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Research Article

CORRELATION BETWEEN SEQUENCING RESULTS ON PLASMA AND ON DRIED BLOOD SPOT FOR THE DETERMINATION OF NON-B SUBTYPES OF HIV TYPE 1 IN N'DJAMENA, CHAD

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ABSTRACT

Objective: The aim of this study was to evaluate the sensitivity of DBS on samples from Chadian patients infected with non-B subtypes of HIV Type 1.

Methods: The blood of 50 patients living with HIV was drawn on a tube with EDTA and 30 of them were randomly put on *Whatman® 903* type DBS. The drying was done overnight at room temperature. Storage was done at -20 ° C. Viral RNA extraction was performed with the *QiAamp Viral RNA* mini kit. The viral load on DBS and on plasma was carried out at LRS CHU-ULg. The determination of the variants was obtained after analysis on the Stanford University/HIV Drug Resistance Database site. The interpretation of the resistances is made in accordance with the algorithm of the ANRS AC11.

Results: An amplification rate of 80% is obtained on the DBS with a detection limit of the blotter is 3.33 $\log_{10}$ copies of RNA/ml. The subtypes obtained on the Protease sequences, which is the least changing region of the virus were retained. CRF02_AG is the most represented both on DBS with 29.17% and on plasma with 30.23%. Sequencing on DBS showed the presence of the K subtype (12.5%) at the level of the Reverse Transcriptase while it is absent in the plasma after sequencing.

Conclusion: An amplification success rate of 80% compared to plasma was observed in this study. However, DBS can be useful for the evaluation of antiretroviral therapy for non-B subtypes.

Key words*:* Sequencing, plasma, DBS, HIV, N'Djamena.

INTRODUCTION

Easily applicable, samples on Dried Blood Spot (DBS) make it possible to collect blood taken from an Ethylene Diamine Tetra Acetic (EDTA) anticoagulant tube or by pricking in the heel or finger, both in town and in decentralized regions [**1**]. They allow samples of dried blood to be stored at room temperature or in the cold, in airtight bags, preventing humidity [**1**]. The viral Ribonucleic Acid (RNA) contained on DBS or plasma can be stored at room temperature or at -70 ° C and is stable for at least one year [**2**].

DBS also has the advantage of being easily routed to laboratories, which therefore promotes decentralization; moreover, the technique of collecting blood on DBS avoids the movement of specialized teams since the sampling can be carried out by any properly trained person [**3**].

Data from the literature indicate that determination of Viral Load (VL) and evaluation of genotypic resistance of HIV-1 by the DBS and plasma is possible; several studies have obtained comparable results even after long-term storage [**4-6**].

DBS is also used for the early diagnosis of HIV-1 infection by DNA PCR, and also for monitoring resistance to treatment in children as recommended by the World Health Organization (WHO) [**7-9**]. In pediatrics, DBS is also used for the screening of metabolic disorders in newborns and for the detection of HIV-1 antibodies [**10-12**]. However, DBS can be used very well for the evaluation of Antiretroviral (ARV) treatment for non-B subtypes [**13**].

The aim of this study was to evaluate the sensitivity of DBS on samples from Chadian patients infected with non-B subtypes of HIV Type 1.

METHODS

The venous blood of 50 People Living with HIV/AIDS (PLHIV) was collected on a tube with EDTA anticoagulant at the elbow and 5 spots of 75 µl (375 µl of blood in total) each were placed on *Whatman® 903* type DBS. The following information was noted on it: the initials of the names of PLHIV, the date of birth, the date of the sample and the identification number of the card. DBS spots were used for each PLHIV. Drying was done overnight at room temperature and protected from light according to the protocols in the literature. The next day, all the DBS were put in a plastic packaging containing two desiccants. The conservation was made at -20 ° C until transport to Belgium for the analyzes in this case the VL and the sequencing, at the HIV/AIDS Reference Laboratory of the University of Liège (LRS CHU-ULg). Out of the 50 samples made on DBS, 30 were chosen randomly for the different analyzes. Viral RNA extraction was performed with the *QiAamp Viral RNA* mini kit. The VL on DBS and on plasma was carried out with the *LRS CHU-ULg* kit.

The determination of the subtypes, the recombinant forms and the resistance mutations were obtained after analysis on the site of Stanford University/HIV Drug Resistance Database (http://hivdb.stanford.edu/).

The interpretation of the resistances was made in accordance with the algorithm of the ANRS AC11 (September 2013) (http://www.hivfrenchresistance.org/).

RESULTS

Forty-four (44) out of 50 samples were successfully amplified on plasma, presenting an 88% amplification success rate. While on DBS, 24 out of 30

samples were successfully amplified, giving an 80% amplification rate. The limit of detection for DBS was 3.33 log_{10} copies of RNA/ml, which is equivalent to 2,150 copies of RNA/ml.

Phylogenetic analyzes were carried out on the Protease region from the 24 samples which had been amplified on DBS; they showed that the Circulating Recombinant Forms (CRF02_AG) was the most represented on both DBS (29.17%) and on plasma (30.23%). Sequencing of the K subtype, from Transcriptase Reverse, was absent on plasma; it was present in 12.5% of the samples sequenced from DBS. The CRF02_AG was followed by the subtypes J (16.67%), D (12.5%) and G (8.33%). The detailed results are presented in Table 1.

Table 1: Comparison of the subtypes detected on plasma vs DBS

DBS/Plasma	Number	Percentage (%)
CRF02_AG/CRF02_AG	7	29,17
CRFF02_AG/J	1	4,17
D/D	3	12,50
D/Und*	1	4,17
F/D	1	4,17
F/J	1	4,17
G/G	2	8,33
J/J	4	16,67
J/Und*	1	4,17
K/G	1	4,17
K/A	1	4,17
K/Und*	1	4,17
Total	**24**	**100**

***Und: undetermined**

The observation for the sequence analyzes is that for the same PLHIV, there may be a subtype on the Transcriptase Reverse and another different on the Protease (Table 2).

Table 2: Resistance mutations detected on Plasma vs DBS

ID	CV Log_{10}/ml	DBS/Plasma	PI Major	PI Minor	INTI	INNTI
2	4,67	K/A	NA	NA	M184V	K103N, Y188F
5	5,46	CRF02_AG/ CRF02_AG	M46I, I54V, V82S	L10V, K20I	A62V, L74V, V75T, M184I	V90I, K103N, V108I, Y181C, H221Y
9	4,81	F/D	NA	NA	M184V, T215F	K101E, V108I, Y181C
12	4,58	D/D	None	L10I	NA	NA
15	4,47	D/D	NA	NA	M41L, T69D, K70R	K103E, V108I
23	5,56	J/J	None	L10I	K65E, M184V	K103N
30	5,98	G/G	None	K20I	M41L, D67E, T69Si, L74V, M184V, L210W, T215Y	A98G, K103N, V108I, P225H
31	5,14	D/NA	None	L10V	T69N, K70R, M184V	V106I, V179D, Y181C
32	5,41	J/J	NA	NA	M41L, D67N, L74V, M184V, T215Y, K219E	V106M, V108I, Y181V, H221Y
35	4,77	G/G	NA	NA	D67N, T69d, V75I, F116Y, Q151M, M184V	K101E, G190A, K238T
48	3,33	K/G	NA	NA	None	None
49	5,19	K/NA	None	L10I, K20I	M184V	V106I
54	5,26	CRF02_AG/ CRF02_AG	None	K20I	K219N	K103N, Y181C
59	5,18	CRF02_AG/ CRF02_AG	None	K20I	None	None
62	5,09	F/J	NA	NA	None	L34A, V35N, E36R, I135V, S162C, K173T, Q174K, I180V, T200A, Q207D, R211K, P226H, F227i, M230V, E233L, P236A, K238R, W239R, T240A, Q242P, P243T, I244L, V245L, P247X, K249E, S251X, N255D, D256X,

ID	CV Log_{10}/ml	DBS/Plasma	PI Major	PI Minor	INTI	INNTI
						I257V, K259Q
65	4,41	J/J	NA	NA	M184V	K103N, Y181C
67	4,63	J/J	None	L10I	D67N, K70R, M184V	Y188L
71	3,88	CRF02_AG/J	None	K20I	NA	NA
79	5,30	J/NA	None	None	NA	NA
92	4,68	CRF02_AG/ CRF02_AG	None	L10I, K20I	NA	NA
93	5,72	D/D	None	None	K70R, M184V, K219Q	K103N, V108I, H221Y
107	4,87	CRF02_AG/ CRF02_AG	None	K20I	M184V	V90I, K101E, V106I, G190A
108	4,39	CRF02_AG/ CRF02_AG	None	K20I	None	V90I
110	5,09	CRF02_AG/ CRF02_AG	None	K20I	M41L, D67N, M184V, T215F	A98G, Y181C

DISCUSSION

The aim of this study was to evaluate the sensitivity of DBS on samples from Chadian patients infected with non-B HIV type 1 subtypes.

Forty-four (44) out of 50 samples were successfully amplified on plasma, representing an 88% amplification success rate; while on DBS, the rate was 80%. Which makes a difference of 8%. Several studies have been carried out as part of the comparison between molecular biology analyzes made from DBS or from plasma. These studies converge or not depending on the type of analysis in question; for the evaluation of resistance to ARVs, Charlotte C and *al*. found that the analysis on DBS is half as sensitive compared to that on plasma; the search for mutations is however identical on DBS and on plasma [**14**]. Regarding the determination of VL, Madhavan V and *al*. and Xiaoning W and *al*. found that the result on plasma is similar to that on DBS, except that the latter becomes somewhat sensitive and unreliable when the viral loads are low [**15,16**]. The difference in amplification success rate observed in this study could be explained by the extraction techniques used; moreover, certain authors, notably Kamangu NE and *al*., have demonstrated that the information provided on DBS is very reliable and can be very useful for researchers in several fields of bioanalysis, including pharmacokinetics, therapeutic monitoring of drugs, toxicokinetic, metabolism, disease diagnosis and even phylogenic studies on HIV [**16-18**]. This is also the observation made by Andrea H and *al*. in his 2018 study on sensitive genotyping of HIV-1 subtypes [**19**]. Some subtypes are more sensitive on DBS than on plasma; viruses archived in lymphocytes on DBS may be different from those present in plasma [**20,21**]. It is for this reason that the data in this present study revealed the presence of the K subtype on DBS and not in plasma.

The DBS detection limit was 3.33 log_{10} copies of RNA/ml, which is equivalent to 2,150 copies of RNA/ml. It corresponds to the 2018 standards for resistance tests using the ANRS method [**21**].

The phylogenetic analyzes carried out on the Protease regions from 24 samples which had been amplified on DBS, showed that the CRF02_AG was the most represented on DBS (29.17%) than on plasma (30.23%). These data do not corroborate those of Kamangu NE and *al*. in his study carried out in 2015 in Kinshasa in the Democratic Republic of Congo (DRC) [**22**]. After sequencing the Protease on 130 samples and the Transcriptase Reverse on 145 samples, in a study carried out on 153 PLHIV, he had found that subtype A was the most represented (22.87%) followed by the Recombinant Circulating Form CRF02_AG (11.11%). It should be noted that in Kinshasa in the DRC, subtype A has predominated the HIV-1 epidemic since its declaration [**23**]. In Chad, the Recombinant Circulating Form CRF02_AG and the subtype J were both in the majority in 2017 [**24**]. Moreover, in certain countries of central Africa in this case Cameroon, Gabon and Equatorial Guinea, it is the Recombinant Circulating Form CRF02_AG which prevails [**25-27**].

The observation for sequence analyzes is that for the same PLHIV, there may be one subtype on Transcriptase Reverse and another different on Protease, while the two enzymes are all derived from the structural gene pol [**28**]. This does not support data from studies by DeLong AK et al. and Melissa JW et al. who had found the same HIV-1 group M subtypes, after phylogenetic analysis using Protease and Transcriptase Reverse [**29,30**]. The difference in the sequences observed in the present study could come from the region of Transcriptase Reverse on the pol gene, which is more variable than that of Protease [**31**].

CONCLUSION

Apart from the use of blotting paper for children for the early diagnosis of HIV-1 by DNA PCR. DBS can be used to assess antiretroviral therapy (genotypic resistance testing) for non-B subtypes. The *QiAamp Viral RNA* mini kit could be used for the extraction of viral RNA for the detection of resistance mutations by sequencing or by ASPCR on DBS.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTION

CA, ENK and DV: conception and design, analysis and interpretation, drafting the article. CA, ENK, FS, DV and AT: conception and design, critical revision of the article, final approval of the article. CA, ENK and FS: collection data. DV and AT: critical revision of the final version of the article, final approval of the article. All the authors have read and agreed to the final manuscript

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