Evaluating Dried Blood Spot Performance in Assessing HIV Treatment Failure in Uganda

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Background

- HIV RNA viral load (VL) testing for patients on antiretroviral therapy (ART) is a key component of patient monitoring.
- The 2013 WHO guidelines use a viral load threshold of 1,000 copies/mL of plasma to indicate treatment failure. However, the costs and logistics of plasma testing have hindered efforts to scale up routine virologic monitoring in sub-Saharan Africa.
- Dried blood spots (DBS) have emerged as a low-cost substitute. Fingerprick DBS does not require a phlebotomist or centrifuge and can be stored at ambient temperature until shipment as nonhazardous material. However, the performance of DBS in large-scale field conditions has not been widely assessed.

Objective

- To evaluate a DBS VL assay to detect VL suppression in a large sample of ART patients under field conditions.

Methods

- Paired plasma and DBS samples were collected from ART patients at nine hospitals and six health centers in Uganda.
- Plasma samples were analyzed with the Roche COBAS AmpliPrep/COBAS TaqMan assay at Joint Clinical Research Centre facilities in Uganda.

Methods (continued)

- Two 50-µL whole blood samples were collected by fingerprick onto DBS cards, which were dried, packaged with desiccant, and stored at room temperature until they could be shipped to the University of Washington for analysis.
- DBS were eluted in 1.3-mL lysis solution, extracted by NucliSENS miniMag, and quantified using the Abbott Real Time HIV-1 m2000rt 1.0-mL protocol with a detection limit of 520 copies/mL.
- DBS performance was assessed by correlation with paired plasma measures as well as sensitivity, specificity, and positive and negative predictive value using the 1,000 copies/mL threshold.
- A logistic regression was used to model the relationship between time spent in ambient air to sensitivity among samples with plasma measures above 1,000 copies/mL

Table 1: DBS and Plasma Contingency Table

	DBS+	DBS-	Total
Plasma +	178	73	251
Plasma -	39	1590	1629
Total	217	1663	1880

Table 2: DBS Assay Characteristics

Sensitivity	70.9	PPV	82
Specificity	97.6	NPV	95.6

Results

- 1,880 valid paired DBS samples were analyzed using the 2-spot elution method.
- DBS samples spent an average of 109 days at ambient temperature before analysis. Days spent in ambient air strongly predicted sensitivity after controlling for plasma VL.
- The correlation between log(10) DBS and log(10) plasma was 0.72. DBS tended to overestimate VL at low plasma levels and underestimate VL at high plasma levels.
- At a threshold of 1,000 copies/mL, DBS samples had sensitivity 70.9%, specificity 97.6%, PPV 82%, NPV 95.6%.

Figure 1: Correlation Between DBS and Plasma Measures of VL

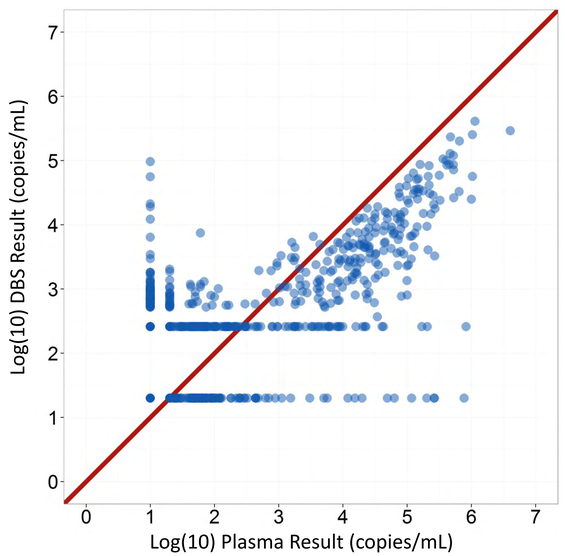
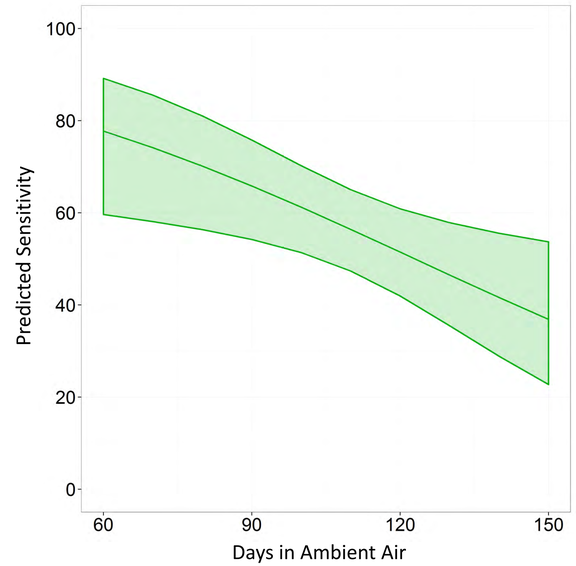


Figure 2: Predicted DBS Sensitivity at Various Days Spent in Ambient Air for a Plasma Sample of 5,000 copies/mL



Conclusions

- DBS measures were correlated with plasma, but the assay had low sensitivity.
- DBS tended to overestimate VL at low plasma levels, possibly due to the detection of viral nucleic acid (RNA & DNA) in whole blood.
- Exploratory analysis suggests that time spent in ambient air contributes to the reduction in sensitivity seen at higher plasma levels.
- Issues with collection, storage, and analysis of DBS samples must first be addressed in a field setting before widespread implementation can be recommended.