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Field Suitability and Diagnostic Accuracy of the Biocentric Open Real-Time PCR Platform for Dried Blood Spot–Based HIV Viral Load Quantification in Eswatini

Bernhard Kerschberger, MD, MPH,[a] Nombuso Ntshalintshali, BSc,[a] Qhubekani Mpala, MSc,[a] Paola Andrea Díaz Uribe, BSc,[a] Gugu Maphalala, BSc, MSc,[b] Sydney Kalombola, ScDip,[a] Addissu Bekele Telila, BSc,[a] Tiwonge Chawinga, BSc,[a] Mukelo Maphalala, BSc,[a] Aditi Jani, MSc,[a] Nomcebo Phugwayo, BSc,[b] Roberto de la Tour, PhD,[c] Nomxolise Nyoni, NDip,[b] Javier Goiri, MD, MPHDC, DTMH,[c] Sindisiwe Dlamini, BSc, MPH,[b] Iza Ciglenecki, MD, MSc,[c] and Emmanuel Fajardo, BSc, MSc[d]

Background: To assess the performance and suitability of dried blood spot (DBS) sampling using filter paper to collect blood for viral load (VL) quantification under routine conditions.

Methods: We compared performance of DBS VL quantification using the Biocentric method with plasma VL quantification using Roche and Biocentric as reference methods. Adults (≥18 years) were enrolled at 2 health facilities in Eswatini from October 12, 2016 to March 1, 2017. DBS samples were prepared through finger-prick by a phlebotomist (DBS-1), and through the pipetting of whole venous blood by a phlebotomist (DBS-2) and by a laboratory technologist (DBS-3). We calculated the VL-testing completion rate, correlation, and agreement, as well as diagnostic accuracy estimates at the clinical threshold of 1000 copies/mL.

Results: Of 362 patients enrolled, 1066 DBS cards (DBS-1: 347; DBS-2: 359; DBS-3: 360) were tested. Overall, test characteristics were comparable between DBS-sampling methods, irrespective of the reference method. The Pearson correlation coefficients ranged from 0.67 to 0.82 ($P < 0.001$) for different types of DBS sampling using both reference methods, and the Bland–Altman difference ranged from 0.15 to 0.30 $\log_{10}$ copies/mL. Sensitivity estimates were from 85.3% to 89.2% and specificity estimates were from 94.5% to 98.6%. The positive predictive values were between 87.0% and 96.5% at a prevalence of 30% VL elevations, and negative predictive values were between 93.7% and 95.4%.

Conclusions: DBS VL quantification using the newly configured Biocentric method can be part of contextualized VL-testing strategies, particularly for remote settings and populations with higher viral failure rates.

Key Words: viral load, Biocentric, DBS, Swaziland, Eswatini

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From the [a]Médecins Sans Frontières (OCG), Mbabane, Swaziland; [b]National Reference Laboratory, Ministry of Health, Mbabane, Swaziland; [c]Médecins Sans Frontières (OCG), Geneva, Switzerland; and [d]Access Campaign, Médecins Sans Frontières, Geneva, Switzerland.

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Correspondence to: Bernhard Kerschberger, MD, MPH, Médecins Sans Frontières (OCG), P.O. Box 18, Eveni, Lot No. 331, Sheffield Road, Industrial Area, Mbabane, Eswatini (e-mail: bernhard.kerschberger@gmail.com).

INTRODUCTION

The World Health Organization (WHO) recommends routine viral load (VL) testing to monitor adherence to antiretroviral therapy (ART) and trigger timely treatment switching.[1] VL monitoring possibly prevents the development of drug resistance and preserves first-line ART regimens.[2,3] As the number of patients receiving ART increases, the demand for VL tests will grow to an estimated 28.5 million by 2021.[4]

VL quantification using plasma samples is the preferred method to quantify VL levels, with whole venous blood requiring shipment to the nearest laboratory for sample preparation within 24 hours.[1] Weak sample transportation systems, however, emerged as an operational barrier in the public sector.[3,5] Although an alternative approach is on-the-spot venepuncture, plasma separation, and cold-chain storage,[6] it may not be feasible in settings lacking trained human resources, technical supervision, and appropriate laboratory equipment. Expansion of VL testing remains suboptimal and programmatic gaps in VL monitoring persist in many countries.[3,7–9]

Dried blood spot (DBS) VL quantification, which uses filter paper to collect capillary or venous whole blood, has

been proposed as a plasma surrogate to overcome infrastructural, logistic, and operational barriers.[1,10] However, performance of DBS VL quantification has shown to be suboptimal or lacking generalizability when performed under strictly controlled laboratory conditions.[11–13] Biocentric has recently standardized its HIV-1 VL assay and made it more automated.[14] To make the assay more RNA-specific and reduce the overestimation of VL with DBS reported in earlier studies,[15,16] Biocentric incorporated a DNase pretreatment step to remove HIV-1 proviral DNA before nucleic acid amplification. We assessed the diagnostic accuracy of this novel method under routine clinical conditions and evaluated its field suitability for DBS sample preparation.

METHODS

Setting

Routine VL monitoring was introduced in Eswatini (formerly Swaziland) in 2012 using 2 commercially available platforms (Biocentric and Roche).[8] Findings reported here are part of a larger study assessing the field suitability of the Biocentric methods for plasma and DBS VL quantification, and DBS early infant diagnostics in comparison with the national reference method (Roche). Details of the setting and of the evaluation of the Biocentric platform for plasma VL quantification are described elsewhere.[14]

VL Platforms

We compared DBS VL quantification using the Biocentric method with plasma VL quantification using 2 different reference methods.

The first reference method (RefM-1) is the COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 Test, Version 2.0 (Roche Molecular Diagnostics, Indianapolis, IN), which operates at the Mbabane laboratory and has a lower limit of detection of 20 copies/mL for plasma VL. It is used for routine plasma VL quantification and is enrolled into the CDC VL testing quality assurance program.

The second reference method (RefM-2) is the Biocentric method using plasma for VL quantification at 2 locations (Mbabane, Nhlangano) and has been described in detail previously.[17–19] It is an open real-time PCR platform for HIV-1 RNA quantification of group M (subtype A–H). The method has been recently standardized for regulatory purposes. It received CE certification by a European Notified Body (British Standards Institution) for plasma testing (but not DBS), and uses the Arrow instrument (DiaSorin, Dublin, Ireland) for automated nucleic acid extraction and the Fluoroclycler instrument (Hein Lifescience, Nehren, Germany) for real-time PCR amplification and detection. The lower limit of detection for plasma VL testing is 416 copies/mL.[20]

Although the same Biocentric platform at both locations was used for DBS VL quantification, sample preparation differed from plasma. In 2017, Biocentric released a newly optimized DBS protocol for VL quantification, which includes a step to remove HIV-1 proviral DNA before amplification, to improve the assay specificity. Briefly, after DBS collection, 2 spots are pretreated with a lysis buffer and proteinase K, and incubated at room temperature in a roller mixer for 1 hour. The sample input is 2 DBS spots, which is equivalent to 100 μL of whole blood and corresponds to approximately 50 μL of plasma. DBS-treated samples are then subject to nucleic acid extraction. The extraction products (eluates) are additionally treated with a recombinant heat-labile dsDNase enzyme (HL-dsDNase; Heat&Run gDNA removal kit from ArcticZyme, Tromsø, Norway) to remove genomic DNA and then amplified.[21] The DNase treatment consists of the following steps: (1) 20 μL of eluate are mixed with 2 μL of HL-dsDNase buffer and 1 μL of HL-dsDNase enzyme; (2) the mixture is incubated for 10 minutes at 37°C; (3) finally, the reaction is stopped by heat inactivation for 7 minutes at 55°C. The mixture is then ready for PCR amplification and detection using the FluoroCycler system.

Study Procedures

Consecutive HIV-infected adults (≥18 years) were enrolled in the study when presenting for ART initiation and follow-up VL testing at 2 locations (Nhlangano Health Centre, Lobamba Primary Care Clinic) from October 12, 2016 to March 1, 2017.[14] Enrolment was irrespective of treatment regimen, ART status, and duration on ART. The study population in Nhlangano Health Centre consisted mainly of patients receiving ART for at least ≥6 months, while the study population in Lobamba Primary Care Clinic also consisted of patients initiating ART (baseline VL).

After study enrolment, a phlebotomist collected 2 ethylenediaminetetraacetic acid (EDTA) tubes from venepuncture. Then, the same phlebotomist prepared a DBS filter card containing 5 spots from the same patient through fingerprick by dropping capillary blood directly onto the paper (DBS-1), without the use of a capillary pipette. Thereafter, a second DBS card was prepared by pipetting 50 μL of whole EDTA blood onto the card using a micropipette (DBS-2). The EDTA tubes were sent within 6 hours to the laboratory, where a laboratory technologist prepared another DBS card by pipetting EDTA blood (DBS-3). DBS samples collected at Nhlangano Health Centre were sent to Nhlangano VL laboratory, and samples from Lobamba Clinic were sent to Mbabane VL laboratory. All DBS cards were dried at room temperature for at least 4 hours or overnight, either in the facility (DBS-1, DBS-2) or in the laboratory (DBS-3). Thereafter, they were stored in zip-locked plastic bags with desiccant packs and frozen at −20°C in freezers of the VL laboratories until the time of testing between March and May 2018. In addition, EDTA blood from the second tube was centrifuged to obtain plasma for VL quantification on RefM-1 and RefM-2 in Mbabane and Nhlangano.[14] Plasma VL quantification on the reference platforms was performed between November 2016 and April 2017.

The manufacturer trained all laboratory technologists on DBS VL quantification for 3 days. DBS VL testing was performed on the Biocentric platforms in Nhlangano and

Mbabane VL laboratories without the involvement of the manufacturer between March 2017 and May 2018. All laboratory technologists were blinded to the VL results on the reference platform.

Statistical Analysis

Patients with at least one paired plasma and DBS result were included in the study. Patients' baseline characteristics were described with frequency statistics, proportions, and medians with interquartile ranges (IQRs). The VL values in copies/mL obtained using DBS on the Biocentric platform were multiplied by a 7.5 correction factor that accounts for lost volume during pretreatment of DBS samples, differences in plasma volumes obtained from 2 DBS spots, the volumes of standards, and a 50% hematocrit correction. No correction factor was used for VL values obtained from plasma on both reference methods. Analyses were performed separately for each DBS-sampling method (DBS-1, DBS-2, DBS-3), and for RefM-1 and RefM-2. First, we calculated the DBS-testing completion rate by dividing the number of successfully tested DBS cards (numerator) by the number of DBS cards collected (denominator). VL results were log-transformed for correlation and agreement analyses. Correlation between plasma and DBS VL results was displayed graphically. The Pearson correlation coefficient and fitted regression line were calculated for VL results above the clinical threshold level (>1000 copies/mL). Then, we plotted Bland–Altman graphs and calculated the mean difference and 95% confidence intervals to assess the difference of paired VL samples against the average.[22] Sensitivity and specificity estimates, and positive predictive values (PPV) and negative predictive values (NPV) were calculated at the clinical threshold level. For predictive values, we assumed a prevalence of 10% and 30% VL elevations (defined as >1000 copies/mL). Finally, the same analyses were performed at lower threshold levels (200, 400, 600 and 800 copies/mL), and viral failure was assessed at 4 higher DBS thresholds (2000, 3000, 4000 and 5000 copies/mL) while keeping the plasma threshold constant at 1000 copies/mL. Analyses were performed with Stata v14.1 (StataCorp, Texas).

Ethics

The Médecins Sans Frontières Ethics Review Board and the Scientific and Ethics Committee of the Ministry of Health of Eswatini approved this study. Informed written consent was obtained from all participants.

RESULTS

Baseline Characteristics

Of 370 patients recruited, 8 were excluded from the study because of age <18 years (n = 3), sub-optimal sample volume at the point of sample collection (n = 3), or blood samples not obtained (n = 2). Of the remaining 362 (97.9%) participants (Table 1), 198 (54.7%) were recruited at Nhlangano, the median age was 36 (IQR: 30–44) years, 111 (30.7%) were men and 305 (84.3%) received a follow-up VL test at a median of 5.0 (IQR: 2.0–7.5) years after ART initiation. Overall, 195 (53.9%) and 290 (80.1%) patients had a VL below the lower limit of detection on Ref-M1 (<20 copies/mL) and RefM-2 (<416 copies/mL), and 99 (27.3%) and 7 (1.9%) patients had a detectable VL <1000 copies/mL, respectively (Table 1). Among all detectable VL test results, the median VL in $\log_{10}$ copies/mL was lower for RefM-1 (1.97, IQR 1.30–4.38) than RefM-2 (4.65, IQR 3.70–5.26), and it was comparable when restricted to VL test results above the clinical threshold of 1000 copies/mL (RefM-1: 4.78, IQR 4.04–5.24; RefM-1: 4.93, IQR 3.96–5.30). Of 305 patients receiving ART, 63.3% and 91.2% had a VL below the lower limit of detection with Ref-M1 and RefM-2, and 93.1% and 92.5% had a VL below 1000 copies/mL.

VL Testing Completion Rate

A total of 1066 DBS card were frozen and potentially available for testing: 347 (32.6%) for DBS-1, 359 (33.7%) for

TABLE 1. Baseline Characteristics of the Study Population (Number and Percentages)

Total enrolled	362
Recruiting facilities	
Nhlangano*	198 (54.7)
Lobamba*	164 (45.3)
Age (median and IQR)	36 (30–44)
Sex and pregnancy status	
Men	111 (30.7)
Nonpregnant women	229 (63.3)
Pregnant women	15 (4.1)
Missing	7 (1.9)
Reason for VL testing	
Baseline VL†	57 (15.8)
Follow-up VL	305 (84.3)
Time on ART, years (median and IQR)	5.0 (2.0–7.5)
VL results on RefM-1, copies/mL	
<20	195 (53.9)
20–415	92 (25.4)
416–999	7 (1.9)
1000–9999	17 (4.7)
≥10,000	51 (14.1)
Median (IQR) for detectable VLs, $\log_{10}$ copies/mL	1.97 (1.30–4.38)
Median (IQR) for VLs >3.00 $\log_{10}$ copies/mL	4.78 (4.04–5.24)
VL results on RefM-2, copies/mL	
<416	290 (80.1)
416–999	7 (1.9)
1000–9999	18 (5.0)
≥10,000	47 (13.0)
Median (IQR) for detectable VLs, $\log_{10}$ copies/mL	4.65 (3.70–5.26)
Median (IQR) for VLs >3.00 $\log_{10}$ copies/mL	4.93 (3.96–5.30)

*Paired, dried blood spot and plasma samples obtained at Nhlangano Health Centre were tested at the laboratory in Nhlangano (LAB-1), and samples obtained at Lobamba Clinic were tested at the laboratory in Mbabane (LAB-2).

†A baseline VL was obtained at the time of ART initiation.

n, number; RefM-1, reference method 1 (Roche, plasma); RefM-2, reference method 2 (Biocentric, plasma); VL, viral load.

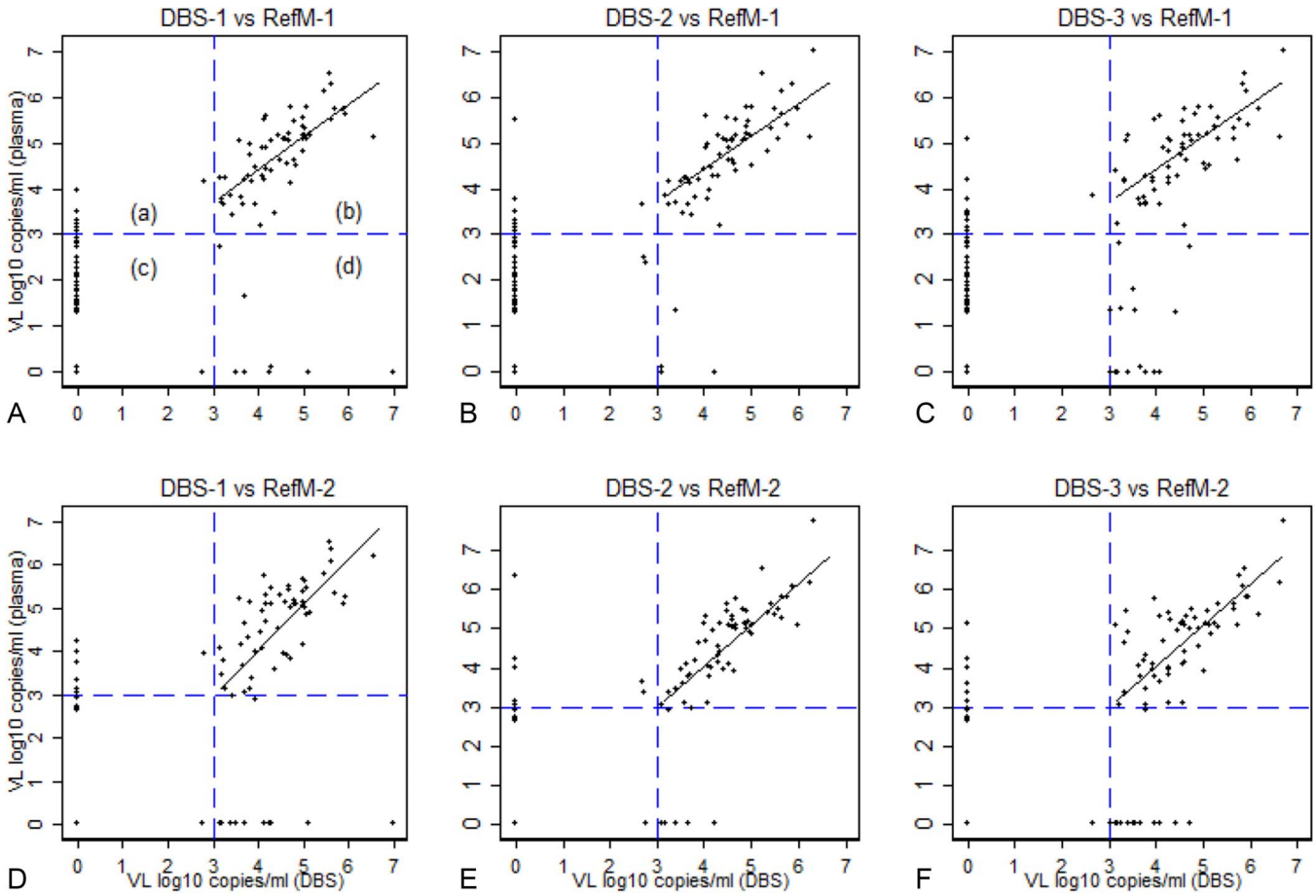

FIGURE 1. Correlation plots between the different types of DBS samples and both reference methods. DBS-1, dried blood spot sampling by finger-prick; DBS-2, dried blood spot sampling with whole venous blood by phlebotomist; DBS-3, dried blood spot sampling with whole venous blood by laboratory technologist; n, number; RefM-1, reference method 1 (Roche plasma) (A, B, C); RefM-2, reference method 2 (Biocentric plasma) (D, E, F). The Pearson correlation coefficient and fitted regression line were calculated for DBS and plasma samples with VL values above the clinical threshold level (>1000 copies/mL). The correlation estimates for each graph are presented in Table 2.

DBS-2, and 360 (33.8%) for DBS-3. After excluding DBS cards with insufficient quality (eg, blood spots did not fill the circle) after defreezing as determined by laboratory technologists, the testing completion rate was 95.9% (n = 347) for DBS-1, 99.2% (n = 359) for DBS-2, and 99.4% (n = 360) for DBS-3.

Correlation and Agreement

Figure 1 shows the correlation graph of paired plasma and DBS values by the DBS-sampling method and reference method. As Table 2 shows, 1.1%–4.4% of DBS test results were downward misclassified and 1.9%–4.4% were upward misclassified. All Pearson correlation coefficients were significant and comparable between DBS samples, ranging from 0.67 to 0.79 for RefM-1 and from 0.67 to 0.82 for RefM-2. The Bland–Altman difference showed high agreement between DBS and plasma VL testing, with differences ranging from 0.21 to 0.30 $\log_{10}$ copies/mL for RefM-1 and from 0.15 to 0.25 $\log_{10}$ copies/mL for RefM-2 (Table 2 and Fig. 2).

Diagnostic Accuracy

Table 2 shows diagnostic accuracy estimates which were overall comparable by the DBS-sampling method and by the reference method [95% confidence interval (CI) intervals overlapped].

Estimates of the receiver operating characteristic (ROC) area under the curve ranged from 0.90 to 0.93 (ROC curve not shown). Sensitivity estimates ranged from 85.3% to 89.2% for RefM-1 and from 87.1% to 89.2% for RefM-2. Specificity estimates ranged from 94.5% to 98.6% for RefM-1 and from 94.6% to 97.6% for RefM-2.

The most favorable PPVs (all ≥87.0%) and NPVs (all ≥93.7%) were at the prevalence of 30% VL elevations irrespective of the DBS-sampling method and reference method. When compared with the prevalence of 10% VL elevations, PPVs were significantly higher for DBS-1 and DBS-2 on both reference methods, and NPVs were significantly lower for DBS-2 and DBS-3 on RefM-1 and for all DBS-sampling methods on RefM-2.

TABLE 2. Test Characteristics (95% CI) of DBS VL Quantification on the Biocentric Platform at the Clinical Threshold Level of 1000 Copies/mL Compared With Plasma on Roche (RefM-1) and Biocentric (RefM-2) Platforms

	RefM-1			RefM-2		
	DBS-1	DBS-2	DBS-3	DBS-1	DBS-2	DBS-3
Total tested	347	359	360	347	359	360
Median (IQR) of detectable VLs, $\log_{10}$ copies/mL	4.3 (3.8 to 5.0)	4.4 (3.7 to 4.9)	4.3 (3.7 to 5.0)	4.3 (3.8 to 5.0)	4.4 (3.7 to 4.9)	4.3 (3.7 to 5.0)
Median (IQR) of VLs >3.00 $\log_{10}$ copies/mL	4.4 (3.8 to 5.0)	4.5 (3.9 to 4.9)	4.3 (3.7 to 5.0)	4.4 (3.8 to 5.0)	4.5 (3.9 to 4.9)	4.3 (3.7 to 5.0)
Downward misclassification, n (%)	9 (2.6)	4 (1.1)	16 (4.4)	8 (2.3)	8 (2.2)	7 (1.9)
Upward misclassification, n (%)	7 (2.0)	8 (2.2)	10 (2.8)	13 (3.7)	7 (1.9)	16 (4.4)
Pearson correlation coefficient	0.74 ($P < 0.001$)	0.79 ($P < 0.001$)	0.67 ($P < 0.001$)	0.69 ($P < 0.001$)	0.82 ($P < 0.001$)	0.67 ($P < 0.001$)
Bland–Altman difference (95% limits of agreement)	0.30 (−0.81 to 1.40)	0.27 (−0.74 to 1.28)	0.21 (−1.13 to 1.54)	0.25 (−1.02 to 1.51)	0.21 (−0.78 to 1.19)	0.15 (−1.27 to 1.57)
Sensitivity	89.2 (79.1 to 95.6)	88.2 (78.1 to 94.8)	85.3 (74.6 to 92.7)	87.1 (76.1 to 94.3)	87.7 (77.2 to 94.5)	89.2 (79.1 to 95.6)
Specificity	96.8 (94.0 to 98.5)	98.6 (96.5 to 99.6)	94.5 (91.3 to 96.8)	95.4 (92.3 to 97.5)	97.6 (95.2 to 99.0)	94.6 (91.3 to 96.9)
ROC area	0.93 (0.89 to 0.97)	0.93 (0.90 to 0.97)	0.90 (0.86 to 0.94)	0.91 (0.87 to 0.96)	0.93 (0.89 to 0.97)	0.92 (0.88 to 0.96)
PPV (at 10%)*	75.6 (61.9 to 85.6)	87.7 (72.9 to 95.0)	63.4 (51.5 to 73.8)	68.0 (55.3 to 78.4)	80.4 (66.2 to 89.5)	64.6 (53.0 to 74.8)
NPV (at 10%)*	98.8 (97.6 to 99.4)	98.7 (97.5 to 99.3)	98.3 (97.0 to 99.0)	98.5 (97.2 to 99.2)	98.6 (97.4 to 99.3)	98.8 (97.5 to 99.4)
PPV (at 30%)*	92.3 (86.2 to 95.8)	96.5 (91.2 to 98.7)	87.0 (80.4 to 91.6)	89.1 (82.7 to 93.3)	94.0 (88.3 to 97.1)	87.6 (81.3 to 92.0)
NPV (at 30%)*	95.4 (91.2 to 97.7)	95.1 (91.1 to 97.4)	93.7 (89.4 to 96.4)	94.5 (90.0 to 97.1)	94.9 (90.6 to 97.3)	95.3 (91.0 to 97.6)

*Prevalence of VL elevation was assumed at 10% and 30%.

DBS-1, dried blood spot sampling by finger-prick; DBS-2, dried blood spot sampling with whole venous blood by phlebotomist; DBS-3, dried blood spot sampling with whole venous blood by laboratory technologist; RefM-1, reference method 1 (Roche, plasma); RefM-2, reference method 2 (Biocentric, plasma).

Diagnostic Accuracy at Different Threshold Levels

For the threshold levels 200, 400, 600, and 800 copies/mL (see S-Table 1a, Supplemental Digital Content, http://links.lww.com/QAI/B339 and S-Table 1b, http://links.lww.com/QAI/B339), sensitivity estimates ranged from 77.2% to 86.6% for RefM-1 and from 81.9% to 87.7% for RefM-2. All specificity estimates remained ≥95% for both reference and all sampling methods. Comparing the DBS thresholds at 2000, 3000, 4000 and 5000 copies/mL while keeping the plasma threshold constant at 1000 copies/mL, sensitivity estimates tended to be lower and specificity estimates slightly higher (see S-Table 1a, Supplemental Digital Content, http://links.lww.com/QAI/B339 and S-Table 1b, http://links.lww.com/QAI/B339). All other estimates (ROC area, NPV, PPV) were also comparable and 95% CI overlapped irrespective of the reference method and sampling method. Considering point estimates only, they tended to be highest at the threshold level of 1000 copies/mL.

Discordant Test Results

For 40 (11.0%) patients, at least one paired DBS VL result (DBS-1 and/or DBS-2 and/or DBS-3) did not concur with the plasma VL result of RefM-1 at the threshold level of 1000 copies/mL (discrepant test result) (Table 3). For most of them (n = 32, 80.0%), only one corresponding DBS VL result was either upward misclassified (n = 23, 57.5%) or downward misclassified (n = 9, 22.5%), with the remaining 2 paired DBS results being concordant with the plasma VL result. In a few patients, all 3 corresponding DBS VL results were either upward (n = 2, 5.0%) or downward misclassified (n = 4, 10.0%).

In comparison with RefM-2, a total of 41 (11.3%) patients had at least one discrepant test result, of which 24 (58.5%) were upward misclassified and 7 (17.1%) were downward misclassified. All 3 corresponding DBS VL results were upward or downward misclassified in 2 (4.9%) and 4 (9.8%) patients (see S-Table 2, Supplemental Digital Content, http://links.lww.com/QAI/B339).

DISCUSSION

DBS VL testing has been suggested as one approach to overcome operational barriers in the scale-up of routine VL monitoring in resource-poor settings.[3] This is the first study to date, evaluating the performance of venous blood and finger-prick DBS VL quantification with the Biocentric method under routine conditions, using a newly-configured system with a more automated nucleic extraction method and a more RNA-specific DBS VL protocol. In the context of DBS sampling, correlation, sensitivity, and specificity were

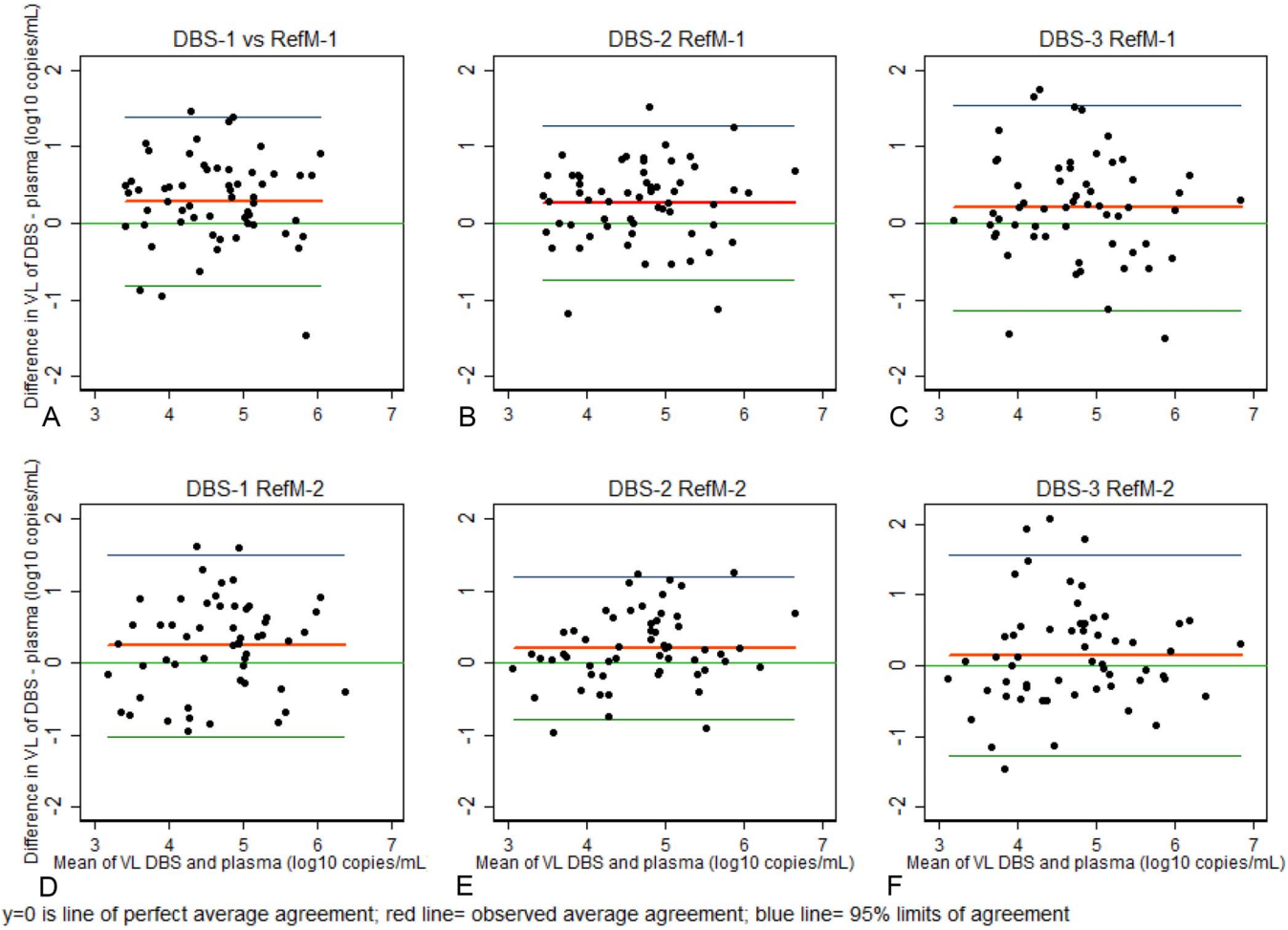

FIGURE 2. Bland–Altman mean difference analysis between the different types of DBS samples and both reference methods. DBS-1, dried blood spot sampling by finger-prick; DBS-2, dried blood spot sampling with whole venous blood by phlebotomist; DBS-3, dried blood spot sampling with whole venous blood by laboratory technologist; RefM-1, reference method 1 (Roche plasma) (A, B, C); RefM-2, reference method 2 (Biocentric plasma) (D, E, F). The Bland–Altman plots were calculated for DBS and plasma samples with VL values above the clinical threshold level (>1000 copies/mL). Bland–Altman statistics (average difference and 95% limits of agreement) for each graph are presented in Table 2.

acceptable and PPVs and NPVs could be high at the clinical decision-making threshold of 1000 copies/mL. We also demonstrated that different DBS sampling procedures could be performed by less-qualified health workers.

Findings in Context

Other studies have shown that VL assays using DBS samples reached sufficiently high sensitivity at the threshold of 3000 copies/mL or greater.[10] Current 2016 WHO guidelines, however, recommend a lower VL-monitoring threshold (1000 copies/mL) for both plasma and DBS VL results.[1] At this threshold, provisional data showed that the Biocentric method using DBS samples achieved high sensitivity (94.9%), but low specificity (55.2%).[23] Similar decreased specificity has been reported with the Roche SPEX protocol, which unselectively amplifies DNA and RNA.[15,24,25]

Overestimation of VL values (low specificity) with the Biocentric assay is a particular problem at low levels of viremia, because the presence of proviral DNA and cell-associated RNA has a greater impact on the results.[24,26] A recent study found that contaminating proviral DNA contributes substantially to higher DBS VL values with the Biocentric assay, reaching 800–1200 DNA copies/10^6 PBMC.[27] Mean DBS VL values with the Biocentric assay decrease significantly after DNase treatment using the Turbo DNase-free enzyme from Ambion LifeScience Technologies, USA.[28,29] In our study, the recently optimized Biocentric protocol treats DBS samples with a different DNase enzyme which uses a smaller volume of reagents and obviates the need for inactivation reagents and centrifugation, therefore simplifying the procedure. Results of a recent DBS study using a newly developed HIV VL assay demonstrated the superiority of HL-dsDNase to remove proviral DNA and improve assay specificity, albeit a reduced sensitivity.[30] In

TABLE 3. Discrepant VL Results Between Plasma VL Quantification With RefM-1 and DBS VL Quantification (in Copies/mL)

RefM-1	DBS-1	DBS-2	DBS-3
0	0	0	2715
0	0	0	1072
0	>7 million	0	0
0	0	0	9829
0	0	0	1553
0	3203	0	0
0	5070	1324	6236
0	>7 million	0	0
0	0	0	1395
0	133,256	0	0
0	0	0	1485
0	17,618	16,725	12,574
<20	0	0	4583
<20	20,531	0	0
<20	0	1241	0
20	0	0	25,541
21	0	0	3638
22	0	2603	0
22	0	0	1118
24	0	0	1766
45	5036	0	0
61	0	0	3236
530	1530	0	0
532	0	*	53,573
597	0	0	1691
1160	0	0	0
1334	0	0	0
1629	0	0	1500
2061	0	0	0
2513	2768	5419	0
2752	24,390	3540	0
3164	0	0	0
4530	4898	506	12,259
5924	1676	0	0
6611	2426	1601	461
9200	0	13,444	9454
13,772	630	3266	8449
14,914	14,528	4613	0
114,007	42,859	76,564	0
325,000	448,905	0	596,734

*VL result not available; 0, VL result is undetectable.

A discrepant VL results was defined as a plasma VL result below the clinical threshold of 1000 copies/mL using the reference method (RefM-1) while the VL DBS result was above this threshold, and vice versa.

DBS-1, dried blood spot sampling by finger-prick; DBS-2, dried blood spot sampling with whole venous blood by phlebotomist; DBS-3, dried blood spot sampling with whole venous blood by laboratory technologist; RefM-1, reference method 1.

this study, irrespective of DBS sampling and reference methods used, specificity estimates remained stable at the clinical threshold of 1000 copies/mL, despite greater variability in sensitivity.

Although diagnostic accuracy at lower threshold levels was comparable with estimates at the 1000-threshold level (95% CIs overlapped), point estimates appeared overall most favorable for the 1000-threshold level. However, robustness of diagnostic performance indicators at lower threshold levels may be limited for RefM-2, because only 7 test results were in the range of 416–999 copies/mL and none were below. Diagnostic test characteristics also did not improve when assessing viral failure at 4 higher DBS threshold levels (2000–5000 copies/mL) while keeping the plasma threshold constant at 1000 copies/mL. Given these findings and analytic limitations, it appears more appropriate to apply the viral failure threshold level of 1000 copies/mL for DBS VL quantification in routine settings. Other studies from routine settings also showed that DBS VL quantification potentially performed best at the viral failure threshold level.[15]

Some VL results were misclassified or discordant. Possible reasons include factors inherent to intra-assay and interassay variation, technical limitations prohibiting DBS approaches reaching the same diagnostic accuracy as plasma RNA quantification, and lot-to-lot variations.[32,33] In addition, different assays vary in their ability to detect RNA of genetically diverse HIV-1 strains,[34,35] with the Biocentric method on plasma performing well in settings with viral diversity.[36] As most misclassifications were upwards, this may indicate the coamplification of cell-associated RNA after DNase treatment, which can contribute to lowering specificity even when using RNA-specific PCR methods.[37] Although results of internal and external quality control activities were passed during the study period, we cannot rule out that mishandling of samples during sample processing could have resulted in cross-contamination leading to false-positive results.

Operational Considerations

VL Testing Approaches

High-quality VL quantification remains crucial to the effectiveness of HIV programs.[4] Despite the potential of DBS supporting expansion of VL monitoring, we believe that DBS VL testing should not replace plasma VL testing if plasma VL testing is feasible. Contextual factors (eg, costs, target population, human resources, and health policies) should guide decisions on which VL methods to place where, and a strategic combination of plasma and DBS VL quantification with point-of-care diagnostics may be required for effective VL expansion.[3] For instance, plasma sampling could be used in facilities near VL laboratories and settings with strong sample transportation systems in place, and DBS sampling and point-of-care in facilities without access. The recent availability of plasma separation devices (dried plasma spots) is also an attractive alternative to overcome the limitations of DBS in accuracy.[38,39]

Human Resources

Suboptimal capacity for sample transportation remains a barrier to decentralizing plasma VL monitoring.[5] This study showed that DBS sample preparation by different health cadres has the potential to achieve similar outcomes. This indicates that DBS sample preparation by finger-prick and/or

EDTA pipetting may be task-shifted to lay cadres (eg, phlebotomists). Previous studies using other VL technologies have shown that task-shifting DBS sample collection to lower-level cadres is feasible.[40]

In this study, throughput volume of DBS samples was reduced because of preparatory steps (eg, cutting of DBS samples) before VL quantification. Although 4 laboratory technologists were able to perform up to 3 runs per day (246 patient samples/day) using plasma samples,[14] only one run per day (82 patient samples/day) was feasible when using DBS samples. Although Biocentric provides preperforated DBS cards, we used the nationally available DBS filter paper requiring manual cutting, which is more labor-intensive and may introduce cross-contamination that could explain some of the discordant results (false positives) between the different sample types assessed.[41] VL testing strategies will need to balance human resource needs and should use most suitable technologies (eg, perforated DBS filter paper) to increase efficiency.

Populations

The suitability of any VL-testing approach for clinical decision-making also depends on the PPV and NPV. This study showed that DBS VL testing with the Biocentric method may be suitable for ART cohorts in which approximately 30% of patients have VL >1000 copies/mL. Low VL suppression rates have been reported for children and adolescents in Eswatini and other settings.[7,42,43] However, rates of VL suppression need to be constantly monitored. If expansion of VL monitoring improves viral outcomes, the performance of DBS to predict VL elevations would be reduced, making VL testing with DBS samples redundant. In addition, DBS sample collection may benefit children from whom whole venous blood collection is complex.

Other Limitations

Improving the laboratory aspects of VL testing alone is unlikely to achieve programmatic effect and the UNAIDS 90-90-90 targets. Other constraints that need to be tackled include health financing constraints, the lack of appropriate policy frameworks allowing for large-scale decentralization of HIV care services, quality of health services, and a health workforce with insufficient capacity to implement the clinical aspects of VL monitoring effectively.[5]

Limitations and Strengths

First, cost-effectiveness of DBS VL testing was not assessed. Cost-effectiveness depends on a range of factors, and the programmatic context plays a major role; for instance, how effectively VL testing is integrated in models of differentiated HIV care and its clinical effectiveness through timely acting on VL results.[44] Second, we did not evaluate how long DBS samples can be kept at ambient temperature before VL quantification. All samples were frozen within 24 hours after DBS preparation, which can limit generalizability as storage conditions may be different in other settings. Although extended periods of DBS storage at more extreme conditions lead to degradation of RNA, a few studies demonstrated reliability of VL quantification using DBS samples with storage at higher temperatures or at room temperatures for extended periods.[32,45,46] Third, DBS samples were frozen at −20°C instead of −80°C and storage time before testing was for some DBS samples more than 1 year, possibly negatively affecting the quality of DBS samples and biasing the comparison with plasma samples which were tested within 3 months of sample preparation.[14] Fourth, findings may not be fully generalizable to ART cohorts, because we also included treatment naïve patients. Finally, although VL quantification was performed in 2 different laboratories, we did not assess interlaboratory reproducibility as the number of quality spots per DBS card was insufficient.

A strength of this study is that DBS samples were prepared in a routine clinical setting and with involvement of trained lay cadres. In addition, we used filter paper that was already routinely available in this context. Thus, the findings of this study may be generalized to similar settings in sub-Saharan Africa. Finally, we were able to compare different DBS-sampling methods and used 2 different reference methods.

CONCLUSIONS

DBS VL quantification using the Biocentric method can be a reliable alternative to plasma VL testing in settings with limited access to blood sample transportation systems and in the absence of point-of-care VL testing. Different types of DBS sampling are feasible and can be performed by less-qualified laboratory staff. DBS-based VL testing can form part of contextualized VL testing strategies to meet the growing demand for VL tests.

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