Design and implementation of an external quality assessment program for HIV viral load measurements using dried blood spots

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Running Title: Quality assessment of HIV viral load from DBS

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Abstract

An external quality assurance program was developed for HIV-1 RNA viral load measurements 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.

Manuscript

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

To assess the quality of 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 RealTime HIV-1 viral load assay, Abbott Diagnostics, Des Plaines, IL, USA, lower limit of detection (LLOD) 40 copies/mL). Cards were dried at ambient temperature for 1-3 days, 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 donors’ HIV serostatus and plasma VL used for the reference DBS (to which the testing and reference labs were blinded) was as follows: HIV seronegative (N=10); HIV seropositive with plasma VL <40 copies/mL (1.6 log_{10}) (N=14); plasma VL ≥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 study participants under informed consent in three field sites. Blood was collected from 179 seropositive donors and confirmed by serial rapid testing or HIV DNA PCR. 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. Cards were stored at ambient temperature until transport to the testing laboratory, within six days of collection, 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 were undetectable (<400 copies/mL) by both laboratories (100% specificity).

Of the 26 reference DBS from subjects with detectable plasma viremia (>4 copies/mL; range 67-10,000,000), 24 DBS samples yielded detectable values (≥ 400 copies/mL) in both labs. One sample (76 copies/mL, plasma) was undetectable in both laboratories, and another sample (>10,000,000 copies/mL, 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 (Figure 1A), 100% of values were within the 95% limit of agreement. Pairwise comparison (Figure 1C) yielded excellent agreement with a significant correlation (P=<0.0001, Pearson r = 0.983, 95% CI 0.961 - 0.993), with 24/26 (96%) having ≤ 0.5 log_{10} difference. Reference samples from HIV seropositives with undetectable plasma VL (<40 copies/mL) had poorer agreement: three of 14 (21%) samples were detectable by both laboratories and 5/14 (36%) were undetectable by both. Six samples showed discordant results with 3 each detected in either laboratory (range 145 – 15,500 copies/mL).

Of the 179 samples collected in the three field sites that tested HIV sero-positive or indeterminate, 27 (15%) randomly selected DBS samples were tested at both laboratories, of which 25 were in the quantifiable range by both laboratories. A Bland-Altman analysis showed 92% (23/25) of values were within the 95% limit of agreement (Figure 1B). Pairwise comparisons of these showed high correlation (Pearson r = 0.92, 95% CI 0.788 to 0.956), (Figure 1D) with 23 (92%) having ≤ 0.5 log_{10} difference.
Overall, these results demonstrate the feasibility for a simple EQA program for DBS VL testing. We demonstrated highly correlative results between two laboratories when testing DBS samples from viremic subjects, despite varying collection conditions and spot sizes inherent in field-collected samples. Although the number of field specimens tested by both laboratories was limited, 27/174 (15%), the agreement in DBS VL results from both the reference panel and the field-collected specimens lent 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 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 amplification of residual DNA in DBS to even a minimal extent may confound interpretation of HIV RNA copy number among subjects with ART-induced virologic suppression. The significant discordance in DBS VL in samples from ART-suppressed subjects underscores the limitations in interpreting 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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References


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

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<tr>
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<th>HIV-1 DBS viral load (copies/mL)</th>
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<tr>
<td></td>
<td>Testing Laboratory (N=25)</td>
</tr>
<tr>
<td>Minimum</td>
<td>400</td>
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<tr>
<td>Maximum</td>
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<tr>
<td>Median</td>
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<tr>
<td>Mean</td>
<td>85,000</td>
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<td></td>
<td>Field collected samples (N=27)</td>
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<tr>
<td>Minimum</td>
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<tr>
<td>Maximum</td>
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<tr>
<td>Median</td>
<td>23,988</td>
</tr>
<tr>
<td>Mean</td>
<td>12,988</td>
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1 DBS VL results shown for samples from HIV seropositive donors with detectable plasma RNA (plasma VL > 40 copies/mL).

2 DBS VL results shown for samples from HIV seropositive or indeterminate donors with unknown plasma RNA levels.
Figure Legend

Figure 1. Agreement and correlation of DBS VL results from laboratory- and field-collected samples tested at two laboratories. A, B: Bland-Altman analysis of log_{10} HIV VL (copies/mL) results from the reference (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 (ARI-UCSF Laboratory of Clinical Virology, San Francisco, CA, USA) from infected subjects with detectable plasma viremia (≥40 copies/mL), or from field-collected DBS from HIV seropositives with unknown plasma VL, respectively. The 95% limits of agreement for results shown in A and B are -0.38 to 0.40 and -0.57 and 0.71, respectively. C, D: Correlation plot of log_{10} HIV VL (copies/mL) results from the reference and test laboratories analyzing DBS samples created in the laboratory or collected from the field, respectively. The distinct symbols (open circles, closed circles, and ×) designate samples collected from 3 different study sites.
Prach et al., Fig 1

A  Laboratory

B  Field

C

D