Performance of dried blood spot specimens prepared under clinical conditions to identify virologic failure among Kenyan children on antiretroviral therapy.

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Background and rationale

- Viral load (VL) testing is recommended by the World Health Organization to monitor antiretroviral treatment (ART) in HIV-infected persons
- Plasma VL testing is currently the gold standard
- Collection of venous blood, especially in young children, can be challenging.
- Separation, storage, and transportation of plasma is resource-intensive.
- Use of DBS may help improve access for VL testing.

Objectives

- Assess the performance of VL testing on DBS prepared under clinical conditions using three simplified spotting modalities with either venous blood or capillary blood

- Assess the diagnostic accuracy of VL on DBS for detecting virologic failure (VF), defined as plasma VL ≥1000 copies/ml

Methods (1)

Study design

- Cross-sectional study in 12, high volume, comprehensive care clinics in Nairobi and Western regions of Kenya

Study population

- Children (<15 years) on ART for ≥6 months attending clinic between May and December, 2013

Sample collection and VL testing

- A plasma sample and three DBS sample types were prepared and tested on Abbott m2000 platform

Data Analysis

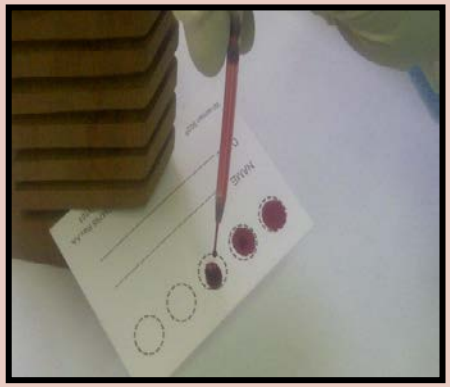
- Correlation, agreement, sensitivity, and specificity were determined for three DBS thresholds (≥1,000, ≥3,000 and ≥5,000 copies/mL) versus plasma (≥1,000 copies/ml)

Methods (2)

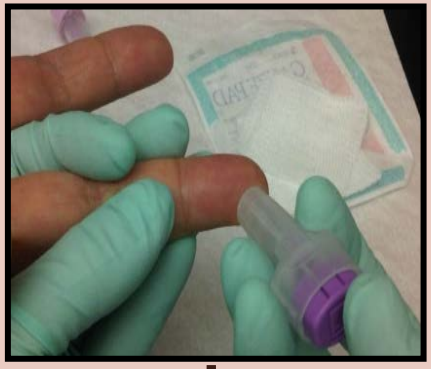
Blood collection and spotting modalities

Venipuncture (Venous)

Venous Blood (V-DBS)

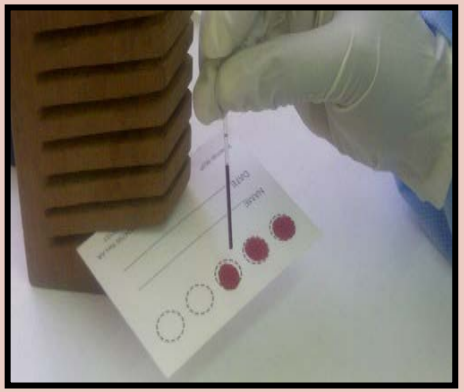


Finger Stick (Capillary)



Directly dropped (D-DBS)

Microcapillary (M-DBS)



Results

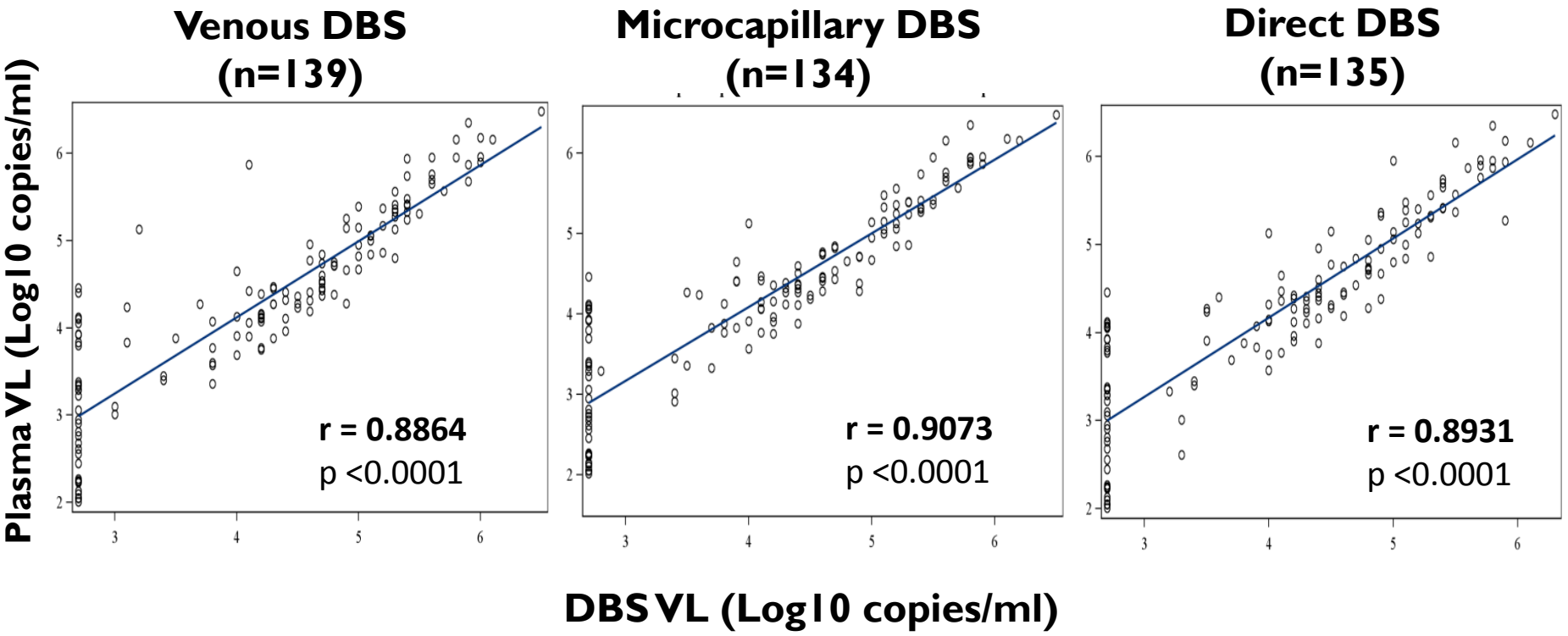
Patient Characteristics	
Total children (% female)	350 (48.3)
Median age in years (IQR)	6.6 (3.8-9.0)
Median duration on ART in months (IQR)	40.9 (24.3-60.0)
Had been on >1 ART regimen (n/N, %) [1]	78/346 (22.5)
ART Regimen [1]	
Nevirapine (NVP)-containing (n/N, %)	242/346 (69.9)
Efavirenz (EFV)-containing (n/N, %)	46/346 (13.3)
Lopinavir/Ritonavir-containing (n/N, %)	51/346 (14.7)
Other (n/N, %)	7/346 (2.0)
Virologic Failure Rates	
Overall (Plasma VL ≥1000 copies/ml) (n/N, %)	122/350 (**34.9**)
Children with VL indication (n/N, %) [2]	30/63 (**47.6**)
Children without VL indication (n/N, %) [2]	92/287 (**32.1**)

[1] Four children missing ART data.

[2] Children were **not** required to have VL indication for enrollment in the study.

Results: VL Performance on DBS

Correlation between DBS and plasma VL results in children using Abbott m2000



Results represent specimens with detectable VL in both Plasma and DBS.

Results: Diagnostic Accuracy

Kappa agreement, Sensitivity, Specificity, and Misclassification by DBS type and threshold compared to plasma on Abbott m2000

Threshold (≥ copies/ml) Plasma:DBS	Sample type (n)	Kappa (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	False Positives (%) (95% CI)	False negatives (%) (95% CI)
1000:1000	V-DBS (341)	0.80 (0.74 - 0.87)	88.5 (81.5 - 93.6)	92.2 (87.9 - 95.4)	7.8 (4.6 - 12.1)	11.5 (6.4 - 18.5)
	M-DBS(334)	0.82 (0.75 - 0.88)	86.4 (78.9 - 92.0)	94.4 (90.5 - 97.1)	5.6 (2.9 - 9.5)	13.6 (8.0 - 21.1)
	D-DBS(342)	0.81 (0.75 - 0.88)	85.8 (78.3 - 91.5)	94.6 (90.7 - 97.2)	5.4 (2.8 - 9.3)	14.2 (8.5 - 21.7)
1000:3000	V-DBS (341)	0.85 (0.79 - 0.91)	84.4 (76.8 - 90.4)	98.2 (95.4 - 99.5)	1.8 (0.5 - 4.6)	15.6 (9.6 - 23.2)
	M-DBS(334)	0.85 (0.78 - 0.91)	84.7 (77.0 - 90.7)	97.7 (94.7 - 99.2)	2.3 (0.8 - 5.3)	15.3 (9.3 - 23.0)
	D-DBS(342)	0.84 (0.78 - 0.90)	82.5 (74.5 - 88.8)	98.6 (96.1 - 99.7)	1.4 (0.3 - 3.9)	17.5 (11.2 - 25.5)
1000:5000	V-DBS (341)	0.85 (0.80 - 0.91)	83.6 (75.8 - 89.7)	99.1 (96.7 - 99.9)	0.9 (0.1 - 3.3)	16.4 (10.3 - 24.2)
	M-DBS(334)	0.83 (0.77 - 0.89)	81.4 (73.1 - 87.9)	98.6 (96.0 - 99.7)	1.4 (0.3 - 4.0)	18.6 (12.1 - 26.9)
	D-DBS(342)	0.83 (0.76 - 0.89)	79.2 (70.8 - 86.0)	99.5 (97.5 - 100.0)	0.5 (0.0 - 2.5)	20.8 (14.0 - 29.2)

Discussion

- Minimizing false negative misclassification may be preferable clinically and programmatically as false negative results may prolong time on ineffective treatment and increase risk for HIV drug resistance

- For all DBS sample collection methods (V, M, and D-DBS) using the Abbott m2000 platform:
  - Strong positive correlation between plasma and all DBS VL
  - High agreement when determining virologic failure
  - Sensitivity and specificity exceed 79% across all cut-offs
  - False negative rates lowest at 1,000 copies/ml DBS cut-off

Limitations

- Convenience sample of urban/peri-urban high volume facilities
- Samples collected by lab technicians or phlebotomists and many sites may not have the same cadres
- DBS evaluated for only one platform (Abbott) while multiple platforms used nationally and regionally

Conclusions

- **V-, M-, and D-DBS using the Abbott platform are comparably accurate and are a practical alternative to plasma** for determination of VF to monitor the therapeutic response to ART among children

- A DBS cut-off of 1,000 copies/ml is optimal and should be considered for VL monitoring using DBS

- Program managers and policy makers may determine which DBS method(s) are most appropriate based on context, resources, and program capacity.

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***DISCLAIMER**: The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the U.S. Centers for Disease Control and Prevention or Government of Kenya.*

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