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Design and Implementation of an External Quality Assessment Program for HIV Viral Load Measurements Using Dried Blood Spots

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An external quality assurance program was developed for HIV-1 RNA viral load measurements taken from dried blood spots using a reference panel and field-collected specimens. The program demonstrated that accurate and reproducible quantitation can be obtained from field-collected specimens. Residual proviral DNA may confound interpretation in virologically suppressed subjects.

HIV-1 RNA viral load (VL) measurements from blood plasma are critical for assessing the response to antiretroviral treatment (ART) in individuals and populations (1–5). Sample preparation and storage, however, require laboratory and cold-chain infrastructure that may be limited when monitoring rural populations in resource-limited countries. These populations are often the most affected by the global pandemic. The collection of finger-prick blood on filter paper as dried blood spots (DBS) is an alternative strategy frequently used in global surveillance studies (6, 7). External quality assurance (EQA) programs for VL testing rely largely on blood plasma and do not effectively assess the unique qualities of the DBS sample matrix, including lower virion input copy number, inconsistent spot size, and the inclusion of proviral DNA, cellular RNA, and virion RNA.

To assess the quality of the VL measurements performed by a designated testing laboratory from DBS collected in a rural field setting for an HIV prevention program in North West Province, South Africa (8), an EQA program was developed and implemented that included two phases. First, to test overall concordance, 50 reference DBS cards were generated in duplicate at a 3rd-party laboratory under standardized conditions using venous EDTA blood (50 μl spots on Munktell filters; Ahlstrom Munktell, Helsinki, Finland) from HIV-seronegative and -seropositive donors with known plasma VL (Abbott RealTi*m*e HIV-1 viral load assay; Abbott Diagnostics, Des Plaines, IL, USA; lower limit of detection [LLOD], 40 copies/ml). The cards were dried at ambient temperature for 1 to 3 days and stored at −80°C in zip-lock bags with desiccant and humidity indicator cards for 3 months, followed by ambient shipment (3-day transit time) to the study testing and reference laboratories. Total HIV nucleic acid was measured at both laboratories from a single DBS using the Cobas AmpliPrep/Cobas TaqMan HIV-1 2.0 test (Roche Applied Sciences, Pleasanton, CA, USA; LLOD, 400 copies/ml). The HIV serostatuses and plasma VL of the donors that were used for the reference DBS (to which the testing and reference labs were blinded) were as follows: HIV seronegative, $n = 10$; HIV seropositive with plasma VL of <40 copies/ml (1.6 $\log_{10}$), $n = 14$; HIV seropositive with plasma VL of ≥40 copies/ml, $n = 25$; and detectable plasma VL of unknown quantity, $n = 1$.

Second, following complete testing and evaluation of the reference cards, DBS cards (Munktell) were collected by finger-prick from the study participants, with informed consent, in three field sites. Blood was collected from 179 seropositive donors and confirmed by serial rapid testing or HIV DNA PCR. The DBS cards were transported in a cooler box to the field office, dried overnight, and packed in zip-lock bags with desiccant and humidity indicators. Within 6 days of collection, the cards were stored at ambient temperature until transport to the testing laboratory, where they were stored at −70°C until VL testing was performed. All seropositive samples from the first site and a random 15% of the DBS cards from sites 2 and 3 were transported at ambient temperature from the testing laboratory to the reference laboratory for VL testing.

Forty-nine of 50 (98%) reference cards produced valid results from both laboratories. All 10 HIV-negative samples had undetectable VL (<400 copies/ml) by both laboratories (100% specificity). Of the 26 reference DBS from subjects with detectable plasma viremia (>40 copies/ml; range, 67 to >10,000,000), 24 DBS samples yielded detectable values (≥400 copies/ml) in both labs. One sample (76 copies/ml in plasma) had undetectable VL by both laboratories, and another sample (>10,000,000 copies/ml in plasma) yielded repeated invalid results in one laboratory. Table 1 shows the mean, median, and range of the DBS VL results from the testing and reference laboratories. In a Bland-Altman analysis (Fig. 1A), 100% of the values were within the 95% limit of agreement. A pairwise comparison (Fig. 1C) yielded excellent agreement with a significant correlation ($P = <0.0001$; Pearson $r = 0.983$; 95% confidence interval [CI], 0.961 to 0.993), with 24/26 (96%) having ≤0.5-$\log_{10}$ difference. The reference samples from HIV-seropositive subjects with undetectable plasma VL (<40

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TABLE 1 Dried blood spot viral load results from the testing and reference laboratories

Outcomes by sample type	HIV-1 DBS viral load (copies/ml) by laboratory	
	Testing	Reference
Reference panel samples[a]	$n = 25$	$n = 26$
Minimum	400	400
Maximum	10,000,000	10,000,000
Median	89,000	112,000
Mean	85,000	79,500
Field collected samples[b]	$n = 27$	$n = 27$
Minimum	400	716
Maximum	169,939	234,000
Median	23,988	13,100
Mean	12,988	12,365

[a] DBS VL results are shown for samples from HIV-seropositive donors with detectable plasma RNA (plasma VL > 40 copies/ml).
[b] DBS VL results shown for samples from HIV-seropositive or indeterminate donors with unknown plasma RNA levels.

copies/ml) had poorer agreement: 3 of 14 (21%) samples had detectable VL by both laboratories, and 5/14 (36%) had undetectable VL by both. Six samples showed discordant results, with 3 each detected in either laboratory (range, 413 to 15,447 copies/ml).

Of the 179 samples collected at the three field sites that tested HIV seropositive or indeterminate, 27 (15%) randomly selected DBS samples were tested at both laboratories, of which 25 had a VL in the quantifiable range by both laboratories. A Bland-Altman analysis showed that 92% (23/25) of the values were within the 95% limit of agreement (Fig. 1B). Pairwise comparisons of these showed a high correlation (Pearson $r = 0.92$; 95% CI, 0.788 to 0.956) (Fig. 1D), with 23 (92%) having a $\leq$0.5-$\log_{10}$ difference.

Overall, these results demonstrate the feasibility of a simple EQA program for DBS VL testing. We demonstrated highly correlative results between two laboratories when testing DBS samples from viremic subjects, despite the various collection conditions and spot sizes inherent in field-collected samples. Although the number of field specimens tested by both laboratories was limited, at 27/174 (15%), the agreement in the DBS VL results from the reference panel and the field-collected specimens lends confidence in the feasibility and accuracy of using DBS for a main study outcome.

However, we also found a high frequency (9/14 [64%]) of reference DBS samples from HIV-positive individuals with undetectable plasma viremia (<40 copies/ml) yielding detectable DBS VL (≥400 copies/ml) by either or both laboratories. These results are consistent with other reports and likely reflect the amplification of cell-associated HIV DNA and RNA in DBS samples in the absence of detectable blood plasma RNA (7, 9, 10). The magnitude of this discrepancy may be reduced using DBS preparation procedures that preferentially select for cell-free HIV RNA (11); however, the amplification of residual DNA in DBS to even a minimal extent may confound the interpretation of HIV RNA copy number in subjects with ART-induced virologic suppression. The significant discordance in DBS VL in samples from ART-suppressed

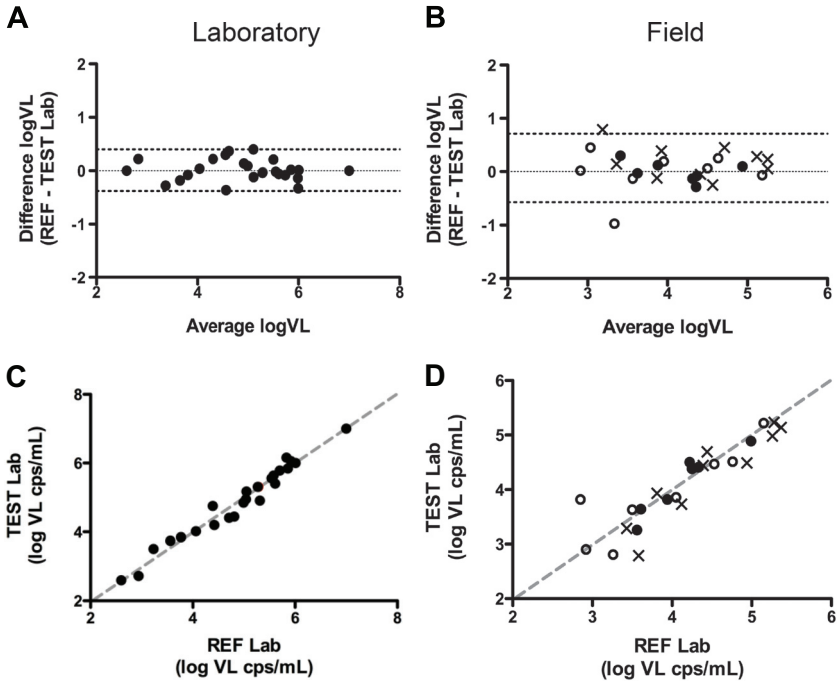

FIG 1 Agreement and correlation of DBS VL results from laboratory- and field-collected samples tested at two laboratories. (A and B) Bland-Altman analysis of $\log_{10}$ HIV VL (copies/ml) results from the reference (REF) (National Institute for Communicable Diseases, Johannesburg, South Africa) and test laboratories (Clinical Laboratory Services, Johannesburg, South Africa) using DBS samples created in duplicate at an independent laboratory (AIDS Research Institute-University of California, San Francisco [ARI-UCSF] Laboratory of Clinical Virology, San Francisco, CA, USA) from infected subjects with detectable plasma viremia (≥40 copies/ml) (A), or from field-collected DBS from HIV-seropositive subjects with unknown plasma VL (B). The 95% limits of agreement for the results are −0.38 to 0.40 (A) and −0.57 and 0.71 (B). (C and D) Correlation plot of $\log_{10}$ HIV VL (copies [cps]/ml) results from the reference and test laboratories analyzing DBS samples created in the laboratory (C) or collected from the field (D). The distinct symbols designate samples collected from 3 different study sites.

subjects underscores the limitations in interpreting the RNA copy number using this sample matrix and testing modality. However, in settings where few patients are virally suppressed, this bias may be minimal.

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