An investigation of fingerstick blood collection for point-of-care HIV-1 viral load monitoring in South Africa

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Background. Viral load (VL) quantification is an important tool in determining newly developed drug resistance or problems with adherence to antiretroviral therapy (ART) in HIV-positive patients. VL monitoring is becoming the standard of care in many resource-limited settings. Testing in resource-limited settings may require sampling by fingerstick because of general shortages of skilled phlebotomists and the expense of venepuncture supplies and problems with their distribution.

Objective. To assess the feasibility and ease of collecting 150 µL capillary blood needed for the use of a novel collection device following a classic fingerstick puncture.

Methods. Patients were recruited by the study nurse upon arrival for routine ART monitoring at the Themba Lethu Clinic in Johannesburg, South Africa. Each step of the fingerstick and blood collection protocol was observed, and their completion or omission was recorded.

Results. One hundred and three patients consented to the study, of whom three were excluded owing to the presence of callouses. From a total of 100 patients who consented and were enrolled, 98% of collection attempts were successful and 86% of participants required only one fingerstick to successfully collect 150 µL capillary blood. Study nurse adherence to the fingerstick protocol revealed omissions in several steps that may lower the success rate of capillary blood collection and reduce the performance of a subsequent VL assay.

Conclusion. The findings of this study support the feasibility of collecting 150 µL of capillary blood via fingerstick for point-of-care HIV-1 VL testing in a resource-limited setting.

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The World Health Organization (WHO) has estimated that about 34 million individuals are infected by the current HIV/AIDS pandemic.[1] Although much progress has been made in controlling this disease, in sub-Saharan Africa approximately 23 million people remain infected,[1] with South Africa (SA) alone contributing about 11 087 cases/100 000 population.[1]

The monitoring of the HIV viral load (VL) in patients receiving antiretroviral therapy (ART) is critical to ensure treatment success, identify problems with treatment adherence, and identify HIV drug resistance to inform the decision to switch to second-line or future third-line therapies.[2] Currently there is much discussion regarding the role of VL monitoring in the care of HIV/AIDS patients. The WHO recommends VL monitoring as the preferred approach because of its ability to identify treatment failure earlier than immunological and clinical modalities.[2] Treatment failure is defined by the WHO as a plasma VL >1 000 copies/mL after two consecutive measurements during a 3-month interval accompanied by adherence support.[2] However, despite the updated WHO recommendations, poor access to VL testing often persists in resource-limited settings owing to simple logistical issues such as the collection and transportation of specimens.

Recently, alternatives such as dried blood spots (DBSs) and point-of-care (POC) devices are being investigated as potential ways to increase access to VL testing in low- and middle-income countries (LMICs). While standard laboratory platforms typically retain high accuracy when utilising the recommended threshold of 1 000 copies/mL, both DBS and POC devices may need to utilise a higher limit (3 000 - 5 000 copies/mL has been suggested) until better sensitivity is established at the lower limit of detection.[2] A study by Viljoen *et al.*[3] in Durban using DBS HIV-1 RNA testing appeared accurate and feasible down to approximately 3 000 copies/mL. In a study conducted in southern India by Neogi *et al.*,[4] DBS HIV-1 RNA testing revealed 100% sensitivity and specificity at 5 000 copies/mL, but only 50% sensitivity with 100% specificity at 1 000 copies/mL. Most recently, when Kleshik *et al.*[5] quantified HIV-1 RNA in single 50 µL DBSs and limited incubation time prior to sample preparation to 30 minutes, a limit of detection of 866 copies/mL was reported.

An important concern regarding the use of DBSs for HIV-1 RNA quantification is the amplification of cell-associated HIV nucleic acid in whole blood, leading to a falsely high VL measurement when compared with the amplification of viral nucleic acid in plasma specimens. Several recent studies using DBSs have shown reasonable correlation for VLs >3 000 copies/mL, but significant over-quantification has been observed in specimens with <3 000 copies/mL.[6-9] Unless this over-quantification is addressed, the usefulness of DBSs for VL monitoring may be limited in samples containing <3 000 copies/mL.

DBSs may serve to improve access to VL monitoring by linking any existing central laboratory infrastructure to regions with poor access to VL testing, where transport delays and centrifugation to plasma are not feasible. However, blood collection and the shipment of DBSs, with subsequent VL quantification and reporting of results, is not easily achieved during the same day in order to impact on patient care on the same visit. Rapid POC testing may address this logistical shortcoming by quantifying VL on site during the same visit. Subsequent ART intervention may then take place during the same day, as patients with poor adherence to treatment or those with newly developed drug resistance are screened earlier in the process.

Shortages of skilled phlebotomists and the expense of venepuncture supplies have contributed to the development of a POC VL quantification device for use in resource-limited settings that may utilise sampling by fingerstick instead of venepuncture. Fingerstick specimens are currently used for a wide range of tests for haematology, chemistry and serology.[10-13] Recently the University of the Witwatersrand, Johannesburg, SA, investigated the feasibility and accuracy of performing multiple point-of-care tests (POCTs) on fingersticks. This study found that capillary blood for up to four POCTs (95 µL) could be obtained from a single fingerstick in 92% of the subjects.[14] A collaboration between the Quidel Corporation and the Northwestern Global Health Foundation (NWGHF), USA, is developing a POC RT-PCR testing platform and VL assay that will require a volume of 150 µL capillary blood to reach a sensitivity with a lower limit of detection of 1 000 copies/mL.[12] The 150 µL whole blood will be converted to plasma using sample preparation materials provided by the NWGHF.[12] A significant barrier to implementing this platform in the future will be overcome if 150 µL capillary blood can be reliably collected following a fingerstick. In order to facilitate the collection of capillary blood for this study, a novel EDTA-treated capillary blood collection device with a capacity of 150 µL was developed.

The aim of this study was to assess: (*i*) the proportion of collection attempts that obtain 150 µL capillary blood using a newly developed fingerstick-based collection device; (*ii*) the number of puncture sites required to obtain 150 µL blood; and (*iii*) study nurse adherence to the fingerstick and blood collection protocol.

Methods

Setting and participants

The study was conducted at the Themba Lethu Clinic at Helen Joseph Hospital, Johannesburg, where a medical student from the Feinberg School of Medicine in Chicago, USA, observed a study nurse perform fingerstick punctures and collect capillary blood specimens from 100 patients having routine blood tests for ART monitoring. Each patient routinely received one venepuncture for blood collection during their visit. Fingerstick punctures were not performed at this clinic for the routine blood tests involved in ART monitoring. For the purposes of this study, a phlebotomist first performed a venepuncture on each patient for their routine blood tests and then one or more fingersticks were performed by the study nurse. The study nurse was highly experienced, with over 1 000 venepunctures and 1 000 fingersticks performed during her career.

Eligible patients were HIV-positive individuals currently receiving ART who had previously been tested for CD4 and/or HIV VL. Primary exclusion criteria included the presence of heavy callouses, severe dehydration, clinically identifiable illness and/or opportunistic infection, and persistently cold fingers after a warming attempt. Suitable participants were recruited from the blood collection room after a phlebotomist had administered venepuncture and collected the requested routine standard-of-care blood specimens. Each patient was asked to sign an informed consent waiver before enrolling in the study and receiving a fingerstick. Ethics approval for this study was granted by the Institutional Review Board at Northwestern University (ID: STU00076689) and the Human Research Ethics Committee at the University of the Witwatersrand (Protocol M120143).

Data collection and measurements

Fingerstick punctures were delivered using a device with a blade depth and width of 2.0 mm and 1.5 mm, respectively. The BD Microtainer Contact-Activated Lancet (BD Diagnostics, USA) was initially used to deliver fingerstick punctures until a stock shortage necessitated the use of the BD Genie Lancet (BD Diagnostics), with identical blade depth and width specifications. The study nurse was not given explicit instructions on how to perform the fingersticks; instead, she was simply asked to perform them according to her usual methods until blood collection was complete. A fingerstick and blood collection protocol checklist was created for the purpose of this study to assess baseline study nurse adherence to the protocol without training or specific instructions provided. The study nurse was blinded with respect to the fingerstick and blood collection protocol checklist used to assess adherence (Appendix 1) for the fingerstick and blood collection protocol in its entirety.

A novel blood collection device capable of holding 150 µL was used to discriminate between successful and unsuccessful collection attempts. The device contained several layers of EDTA-treated membrane strips designed to wick exactly 150 µL capillary blood. A complete collection was described to the nurse as the moment when both the front and rear of the membrane strips in the collection device appeared solid red in colour. Each step of the protocol checklist was observed, and completion or omission of any step was recorded on a template for every patient. A stopwatch was started immediately after the fingerstick to time the duration of the fingerstick procedure followed by blood collection. When more than one fingerstick was necessary, the study nurse obtained verbal consent before proceeding with each additional fingerstick. The result of each collection attempt was recorded. The study nurse performed translations as needed. Any unique insights offered by her were documented.

Results

A total of 132 patients were approached for participation in this study. Twenty-nine patients refused to give consent: 18 offered no reason for their refusal to do so, 7 stated that they were in a hurry, 2 did not want additional tests performed, and 2 stated that they were scared of receiving a fingerstick. Of the remaining 103 patients, 3 were excluded from the study because of the presence of callouses and/or extremely thick skin, self-described as relating to their respective occupations. One hundred remaining patients participated in the study.

Ninety-eight out of 100 collection attempts were successful, and 86% required only one fingerstick to successfully collect 150 µL of capillary blood (Table 1). The two failed collection attempts were in adult men without callouses, exceptionally thick skin

Table 1. Number of fingersticks required to obtain 150 µL blood

Fingersticks received, *n*	Patients (*N*=100), *n*
1	87*
2	10
3	2
4	1†

*One collection attempt failed to obtain 150 µl blood.
†The combined collection from four fingersticks failed to produce 150 µL blood.

Table 2. Nurse adherence to protocol

Protocol steps	Adherence, % (*N*)
Pair of gloves worn by nurse	0 (0)
Patient sitting	100 (100)
Patient's fingers warmed in advance	7 (7)
Puncture site disinfected with alcohol pad	86 (101)
First drop of blood wiped away	5 (109)
Hand positioned palm down	100 (100)
Hand positioned below elbow	56 (65)
Collection device held above skin; scraping avoided	96 (104)
Gentle pressure applied; strong milking avoided	95 (103)
Pressure applied after collection	100 (100)

or persistently cold fingers after a warming attempt. After four consecutive fingersticks were conducted on the first patient, he refused additional attempts. In the second patient, slow blood flow was observed after the first fingerstick. He declined to give consent to perform any additional fingersticks. Neither patient exhibited a negative response to receiving their fingerstick(s), as neither was observed to wince in pain, pull away or cry out. Neither patient exhibited physical signs of dehydration, but further questioning revealed a history of possible low fluid intake.

The mean time to perform one fingerstick followed by a successful collection was 76 seconds (range 27 - 225). Study nurse adherence to the fingerstick and blood collection protocol is summarised in Table 2.

Discussion

The successful monitoring of VL in patients receiving ART is critical in identifying treatment failure resulting from adherence issues or the development of HIV drug resistance. The world's largest population of HIV-positive individuals resides in sub-Saharan Africa, yet this region has variable and often limited access to VL testing. Development of a simple, cost-effective and readily accessible VL assay with high sensitivity is therefore needed. Currently, DBSs and novel POC platforms are being investigated as opportunities to expand access to VL monitoring in LMICs. Failure to access VL testing is frequently due to simple issues such as sample collection and transport. This study aimed to assess the feasibility of collecting 150 µL of capillary blood following a fingerstick puncture for use in a POC rapid RT-PCR testing platform and VL assay.

Although 86% of collection attempts successfully achieved a complete collection from a single fingerstick, the study nurse adherence to the protocol (Table 2) revealed omissions in several key steps that may adversely affect the success of capillary blood collection and/or the sensitivity of a subsequent VL assay. However, it should be noted that fingerstick device training may occur in an informal manner that fails to emphasise strict adherence to every step of the detailed manufacturer's protocol for fingerstick blood collection. Notably, with 0% adherence, the study nurse was never observed wearing a pair of gloves during this study. While wearing gloves ultimately has no effect on the success of blood collection, repeated omission of this step may inform the subsequent design of a blood collection device that minimises the risk of healthcare worker contact with the collected blood specimen.

The two most commonly omitted steps that may adversely affect the success of blood collection were: (*i*) patient's fingers warmed in advance by any method; and (*ii*) positioning of the patient's hand below elbow level, with 7% and 56% adherence, respectively (Table 2). Occasionally a patient with cold fingers was asked to rub their hands together quickly to generate heat. A warm cloth, which would have been ideal, was not readily available for the purpose of warming fingers. Placement of the patient's hand below the level of their elbow also presented a significant challenge in many cases. Patients were seated in a chair rather than on an elevated examination table because they were subjected to phlebotomy immediately before fingerstick testing, and the routine practice in this setting was for phlebotomy to be done with the patient in a chair. Placing the patient's hand below the level of their elbow while seated in a chair meant that the study nurse would have to bend over and painstakingly reach down in order to perform the fingerstick and observe progress in filling the collection device. Lastly, the first drop of blood was wiped away from the puncture site in only 5% of all fingersticks performed in this study (Table 2). It is hypothesised that the first drop of blood may contain interstitial fluid that could adversely affect the results of a subsequent VL assay, but this has yet to be confirmed.

Omissions in potentially important steps of the protocol suggest that when a POC VL platform and novel VL assay are first introduced to clinics, supplemental quick reference materials and/or brief maintenance training may improve the quantitative performance of a POC VL assay. When training healthcare personnel or preparing a protocol checklist for them in the future, special attention should be given to those frequently omitted steps that may adversely affect the outcome of a subsequent assay. The need for ongoing quality monitoring and training has been reported for performing rapid HIV testing and is a critical component of successful diagnostics.[15] Additionally, all necessary fingerstick materials should be conveniently located to facilitate optimal adherence to the manufacturer's fingerstick protocol.

The unique perspective of the study nurse highlighted several important benefits of performing a fingerstick over venepuncture. First, nurses or community healthcare workers with minimal training can perform fingersticks, potentially resulting in increased access to VL monitoring for patients. Nursing assistants in SA, for instance, receive 1 year of formal training and are not qualified to perform venepuncture on patients. A POC VL quantification assay relying on fingersticks rather than venepuncture could be widely utilised by this workforce. Second, fingersticks result in fewer blood spills and so decrease biohazard risk to healthcare workers, and require far less blood than venepuncture. In some cases a dehydrated and/or sick patient will provide an insufficient quantity of blood by venepuncture, requiring the test(s) to be completed again at a later time. Fingerstick blood collection may be more successful than venepuncture in certain patients. Finally, fingersticks require less counter space, fewer waste bins and less disposal of packaging materials.

The transition to fingerstick blood collection for VL testing may initially complicate the workflow in clinics that require other routine tests for ART monitoring. Venepuncture blood collection is often used for a variety of laboratory tests, including but not limited to CD4, a full blood count, liver function tests, and haemoglobin, creatinine, cholesterol and triglyceride measurements. A transition to fingerstick blood collection for VL testing would initially require phlebotomists to perform one or more fingersticks in addition to venepuncture for most patients. The overall utility of fingerstick blood collection would therefore increase if multiple POC tests could be performed simultaneously for ART monitoring.

Conclusions

Capillary blood collection was highly successful in this study, with the vast majority of patient encounters yielding 150 µL blood after only one or two fingersticks. The widespread implementation of a POC VL assay in a resource-limited setting would not be hindered by the ability to collect the targeted volume of 150 µL capillary blood when using the appropriate lancet, but would require training and ongoing quality monitoring.

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Appendix 1

Fingerstick and blood collection protocol

1. Assemble materials
- Disposable gloves
- 70% isopropyl alcohol pads
- Lancets
- Blood collection device
- Sterile gauze pad
- Warming device (moist towel or sodium acetate hand warmer)

2. Wash hands and put on pair of disposable gloves
3. Position patient and select the fingerstick puncture site
- Patient should be sitting or lying down
- Patient should have their hand in a downward position, allowing gravity to increase blood supply to the hand
- Middle or ring finger is preferable; fifth finger should not be punctured, because tissue depth is insufficient to prevent bone injury

4. Warm the site
- Use a warm, moist towel or other appropriate warming device (not exceeding 40°C/105°F) for 3 minutes; alternatively, have the patient vigorously rub their hands together to generate heat

5. Disinfect the site
- Cleanse the site using a 70% isopropyl alcohol pad
- Allow the site to air dry in order to provide effective disinfection and to prevent possible haemolysis or erroneous results from residual alcohol

6. Perform the puncture
- Have the patient hold their hand below elbow level
- Turn the patient's hand palm down
- Hold the lancet with two fingers
- Position the lancet firmly against the puncture site
- Press lancet against puncture site until release mechanism is activated

7. Discard used lancet into a sharps container
8. Collect the blood specimen
- Wipe away the first drop of blood, as this drop may contain an excess of tissue fluids that may cause erroneous results
- Position the collection device directly beneath the puncture site and avoid scraping across skin
- Gently apply intermittent pressure along finger capillaries and open the puncture slightly to maximise blood flow
- Avoid strong repetitive pressure or 'milking', as this may cause haemolysis or tissue fluid contamination of the specimen
- Blood collection is complete when both sides of the collection device appear solid red in colour

9. Cover the puncture site and dispose of all materials
- Wipe the site dry and apply direct pressure with a sterile gauze pad until bleeding has stopped
- Place all used materials in appropriate biohazard containers