

Sensitivity and specificity of dried blood spots for HIV-1 viral load quantification

A laboratory assessment of 3 commercial assays

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Abstract

The use of dried blood spots (DBS) instead of plasma as a specimen type for HIV-1 viral load (VL) testing facilitates the decentralization of specimen collection and can increase access to VL testing in resource-limited settings. The performance of DBS for VL testing is lower, however, when compared to the gold standard sample type plasma. In this diagnostic accuracy study, we evaluated 3 VL assays with DBS.

Participants were recruited between August 2012 and April 2015. Both plasma and DBS specimens were prepared and tested for HIV-1 VL with the Roche CAP/CTM HIV-1 test v2.0, the Abbott RealTime HIV-1, and the bioMérieux NucliSENS EasyQ HIV-1 v2.0. Sensitivity and specificity to detect treatment failure at a threshold of 1000cps/mL with DBS were determined.

A total of 272 HIV-positive patients and 51 HIV-negative people were recruited in the study. The mean difference or bias between plasma and DBS VL was <0.5 log cps/mL with all 3 assays but >25% of the specimens differed by >0.5 log cps/mL.

All 3 assays had comparable sensitivities around 80% and specificities around 90%. Upward misclassification rates were around 10%, but downward misclassification rates ranged from 20.3% to 23.6%. Differences in between assays were not statistically significant ($P > 0.1$).

The 3 VL assays evaluated had suboptimal performance with DBS but still performed better than immunological or clinical monitoring. Even after the introduction of the much-anticipated point-of-care VL devices, it is expected that DBS will remain important as a complementary option for supporting access to VL monitoring, particularly in rural, resource-limited settings. Manufacturers should accelerate efforts to develop more reliable, sensitive and specific methods to test VL on DBS specimens.

Abbreviations: ART = antiretroviral therapy, CAP/CTM = COBAS AmpliPrep/COBAS TaqMan, DBS = dried blood spot, ITM = Institute of Tropical Medicine, LOQ = limit of quantification, VL = viral load.

Keywords: decentralization, diagnostic accuracy, dried blood spots, HIV-1, point-of-care, viral load

1. Introduction

The World Health Organization currently recommends viral load (VL) monitoring as the preferred approach to determining the effectiveness of antiretroviral therapy (ART) for people living with HIV.^[1] Routine VL monitoring allows for earlier detection of treatment failure compared to clinical or immunological monitoring, thereby reducing morbidity and mortality, avoiding

accumulation of resistance mutations, and identifying patients in need of adherence support.^[2–7] In resource-limited settings, however, access to viral load monitoring is generally limited.^[8] Barriers to VL testing in resource-limited settings include the high cost, the need for substantial laboratory infrastructure, efficient specimen transport systems, and implementation of quality assurance measures.^[3,9–11] As a result, most people living with HIV in resource-limited settings, where the needs are greatest, lack access to VL testing,^[12] and clinical and immunological monitoring is still commonly used, despite reduced accuracy for identifying treatment failure.^[8]

The complexity of specimen collection, storage, and transport for VL testing is mainly related to the use of blood plasma. In resource-limited settings, dried blood spots (DBS) are being introduced as an alternative specimen type to plasma. DBS can be collected using capillary blood from a finger prick, carry no risk of infection, and can be stored and transported at ambient temperature, thus avoiding the need for phlebotomists, cold chain, and same-day transport to a VL testing facility. Using DBS instead of plasma as a specimen type facilitates the decentralization of specimen collection while maintaining high-throughput centralized laboratory testing.^[13–15]

Nevertheless, most commercially available VL assays have not been validated for use with DBS and concise locked-down manufacturer protocols for specimen preparation, storage, and processing are often lacking. Several studies comparing DBS with plasma for VL testing have been published but due to the lack of standardization in specimen preparation, storage and processing,

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these studies are difficult to compare.^[16,17] In addition, most evaluations have been carried out either on a single or on 2 different assays, limiting the possibility to compare across assays. There is need for more reliable, standardized, quality assured, and validated methods of HIV-1 VL testing on DBS.

This study summarizes the results of a diagnostic accuracy study with 3 commercially available WHO prequalified HIV-1 VL assays on DBS specimens.

2. Methods

2.1. Study population and setting

Participants were recruited at the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, and the University Hospital in Ghent, Belgium. All consenting HIV-positive patients ≥ 18 years presenting for VL monitoring as well as people seeking an HIV test between August 2012 and April 2015 were eligible to participate.

2.2. Study procedure

Venous whole blood was collected in EDTA vacutainers, DBS specimens were spotted, and plasma was prepared from the remaining blood by centrifugation at $1100 \times g$ for 20 minutes. Plasma was stored at -80°C until testing. Plasma specimens were tested for VL with the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 test v2.0 (Branchburg, NJ) at the AIDS Reference Laboratories of the ITM in Antwerp and the University Hospital in Ghent. Results from this assay were used as the reference results in this study. All DBS specimens were tested at the ITM in Antwerp with the Roche CAP/CTM, the Abbott RealTime HIV-1 (Des Plaines, IL), and the bioMérieux NucliSENS EasyQ HIV-1 v2.0 (Marcy-l'Etoile, France).

2.3. Dried blood spot preparation

DBS were prepared by spotting 5 times $70 \mu\text{L}$ (Roche and Abbott) or $50 \mu\text{L}$ (bioMérieux) of EDTA venous whole blood on Whatman 903 filter paper cards (Whatman, Maidstone, UK). The blood was left to dry overnight, packed in separate zip-lock bags with desiccant sachets and a humidity indicator card, and stored at -20°C until testing.

2.4. Dried blood spot processing

Each manufacturer supplied different DBS processing instructions.

2.4.1. Roche: (Free virus elution "FVE" protocol). One entire blood spot was cut out with individual scissors, transferred to a S-tube, $1000 \mu\text{L}$ of sterile phosphate buffered saline (PBS) was added and incubated at room temperature for 1 hour without shaking. After incubation, tubes were gently tapped to homogenize the solution and then loaded on the CAP instrument without removing the filter paper.

2.4.2. Abbott. One circle of 12 mm was punched out with a punching device (bioMérieux, Marcy-l'Etoile, France), transferred to an empty master mix tube, $1300 \mu\text{L}$ of Abbott *mWash* 1 buffer^[18] was added and incubated at room temperature for 20 minutes without shaking. After the incubation, tubes were loaded on the m2000sp instrument without removing the filter paper.

2.4.3. bioMérieux. Two circles of 12 mm were punched out with a punching device (bioMérieux, Marcy-l'Etoile, France), transferred to a 15 mL tube containing 2 mL lysis buffer and incubated at room temperature for 30 minutes on a roller-mixer. After incubation, the tubes were centrifuged for 15 seconds and all liquid was transferred to the disposables and loaded on the EasyMAG instrument.

2.5. Outcome measures

Bias, sensitivity, specificity, and misclassification rates at a threshold of 1000 cps/mL were used as outcome measures. The threshold of 1000 cps/mL was chosen in accordance with the current WHO guidelines for defining treatment failure.^[1] In addition, sensitivity and specificity were determined using the respective limit of detection of each assay. For each of the 3 assays, DBS VL results were compared to an accepted reference value. Here, the reference value was defined as the result obtained using 1.0 mL of EDTA plasma as a specimen with the Roche CAP/CTM.

2.6. Data analysis

To compensate for the fact that DBS contains whole blood instead of plasma, with $\sim 50\%$ red blood cells, Roche recommends that DBS results be multiplied by 2, whereas bioMérieux recommended using a factor of 2.29 for comparison with plasma. Abbott has incorporated such a conversion factor in the assay software itself.

The Roche CAP/CTM, the Abbott RealTime, and NucliSENS EasyQ assays report results in cps/mL, where 1 copy corresponds to 1.7, 1.74 and 2.08 international units respectively. Therefore, Abbott and bioMérieux results were corrected with a factor of 1.02 ($=1.74/1.7$) and 1.22 ($=2.08/1.7$) respectively before comparison with Roche results.

Sensitivities and specificities were compared with a 2-sided Chi-squared test in Graphpad Prism 7.0 (La Jolla, CA). *P*-values < 0.05 were considered significant.

Ethics approval was obtained from the Institutional Review Board of the ITM and the University Hospital in Antwerp (Wilrijkstraat 10, 2650 Edegem Belgium).

3. Results

A total of 272 HIV-positive patients and 51 HIV-negative people were recruited in the study. The average age was 44.0 years (IQR: 32.2 – 49.6) and 79.1% were male. Fifty-one had an undetectable plasma VL, 75 had a detectable VL < 1000 cps/mL, and 146 had a VL > 1000 cps/mL. Subtype information was available for 64% of patients, of which 35% were infected with subtype B, 46% with recombinant forms, and 19% with other subtypes.

Bias between the results on DBS and the reference value was calculated for specimens with a plasma viral load > 1000 cps/mL. For specimens with an undetectable VL on DBS, an arbitrary value was assigned, corresponding to half of the limit of detection. Similarly, for specimens with a detectable VL below the limit of quantification (LOQ), half of the LOQ was assigned.^[19] In Table 1, the resulting bias is named "bias 1." Bias was also calculated after excluding all specimens with an undetectable viral load on DBS and was named "bias 2." All biases were < 0.5 log cps/mL. Nevertheless, 26%, 27%, and 26% differed by > 0.5 log cps/mL on Roche, Abbott and bioMérieux, respectively.

Table 1**Bias (in log cps/mL) between dried blood spot and plasma viral load results for samples with a plasma viral load >1000 cps/mL.**

	Roche CAP/CTM HIV-1 v2.0	Abbott RealTime HIV-1 test	bioMérieux NucliSENS EasyQ HIV-1 v2.0
Bias 1	-0.16 SD=0.50	-0.30 SD=0.39	-0.26 SD=0.50
Bias 2	0.08 SD=0.30	-0.27 SD=0.41	-0.22 SD=0.46
Samples with bias >0.5 log cps/mL	26%	27%	26%

"Bias 1" includes all samples whereas "bias 2" excludes samples with undetectable DBS VL results. CAP/CTM = COBAS AmpliPrep/COBAS TaqMan, DBS = dried blood spot, SD=standard deviation, VL = viral load.

Sensitivities, specificities, and percentages of false treatment failures and successes were calculated at a threshold of 1000 cps/mL. All 3 assays had sensitivities around 80% and specificities around 90% (Table 2). Upward misclassification rates were around 10%, but downward misclassification rates ranged from 20.3% to 23.6% (Table 2). Differences in between assays were not statistically significant ($P > 0.1$).

DBS specimens from 51 HIV negative people were tested. None gave a positive signal when tested with bioMérieux. However, 5 had a detectable result below the LOQ when tested with Roche and 2 had a detectable result above 1000 cps/mL when tested with Abbott. When retested, none of the 5 were detectable with Roche, whereas 1 of the 2 remained detectable above 1000 cps/mL with Abbott.

All plasma and DBS VL results are listed in a table as Supplemental Digital Content 1, <http://links.lww.com/MD/B429>.

4. Discussion

In this study, the mean difference between plasma and DBS VL was <0.5 log cps/mL, but 26% (Roche and bioMérieux) and 27% (Abbott) of individual specimens did differ by >0.5 log cps/mL. Sensitivities and specificities to detect treatment failure at a threshold of 1000 cps/mL were comparable across the 3 assays and similar to previously published studies.^[16] The false positive results on Roche and Abbott when testing HIV negative DBS specimens are an issue of concern. Overall, the results of this study suggest a need to improve the sensitivity of VL testing on DBS.

We found that DBS VL systematically underestimated plasma VL, resulting in many false treatment successes. In clinical practice, this means that when DBS is used to test VL, a small but important proportion of treatment failures would go unnoticed. Especially in settings where patients are monitored just once per year, this is an issue of concern. VL assays should be further optimized for better performance with DBS with a focus on increasing its sensitivity. Further research and development will need to determine whether this is best done by increasing input blood volume, improving blood elution from the filter paper, or by applying better correction factors to account for, for example, hematocrit.

Despite these concerns, the results of our study show that DBS VL for adults performs better than immunological or clinical

monitoring. A systematic review of the performance of immunologic and clinical criteria for detecting virological failure found that sensitivity was 55% for immunological criteria and 11% for clinical criteria.^[8] This suggests that in settings where DBS is the only viable means to monitor VL, it will still provide a more accurate assessment of treatment success than immunological or clinical monitoring alone. Based on the findings of a large systematic review of the performance of DBS, which included the results of this study, the latest WHO guidelines for antiretroviral therapy recommend the use of DBS as an option for improving access to VL monitoring.^[1]

This study has a number of strengths and limitations. Strengths include the fact that all evaluations were done in the same laboratory, on the same patient specimens by the same 2 operators whom had several years of experience with VL testing. However, the operators were not blind to the results of the reference test. As this evaluation used blood specimens collected from routine monitoring visits, limited volumes were available and all DBS results obtained with 3 different assays were compared to plasma results obtained from the Roche CAP/CTM v2.0 assay only. Inherent differences between the different assays warrant caution when comparing data between assays.^[20-24]

In addition, the VL assays from Roche and the protocol in use at the time for Abbott had not been fully validated by the manufacturers for DBS and no official instructions for use were available except for the NucliSENS EasyQ v2.0 assay. Since then, Roche has adapted its DBS processing instructions and Abbott has obtained CE approval and WHO prequalification status for a revised DBS protocol.

Another limitation of this evaluation was that DBS specimens were stored at -20°C, whereas ambient temperature would have been more relevant to the context in which DBS will be used. This was necessary because of the length of time needed to reach a large enough sample size of the different VL categories before specimens could be tested. This may have affected the results for the Roche assay, for which the recently developed FVE protocol was used. According to the manufacturer, DBS specimens should never be frozen or refrigerated for extended periods of time when using FVE, as this might release cell-associated viral RNA and over-estimate the results. Finally, these results are limited to adults. There is a need to also evaluate DBS to increase access to

Table 2**Sensitivity, specificity, false failures, and false successes for dried blood spot VL testing as compared to plasma VL testing.**

	Roche CAP/CTM HIV-1 v2.0% (95% CI)	Abbott RealTime HIV-1 test % (95% CI)	bioMérieux NucliSENS EasyQ HIV-1 v2.0% (95% CI)
Sensitivity	80.8 (73.3-86.7)	76.0 (68.1-82.5)	76.7 (68.9-83.1)
Specificity	87.3 (79.9-92.3)	89.7 (82.7-94.2)	92.9 (86.5-96.5)
False failures	11.9 (7.2-18.9)	10.5 (5.9-17.6)	7.4 (3.7-14.0)
False successes	20.3 (14.1-28.2)	23.6 (17.2-31.4)	22.5 (16.3-30.2)

CAP/CTM = COBAS AmpliPrep/COBAS TaqMan, CI=confidence interval, VL = viral load.

VL monitoring in children, given the very low sensitivity and specificity of clinical and immunological monitoring.

In conclusion, the 3 VL assays evaluated had sub-optimal performance with DBS, although performance was better than immunological or clinical monitoring. Even after the introduction of the much-anticipated point-of-care VL devices, it is expected that DBS will remain important as a complementary option for supporting access to VL monitoring, particularly in rural, resource-limited settings. Manufacturers should accelerate efforts to develop more reliable, sensitive, and specific methods to test VL on DBS specimens.

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