



The feasibility of fingerstick blood collection for point-of-care HIV-1 viral load monitoring in rural Zambia

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Abstract

Viral load monitoring for HIV treatment is recommended but not feasible in many settings. A point-of-care test using capillary blood would increase access but may require up to 200 μ L of blood to achieve a lower limit of detection of 1000 copies/mL. This cross-sectional study evaluated the feasibility of collecting 200 μ L of capillary blood as well as blood collection preferences among adults in rural Zambia. Adults seeking HIV counseling and testing at Macha Hospital were recruited in 2015. Capillary blood was collected in four 50 μ L tubes. Blood collection was categorized as complete (200 μ L collected), partial (all tubes filled but <200 μ L obtained due to collection techniques), or incomplete (1-4 tubes attempted; <200 μ L obtained due to insufficient blood flow). One fingerstick was required for 90% of the 201 participants. A median blood volume of 196 μ L was collected. Complete, partial and incomplete collection was achieved in 34%, 59% and 6% of participants. The majority of participants (95%) preferred fingerstick over venous blood collection. A point-of-care viral load test requiring up to 200 μ L of blood is feasible in a rural setting but would require training and supervision to ensure that sufficient blood was collected.

Keywords: sub-Saharan Africa, HIV, viral load testing, point-of-care test, capillary blood

Introduction

In 2017, an estimated 25.7 million people in sub-Saharan Africa were living with HIV, of whom approximately 15.3 million were receiving antiretroviral therapy (ART) (WHO, 2018). The World Health Organization (WHO) recommends that all individuals receiving ART be monitored for treatment response. Viral load monitoring is preferable to CD4+ T-cell count or clinical status as it provides an earlier and more accurate indication of treatment failure, thereby reducing the accumulation of drug resistance mutations and improving clinical outcomes (WHO, 2016). Routine viral load monitoring is recommended 6 and 12 months after starting treatment and then yearly thereafter. Failure is defined as two measures exceeding 1 000 copies/mL within a 3-month interval with adherence support provided between measures (WHO, 2016).

Measuring viral load requires a level of laboratory capacity and expertise not widely available in sub-Saharan Africa, so that viral load testing has primarily been available in centralized labs in large urban centers. The use of dried blood spots with sample collection by fingerstick has increased access to viral load testing for rural and remote areas by eliminating the need for a phlebotomist to collect venous blood and simplifying sample storage, processing and transport. However, the need to transport samples to a centralized laboratory can lead to long turnaround times for results (Boillot et al., 2016, Lofgren et al., 2009) and several assays have been found to have lower sensitivity and specificity using dried blood spots compared to plasma samples (WHO, 2016).

A point-of-care test that could be performed by healthcare workers at facilities providing HIV care and treatment and that used blood collected by fingerstick would increase access to testing and make routine viral load monitoring feasible for HIV-infected patients living in rural and remote areas. In collaboration with Quidel Corporation (USA), the Northwestern Global Health Foundation (USA) is developing a point-of-care test that will require up to 200 μ L (with a target blood volume of 165 μ L) of capillary blood to reach a lower limit of detection of 1 000 copies/mL. Prior studies conducted in urban clinics in South Africa have demonstrated the feasibility of collecting 95 and 150 μ L of capillary blood from study participants (Gous et al., 2013, Maiers et al., 2015). Little is known about the feasibility of collecting capillary blood from patients in rural areas, where they may be more likely to be engaged in subsistence farming, fishing, mining, and other labor-intensive activities. These activities may lead to the formation of calluses on the finger tips which could impact the success of fingerstick blood collection by reducing the penetration depth of the lancet and impairing access to capillary blood vessels. Understanding the feasibility of collecting the desired volume of blood in different

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settings and the potential factors affecting blood collection by fingerstick will aid in the development of point-of-care diagnostic platforms.

This cross-sectional study was conducted in a rural hospital in southern Zambia to evaluate the feasibility of collecting 200 μL of capillary blood and preferences for blood collection among men and women receiving HIV counseling and testing.

Methods

Study Design and Settings

This cross-sectional study was conducted at Macha Hospital in a rural area of Choma District, Southern Province Zambia. The catchment area of Macha Hospital is populated by subsistence farmers living in small, scattered homesteads. The hospital compound consists of separate inpatient wards, including wards for male, female, maternity, pediatric, and TB/leprosy patients, as well as outpatient facilities, including a primary healthcare center, general outpatient clinic, dental clinic, eye clinic, HIV/ART clinic, pharmacy and clinical laboratory. The hospital serves as a district-level referral hospital for rural health centers within an 80 km radius, covering a population of approximately 150,000 people.

HIV counseling and testing services are available in several places at the hospital, including the male, female, maternity and pediatric wards and the primary healthcare center. HIV counseling and testing is performed by trained counselors. Blood is collected by fingerstick (50 μL) into a standard capillary tube provided in the kit for the HIV rapid test (Abbott Determine[®], Abbott Park, IL).

Data Collection and Measurements

This study was conducted between January 28, 2015 and April 30, 2015 in the wards for male, female and maternity patients of Macha Hospital and at the voluntary counseling and testing clinic of the primary healthcare center. At the beginning of the study, all counselors involved in HIV counseling and testing were told about the study and a questionnaire was administered to collect information on their education and experience with blood collection. No training on blood collection techniques was provided to counselors and they were requested to continue performing blood collection according to their usual methods.

All adults (≥ 16 years of age) requesting HIV counseling and testing were eligible for enrollment in the study. The target enrollment was 200 adults - 100 men and 100 women. This sample size was selected based on a desired width of the 95% confidence interval of $\pm 5\%$ for the proportion of participants providing 200 μL of capillary blood, which was estimated to be 95%, and 80% power to detect a potential difference between men and women of 10%. Successive adult male and female patients seeking HIV counseling and testing were approached for enrollment until the target enrollment was met (one additional participant was enrolled on the last day due to recruitment in multiple locations). Oral informed consent was obtained from each individual to participate in the study. After enrollment, a questionnaire was administered by study staff to collect demographic information and prior experience with and preference for blood collection by venipuncture or fingerstick. Blood was then collected by fingerstick by the counselors using standard lancets (Becton Dickinson, Microtainer Contact-Activated Lancet, 1.5 mm x 2.0 mm) and capillary tubes (Innovative Med Tech, plastic capillaries with EDTA). Blood was collected in four 50 μL capillary tubes to aid in measuring the exact volume collected, with the goal of collecting a total volume of 200 μL . Counselors were instructed to completely fill all four capillary tubes. Up to three fingersticks were attempted for each participant if less than 200 μL was collected on the first attempt. Permission was sought from the participant before each additional attempt.

Study staff observed blood collection and documented adherence of the counselors to standard practice (Maiers et al., 2015). After blood collection, a photograph was taken of the capillary tubes and the participant's fingertips, and the participant was again asked about their preference for blood collection methods.

All data from questionnaires and observations were collected electronically on tablets using Open Data Kit software (www.opendatakit.org).

The photographs of the capillary tubes were evaluated by investigators (MJF) and the total volume of blood collected was calculated. The extent of blood collection was categorized for each participant as 'complete' (all four tubes were filled; 200 μL was obtained), 'partial' (all four tubes were filled; $< 200 \mu\text{L}$ was obtained due to collection techniques - e.g. air bubbles, failure to fill the tube to the top), or 'incomplete' (1-4 tubes were attempted; $< 200 \mu\text{L}$ was obtained due to insufficient blood flow from the finger) (Figure 1). Data were entered into Microsoft Excel (2013).

Statistical Analysis

Descriptive statistics, including chi-square tests for categorical variables and Wilcoxon rank-sum tests for continuous variables, were used to determine differences in the characteristics, blood collection preferences and outcomes (number of fingersticks and volume of blood collected) between men and women enrolled in the study. Participant (age, sex, occupation) and counselor (years of experience, number of fingersticks per week, preference for blood collection methods) characteristics associated with incomplete blood collection were evaluated using log-binomial regression to estimate prevalence ratios and 95% confidence intervals. For the evaluation of counselor characteristics, clustering by counselor ID was included in the models to account for the multiple blood draws per counselor. Variables marginally associated ($p < 0.2$) with incomplete blood collection were included in a final multivariable model. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina) and STATA version 14.2 (College Station, Texas).

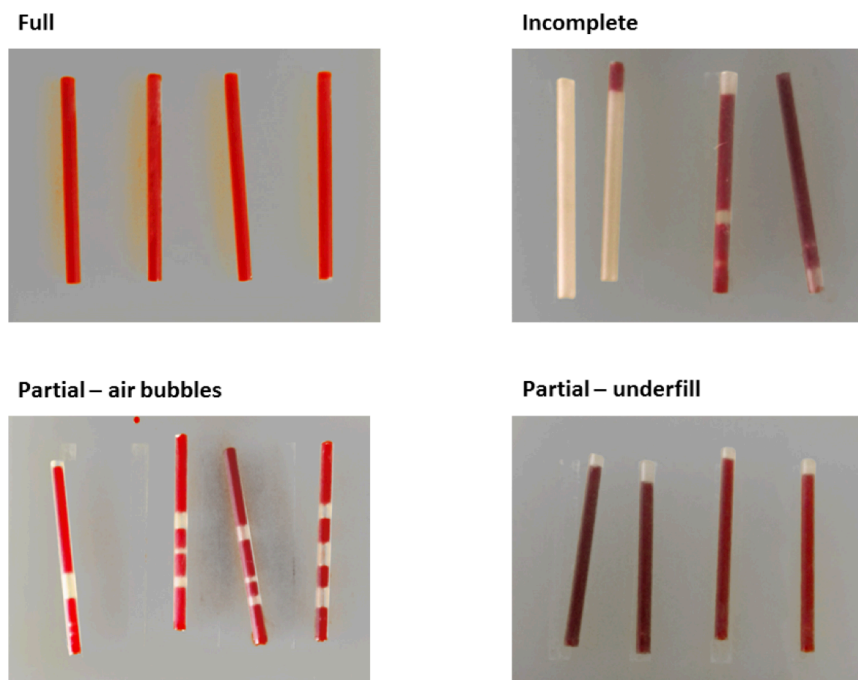


Figure 1. Examples of full, partial and incomplete blood collection

Results

Nine counselors participated in the study and performed the blood collection. Most (7/9; 78%) had completed grade 12 and all had received additional training (8/9; 89% in psychosocial counseling) for a median of 1 year (interquartile range [IQR]: 0.6, 1.5). Counselors had a median of 6 years (IQR: 5, 7) experience with blood collection and performed a median of 30 fingersticks (IQR: 15, 50) and 5 venipunctures (IQR: 2, 12) in a typical week. More than half (5/9; 56%) preferred collecting blood by fingerstick, while 33% (3/9) preferred venipuncture and 11% (1/9) had no preference.

A total of 202 individuals seeking HIV counseling and testing were approached and 201 individuals (101 men and 100 women) agreed to participate in the study (Table 1). The median age of participants was 29 years and 75% (151/201) were farmers. Among participants whose primary occupation was not farming ($n=50$), 58% (29/50) were involved in farming (men: 19/28, 68%; women: 10/22, 45%; $p=0.11$). Most (184/201; 92%) participants previously had blood collected by fingerstick but only half (109/201; 54%) by venipuncture. Most (134/201; 67%) participants expressed a preference for blood collection by fingerstick prior to the blood draw. Women had significantly more experience with blood collection by venipuncture (65% vs. 44%; $p=0.004$) than men. While non-significant, women also had more experience with blood collection by fingerstick (95% vs. 88%; $p=0.08$).

Table 1: Characteristics of study participants and preferences for blood collection

Characteristics at study enrollment	Total N=201	Men N=101	Women N=100	p-value ^a
Age in years: median (IQR)	29 (24, 39)	28 (23, 39)	30 (24, 39)	0.48
Primary occupation- farming: n (%)	151 (75)	73 (72)	78 (78)	0.17
Ever had a fingerstick: n (%)	184 (92)	89 (88)	95 (95)	0.08
Ever had venipuncture: n (%)	109 (54)	44 (44)	65 (65)	0.004
Preference for blood collection: n (%)				0.10
Fingerstick	134 (67)	73 (72)	61 (61)	
Venipuncture	15 (7)	4 (4)	11 (11)	
No preference	52 (26)	24 (24)	28 (28)	

^a p-value from chi-square test for categorical variables from Wilcoxon rank-sum test for continuous variables

For the majority (180/201; 90%) of participants, only one fingerstick was performed. The median volume of blood collected was 196 μL (IQR: 180, 200; range: 73, 200). Counselors collected 200 μL of blood from 34% (69/201) of participants (Table 2), with no differences observed by sex. Among participants from whom 200 μL was obtained, 96% (66/69) only required one fingerstick and 4% (3/69) required two fingersticks. Among the 59% (119/201) of participants with only partial blood collection, 27% (32/119) had tubes that were underfilled, 28% (33/119) had tubes that contained air bubbles, and 45% (54/119) had tubes that both contained air bubbles and were underfilled. Among those with partial blood collection, the median volume of blood collected was 187 μL (IQR: 178, 196; range: 143, 199). One, 2 and 3 fingersticks were performed for 90% (107/119), 8% (9/119) and 3% (3/119) of participants with partial blood collection, respectively. Only 6% (13/201) of participants had incomplete blood collection (median volume of blood collected: 143 μL ; IQR: 95, 152; range: 73, 190). One, 2 and 3 fingersticks were performed for 54% (7/13), 38%

(5/13) and 8% (1/13) of participants with incomplete blood collection, respectively. Among all participants, 90% (180/201) provided 165 µL of blood, the target for the point-of-care test.

Table 2: Outcome of blood collection in rural Zambia, 2015

Blood collection	Total N=201	Men N=101	Women N=100	p-value^b
No. of fingersticks performed: n (%)				0.96
1	180 (90)	91 (90)	89 (89)	
2	17 (8)	8 (8)	9 (9)	
3	4 (2)	2 (2)	2 (2)	
Volumes of blood collected: median (IQR)	196 (180, 200)	193 (177, 200)	197 (183, 200)	0.48
Extent of blood collected: n (%)				0.66
Complete (200 µL)	69 (34)	33 (33)	36 (36)	
Partial (<200 µL) ^a	119 (59)	60 (59)	59 (59)	
Incomplete (<200 µL) ^a	13 (6)	8 (8)	5 (5)	
After blood collection				
Preference for blood collection: n (%)				0.28
Fingerstick	190 (95)	98 (97)	92 (92)	
Venipuncture	8 (4)	2 (2)	6 (6)	
No preference	3 (1)	1 (1)	2 (2)	

^aPartial: all four tubes filled but <200 µL collected due to collection techniques (e.g. air bubbles, underfilled tubes); Incomplete: 1-4 tubes attempted but <200 µL collected due to insufficient blood flow from the finger

^bp-value from chi-square test for categorical variables and from Wilcoxon rank-sum test for continuous variables

Table 3: Participant and counselor characteristics associated with incomplete blood collection

Participant characteristics	Total N	Incomplete blood collection	Crude Prevalence ratio (95% confidence interval)	Adjusted Prevalence ratio (95% confidence interval)
Sex: n (%)				
Men	101	8 (8)	1.0	
Women	100	5 (5)	0.6 (0.2, 1.9)	
Age in years: n (%)				
≤35	132	6 (4)	1.0	1.0
>35	63	7 (11)	2.6 (0.9, 7.3)	2.4 (0.8, 6.6)
Primary occupation: n (%)				
Farming	151	12 (8)	4.0 (0.5, 29.8)	3.6 (0.9, 15.2)
Other	50	1 (2)	1.0	1.0
Counselor characteristics^a				
Number of fingersticks performed per week: n (%)				
≤30	123	11 (9)	1.0	1.0
>30	77	2 (3)	0.3 (0.1, 1.0)	0.2 (0.1, 0.8)
Prefers fingersticks to venipuncture: n (%)				
Yes	106	8 (8)	1.0	
No (including no preference)	94	5 (5)	0.7 (0.1, 4.0)	
Years of experience with fingersticks: n (%)				
<5	81	6 (7)	1.3 (0.3, 4.9)	
≥5	119	7 (6)	1.0	

^aThe counselor ID was not recorded for one participant.

In the unadjusted analysis, no participant characteristics were significantly associated with incomplete blood collection (Table 3), although participants with farming as a primary occupation (8% vs. 2%; p=0.18) and older than 35 years of age (11% vs. 4%; p=0.08) were more likely to have incomplete collection. In addition, no counselor characteristics were significantly associated with incomplete blood collection, although counselors who performed ≤30 fingersticks per week were more likely to have incomplete blood collection (9% vs 3%; p=0.05). After adjusting for age, farming and number of fingersticks performed per week, number of fingersticks performed per week was significantly associated with incomplete blood collection (adjusted prevalence ratio: 0.2; 95%

confidence interval: 0.1, 0.8).

There was variation in the abilities of the counselors to collect 200 µL of blood (Figure 2) but no trend towards improvement in blood collection throughout the study period. Counselors adhered to most procedures in standard practice for blood collection, although correct positioning of the hand with the palm down and below the elbow was uncommon (Table 4).

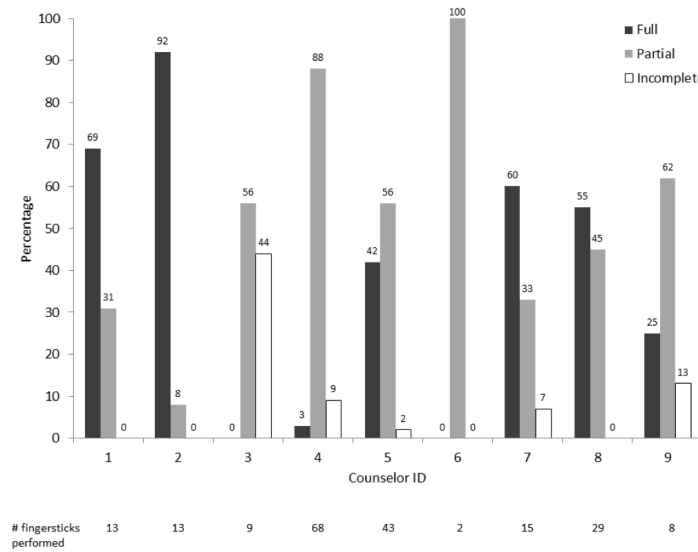


Figure 2. Percentage of full, partial and incomplete blood draws by counselor

ID: identifier. Note: The counselor ID was not recorded for one participant. Counselors 3, 4, 6, 7 and 8 reported performing ≤30 fingersticks per week.

Table 4: Adherence to standard practice for blood collection

Procedure	N (%)
Two gloves worn by nurse	198 (99)
Patient sitting	201 (100)
Puncture site disinfected with alcohol	200 (99)
First of drop of blood wiped away	178 (89)
Hand positioned palm down	4 (2)
Hand position below the elbow	26 (13)
Collection device held above skin, scraping avoided	143 (71)
Gentle pressure applied, strong milking avoided	156 (78)
Pressure applied after collection	199 (99)

After blood collection, 95% (190/201) of participants expressed a preference for blood collection by fingerstick compared to venipuncture, with no difference by sex (Table 2). Among participants who preferred blood collection by fingerstick (n=187), 88% (164/187) and 73% (137/187) stated that their preference would remain unchanged even if two or three fingersticks were required, respectively.

Discussion

In this study of men and women seeking HIV counseling and testing at a rural clinic in Zambia, 200 µL of capillary blood was collected from only 34% of participants. However, 90% of participants provided 165 µL of blood, the target for the point-of-care test. Among participants who did not provide 200 µL of blood, the majority provided <200 µL of blood due to collection techniques. Most participants with full blood collection only required one fingerstick and preference for blood collection by fingerstick among all participants was high and increased after blood collection. The proficiency of blood collection, and therefore the total volume of blood collected, varied by counselor. Counselors performing more fingersticks per week were less likely to have incomplete blood collection.

While other point-of-care tests are routinely performed with capillary blood in sub-Saharan Africa, including for HIV rapid diagnosis, CD4+ T-cell count and malaria rapid diagnosis, few require a large volume of blood. Few studies have been performed documenting the feasibility of blood collection by fingerstick in sub-Saharan Africa and most have been conducted in urban areas.

One study, conducted among 103 HIV-infected adults receiving ART in Johannesburg, South Africa, found that three individuals had sufficient callouses or thick skin to preclude successful blood collection (Maiers et al., 2015). Among the remaining 100 participants, 98% successfully provided 150 μ L of capillary blood, the target volume for the study, and 87% only required one fingerstick (Maiers et al., 2015). In contrast, this study was conducted in a rural area, where farming is common and the presence of callouses and thick skin may be more prevalent. Despite these factors, a similar proportion of individuals had successful (complete or partial) blood collection and only required one fingerstick. This finding is encouraging and suggests that a point-of-care viral load test requiring 150- 200 μ L of capillary blood could be successfully performed on most individuals in similar urban and rural settings.

More than half of participants provided less than 200 μ L of blood due to collection techniques, that resulted in air bubbles and underfilled tubes. There was considerable variability by counselor, with the percentage of partially filled tubes ranging from 8% to 100%. No specific training was provided in blood collection as all counselors had years of experience and collected blood by fingerstick in standard capillary tubes every week for HIV rapid testing. Their variable proficiency in collecting blood in the capillary tubes was surprising but has also since been observed in this setting with heelstick blood collection for point-of-care early infant diagnosis (Sutcliffe et al., 2016) and in other studies with fingerstick blood collection (Pinto et al., 2015). This finding emphasizes the importance of training in blood collection techniques when a new platform or assay is introduced. Product developers should also carefully consider the blood collection devices and how simple they will be to use by all target users. This will ensure that sufficient blood volume is consistently collected and valid test results are obtained.

Most participants preferred blood collection by fingerstick compared to venipuncture before the study and that preference increased after the study, as a majority of participants initially expressing no preference changed their response to fingerstick. This finding is consistent with other studies that compared both capillary and venous blood collection and determined that participants reported fingerstick to be less painful than venipuncture (Pinto et al., 2015, Woods et al., 2004). In addition to being more acceptable to patients, fingerstick blood collection was preferred by healthcare workers as it was easier to perform, required less training and equipment than phlebotomy and, in some cases, was more successful in patients who were ill or dehydrated (Maiers et al., 2015, Pinto et al., 2015). Consequently, a point-of-care viral load test using capillary blood could be performed by healthcare workers with minimal training and is more likely to increase access to viral load monitoring for HIV-infected patients receiving care and treatment in all levels of healthcare facilities, particularly those in rural and remote areas.

This study had several limitations. First, men and women of unknown HIV infection status were enrolled, instead of HIV-infected individuals receiving ART who would be the target for a point-of-care viral load test. Preferences for blood collection method may be different among HIV-infected individuals who are more likely to be experienced with venipuncture from the routine lab tests required for HIV monitoring. In addition, HIV-infected individuals may be more likely to be acutely ill which may impact the volume of blood collected. However, in the HIV clinic in this setting, HIV-infected individuals do not routinely have blood collected by fingerstick and they would also have undergone venipuncture for routine care, therefore, it was not considered acceptable to include them in this study. Second, it was not possible to objectively determine whether callouses and thick skin were responsible for the incomplete blood collection. Third, the objective of this study was to measure the quantity and not quality of blood collected. There may be other factors related to blood quality, such as interstitial fluid, that could also impact a point-of-care viral load test. Fourth, the study used a standardized protocol and supplies for blood collection. Other protocols or supplies, such as the type of lancets or capillary tubes, could impact the quantity of blood collected but were not evaluated in this study. Lastly, this study was conducted at one clinic in southern Zambia and the results may not be generalizable to populations in other rural areas, particularly if other forms of employment are prevalent that impact fingerstick blood collection.

Conclusion

Most men and women in this rural setting in southern Zambia expressed a preference for capillary compared to venous blood collection. The desired volume of 200 μ L of capillary blood was collected from only one third of participants due to the variable proficiency in blood collection by counselors. Few participants were unable to provide 200 μ L of capillary blood due to insufficient blood flow. A point-of-care viral load test that requires as much as 200 μ L of capillary blood is feasible in a rural setting but would require training and supervision on blood collection to ensure that sufficient blood was collected to perform the test successfully.

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Ethics Approval

The study was approved by the Institutional Review Boards of the Johns Hopkins Bloomberg School of Public Health and the Macha Research Trust.

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