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Performance of dried blood spot (DBS) PUNCHER and dried blood spots to measure HIV-1 viral load

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The use of plasma is an obstacle to realize HIV-1 viral load in sub-Saharan Africa. In this context, the dried blood spot (DBS) is an interesting tool for sample collections. This approach was tested using a DBS hole-punch device (PUNCHER). Plasma and DBS samples were obtained from 102 patients, comprising 17 HIV-1 negative patients and 85 HIV-1 infected patients. The PUNCHER's performance used to cut DBS was evaluated with the following criteria: ease of use, time savings and safety. VL was measured in parallel on plasma and DBS samples using NucliSENS EasyQ HIV-1. The correlation between plasma and DBS results was strong (R = 0.91; P < 0.001). The mean difference (± standard deviation) was -0.59 ± 0.52 $\log_{10}$ copies/ml. The sensitivity and specificity of DBS were 91.3% (n= 74) for the 81 VL detectable samples and 100% for the 21 VL undetectable samples, respectively. On a scale of 10, the PUNCHER's performance scored 9.3 for ease of use, 8.6 for time savings and 10 for safety. PUNCHER is highly efficient at cutting DBS, and the VL resulting from DBS correlated well with those obtained from plasma.

Key words: Puncher, dried blood spot (DBS), viral load, performance.

INTRODUCTION

In developed countries, viral load (VL) is an essential assay for monitoring the human immunodeficiency virus type 1 (HIV-1) infection, especially for evaluating the efficacy of antiretroviral treatment (ART) (Mellors et al., 1997; Yilmaz, 2001). In sub-Saharan Africa, the use of VL monitoring to detect treatment failure is the major challenge to improve HIV management. Access to this assay faces several obstacles: cost of equipment and reagents, availability and stability of energy. If VL measuring equipment exists in these countries, it is more often only available in reference laboratories far from peripheral sites, posing the problem of accessibility to people living with HIV. Then, the use of dried blood spot (DBS) represents an alternative for the collection and transport of samples as compared to plasma, which requires more restrictive conditions, transport without delay and storage at -80°C (Cassol et al., 1997; Brambilla et al., 2003; Alvarez-Muñoz et al., 2005; Kane et al, 2008 ; Johannessen, et al 2009; Arredondo et al, 2012). In most laboratories, DBS are cut with a pair of scissors,

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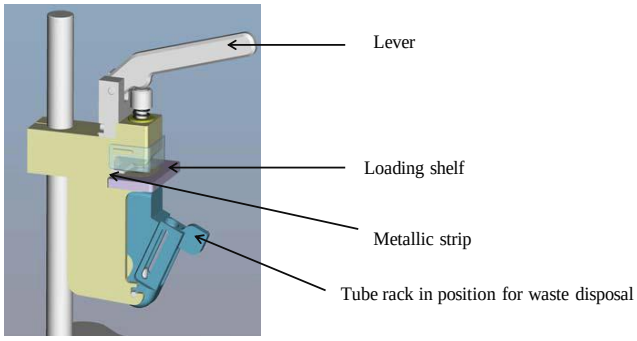

Figure 1. Puncher –bioMérieux.

with a risk of injury while cutting or decontaminating the scissors. In addition, discomfort from the long use of scissors limits the number of DBS that are cut.

The objectives of this study were, to evaluate the performance of the puncher, a new method of cutting DBS (weight = 4.6 kg; length = 48.5 cm; width = 21 cm; Figure 1; http://www.biomerieux-diagnostics.com) according to 7 criteria and to investigate the performance of DBS in HIV-1 RNA quantification against the standard plasma viral load assay.

METHODS

A total of 102 patients, comprising 17 HIV-1 negative patients and 85 infected patients (58% of whom were antiretroviral therapy naive), were recruited at Sylvanus Olympio University Hospital in Lomé (Togo) over a period of six months, January to June 2013. After obtaining written informed consent, five milliliters of whole blood was drawn from each patient by venipuncture and collected in tubes with EDTA. DBS were prepared by dispensing 50 µl of blood per spot (5 spots per card) onto filter paper cards (Whatman no. 903; Schleicher and Schuell, BioScience GmbbH, Barcelona, Spain). The spotted filter papers were allowed to dry at room temperature for 4 to 6 h in a hood. The DBS were stored in zip-lock plastic bags with a silica gel desiccant at room temperature for 15 days before further processing and assaying. The remaining blood sample was centrifuged at 1500 xg and plasma was stored at -80°C until testing.

The DBS were cut with the PUNCHER according to the manufacturer's instructions. During the use of the PUNCHER, the following performance-related parameters were evaluated: speed of handling, number of manipulations to cut the spots, speed of positioning the card; risk of contamination associated with handling. To avoid contamination of one sample by another, 3 white spots of the same blotting paper were cut after punching 2 spots of a DBS sample before moving on to the next sample. Seventeen (17) HIV-1 negative samples were included in the study to evaluate the contamination risk by using the PUNCHER. Criteria for PUNCHER evaluations were defined taking into account the difficulties associated with using scissors to cut DBS. The use of the PUNCHER was carried out by one lab technician. For each parameter, a score between 1 and 10 was awarded after each sample: 10 meaning completely in line with expectations.

HIV-1 RNA isolation from DBS and plasma was performed using 100 µl (2 spots) and 500 µl of samples, respectively. HIV-1 RNA was extracted from the same patient in plasma and DBS according to the NucliSENS miniMAG procedure. VL was measured by NucliSENS HIV-1 EasyQ version 2.0 (bioMérieux, Lyon, France). All RNA values are reported as $\log_{10}$-transformed copy numbers of HIV RNA per ml of DBS or plasma. Viral load was stratified into three levels (undetectable VL, VL < 5000 copies/ml and VL > 5000 copies/ml). Sensitivity and specificity of DBS viral load using plasma assay as the gold standard was assessed at all three viral load strata. Pearson correlation analysis was performed, as well as Bland-Altman analysis to examine the level of agreement between the two tests (Bland et al., 1986). Bland-Altman analysis was performed using MedCalc version 9.5.0.0 (MedCalc Software, Mariakerke, Belgium). With the given sample size that was used for Bland Altman analysis, the 95% CI for the limits of agreement were ± 0.11 $\log_{10}$ copies/ml. This narrow range in the precision of the limits of agreement was deemed to be clinically acceptable. Differences were considered significant only when P values were <0.05. The Ministry of Health of Togo (No. 0411/2012/MS/CAB/ DGS/DPLET/CBRS) and National ethic committee of Togo (Comité de bioéthique pour la recherche en santé, No. 001/2012/CBRS) approved the study.

RESULTS

The plasma results showed that all HIV-1 negative samples (n=17) were undetectable for VL results. From 85 infected patients, 81 (95.3%) were detectable VL (Table 1), including 24 VL < 5000 copies/ml and 57 VL > 5000 copies/ml. The correlation between DBS and plasma was high (R = 0.91; P < 0.001) (Figure 2). The mean difference and standard deviation in samples with a VL < 4 $\log_{10}$ (n = 30) was 0.65 (0.40); 0.60 (0.25) for samples between 4-5 $\log_{10}$ (n = 26) and 0.59 (0.15) for samples with a VL > 5 $\log_{10}$ (n = Bland-Altman plot illustrates the agreement between plasma and DBS (Figure 3). The mean difference for quantitative 25). Overall, 49 pairs of plasma and DBS (49%) had a difference of more than 0.5 log copy, and for 14 pairs of samples (14%), the difference was greater than 1 $\log_{10}$ copy. The corresponding data between measurements (DBS minus plasma) was -0.59 $\log_{10}$ copy (standard deviation, 0.52 $\log_{10}$ copies/ml). The sensitivity of DBS was 91.3% (n= 74) for the 81 VL detectable samples. Ten results from DBS were < 5000 copies/ml among patients who had VL > 5000 copies with plasma; for 6 of these 10 samples, the VL from plasma was between 5000 and 7000 copies/ml and for 4 samples the VL was > 11000 copies/ml. The specificity of DBS versus plasma was 100% for the 21 VL undetectable samples (17 HIV-1 negative samples and 4 HIV-1 positive samples).

On a scale of 10, PUNCHER scored 9.3 for ease of use, 8.6 for time savings (Table 2). Concerning the rapidity of cutting, 124 DBS corresponding to 62 patients were cut in one hour using the puncher. As compared to archives data (unpublished data), 88 DBS corresponding to 44 patients were cut using scissors in the laboratory. No contamination was observed, all 17 negative samples were found negative although randomly tested between samples with high viral loads. The absence of contamination proved the efficacy of the decontaminating protocol.

Table 1. Summary of the HIV-1 viral load results of 102 samples from plasma and DBS.

		Plasma			Total
		Undetectable	VL* < 100 copies	Quantifiable	
DBS	Undetectable	19	3	3	25
	VL* <100 copies	0	0	1	1
	Quantifiable	2	1	73	76
Total		21	4	77	102

*Viral load.

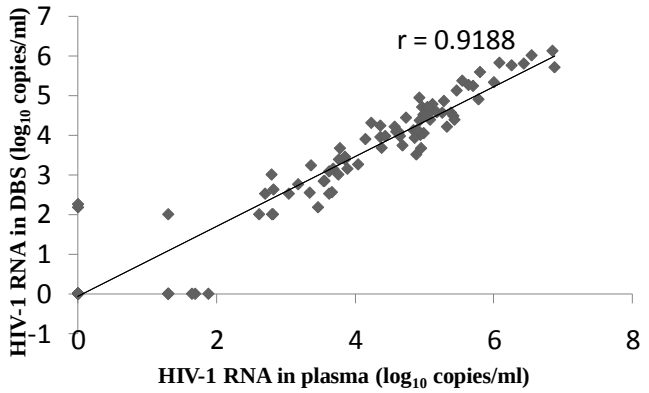

Figure 2. Linear regression comparing HIV type 1 RNA levels obtained by testing 102 paired plasma and DBS

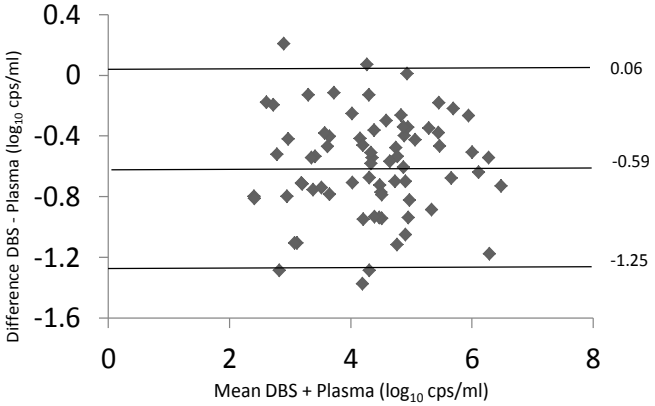

Figure 3. Bland and Altman analysis of viral load values comparing DBS versus plasma (n = 73) using NucliSENS EasyQ HIV-1 assay.

DISCUSSION

The use of DBS specimen as source for diagnostic test has become increasingly popular in recent years. DBS has been used to identify genetic and metabolic disorders in neonates, detection of HIV-1 antibody, and HIV-1 DNA for infant diagnosis of HIV infection. The WHO recommends the use of DBS for HIV drug resistance surveillance for monitoring transmitted drug resistance in resources limited settings (WHO, 2010).

In this study, a new tool to cut DBS replacement scissors wa evaluated in order to validate its use in routine practice. As compared to scissors, the performance of the puncher concerning ease of use, time savings and rapidity of cutting was better. But the data obtained for the scissors are archive data.

The VL results were divided into two groups based on the new WHO recommendations; patients on high active antiretroviral therapy for at least 4 weeks and with a VL > 5000 copies/ml are considered to be in treatment failure (WHO, 2012). The results showed a strong correlation between plasma and DBS. But the small sample size included in this study may limit the accuracy of the results; however, significant correlation and limits of agreement of two assay methods found in this study reinforce the usefulness and feasibility of utilizing DBS as method for clinical viral load monitoring of patients on ART or HIV-1 early diagnosis.

Previous data report correlations range from 78 to 99% between DBS and plasma according to different analyzers for the measurement of VL, and sometimes with different extraction methods (Brambilla et al., 2003; Johannessen et al., 2009; Garrido et al., 2009; Marconi et al., 2009; Mbida et al., 2009; Hamers et al., 2009; Bertagnolio et al., 2010; Johannessen et al., 2011b; Neogi et al., 2012). In this study, DBS were stored at room temperature (28 to 35°C) for 15 days before being handled, and were never frozen. 15 days was set as storage time at room temperature for DBS in this method to be used by the HIV care centers; which gives a time to transport DBS to the national reference laboratory.

HIV-1 RNA quantification from DBS has shown good stability under different temperature and storage conditions ranging from ambient to -70°C (Cassol et al., 1997; Brambilla et al., 2003; Alvarez-Muñoz et al., 2005; Kane et al., 2008; Marconi et al., 2009; Monleau et al., 2010). Nevertheless, subsequent studies should define the DBS VL values at which the patient will be considered either in remission or in virological failure.

In conclusion, the performance of the PUNCHER is excellent. It can therefore be recommended tolaboratories

Table 2. Performance of the DBS PUNCHER.

Parameter	Total no. of points for 102 DBS punches*	Score out of 10
Ease of use		
Handling	966	9.6
Number of manipulations to punch out the spots	890	8.9
Time savings		
Speed of handling	909	9.1
Speed of positioning the card	829	8.2
Manipulation time (card to lysis tube)	867	8.7
Safety		
Risk of contamination associated with handling	1000	10
Method of decontamination	908	9

*Each DBS Punched was scored between 1 and 10; 1 means does not meet expectations and 10 means completely in line with expectations.

handling DBS to avoid the disadvantages and risks related to the use of a pair of scissors. The results is recommended also, because of its good performance for measuring viral load in tropical climatic conditions. DBS can be used as an alternative sampling method for viral load monitoring in resource-limited settings. Further studies of operational research to apply these findings within a clinical setting on a large scale will be useful.

Conflict of interest

The authors have not declared any conflict of interest.

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