Performance of the bioMérieux DBS Puncher and dried blood spots to measure HIV-1 viral load by real-time NucliSENSEasyQ HIV-1 assay

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Running title: bioMérieux Puncher for cutting DBS

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Abstract

Background. The latest World Health Organization recommendations request viral load (VL) testing, if possible, for monitoring HIV-1 infections. However, the use of plasma is an obstacle to realize this test in sub-Saharan Africa. In this context, the dried blood spot (DBS) is an interesting tool for sample collections. The objective of this study was to assess the performance of, the bioMérieux PUNCHER a new tool for cutting DBS.

Methods. Plasma and DBS samples were obtained from 102 patients, with 17 HIV-1 negative patients and 85 HIV-1 infected patients (58% were antiretroviral therapy naïve). DBS were stored at room temperature for 15 days before testing. The PUNCHER’s performance used to cut DBS was evaluated with following criteria: contamination, time of cut and VL measurement. VL was measured in parallel on plasma and DBS samples using NucliSENSEasyQ HIV-1.

Results. No contamination was observed with negative samples. Sixty two DBS were cut in one hour. The correlation between plasma and DBS results was strong (R = 0.91; P < 0.001). According to Bland-Altman approach the mean of differences (± 1.96 standard deviation) was [-0.59 ± 0.65] log_{10} copies/ml.

Conclusions. PUNCHER is highly efficient at cutting DBS, and the VL results from DBS correlated well with those obtained from plasma. bioMérieux puncher can be used to cut DBS for HIV-1 diagnosis and virological monitoring.
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 (ARV) treatment [1-2]. In sub-Saharan Africa, the use of VL monitoring to detect treatment failure is the major challenge to improve HIV management. Several factors limit access to viral load testing: cost of equipment and reagents, availability and stability of energy. If VL measuring equipment exists in these countries, it is often available in reference laboratories far from peripheral sites. In these conditions, the use of Dried Blood Spot (DBS) represents an alternative for the collection and transport of samples compared to plasma, which requires more restrictive conditions, transport without delay and storage at -80 °C [3-8]. In most laboratories, DBS are cut with a pair of scissors, 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 objective of this study was to assess the performance of, the bioMérieux PUNCHER a new tool for cutting DBS.

Methods

A total of 102 patients, including 17 HIV-1 negative patients and 85 infected patients (58% of them were antiretroviral therapy naïve), were recruited at Sylvanus Olympio University Teaching Hospital in Lomé (Togo). 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 GmbH, Barcelona, Spain). The spotted filter papers were allowed to dry at room temperature for 4 to 6h 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 1500g and plasma was stored at –80°C until testing.
The DBS spots were cut with the new tool: bioMérieux PUNCHER (weight = 4.6 kg; length = 48.5 cm; width = 21 cm; see the detail in http://www.biomerieux-diagnostics.com) according to the manufacturer’s instructions. Three criteria were used to assess the new tool:

- Risk of contamination: to evaluate the absence of contamination of a 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. Negative samples were included at random among those obtained from infected patients.

- Time of cut: to appreciate the number of spots able to be cut in one hour, the same lab technician used in first time scissors and after the bioMérieux PUNCHER.

- VL measurement with DBS versus Plasma: HIV-1 RNA isolation from DBS and plasma was performed using 100 μl (2 spots cut by bioMérieux Puncher) and 500 μl of sample respectively. HIV-1 RNA was extracted from the same patient in plasma and DBS according to the NucliSENSminiMAG 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. Concordance between the various sample types was assessed by using the Bland-Altman plot [9]. Differences were considered significant only when P values were <0.05.

The Ministry of Health of Togo (N° 0411/2012/MS/CAB/DGS/DPLET/CBRS) and National ethic committee of Togo (Comité de bioéthique pour la recherche en santé, N° 001/2012/CBRS) approved the study.

**Results and discussion**

All 17 negative samples were found negative. No contamination was observed although negative samples were cut randomly after samples with high viral loads. Buckton et al. and Driver et al. [10-11] reported no contamination with the use of office hole-punch and manual
hole-punch. The absence of contamination proved the efficacy of the decontaminating protocol. Compare to the protocol used by Buckton et al [10] which clean the punch by cutting 5 filter paper spots, this study showed that even cut three spots, the decontamination is efficient. Several studies have reported different methods to excise HIV DBS: sterile disposable blade [12], razor blade, sterile scissors (6) which are timing-consuming and potentially hazardous. Concerning the rapidity of cutting, 124 DBS corresponding to 62 patients were cut in one hour using the puncher compared to 88 DBS corresponding to 44 patients were cut using scissors. The use of this puncher provides a gain of time for routine lab with large series, a comfort and it is easy to use.

For VL results, plasma samples were distributed as follows: 21 were undetectable including all negative samples and 81 had detectable VL, with 24 VL < 5000 copies/ml and 57 VL > 5000 copies/ml. The VL obtained with DBS showed 25 undetectable and 77 detectable with 30 VL < 5000 copies/ml and 47 VL > 5000 copy/ml (Table1). The correlation between DBS and plasma was high ($R = 0.91; P< 0.001$) (figure 1). The mean of differences according to Bland-Altman approach was $-0.59\log_{10}IC_{0.05}$ [-1.24 - 0.06] $\log_{10}$. Overall, 68 pairs of plasma and DBS (93%) had a mean of differences which is in the interval of confidence. The corresponding Bland-Altman plot illustrates the agreement between plasma and DBS cut with bioMérieux puncher (Figure 2). The results showed good correlation between plasma and DBS. In fact previous data which used others tools to cut DBS report correlations ranging from 78 to 99% between DBS and plasma according to different analyzers for the measurement of VL, and sometimes with different extraction methods [4,7,12-17]. A good correlation exists between plasma and DBS independently of cut protocol. The challenge is to choose the tool which gives more comfort to the lab technician and gain of time.

Conclusions
The performance of the PUNCHER is excellent. It can therefore be recommended to laboratories handling DBS to avoid the disadvantages and risks related to the use of a pair of scissors.

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Competing Interests

The authors declare no conflicts of interest.

Authors’ contributions

AYD, MS, SD and MPD participated in the design of the study. AE, AK and AE carried out the viral load assays and the evaluation of the puncher. AE, AK, TN and OM enrolled patients and collected data on patient history. AYD, MS and MPD coordinated the study. AYD, MS and MPD drafted the manuscript. All authors read and approved the final manuscript.
References


Figure 1. Linear regression comparing HIV type 1 RNA levels obtained by testing 102 paired plasma and DBS
Figure 2. Bland and Altman analysis of viral load values comparing DBS versus plasma (n = 73) using NucliSENS EasyQ HIV-1 assay