Dried Blood Spot HIV-1 RNA Quantification Using Open Real-Time Systems in South Africa and Burkina Faso

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Abstract: There is an urgent need to assess the accuracy/feasibility of using dried blood spots (DBS) for monitoring of HIV-1 viral load in resource-limited settings. A total of 892 DBS from HIV-1–positive pregnant women and their neonates enrolled in the Kesho Bora prevention of mother-to-child transmission trial conducted in Durban (South Africa) and Bobo-Dioulasso (Burkina Faso) between May 2005 and July 2008 were tested for HIV-1 RNA. The combination Nuclisens extraction method (BioMérieux)/Generic HIV Viral Load assay (Biocentric) was performed using one DBS (in Durban) versus 2 DBS (in Bobo-Dioulasso) on 2 distinct open real-time polymerase chain reaction instruments. DBS HIV-1 RNA results were compared with plasma HIV-1 RNA and HIV serology results used as the gold standards. The limits of detection of assays on DBS were 3100 and 1550 copies per milliliter in Durban and Bobo-Dioulasso, respectively. DBS HIV-1 RNA values correlated significantly with plasma levels (n = 327; $R = 0.7351$) and were uniformly distributed according to duration of DBS storage at −20°C (median duration, 280 days). For early infant diagnosis, the sensitivity and specificity were 100% (95% confidence interval: 97.2 to 100.0 and 96.5 to 100.0, respectively). HIV-1 viral load kinetics in DNase-pretreated DBS were similar to those obtained in plasma specimens among 13 patients receiving antiretroviral treatment. HIV-1 RNA findings from serial infant DBS collected prospectively (n = 164) showed 100% concordance with HIV serology at 18 months of life. Our findings strongly advocate the implementation of DBS HIV-1 RNA testing in remote areas from low-income and middle-income countries.

Key Words: DBS, HIV-1 RNA quantification, real-time PCR, sub-Saharan Africa

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INTRODUCTION

At present, laboratory capacity for biological monitoring of HIV-1 infection in sub-Saharan Africa remains insufficient.[1] Without individually scheduled HIV-1 RNA viral load (VL) measurements (as done in developed countries), the recent gains in HIV treatment in developing countries might fade in the coming years, given the important risks of virological failure and subsequent spread of HIV-1 drug-resistant strains.[2]

To make HIV-1 RNA VL measurements more accessible in Africa, dried blood spots (DBS) may be a "field-friendly" tool for sample collection and transport from remote resource-limited settings to central testing laboratories. Compared with standard plasma specimens, DBS offers a simplified sampling method eliminating many logistical and technical limitations, as they are much easier to collect, transport, and store.[3–4] However, studies evaluating the usefulness and reliability of filter papers focused mainly on the role of DBS in public health HIV-1 drug resistance surveillance.[5] Studies using DBS for clinical HIV-1 RNA VL monitoring on an individual patient basis are fewer, and testing was mainly performed in laboratories in developed countries where DBS were shipped,[6–11] prohibiting technology transfer to low-income countries. Studies performed in African laboratories remain scarce, with limited sample sizes.[12–13] Apart from an assay developed by Mehta et al,[8] expensive Food and Drug Administration–approved HIV-1 RNA tests on closed platforms have been used, further reducing the potential for implementation in low-income countries.

We report the accuracy and feasibility of using long-term stored DBS for HIV-1 RNA VL measurements in 2 African laboratories. We used the combination of the Nuclisens extraction method (BioMérieux) and the Generic

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All authors declare that the answers to the questions on your competing interest from all are no and therefore have nothing to declare.

The study protocol was reviewed and approved by the World Health Organization ethics committee and by the National and Institutional Ethical Review Boards in Burkina Faso and South Africa. A written informed consent was obtained from all participants.

*See appendix.

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HIV Viral Load assay (Biocentric) performed on 2 distinct open real-time polymerase chain reaction (PCR) instruments. The rationale for the choice of this combination was the excellent performance of the BioMérieux technique for DBS extraction[14,15] and the affordability of the Biocentric assay which accurately amplified non–B HIV-1 subtypes.[16]

SUBJECTS AND METHODS

Study Design and Selection Criteria for Specimens

Our study was carried out at two African sites, Bobo-Dioulasso (Burkina Faso) and Durban (South Africa), where the Kesho Bora trial aimed at preventing mother-to-child transmission of HIV-1 was conducted in 2005–2010.[17] Written informed consent was obtained from all participants. The protocol was approved by the World Health Organization (WHO) ethics committee and by the Institutional and National Review Boards in both countries.

The study consisted of 2 parts in which a total of 892 DBS and 69 dried plasma spots (DPS), collected within the Kesho Bora trial, were tested for HIV-1 RNA (Table 1). Part 1 was a large-scale retrospective survey evaluating VLs obtained with DBS and DPS, collected at both sites between May 2005 and July 2008. Results were compared with those obtained on paired plasma specimens used as the gold standard. All maternal DBS collected at enrollment in Durban were assessed, whereas in Bobo–Dioulasso, cases were selected in a blinded manner. DBS from all HIV-1–infected children in Bobo–Dioulasso, taken between 6 weeks and 18 months, were tested for HIV-1 RNA. For each infected child, 3 uninfected infants were randomly selected. Their DBS samples, taken between 6 weeks and 12 months, were also tested for HIV-1 RNA. Forty-six pregnant women with CD4 count <200 cells per cubic millimeter at inclusion received highly active antiretroviral therapy (HAART) in Bobo–Dioulasso.[18] From this group, we selected sequential DBS from all women (n = 4) who showed moderate (>300 copies/mL) or major (>5000 copies/mL) plasma viral rebounds at month 6 and/or 12 of follow-up. We also randomly selected 9 women who had a successful treatment response according to their plasma values. Part 2 of the study, from August 2008 to April 2010, was a prospective cohort study in Durban, where all field-based DBS collected consecutively in infants were used prospectively for the early diagnosis of pediatric HIV-1 infection and finally compared with HIV serology results (SD BIOLINE HIV 1/2 3.0, Standard Diagnostics, Inc. Kyonggi-do, Korea) obtained at 18 months of life.

DBS/DPS Collection

In part 1, DBS were prepared in reference laboratories using venous blood collected by venipuncture in 5.0 mL EDTA-anticoagulated tubes. Briefly, 5 spots of whole blood (50 μL each) were spotted onto filter specimen collection paper (Whatman no. 903; formely SS903, Schleicher & Schull, Kenne, NH), dried overnight at room temperature, placed in individual zip-lock bags containing a silica desiccant, and stored at −20°C until further testing. The remaining blood sample was centrifuged, and plasma was used for preparing DPS (50 μL each). Remaining plasma was stored at −80°C until further testing.

In part 2, DBS were prepared by health professionals in 2 rural antenatal clinics in South Africa (KwaDabeka and KwaMsane), from finger or heel prick. Samples were processed in a similar way as above and transported by road at ambient temperature within 24 hours to the reference laboratory in Durban.

HIV-1 Molecular Techniques

All assays were performed at the Africa Centre Virology Laboratory for specimens from South Africa and at the

TABLE 1. Selected Populations and Samples Tested

	Tested Subjects (n)	Tested Samples			Tests/Samples for Comparison
		DBS (n)	DPS (n)	Timing	
Part 1					
HIV-1–positive mothers					
Untreated	353	353	69*	Incl	HIV-1 RNA/PL
Treated with HAART†	13	82 (41 × 2‡)	—	Incl, delivery, M6, M12	HIV-1 RNA/PL
HIV-1–exposed infants†					
HIV-1 uninfected	105	105	—	W6, M3, M12	HIV-1 RNA/PL
HIV-1 infected	33	106	—	D2, D15, W6, M3, M6, M9, M12, M18	HIV-1 RNA/PL
Part 2*					
HIV-1–exposed infants					
HIV-1 uninfected	153	220	—	W6, M3, M5, M9, M12	HIV-1 Ab/PL
HIV-1 infected	11	26	—	D2, D15, W6, M3, M5, M9, M12, M18	HIV-1 Ab/PL
Total	668	892	69	—	—

Kesho Bora PMTCT trial. Bobo-Dioulasso and Durban (2005–2010).
*Evaluation conducted in Durban only.
†Evaluation conducted in Bobo–Dioulasso only.
‡41 DBS measurements without DNase pretreatment, and 41 DBS measurements with DNase pretreatment.
PL, plasma; Incl, inclusion; D, day; W, week, M, month.

Virology Laboratory of Centre Muraz for samples from Burkina Faso. The Nuclisens miniMag extraction method (BioMérieux, Boxtel, The Netherlands) was used for DBS/DPS RNA extraction.[14] To assess the sensitivity of DBS HIV-1 RNA VL testing according to the number of spots used, the pre-extraction procedure differed between laboratories: in Durban, 1 spot of DBS or DPS was eluted in 9.0 mL of Nuclisens lysis buffer[19] compared with two spots in 2.0 mL of the same buffer in Bobo–Dioulasso.[12] Samples were rotated for 60 minutes and subsequently processed according to the manufacturer's instructions. For paired plasma specimens, RNA was isolated from 200 mL of plasma using the QIAGEN procedure (QIAamp Viral RNA Mini Kit, Qiagen, Courtaboeuf, France).

HIV-1 RNA was quantified in all extracts using the long-terminal repeat–based Generic HIV Viral Load assay (Biocentric, Bandol, France), with a limit of detection (LOD) of 300 copies per milliliter for plasma using an input volume of 200 μL.[16,20] For standardization in DBS results, customized DBS standards were prepared as follows: the liquid standard with a known HIV-1 RNA concentration (6,200,000 copies/mL) (Optiquant quantification panel HIV RNA N°6 (Acrometrix Inc, CA) included in the kit) was diluted 1:1 with HIV-seronegative blood, and spotted onto filter papers (each spot 50 μL). A DBS for the low-positive control (LPC) included in the kit (quantified at 6200 copies/mL) was similarly prepared. DBS standard and LPC were then extracted together with DBS clinical specimens. Extracted DBS standard was serially diluted (10-fold) to concentrations from 3,100,000 to 310 copies per milliliter. DPS standard and LPC were spotted onto filter papers and extracted with DPS clinical specimens. Extracted standard DPS was 10-fold diluted. All DBS values from clinical specimens were corrected for hematrocrit as follows: result in copies per milliliter of blood × 100/100—hematocrit.[9] For patients receiving HAART, we compared DBS results obtained with or without prior DNase treatment (DNase I, Applied Biosystems/Ambion Inc, Austin, TX). Treatment with DNase may prevent co-extraction and co-amplification of proviral HIV-1 DNA, due to the presence of white blood cells in whole blood, which can interfere on HIV-1 RNA levels. Amplification and quantification were carried out with the MiniOpticon (BioRad, Marne-La-Coquette, France) in Durban and ABI PRISM 7000 (Applied BioSystems, Foster City, CA) in Bobo–Dioulassso.

Statistical Analyses

In each laboratory, the DBS standard serial dilutions were tested in >10 independent runs to determine the analytical sensitivity and linearity of the DBS assay.[21] Repeatability (intra-assay variance) was assessed by testing DBS-LPC in 10 replicates in the same run. Reproducibility (interassay variance) was calculated by testing DBS-LPC in >10 separate runs. Clinical sensitivity was calculated as the number of positive results divided by the total number of plasma HIV-1 RNA VL results ≥300 copies per milliliter from infected subjects. Clinical specificity was calculated as the number of negative results divided by the total number of negative results from uninfected individuals with plasma VL measurement <300 copies per milliliter. Spearman correlation coefficients were calculated to determine the relationship between HIV-1 RNA concentrations in DBS versus plasma specimens or in DPS versus plasma samples, and between $CD4^+$ T-cell counts and DBS HIV-1 RNA levels. The Bland–Altman method[22] was used to assess the agreement between HIV-1 RNA values obtained with DBS versus plasma and with DPS versus plasma, and to study the impact of duration of DBS storage on DBS assay accuracy. For monitoring HAART efficiency, individual DBS results were compared with plasma results and analyzed according to a threshold of 5000 copies per milliliter. The WHO currently recommends this value for conservation of first-line HAART (or a switch to second-line regimen) in resource-limited settings.[23] These data were also analyzed by using a Bland–Altman representation. If plasma and DBS samples were undetectable (<300 and <1550 copies/mL, respectively), the difference (d) was assigned to zero.

RESULTS

Retrospective Laboratory Study on DBS Performance

Analytical Sensitivity, Reproducibility, and Repeatability of the DBS Assays

In both configurations (1 DBS with the MiniOpticon versus 2 DBS with the ABI PRISM), the assay was shown to be linear over the entire range of 3,100,000–3100 copies per milliliter, with detection rates of 100% at 3100 copies per milliliter (Fig. 1). The fitted slope was marginally greater in Durban. At 310 copies per milliliter (dotted sections of the lines), the assays' sensitivities decreased to 38% with 1 spot and 45% with 2 spots. To estimate the LOD more precisely, 10 additional measurements at 1 550 and 775 copies per milliliter were performed (2-fold dilutions of 3100 copies/mL). Using 1 spot yielded detection rates of 70% and 40%, versus 100% and 70% using 2 spots. Therefore, LODs were set at 3100 and 1550 copies per milliliter in Durban and Bobo–Dioulasso, respectively.

In Durban, the DBS-LPC yielded repeatability and reproducibility mean values of 3.42 (SD: ±0.27) and 3.47 (SD: ±0.43) $\log_{10}$ copies per milliliter, with coefficients of variation of 7.9% and 12.3%, respectively. In Bobo–Dioulasso, they were 3.65 (SD: ±0.23) and 3.32 (SD: ±0.30) $\log_{10}$ copies per milliliter, with coefficients of variation of 6.3% and 8.9%.

Clinical Sensitivity of the DBS Assay Compared With Plasma Specimens

Overall, of 353 positive (≥300 copies/mL) maternal plasma samples obtained at inclusion from 353 pregnant antiretroviral (ARV)-naive women, 327 paired DBS [327 of 353, 92.6%, 95% confidence interval (95% CI): 89.5 to 95.0] tested positive (Table 2). The sensitivity decreased significantly (χ^2 test, $P < 0.001$) for HIV-1 RNA levels approximately equal to or below the assays' LODs. Contrary to Bobo–Dioulasso, where the sensitivity was 100% from 3.51 $\log_{10}$ copies per milliliter, there were 7 discordant pairs in Durban (plasma positive range, from 3.53 to 4.47 $\log_{10}$ copies/mL but

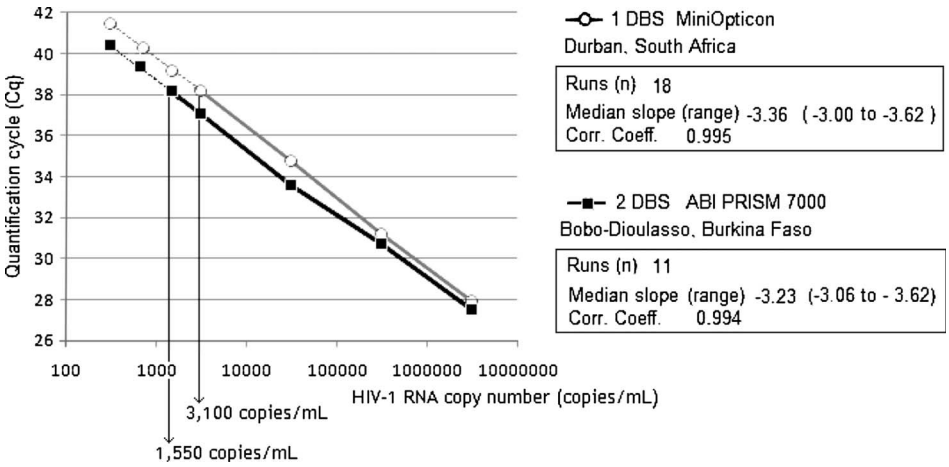

FIGURE 1. Standard curves of the DBS assays using dilutions of known HIV-1 RNA concentrations. The quantification cycles (Cq),[21] which are the number of cycles before the fluorescence passes a fixed limit were plotted against theoretical standard concentrations. For each dilution, the median Cq values are represented by white circles (for Durban) and black squares (for Bobo–Dioulasso).

DBS negative); 4 of which had DBS VL approximating 3100 copies per milliliter.

For 327 concordantly positive results, plasma and hematocrit-corrected DBS levels were well correlated (Fig. 2A) (R = 0.7351; $P < 0.001$). The overall mean difference (d) in the HIV-1 RNA values obtained with DBS and plasma samples was +0.28 $\log_{10}$ copies/mL (Fig. 2B). When considering low-level viremia (<4.0 $\log_{10}$ copies/mL), d reached +0.39 $\log_{10}$ copies per milliliter. As shown in Figure 3, DBS levels were negatively correlated with absolute $CD4^+$ T-cell counts ($R = -0.3861$; $P < 0.001$).

Data comparing DPS with plasma from 69 consecutively enrolled women in Durban revealed a lower sensitivity (62 DPS positive, sensitivity = 89.9%) (Table 2) compared with DBS but with good correlation (Fig. 2C) (R = 0.7683; $P < 0.001$). The overall d between DPS and plasma results was +0.07 $\log_{10}$ copies per milliliter (Fig. 2D). Thus, we decided to focus our work on field-friendly DBS only.

Impact of DBS Storage Duration on the Reliability of the DBS Assay Results

The 327 maternal DBS with concordant positive plasma results were stored for a median duration of 280 days (range: 1–1599 days) at −20°C. No statistically significant difference was observed in the mean VL difference (d) (DBS minus plasma results) obtained between short/medium-term (≤280 days; n = 163; d = +0.30 $\log_{10}$/mL) and long-term (>280 days; n = 164; d = +0.26 $\log_{10}$/mL) stored DBS and plasma specimens (Wilcoxon signed rank test, P = 0.65) (Fig. 4).

Clinical Evaluations

Early Diagnosis of HIV-1 Infection in Children

In Bobo–Dioulasso, 106 samples from 33 HIV-1–infected children had detectable RNA in plasma and paired DBS, leading to a DBS sensitivity of 100% (106 of 106, 95% CI: 97.2 to 100) at 6 weeks (n = 20), 3–6 months (n = 34), and 9–18 months (n = 52) of life. In 105 HIV-1–uninfected children, all DBS collected at 6 weeks (n = 94), 3–6 months (n = 4), and 12 months (n = 7) of life were concordantly negative with plasma, yielding a DBS specificity of 100% (95% CI: 96.5 to 100).

Kinetics of DBS Measurements Among Patients Who Received HAART

Nine women (numbered 1–9, Fig. 5) were treated successfully with HAART and maintained plasma HIV-1 RNA levels <5000 copies/mL during their entire follow-up (except

TABLE 2. DBS and DPS Clinical Sensitivity Among Untreated Pregnant Women Enrolled in the Kesho Bora Trial in Durban and Bobo–Dioulasso (2005–2008)

Plasma HIV-1 VL RNA Class (in $\log_{10}$/mL)	Clinical Sensitivity, n/N (%)			
	Durban		Bobo–Dioulasso	Total
	DPS*	DBS†	DBS‡	DBS
2.5–3.0	0/2 (0)	5/11 (45.5)	2/4 (50)	7/15 (46.6)
3.01–3.5	3/5 (60.0)	22/28 (78.6)	8/13 (61.6)	30/41 (73.2)
3.51–4.0	12/14 (85.7)	48/51 (94.1)	12/12 (100)	60/63 (95.2)
4.01–4.5	25/26 (96.2)	69/73 (94.5)	25/25 (100)	94/98 (95.9)
4.51–5.0	14/14 (100)	41/41 (100)	30/30 (100)	71/71 (100)
>5.0	8/8 (100)	21/21 (100)	44/44 (100)	65/65 (100)
Total	62/69 (89.9)	206/225 (91.6)	121/128 (94.5)	327/353 (92.6)

*The sensitivity for DPS was calculated using a theoretical LOD equal to 1200 copies/mL.
†The sensitivity for DBS was calculated using the LOD experimentally determined in Durban (ie, 3100 copies/mL).
‡The sensitivity for DBS was calculated using the LOD experimentally obtained in Bobo–Dioulasso (ie, 1550 copies/mL).

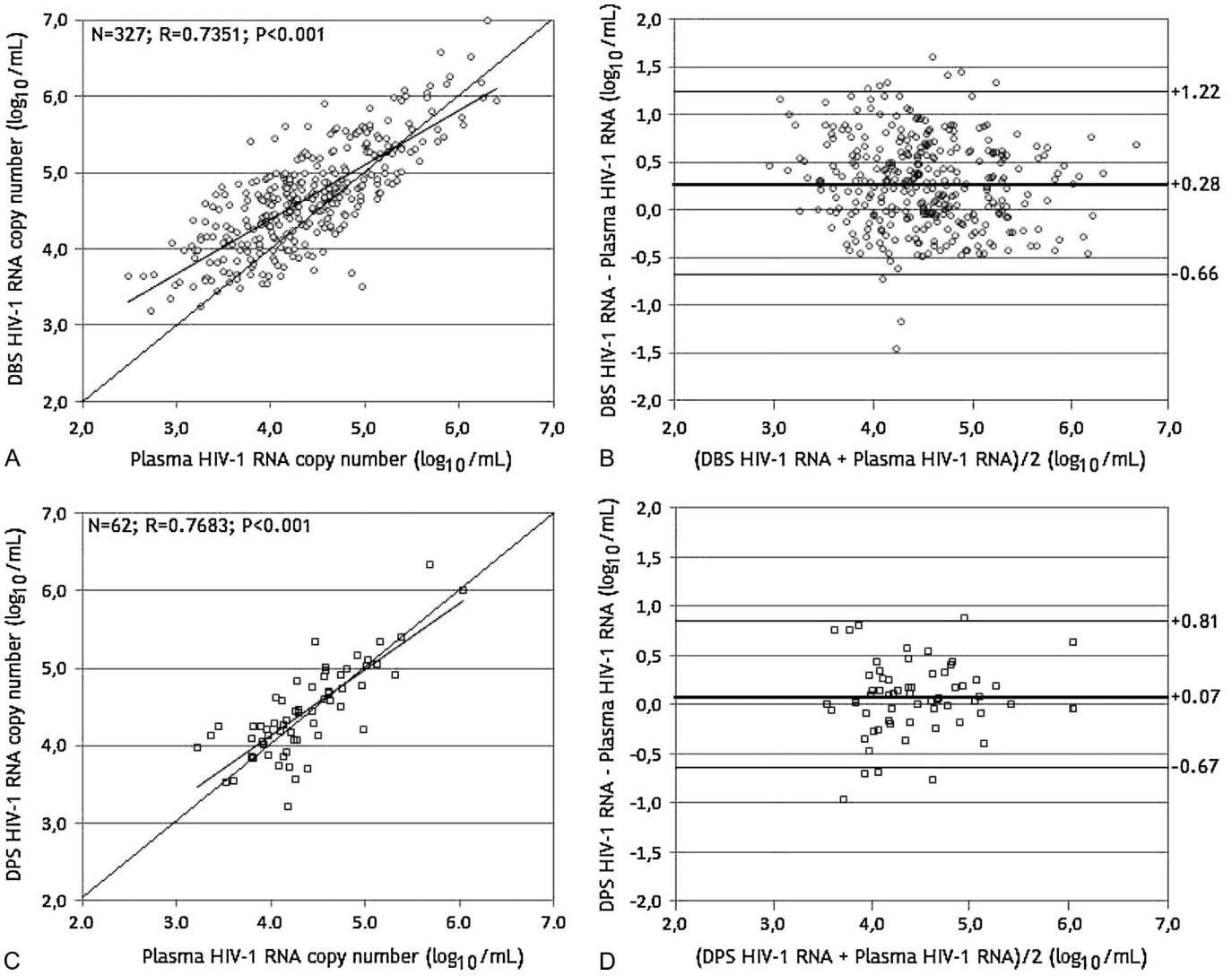

FIGURE 2. Correlation and difference (Bland–Altman representation) between plasma HIV-1 RNA concentrations and HIV-1 RNA measurements obtained by either DBS (white circles) or DPS (white squares). Kesho Bora trial, 2005–2008. For the correlations (A, C), the fitted regressions are represented by solid lines. For the Bland–Altman representations (B, D), the mean differences and the 95% CIs are represented by solid lines.

for woman 9 at delivery). In 17 DBS taken during follow-up, and not pre-treated with DNase, 10 (10 of 17, 59%) HIV-1 VL values were discordantly >5000 copies per milliliter. After DNase treatment that prevented HIV-1 proviral DNA co-amplification, all (17 of 17, 100%) DBS values became concordant (<5000 copies/mL) with plasma levels.

Four women (numbered 10–13, Fig. 5) failed ARV treatment according to their plasma HIV-1 RNA results (viral rebound at month 6 and/or 12 after an initial decrease at delivery). DBS treated or not with DNase showed similar HIV-1 VL kinetics as those obtained for plasma specimens.

The mean difference (d) in $\log_{10}$ copies per milliliter between DBS HIV-1 VL results and plasma values obtained from the 28 samples taken during follow-up is summarized in Figure 6. The overall d between crude DBS results and plasma values was almost +1.0 $\log_{10}$ copies per milliliter (Fig. 6A). This difference was particularly marked for samples showing undetectable (<300 copies/mL) plasma HIV-1 RNA values. When considering DBS pre-treated by DNase, the overall d with plasma concentrations was nil, strongly suggesting that the DBS enzymatic treatment allowed HIV-1 RNA levels not to be affected by coquantification of HIV-1 proviral DNA (Fig. 6B).

Prospective Field Study on DBS Feasibility

In Durban, from August 2008 to April 2010, 220 DBS samples were negative for HIV-1 RNA in 153 infants at 6 weeks of life or thereafter. All of them were HIV antibody negative at months 18 of life. By testing DBS, 11 children were diagnosed as HIV-1 infected, including 6 cases of postnatal transmission by breastfeeding. All of these were confirmed positive by HIV serology at 18 months of life.

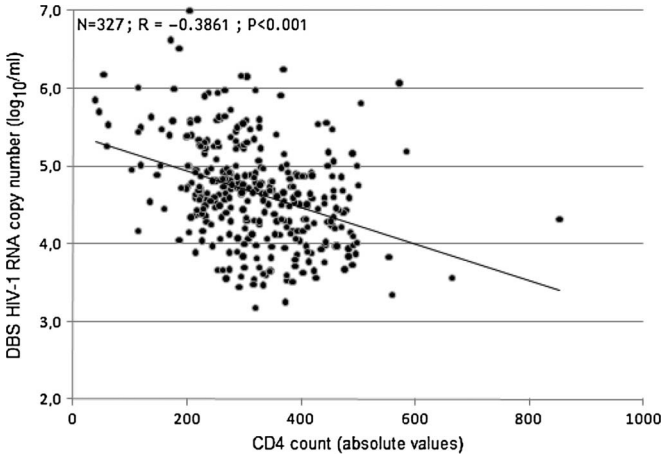

FIGURE 3. Correlation between $CD4^+$ T-cell counts and DBS HIV-1 RNA levels. Kesho Bora trial, 2005–2008 (n = 327). The fitted regression between CD4 and DBS HIV-1 RNA levels is indicated by a solid line.

DISCUSSION

Our open real-time systems using DBS showed excellent performance characteristics for HIV-1 RNA VL measurements in South Africa and Burkina Faso. DBS HIV-1 RNA levels displayed good concordance with plasma values and were inversely correlated with $CD4^+$ T-cell counts. Long-term stored DBS (as long as 4 years) at −20°C was accurate as a repository. Under basic field conditions, DBS were useful for clinical applications such as early infant diagnosis. Monitoring the efficiency of HAART was achieved after a prior DNase treatment step. The cost per test was ~US $12 which made this strategy cost effective.

This large-scale study was carried out in 2 African laboratories, reflecting real-life conditions of HIV-1 RNA monitoring and the relevance of its implementation to similar settings, despite difficulties in human resources, reagents supply, and laboratory infrastructure/maintenance. As previously demonstrated,[8,24,25] the use of robust and highly flexible real-time PCR instruments was a significant advantage, compared with the restrictions of expensive closed platforms. For instance, the long-life light-emitting diode–based MiniOpticon, used in Durban, was affordable (~US $20,000) and required no maintenance.

Compared with liquid plasma-based methods, DBS nevertheless have some disadvantages. First, the extraction of nucleic acids from DBS was manual and required extra hands on time, including their excision with scissors and a potential DNase pretreatment step. Future operational research studies should be directed toward automation of both DBS excision (with automated punchers, instead of scissors) and nucleic acid extraction by using automated extractors (such as the EasyMag from BioMérieux), to increase throughput.

Second, as found by others,[10–13,15] our study revealed a reduced DBS sensitivity in comparison with plasma. However, whatever the number of DBS used (1 or 2), the sensitivities of our assays fit with the national guidelines in South Africa and Burkina Faso which recommend, in accordance with WHO guidelines,[23] an ARV therapy switch above a level >5000 copies per milliliter. Thus, in our clinical context where VL measurements are required in conjunction with targeted adherence monitoring for conserving first-line ARV drugs regimen,[26] both methods are acceptable and show distinct advantages: given its better sensitivity (LOD ~1000 copies/mL), the use of 2 DBS represents the standardized extraction protocol, currently recommended by BioMérieux. Tubes containing 2.0 mL of lysis buffer are ready made and seem more convenient than handling 9.0 mL. Using only 1 DBS, as historically performed at the Africa Center Virology Laboratory in Durban,[19,27] allows usage of additional spot for other purposes (such as HIV serology, ultrasensitive p24 antigen, HIV DNA PCR or resistance genotyping).

Seven of 186 (3.7%) DBS false negative results were obtained in clinical specimens from Durban, despite corresponding plasma results higher than the LOD. These discrepancies could be explained by impaired efficiency

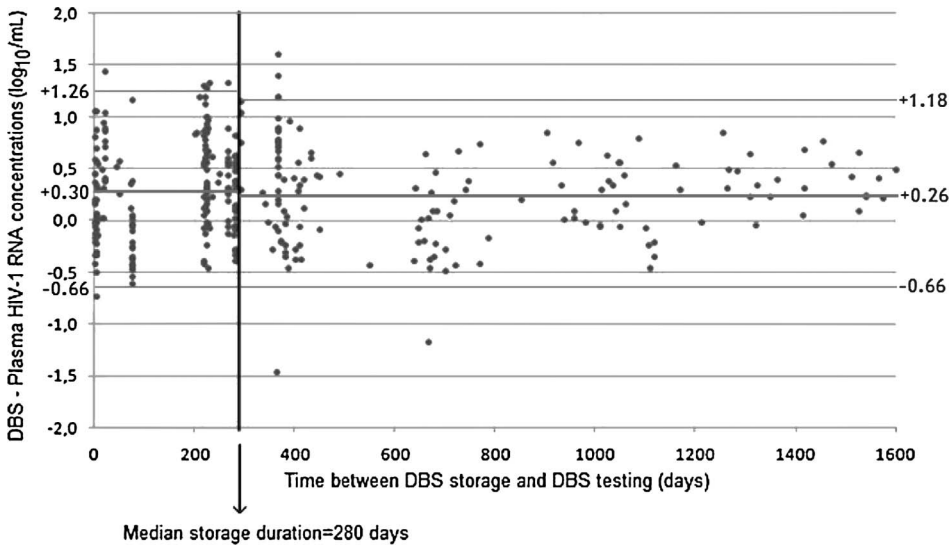

FIGURE 4. Impact of duration of DBS storage on HIV-1 RNA levels. Kesho Bora trial, 2005–2008 (n = 327).

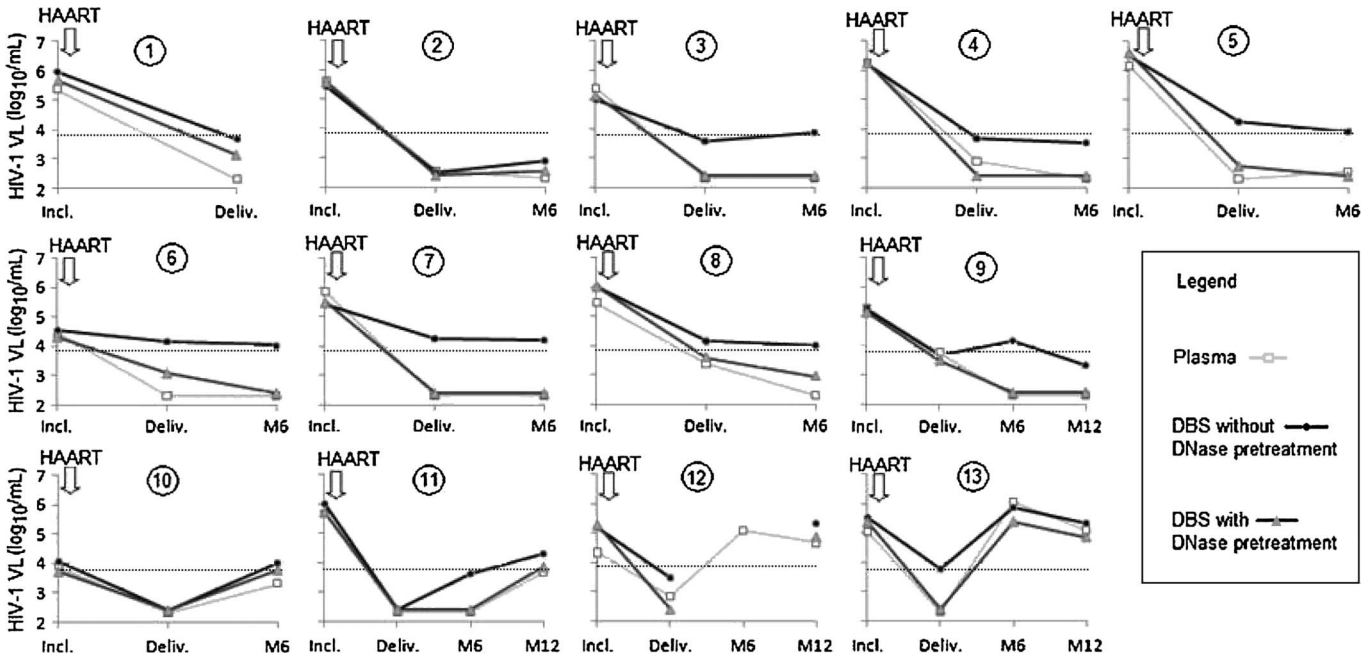

FIGURE 5. Individual kinetics of HIV-1 VL measurements obtained by using plasma specimens, DBS without DNase treatment, and DBS with DNase treatment among 13 patients from Bobo–Dioulasso receiving HAART. For each patient, the threshold of 5000 copies per millimiter is indicated by a dotted line. The median time between inclusion and delivery was 2 months. Patients 1–9: plasma virological success; Patients 10–13: plasma virological failure (moderate for patients 10 and 11 and major for patients 12 and 13).

of nucleic acid extraction (in our experience, some silica-based reagents were substandard) and nucleic acid degradation during DBS preparation and/or storage.[28] Given that the Generic HIV Viral Load assay amplifies a small HIV-1 long-terminal repeat fragment (123 base pairs), it is less likely to be affected by degradation than techniques amplifying longer ones.

Third, because the Nuclisens extraction method is not RNA specific but isolates cell-associated DNA as well, archived proviral HIV-1 DNA may interfere with results generated by DBS, yielding false-positive results as previously documented.[7] In our study, an overall +0.3 $\log_{10}$ copies per milliliter difference was obtained between DBS and plasma levels from untreated subjects. The impact of DNA was more significant in low-viremia specimens where positive DBS values could be due to the presence of detectable HIV-DNA. The presence of HIV-1 DNA in crude DBS interfered with

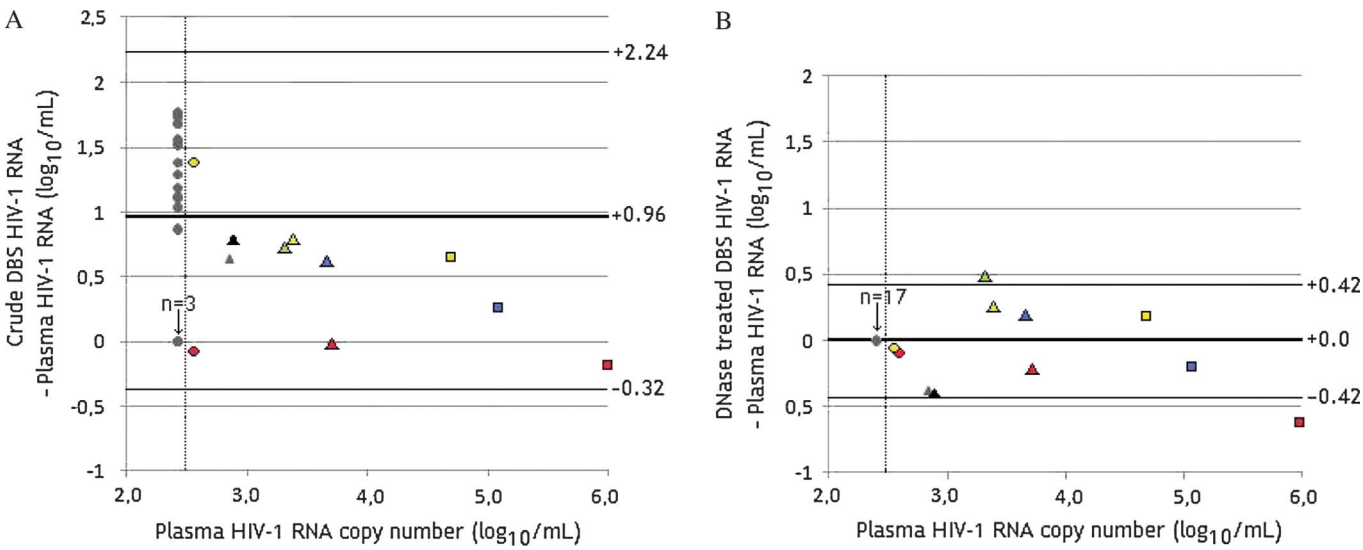

FIGURE 6. Difference between the viral loads measured on DBS and plasma specimens among 28 samples taken during the follow-up of 13 ARV-treated patients from Bobo–Dioulasso. A, DBS not pretreated by DNase. B, DBS pretreated by DNase. Undetectable (<300 copies/mL) plasma specimens (n = 17) are represented by gray circles. Detectable (>300 copies/mL) plasma samples (n = 11) are depicted by distinct colored symbols.

monitoring of successfully ARV-treated patients (with undetectable plasma results), whereas this interference was not significant in individuals with plasma virological failure. The specificity of our assay was very low (~40% at a threshold of 5000 copies/mL) when using crude DBS in ARV-treated patients showing plasma virological success. In addition, the difference between crude DBS and plasma results was high (oscillating from +0.87 to +1.76 $\log_{10}$ copies/mL). Thus, in our survey, performing a prior DNase treatment step on DBS was a prerequisite for accurate monitoring of HAART efficiency. After enzymatic treatment, DBS specificity reached 100%, and the difference between DBS and plasma values was virtually nil. Other solutions may be to use DPS, to perform extraction with the Abbott method which is more RNA specific than the BioMérieux technique,[13] or to resort to the NASBA technology which is designed specifically for RNA, as reported recently by Johannessen et al.[11]

We have shown in 2 African countries that DBS HIV-1 RNA measurements, using open real systems and long-term stored spots, are reliable and feasible. These data should prompt other reference laboratories from similar settings to revisit and expand DBS HIV-1 RNA monitoring strategies. They should also help to strengthen the commitment of health care providers, physicians, and all public health stakeholders who are not sufficiently aware of this affordable, simple, and robust sampling method, ideal for HIV-1 infection monitoring in remote areas from middle-income (such as South Africa) and low- income (such as Burkina Faso) countries. Considering the difference between DBS and DPS/plasma values, clinicians should be informed that it is recommended not to switch between these 2 formats of testing during monitoring of efficiency of HAART. It is our view that plasma specimens should remain the gold standard for adults living in African cities where tertiary reference laboratories and transportation facilities are available. However, in remote rural areas, besides rapid VL testing strategies (such as the BioHelix Express Strip (BESt) and lab-in-a-tube (Liat) technologies)[29–30] which need to be further evaluated, DBS can pave the way for expanded access to HIV-1 VL testing for millions of ARV-treated adults and babies born to seropositive mothers. Such efforts are imperative to meet the high demand encountered presently in sub-Saharan African countries endemic for HIV-1.

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