Evaluation of Qualitative and Semi-quantitative HIV Point-of-care Nucleic Acid Tests

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Abstract

Background: Point-of-care (POC) nucleic acid tests (NAT) could detect acute HIV infection, resolve discordant screening results, monitor patients taking pre-exposure prophylaxis (PrEP), and identify virologic failure among persons with HIV infection (PWH) on antiretroviral treatment (ART). Real-time communication of POC NAT results can improve HIV prevention and care. While the SAMBA II POC NAT is used in Europe and Africa, it has not been evaluated in the US.

Methods: From June 2018 to September 2019, PWH and persons testing for HIV participated in Project DETECT, a study evaluating POC HIV tests in real-time. From June 2018 to March 2019, the SAMBA II Whole Blood Qual test [limit of detection (LOD) 400 copies/mL] was used for all participants. From April to August 2019, the Qual test was used on participants who had never been on ART and the SAMBA II Leukodepleted Whole Blood Semi-Q (LOD 1000 [range 500-2000] copies/mL) test was used for PWH who had started ART. Both whole blood (WB) tests were performed on unprocessed venipuncture (VP) and finger-stick (FS) WB. Results from WB tests and the SAMBA II Plasma Semi-Q test performed on frozen plasma from PWH were compared to Abbott RealTime HIV-1 PCR results (PCR) on plasma. Sensitivity, specificity, and concordance between tests were calculated.

Results: SAMBA was used in 292 visits among 249 participants; 180 (62%) visits were from PWH, and 112 (38%) from persons testing HIV-negative. 58 PWH had undetectable RNA levels by PCR, 27 had detectable but unquantifiable levels, and 95 had quantifiable RNA levels with a median 7487 (IQR 576-89630) copies/mL. The Qual test was used at 224 visits. Sensitivity of the Qual test among PWH with plasma RNA >400 copies/mL was 92% in VP and 97% in FS WB [Table]. Sensitivity of the Semi-Q test among ART-treated participants with RNA >1000 copies/mL was 100% in VP and FS WB. Specificity of the Qual test in ART-naive participants with RNA <400 copies/mL and persons testing HIV-negative was 100% in VP (n=111) and FS (n=9) WB. Four (8%) of 52 persons on ART with plasma RNA <1000 copies/mL (<20, 469, 608, and 949 copies/mL) had VP WB Semi-Q test results reported as >1000 copies/mL. Among PWH, the plasma Semi-Q had 96% and 95% concordance to WB and PCR results, respectively.

Conclusions: SAMBA's high concordance in this population suggests a role for POC NATs when starting and monitoring PrEP, detecting acute infection, and for monitoring potential virologic failure among PWH on ART.

BACKGROUND

> POC HIV tests currently approved by the US Food and Drug Administration (FDA) have relatively long window periods for detecting HIV compared to lab-based tests like pooled NATs and fail to detect people with acute HIV infection (AHI) when levels of circulating virus and risk of onward transmission are both elevated. [1]

> By providing real-time results, POC NATs can be useful in diagnosing AHI, identifying PrEP failures, monitoring patients on ART, and as a confirmatory or supplemental POC test.

> The SAMBA (**S**imple **AM**plification **B**ased **A**ssay), a POC NAT developed by Diagnostics for the Real World (DRW), has been used in several countries outside of the US as an HIV diagnostic test and ART adherence monitoring tool. [2,3]

> Project DETECT evaluated the performance of POC HIV tests, including SAMBA, among participants who were HIV-negative or of unknown status and those diagnosed with HIV. [4]

SAMBA

> Fully automated sample-in, result-out, CE-marked system. [2,3]

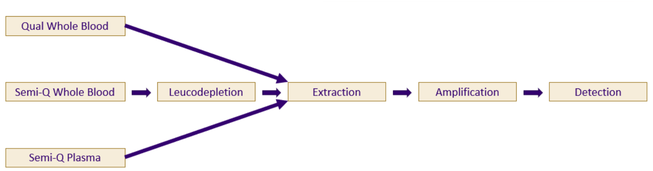
> Not FDA-approved; for investigational use only.

> Process is same across test type but concentration of reagents and time differ depending on the sample type.

> Semi-Q Whole Blood test has leucodepletion step which removes 3 logs of CD4+ cells which includes intracellular RNA and pro-viral DNA.[3]

> Leucodepletion eliminates the need for centrifugation.





METHODS

> Enrolled participants in Project DETECT from June 2018-September 2019 in Seattle, WA to compare POC HIV tests using unprocessed whole blood (WB), oral fluid (OF), and previously-frozen plasma.[4]

> Participants with discordant test results at the first study visit were invited to enroll in serial follow-up.

> SAMBA results were compared against plasma HIV-1 RNA results using Abbott RealTime HIV-1 RNA quantitative viral load assay (Abbott Molecular Diagnostics, Des Plaines, IA, USA)

Test	Specimens Tested	Approved Limit of Detection	Time to result	Targets	Testing Population
SAMBA II HIV-1 Qualitative Whole Blood Test	150 µL unprocessed venipuncture & finger stick whole blood	400 copies/mL	120 minutes	HIV RNA & proviral DNA	»6/18-3/19: All participants (n=177) »4/19-9/19: Participants who are HIV-negative or of unknown status and HIV-positive participants who are ART-naïve (n=66)
SAMBA II HIV-1 Semi-quantitative Whole Blood Test	150 µL unprocessed venipuncture & finger stick whole blood	1000 copies/mL (±0.3 log)	90 minutes	HIV RNA	»4/19-9/19: Participants who are HIV-positive and have used or are using ART (n=86)
SAMBA II HIV-1 Semi-quantitative Plasma Test	300 µL previously-frozen plasma	1000 copies/mL (±0.3 log)	90 minutes	HIV RNA	»11/18-3/19: subset of HIV-positive participants (n=8) »4/19-9/19: All HIV-positive participants (n=84)

RESULTS

Table 1. Participant characteristics, Project DETECT, 6/2018-9/2019, N=280

	n (%)
Age – median [IQR]	37 [29-49]
Race/ethnicity	
American Indian or Alaskan Native	5 (1.8)
Asian or Pacific Islander	9 (3.2)
Black or African American	65 (23.2)
Hispanic or Latinx	45 (16.1)
Multiracial	11 (3.9)
White	135 (48.2)
Unknown/refused	10 (3.6)
Gender identity	
Cisgender man	254 (90.7)
Cisgender woman	18 (6.4)
Non-binary or genderqueer	4 (1.4)
Transgender woman	4 (1.4)
HIV status	
HIV-negative	125 (44.6)
HIV-positive	155 (55.4)

Table 2. Visit characteristics among HIV-negative participants, Project DETECT, 6/2018-9/2019, N=128

	n (%)
On PrEP at visit	43 (33.6)
Not on PrEP at visit	85 (66.4)

Table 3. Visit characteristics among HIV-positive participants, Project DETECT, 6/2018-9/2019, N=202

	n (%)
On ART at visit	152 (75.2)
Not on ART at visit	50 (24.8)
Undetectable HIV RNA	66 (32.7)
Detectable but not quantifiable (<40 copies/mL)	31 (15.3)
Detectable and quantifiable (>40 copies/mL)	105 (52.0)
Median [IQR] (copies/mL)	7487 [608-91540]
<1000 copies/mL	132 (65.3)
≥1000 copies/mL	70 (34.7)

Table 4. Cumulative sensitivity of SAMBA POC NAT HIV-1 results at increasing plasma HIV-1 RNA thresholds by Abbott RealTime HIV-1 PCR among participants currently on or with a history of ART at the time of visit

Plasma HIV-1 viral load threshold (copies/mL)	SAMBA II Qual Whole Blood Test		SAMBA II Semi-Q Whole Blood Test		SAMBA II Semi-Q Plasma Test [b]
	VP WB	FS WB [a]	VP WB	FS WB [a]	
All samples	85% (69/81)	65% (15/23)	29% (25/86)	21% (6/28)	26% (20/77)
Detectable HIV-1 RNA	89% (40/45)	88% (14/16)	43% (24/56)	26% (6/23)	41% (20/49)
≥200	96% (22/23)	90% (9/10)	86% (24/28)	75% (6/8)	68% (19/28)
≥400 [c]	96% (22/23)	90% (9/10)	92% (24/26)	75% (6/8)	73% (19/26)
≥1000 [d]	100% (17/17)	100% (7/7)	100% (19/19)	100% (5/5)	95% (18/19)
≥2000	100% (15/15)	100% (6/6)	100% (19/19)	100% (5/5)	100% (18/18)
≥3000	100% (14/14)	100% (5/5)	100% (17/17)	100% (4/4)	100% (16/16)

Table 5. Cumulative sensitivity of SAMBA POC NAT HIV-1 results at increasing plasma HIV-1 RNA thresholds by Abbott RealTime HIV-1 PCR among ART-naive participants at the time of visit

Plasma HIV-1 viral load threshold (copies/mL)	SAMBA II Qual Whole Blood Test		SAMBA II Semi-Q Plasma Test [b]
	VP WB	FS WB [a]	
All samples	91% (31/34)	100% (26/26)	100% (15/15)
Detectable HIV-1 RNA	91% (31/34)	100% (26/26)	100% (15/15)
≥200	91% (31/34)	100% (26/26)	100% (15/15)
≥400 [c]	91% (31/34)	100% (26/26)	100% (15/15)
≥1000 [d]	91% (30/33)	100% (26/26)	100% (15/15)
≥2000	91% (28/31)	100% (24/24)	100% (14/14)
≥3000	91% (28/31) [e]	100% (24/24)	100% (14/14)

Abbreviations: POC=Point-of-care, NAT=nucleic acid test, PCR=polymerase chain reaction, ART=antiretroviral therapy, VP=venipuncture, WB=whole blood, FS=finger stick

a. Unprocessed FS WB only obtained from participants with acute HIV infection or those enrolled in Project DETECT extended follow-up. Acute HIV infection is defined as having a first positive HIV test within 90 days of the Project DETECT study visit.

b. VP WB is collected in EDTA tubes, centrifuged within 24 hours of blood draw, and plasma is stored as 1 mL aliquots at -70˚C in the Public Health – Seattle King County laboratory.

c. SAMBA II Qual Whole Blood Test limit of detection.

d. SAMBA II Semi-Q Whole Blood Test and SAMBA II Semi-Q Plasma Test limit of detection.

e. Three participants with plasma HIV-1 viral loads >3000 copies/mL and negative SAMBA II Qual results using VP WB described in Table 6.

> Sensitivity at LOD of SAMBA II Qual WB test among ART-naïve participants:
- ➡ 91% using venipuncture whole blood
- ➡ 100% using finger stick whole blood

> Sensitivity at LOD of SAMBA II Semi-Q WB test among participants on or with a history of ART:
- ➡ 100% using venipuncture whole blood
- ➡ 100% using finger stick whole blood

> Sensitivity at LOD of SAMBA II Semi-Q Plasma test among all participants:
- ➡ 97% (33/34) using previously-frozen plasma

> **Specificity** of SAMBA II Qual Whole Blood Test among HIV-negative participants:
- ➡ 100% (n=128) using venipuncture whole blood
- ➡ 100% (n=8) using finger stick whole blood

Table 6. Detailed case reports for result discrepancies between SAMBA POC NAT HIV-1 results and Abbott RealTime HIV-1 RNA plasma viral load

SAMBA II Test	SAMBA II Result	Plasma HIV-1 RNA (copies/mL)	EIA	VP WB Other POC Tests	FS WB Other POC Tests	OF POC	ART Use
Qual VP WB	Negative	59,880	Positive	DPP and Determine Ag/Ab Negative	N/A	Concordant Positive	ART-naïve
Qual VP WB	Negative [a]	696,400	Positive	Concordant Positive	Concordant Positive	Concordant Positive	ART-naïve
Qual VP WB	Negative	>10,000,000	Positive	Concordant Negative	N/A	Concordant Negative	ART-naïve
Semi-Q VP WB	>1000	Undetectable	Positive	Concordant Positive	N/A	Concordant Positive	On ART 1 year
Semi-Q Plasma	>1000	53	Positive	Concordant Positive	N/A	Concordant Positive	On ART 6 years

a. Participant had a positive SAMBA II Qual result using finger stick whole blood.

Table 7. Concordance of SAMBA II HIV-1 tests with plasma HIV-1 RNA by Abbott RealTime HIV-1 PCR

Type of Test	Specimens Tested	Total Result Concordance at Approved Limit of Detection (1000 copies/mL)	Total Result Concordance within Approved Range of Detection (1000 copies/mL ±0.3 log)
SAMBA II HIV-1 Semi-quantitative Whole Blood Test	Unprocessed venipuncture whole blood	93% (80/86)	98% (84/86)
SAMBA II HIV-1 Semi-quantitative Whole Blood Test	Unprocessed finger stick whole blood	96% (27/28)	100% (28/28)
SAMBA II HIV-1 Semi-quantitative Plasma Test	Previously-frozen plasma	97% (89/92)	99% (91/92)

CONCLUSION

> SAMBA is a true point-of-care test with high sensitivity using unprocessed whole blood which shows potential for detecting acute HIV infection.

> The high specificity and ability of SAMBA II Qual test to detect pro-viral DNA could be useful in PrEP starts and monitoring, and identifying PrEP failures presenting with low-level viremia.

> Anecdotally, integration of SAMBA into study testing algorithm did not cause interruption.

> Long processing time (90-120 minutes) could be limitation in non-research setting.

> Current limit of detection for Semi-Q test is 1000 copies/mL, which corresponds to the level at which drug resistance testing can be performed, but is higher than the current standard of what is considered "undetectable" in the US (200 copies/mL)

CONFLICTS OF INTEREST

> Two SAMBA II machines were loaned to Project DETECT for investigational use.

> Co-authors Assennato, Ritchie, Goel, and Lee are employed by DRW, the manufacturers of the SAMBA POC NAT.

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