequenced by the Sanger sequencing method and purified by the ExoSAP-IT technique to eliminate the residual products of the PCR and sequenced by amplifying the purified fragment [13].

Pairing of the resulting fragments (sense and antisense) was done with the Vector NTI Advance[®] 11.5 software (Invitrogen, Life Technologies) and compared with various databases to identify subtypes of HIV-1 [13] [14].

2.4. Ethical Clearance

The present study was approved by the university ethical comity and had received consent from the center where samplings were done. The supervising team had seen that no harm was done to the patients during blood collection.

2.5. Statistics

The Friedman test was used to analyze the differences of groups of variants.

3. Results

Fifty (50) patients infected with HIV Type 1 were selected for this study. Samples of DPS as well as plasma were sequenced successfully. Protease and Reverse Transcriptase were amplified and sequenced respectively for 44 (88%) and 48 (96%) with the liquid samples and 40 plasma (80%) and 45 (90%) with the DPS

(Table 1).

3.1. Viral Load and Subtyping

The results of Viral Loads (VL) were in the range of 2.5 log₁₀ and 6.5 log₁₀. Ten patients (20%) had a VL under 3.0 log₁₀ while 32 (64%) had VL included between 3.0 log₁₀ and 6.0 log₁₀. Only 8 patients (16%) had a VL greater than 6.0 log₁₀.

From samples taken from the liquid plasma, subtype A is dominant with 12 cases (25%); followed by CRF02_AG (14.6%), C (10.4%), G (10.4%), K (8.3%), D (8.3%), H (6.25%) and J (6.25%) as presented in Table 2.

For samples collected on DPS, the subtype A is dominant with 10 cases (22.2%); followed by CRF02_AG (17.8%), C (8.9%), D (8.9%), G (8.9%), H (6.7%), J (6.7%), K (6.7%) and the CRF01_AE (6.7%) (Table 2).

3.2. Correlation of Results

The results of sequencing are comparable for plasma samples and DPS. For the Protease, the results are comparable to the 40 amplified samples on DPS. At the Reverse Transcriptase, 2 samples (4.4%) of the 45 amplified on DPS have presented different results; one subtype A sample from liquid plasma was presented as CRF02_AG from DPS, and another one subtype A sample from liquid plasma was presented as CRF01_AE from DPS. The correlation coefficient (R^2) between the two methods is good ($R^2 = 0.903$, $p < 0.001$).

Table 1. Results of amplification.

Amplification	Plasma liquid		<i>Dried Plasma Spot</i> (DPS)	
	Protease	TR	Protease	TR
Positive	44 (88%)	48 (96%)	40 (80%)	45 (90%)
Negative	6 (12%)	2 (4%)	10 (20%)	5 (10%)

Table 2. Frequencies of HIV type 1 subtypes.

HIV-1 Subtypes	Frequency on Liquid Plasma n = 48 (%)	Frequency on DPS n = 45 (%)
A	12 (25%)	10 (22.2%)
C	5 (10.4%)	4 (8.9%)
D	4 (8.3%)	4 (8.9%)
F	1 (2.1%)	1 (2.2%)
G	5 (10.4%)	4 (8.9%)
H	3 (6.25%)	3 (6.7%)
J	3 (6.25%)	3 (6.7%)
K	4 (8.3%)	3 (6.7%)
CRF01_AE	2 (4.2%)	3 (6.7%)
CRF02_AG	7 (14.6%)	8 (17.8%)
U	2 (4.2%)	2 (4.4%)

4. Discussion

The objective of this study was to compare the results of sequencing done from Liquid Plasma and Dried Plasma Spot (DPS) for samples of HIV-1 non-B. Fifty (50) HIV positive samples were collected, amplified and sequenced using various techniques of Molecular Biology.

Protease and Reverse Transcriptase were amplified and sequenced respectively for 44 (88%) and 48 (96%) samples with the Liquid plasma, while 40 (80%) and 45 (90%) with the DPS. This gives an average amplification higher than 80% for both. For DPS, all samples with a Viral Loads (VL) of less than $3.0 \log_{10}$ have not amplified for the Protease. These data confirm the literature on amplifications of DBS and DPS [5] [15] [16]. Various research groups have reported detection limit of amplification in the range of $3.0 \log_{10}$ and $3.33 \log_{10}$ with the use of blotting paper [5] [9] [15] [16]. With 20% of the samples having a VL of less than $3.0 \log_{10}$, the results obtained by sequencing in this study meet the standards and confirmed the literature.

In this study population, the subtype A is dominant with 12 cases (25%); followed by CRF02_AG (14.6%), C (10.4%), G (10.4%), K (8.3%), D (8.3%), H (6.25%) and J (6.25%). This is consistent with what the literature presents in terms of the subtypes circulating in Kinshasa where the subtype A prevails [13].

The results of sequencing are comparable for plasma samples and DPS. For the Protease, the results are comparable to the 40 amplified samples. This may be justified because the sequence of the Protease, by its size (± 124 nucleotides), is the least changing region to the HIV Type 1 genome [17], thus implying a uniformity of result of sequencing on plasma and DPS. For the Reverse Transcriptase, 2 samples (4.4%) of the 45 amplified have presented different results; 1 sample of subtype A from liquid plasma was presented as CRF02_AG from DPS and one other subtype A sample from liquid plasma was presented as CRF01_AE from DPS. The correlation coefficient (R^2) between the two methods is good ($R^2 = 0.903$, $p < 0.001$). Nevertheless, the difference between subtype A and CRF_01_AE and 02_AG is not large [18]. Several techniques have frequently assimilated and do not give a clear difference [18]. Hence, the results of Protease are most often considered as reverse transcriptase in the case of sequencing of HIV [18].

5. Conclusion

The Liquid Plasma and the Dried Plasma Spot (DPS) give highly correlated results for sequencing strains of HIV type 1 non-B. The Viral Loads below $3.00 \log_{10}$ do not provide sequencing results on DPS. These results support the use of DPS for sequencing in countries with limited resources in cases of high viral load greater than $3.00 \log_{10}$.

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