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# Pooled HIV-1 Viral Load Testing Using Dried Blood Spots to Reduce the Cost of Monitoring Antiretroviral Treatment in a Resource-Limited Setting

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**Abstract:** Rollout of routine HIV-1 viral load monitoring is hampered by high costs and logistical difficulties associated with sample collection and transport. New strategies are needed to overcome these constraints. Dried blood spots from finger pricks have been shown to be more practical than the use of plasma specimens, and pooling strategies using plasma specimens have been demonstrated to be an efficient method to reduce costs. This study found that combination of finger-prick dried blood spots and a pooling strategy is a feasible and efficient option to reduce costs, while maintaining accuracy in the context of a district hospital in Malawi.

**Key Words:** HIV, viral load, pooling, DBS, resource-limited setting, finger prick, operational research

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## INTRODUCTION

The World Health Organization recommends routine HIV viral load (VL) monitoring of patients on antiretroviral therapy (ART).<sup>1</sup> As clinical and immunologic criteria for treatment failure have comparatively low sensitivity and positive predictive values (PPVs),<sup>2–5</sup> VL monitoring enables earlier detection of ART failure, preventing emergence of drug resistance, enabling

timely switching to second-line regimens (if indicated), and preventing inappropriate switching to second-line regimens.<sup>6–8</sup>

In most resource-limited settings, VL monitoring is not currently available because of logistic and cost constraints.<sup>9</sup> Logistic constraints include the need for an efficient sample transport system, a reliable cold chain, and phlebotomists to draw venous blood (VB) for plasma, the standard sample type for VL testing. Use of dried blood spots (DBS) prepared from either finger prick (FP) or ethylenediamine tetraacetic acid–anticoagulated VB provide a practical alternative to plasma testing.<sup>10,11</sup> DBS samples can be stored at room temperature for at least 1 month.<sup>10</sup> Using FP DBS does away with the need for phlebotomy, enabling task shifting to lower staff cadres, where skilled human resources are scarce. A recent study conducted in Malawi found good correlation between VL results from plasma and DBS using the NucliSENS EasyQ HIV-1 v2.0 assay (bioMérieux, Marcy l’Etoile, France).<sup>11</sup>

Other recent studies have shown that pooled testing methods can achieve substantial cost reductions without compromising test accuracy.<sup>12–17</sup> Testing VL on pooled DBS samples could reduce logistic and cost constraints, restricting access to VL testing, if shown to have satisfactory accuracy compared with individual plasma testing.

This study aimed to determine the efficiency, cost savings, and accuracy of pooled VL testing compared with individual plasma testing using 2 mini pooling methods, using pooled FP DBS, VB DBS, and plasma samples.

## METHODS

### Setting

The study was carried out from August to November 2012 at the district hospital in Thyolo, a rural district in southern Malawi. The district has a population of approximately 620,000 and an HIV prevalence of 14.5%.<sup>18</sup> At the end of 2012, 35,000 patients were on ART, of whom 0.2% were on a second-line regimen.<sup>18</sup>

The VL testing laboratory at Thyolo District Hospital became operational in April 2011. VL monitoring replaced CD4 monitoring of patients attending the hospital ART clinic and was later extended to patients attending other health

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facilities in the district. VL monitoring coverage was approximately 15% by the end of 2012. Patients with a VL  $\geq 1000$  copies per milliliter receive an adherence intervention and a repeat VL test 3 months later. Patients with a repeat VL  $\geq 5000$  copies per milliliter are considered for second-line ART.

### Study Population

Three hundred fifty patients participated in the study. All HIV-positive patients older than 18 years, on ART for at least 6 months, and having VB drawn as part of their clinical management were eligible to participate. Ethics approval was obtained from the Malawi National Health Sciences Research Committee and the Médecins Sans Frontières Ethics Review Board. All participants provided written informed consent.

### Sample Collection and Preparation

Each participant provided an FP DBS sample and a tube of VB. A laboratory technician drew blood in to a BD Vacutainer EDTA tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ); this was used to prepare VB DBS and plasma samples. Laboratory technicians prepared 5 FP DBS and 5 VB DBS samples per participant by applying 50  $\mu\text{L}$  of blood on Whatman 903 filter paper cards (Whatman, Maidstone, United Kingdom). DBS samples were dried overnight and stored at room temperature in separate plastic bags with desiccants and humidity indicator cards until tested. Within 3 hours of collection, after preparing VB DBS samples, the remainder of the EDTA blood was centrifuged at 3000g for 10 minutes. Plasma was transferred to sterile polypropylene tubes and stored at  $-20^{\circ}\text{C}$  until testing.

### Pooling Strategies

The “minipool” and “minipool + algorithm” strategies used pools of 5 samples, as previously described.<sup>13</sup> Seventy sets of minipools were constituted from the 350 sets of samples. Plasma pools were constituted by pooling 100  $\mu\text{L}$  of plasma from 5 patients, for a total volume of 500  $\mu\text{L}$ . DBS pools were constituted using 2 circles of 50- $\mu\text{L}$  of blood per patient eluted in 2-mL lysis buffer and pooling 400- $\mu\text{L}$  eluates from 5 patients. Pooled samples were analyzed according to standard operating procedures.

As each pool contained 5 samples, a pool VL threshold of 1000 copies per milliliter was used to assess the accuracy and efficiency of pooling for identifying patients with a VL  $\geq 5000$  copies per milliliter and a pool VL threshold of 200 copies per milliliter was used to assess the accuracy and efficiency of pooling for identifying patients with a VL  $\geq 1000$  copies per milliliter. These thresholds were chosen because they were in clinical use.

### Minipool Strategy

Using the minipool strategy, pools with a VL result above the threshold were deconvoluted by testing individual samples from each patient represented in the pool.

### Minipool + Algorithm Strategy

Using the minipool + algorithm strategy, pools with a VL result above the threshold were deconvoluted by serial testing of individual samples from patients represented in the pool, in random order, and subtracting individual test results from the pool result until the VL of the pool had been accounted for.

For both pooling strategies, pools with a VL result less than the threshold were not deconvoluted, and all patients with samples in these pools were considered to have a VL lesser than the individual threshold.

### VL Assay

Quantitative HIV-1 RNA VL testing was performed with the NucliSENS EasyQ v2.0 assay (bioMérieux). The assay has a lower limit of detection of 50 copies per milliliter using a plasma input volume of 0.5 mL, and 800 copies per milliliter using a DBS input volume of 0.1 mL.

### Statistical Analyses

VL in individual plasma samples served as the reference for all calculations of accuracy and efficiency. Accuracy was determined by calculating sensitivity, specificity, PPV, and negative predictive value (NPV). Relative efficiency was defined as 1 minus the average number of tests performed divided by the number of samples. Cost savings were calculated with a reagent price of \$30 per test, the local price at the time of the study. Exact binomial 95% confidence intervals were calculated. *P* values were calculated using 2-sample tests of proportion. Statistical tests were 2 sided at  $\alpha = 0.05$ . All analyses were performed using Stata version 11 (StataCorp, College Station, TX).

## RESULTS

Of the 350 participants in the study, 70.5% were women and 16.3% had suspected treatment failure identified on clinical grounds. Their median age was 38 years [interquartile range (IQR): 30–46], and median time on ART was 37 months (IQR: 18–65).

Of the participants, 82.0% had an undetectable individual plasma VL, 10.0% had a detectable VL  $< 1000$  copies per milliliter and 8.0% had a VL  $\geq 1000$  copies per milliliter, including 6.6% with a VL  $\geq 5000$  copies per milliliter. Compared with patients having routine VL monitoring, participants with clinically suspected treatment failure were less likely to have a plasma VL  $< 1000$  copies per milliliter (86.0% vs. 93.2%; *P* = 0.066), and more likely to have a plasma VL  $\geq 5000$  copies per milliliter (12.3% vs. 5.5%; *P* = 0.057).

### Efficiency of Pooled Testing

The efficiencies of the 2 minipooling strategies with each sample type are shown in Table 1. For example, with FP DBS and a 5000 copies per milliliter threshold, mini pooling would reduce the number of tests required by 51.4% compared with testing individual plasma samples. There were no

**TABLE 1.** Relative Efficiencies of Different Pooling Methods, With Different Sample Types, at Thresholds of 1000 and 5000 copies per milliliters

Sample Type	1000 copies/mL			5000 copies/mL		
	Mini pool (95% CI)	Mini pool + Algorithm (95% CI)	Relative Efficiency, <i>P</i>	Mini pool (95% CI)	Mini pool + Algorithm (95% CI)	Relative Efficiency, <i>P</i>
Plasma	44.3 (39.0–49.7)	51.4 (46.1–56.8)	0.059	48.6 (43.2–53.9)	54.0 (48.6–59.3)	0.151
FP DBS	28.6 (23.9–33.6)	34.6 (29.6–39.8)	0.088	51.4 (46.1–56.8)	54.6 (49.2–59.9)	0.405
VB DBS	32.9 (28.0–38.1)	35.7 (30.7–41.0)	0.426	51.4 (46.1–56.8)	53.4 (48.0–58.7)	0.596

CI, confidence interval; FP DBS, finger prick dried blood spot; VB DBS, venous blood dried blood spot.

statistically significant differences in the efficiency of the 2 algorithms ( $P > 0.05$ ), although the minipool + algorithm was slightly more efficient than the minipool strategy at a 1000 copies per milliliter threshold with pooled plasma and FP DBS samples. Pooling plasma was more efficient than pooling DBS at the 1000 copies per milliliter threshold ( $P < 0.002$ ), but similar at the 5000 copies per milliliter threshold ( $P \geq 0.45$ ). There were no statistically significant differences in efficiency between FP DBS and VB DBS samples ( $P \geq 0.22$ ).

### Accuracy of Pooled Testing

The accuracies of the 2 minipool strategies in combination with each sample type are shown in Table 2. NPV ranged from 97.3% to 100%, and PPV ranged from 96.2% to 100%, despite the limited sensitivity (range, 60.9%–69.6%) of pooled DBS samples at the 5000 copies per milliliter threshold.

### DISCUSSION

This study combined the use of DBS and pooling strategies for quantitative HIV-1 VL determination. In Thyolo, approximately 23,000 VL tests are currently required per year to attain full VL monitoring coverage in accordance with the national guidelines, which recommend VL testing 6 and 24 months after starting ART and every 24 months thereafter. At a reagent price of \$30 per test and a 5000 copies per milliliter threshold, pooling DBS samples would save \$345,000 per year by reducing the number of tests required by approximately 50%. At a 1000 copies per milliliter threshold, pooling DBS samples would reduce the number of tests required by approximately 30%, saving \$207,000 per year. As the cost of VL reagents varies across countries in the region, ranging from \$11 to \$90 per test<sup>19</sup> because of differences in failure rates, cost savings would vary according to the setting. For if too many positives “poison” your pools, the saving will also be diminished. In each context, potential costs and benefits need to be balanced when choosing thresholds. Although raising the threshold produces greater savings,

**TABLE 2.** Accuracy of Minipooling and Minipooling + Algorithm Strategy for Detecting an Elevated Viral Load (VL) at 1000 and 5000 copies/mL Thresholds as Compared With Individual Plasma VL (N = 350)

Pooled Sample Type	VL Threshold (copies/mL)	Pooling Strategy	Individual VL Above Threshold (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Plasma	1000	Minipool	8.0	96.4 (81.7 to 99.4)	100 (98.9 to 100)	100 (87.2 to 100)	99.7 (98.3 to 100)
FP DBS	1000	Minipool	8.0	78.6 (59.0 to 91.7)	100 (98.9 to 100)	100 (84.6 to 100)	98.2 (96.1 to 99.3)
VB DBS	1000	Minipool	8.0	89.3 (71.8 to 97.7)	99.7 (98.3 to 100)	96.2 (80.4 to 99.9)	99.1 (97.3 to 99.8)
Plasma	5000	Minipool	6.6	100 (85.2 to 100)	100 (98.9 to 100)	100 (85.2 to 100)	100 (98.9 to 100)
FP DBS	5000	Minipool	6.6	69.6 (47.1 to 86.8)	100 (89.9 to 100)	100 (79.4 to 100)	97.9 (95.7 to 99.2)
VB DBS	5000	Minipool	6.6	60.9 (38.5 to 80.3)	100 (98.9 to 100)	100 (76.8 to 100)	97.3 (95.0 to 98.8)
Plasma	1000	Minipool + algorithm	8.0	92.9 (76.5 to 99.1)	100 (98.9 to 100)	100 (86.8 to 100)	99.4 (97.8 to 99.9)
FP DBS	1000	Minipool + algorithm	8.0	78.6 (59.0 to 91.7)	100 (98.9 to 100)	100 (84.6 to 100)	98.2 (96.1 to 99.3)
VB DBS	1000	Minipool + algorithm	8.0	89.3 (71.8 to 97.7)	99.7 (98.3 to 100)	96.2 (80.4 to 99.9)	99.1 (97.3 to 99.8)
Plasma	5000	Minipool + algorithm	6.6	95.7 (78.1 to 99.9)	100 (89.9 to 100)	100 (84.6 to 100)	99.7 (98.3 to 100)
FP DBS	5000	Minipool + algorithm	6.6	69.6 (47.1 to 86.8)	100 (89.9 to 100)	100 (79.4 to 100)	97.9 (95.7 to 99.2)
VB DBS	5000	Minipool + algorithm	6.6	60.9 (38.5 to 80.3)	100 (89.9 to 100)	100 (76.8 to 100)	97.3 (95.0 to 98.8)

NPV, negative predictive value; VB DBS, venous blood dried blood spot.

it also reduces the sensitivity of pooled VL testing at detecting treatment failure.

Several factors need to be considered when choosing between the minipool and minipool + algorithm strategies. Although the minipool + algorithm had a slightly higher efficiency than the minipool strategy at the 1000 copies per milliliter threshold, this advantage of the minipool + algorithm is offset by several disadvantages, including the greater complexity of the algorithm; a greater probability of false-negative individual VL results in pools with more than 1 sample above the individual threshold because of interassay variability, and a longer average run time because serial testing of individual samples used in deconvolution of positive pools requires up to 5 extra runs compared with 1 extra run using the mini pooling strategy. Despite slightly lower cost-savings, implementation of the minipool strategy may be operationally more feasible than implementing the minipool + strategy in resource-limited settings.

The use of DBS samples may enable task shifting to lower cadres, resulting in human resource allocation efficiencies. The prevalence of treatment failure in the patient population also needs to be considered when developing algorithms for pooled testing. Pooling methods become less efficient as the prevalence of treatment failure increases.

Previous pooling studies have found similar efficiencies using plasma or dried plasma spots as the sample type.<sup>12–17</sup> One study that used pooled VB DBS found lower efficiencies and NPVs with pooled VB DBS than with plasma.<sup>15</sup> In contrast to other studies, our study compared VL results from both FP DBS and VB DBS pools with individual plasma VL results.

Our findings are important as they address 2 of the major obstacles to the rollout of routine VL testing in resource-limited settings, namely the high cost of individual VL testing and the logistic difficulties associated with sample collection and transport.<sup>9</sup> Although point-of-care VL tests may play an important role in increasing access to VL monitoring in the future,<sup>20</sup> the use of DBS, tested at a district or central laboratory, is a feasible alternative to plasma testing and maybe more suitable and cost efficient than point-of-care VL testing in some settings, particularly those requiring high-throughput testing.

Strengths of this study include the sample size and using the same laboratory technicians to perform all tests in 1 laboratory. In addition, our findings demonstrate that constituting, testing, and resolving of positive pools are feasible in a rural district hospital laboratory. Implementing pooling strategies will help with the scale-up of routine VL monitoring, which in turn will increase costs saved as a result of pooling.

VL monitoring provides a direct measurement of response to ART and is more reliable than CD4 monitoring at detecting treatment failure. As more patients are initiated on ART at higher CD4 thresholds, as a result of changes in ART guidelines, VL monitoring will become the only reliable method of determining response to treatment. This study demonstrates that the use of FP DBS or VB DBS combined with pooled testing using the NucliSENS assay and provides a feasible option to facilitate the rollout of routine VL monitoring in resource-limited settings.

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