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Evaluation of SAMBA II: a qualitative and semi-quantitative HIV point-of-care nucleic acid test

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Abstract

Background: Point-of-care (POC) nucleic acid tests (NAT) have potential to diagnose acute HIV infection and monitor persons taking pre-exposure prophylaxis (PrEP) or antiretroviral treatment (ART). POC NATs have not yet been evaluated in the US.

Methods: From June 2018-March 2019, we conducted a cross-sectional evaluation of the SAMBA II POC NAT. PWH and persons testing for HIV were tested with the SAMBA II qualitative (Qual) whole blood (WB) test. From April-September 2019, the Qual test was used on persons who were ART-naïve, and SAMBA II semi-quantitative (Semi-Q) WB was used with ART-experienced PWH. Both were performed on unprocessed venipuncture (VP) and, when indicated by protocol, fingerstick (FS) WB and plasma. SAMBA results were compared to Abbott RealTi*m*e HIV-1 PCR results on plasma. We calculated sensitivity, specificity, and concordance between tests.

Results: SAMBA was used in 330 visits among 280 participants: 202 (61.2%) visits from PWH, and 128 (38.8%) from HIV-negative persons. Qual test sensitivity with ART-naïve participants was 91.4% (32/35, 95% CI: 77.6–97.0%) using VP WB and 100% (27/27, 95% CI: 87.5–100%) using FS WB. Specificity was 100% using both specimen types. Concordance between the gold standard

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Conflicts of Interest

[Authors Sonny Michael Assennato, Allyson Ritchie, Neha Goel, and Helen Lee are employed by Diagnostics for the Real World (DRW), the creators of the SAMBA II test. DRW provided two SAMBA II machines for investigational use. For the remaining authors, none were declared.]

and Semi-Q at 1000 copies/mL among PWH on ART was 97.7% (86/88, 95% CI: 92.1–99.4%) and 100% (30/30, 95% CI: 88.7–100%) using VP and FS WB, respectively.

Conclusion: The SAMBA II POC NATs showed high sensitivity, specificity, and concordance with the gold-standard assay, indicating its potential use in diagnostics and monitoring. Future work will evaluate POC NAT implementation in the US.

Keywords

point-of-care; nucleic acid test; acute HIV infection; HIV testing; ART monitoring

Introduction:

At the end of 2018, 1.2 million people in the United States (US) were living with HIV, and an estimated 14% of which did not know of their positive (+) HIV status.[1,2] In order to achieve the first 95 of the Joint United Nations Programme on HIV/AIDS (UNAIDS) 95–95-95 goals,[3] current gaps in HIV testing must be addressed. Point-of-care (POC) HIV tests can offer a decentralized solution to providing more people with their test results and increase individuals' awareness of HIV status.[4–7]

There are several POC HIV tests approved for use by the US Food and Drug Administration (FDA), but many of these antibody- or antigen-based tests are limited by relatively long window periods for detecting HIV when compared to laboratory-based tests.[8–18] The inability to detect persons with acute HIV infection when levels of circulating virus are high increases the risk of onward HIV transmission.[19–22] Additionally, identifying persons with early HIV infection can expedite initiation of antiretroviral therapy (ART), which is associated with the delay of HIV progression,[23–28] decreased HIV transmission, and longer survival.[29,30]

The use of POC nucleic acid tests (NAT) for detection of acute HIV infection and early infant diagnosis is increasing globally.[31–33] To date, several POC NATs, including the Alere q HIV-1/2 Detect and HIV-1/2 VL plasma ("m-PIMA HIV-1/2 VL") assays (Alere Technologies GmbH, Jena, Germany), Xpert HIV-1 Qual and Viral Load Assays (Cepheid AB, Solna, Sweden), and the Aptima HIV-1 Quant Dx Assay (Hologic, Inc., San Diego, USA) have received World Health Organization (WHO) pre-qualification.[34] POC NATs have shown promise in expediting the receipt of HIV testing results and ART initiation, especially in resource-limited settings.[7,35–37] By shifting the burden away from centralized laboratories, POC NATs can reduce the time, cost, and resources it takes to transport, store, and process specimens for laboratory-based NATs.[38] Evaluations of POC NATs outside the US have shown high sensitivity and specificity.[34] Additionally, sustained viral suppression and retention in HIV care coincided with implementation of POC NATs in South Africa, indicating potential for ART monitoring.[7]

The Simple Amplification Based Assay version II (SAMBA II), developed by Diagnostics for the Real World (DRW),[39,40] is a fully automated sample-in, result-out POC HIV-1 NAT. The Conformité Européenne (CE)-marked SAMBA II Qualitative (Qual) whole blood test can detect plasma ribonucleic acid (RNA), intracellular RNA, and pro-viral

deoxyribonucleic acid (DNA) for the diagnosis of HIV infection. The CE-marked SAMBA II Semi-quantitative (Semi-Q) is a semi-quantitative whole blood test that was developed as an ART monitoring tool.[40,41] The Semi-Q has an internal leukodepletion step, which eliminates the need for centrifugation to isolate viral RNA in plasma, making the Semi-Q a true POC NAT.[42] In previous studies, the SAMBA II Qual and Semi-Q tests have also shown high sensitivity, specificity, and concordance with laboratory-based polymerase chain reaction (PCR) assays.[39–41,43]

To date, the SAMBA II Qual and Semi-Q POC NATs have not been evaluated in the US. We evaluated the SAMBA II Qual and Semi-Q whole blood and plasma tests as part of the Diagnostic Evaluation To Expand Critical Testing Technologies (Project DETECT), a Centers for Disease Control and Prevention (CDC)-funded evaluation of multiple POC HIV tests.[44,45] We sought to determine the sensitivity, specificity, and concordance of the SAMBA tests with laboratory-based PCR assays as a diagnostic test in HIV-negative or newly diagnosed persons and as a monitoring tool among persons on ART.

Methods:

Population

Project DETECT methods have been described in detail elsewhere.[44] Briefly, Project DETECT was a cross-sectional, prospective study designed to evaluate the performance of POC HIV tests in real-time with unprocessed anticoagulated venipuncture (VP) whole blood (WB), fingerstick (FS) WB, and oral fluid (OF) specimens. Project DETECT enrolled a convenience sample of 1) HIV-negative participants who were seeking HIV testing at the Public Health – Seattle & King County (PHSKC) Sexual Health Clinic (formerly the PHSKC STD Clinic), 2) participants with known HIV infection (diagnosed > 90 days prior) recruited from a large HIV care clinic, with oversampling of persons with detectable HIV-1 RNA levels, and 3) participants who had suspected acute HIV infection or who were newly diagnosed (<90 days prior) with any stage of HIV infection. Suspected acute HIV infection was defined as participant-reported potential HIV exposure, symptoms consistent with acute HIV infection, and/or a recent reactive HIV test.[44]

A 4mL tube of anticoagulated (EDTA) WB was used to perform all POC tests, including SAMBA II and Determine HIV-1/2 Antigen/Antibody (Ag/Ab) Combo (Abbott Molecular Inc., Des Plaines, IL) tests. Dried blood samples (DBS) were also spotted from this same tube, dried, and stored for future testing. SAMBA II tests were incorporated into Project DETECT in June 2018 and participants were recruited through the end of the study contract period in September 2019. To assess test sensitivity during seroconversion, participants with discordant HIV test results were offered enrollment into longitudinal follow-up for up to one year of serial visits. Participant demographics and ART or pre-exposure prophylaxis (PrEP) use were self-reported.

All participants provided written or verbal consent and were compensated $40 or $50, depending on procedures conducted during the study visit. Project DETECT was approved by the University of Washington Human Subjects Division (#00001637).

SAMBA Testing

All participants were tested using SAMBA with the VP WB sample. A subset of participants who were newly diagnosed with HIV, participants with suspected acute infection, and those enrolled in the longitudinal arm of the study also had SAMBA performed on FS samples. From June 2018 through March 2019, all participants, including those on ART, were tested using the SAMBA II Qual whole blood test, which has a limit of detection of 400 copies/mL of RNA. Although the SAMBA II Qual whole blood test is only indicated as a diagnostic tool due to its ability to detect DNA, it was used on participants who were previously known to be positive in our initial evaluation phase to determine its sensitivity and because the SAMBA II Semi-Q whole blood test was not yet available for our use. From April through September 2019, ART-experienced PWH were tested with the SAMBA II Semi-Q whole blood test, and the SAMBA II Qual test was only used for participants without a prior diagnosis of HIV infection and PWH who were ART-naïve at the time of the study visit. The SAMBA II Semi-Q whole blood test reports results as less than or greater than 1000 copies/mL. All SAMBA II testing was conducted by Project DETECT staff in a study-specific exam room. The POC NAT was conducted before laboratory-based results were available, therefore, the SAMBA II test operators were not aware of the laboratory-based HIV-1 RNA levels.

Any SAMBA II Qual or Semi-Q test that did not return a valid result on the reader device was considered nonvalid and repeated if remaining VP WB was available; if no VP WB remained or if the nonvalid result was from a FS WB sample, we reported the final test result as nonvalid. Results of “aborted”, “no result”, “read failure”, and “invalid” from the reader device were considered nonvalid for the purposes of this evaluation. The product characteristics and testing population, and participant testing flow are described in Table 1 and in the Supplemental Digital Content 1 figure, respectively.

The SAMBA II Semi-Q plasma test was performed by research staff using frozen plasma specimens from a subset of participants who were HIV-positive with stored plasma prior to April 2019 and on all PWH from April through the end of September 2019 in order to increase the sample size for the concordance evaluation. Any nonvalid SAMBA II Semi-Q plasma test was repeated with remaining plasma until a valid result was produced.

Laboratory Testing

Laboratory-based NAT was performed in real time for all participants using Abbott RealTi*m*e HIV-1 assay (Abbott Molecular Inc., Des Plaines, IL), the standard NAT used by the PHSKC Laboratory.[46] Individual NATs were run for any participant with at least one reactive POC test among the panel of POC tests used in Project DETECT;[44] plasma from participants with concordant negative POC results were pooled in groups of ten. The remaining plasma was stored as 1mL aliquots at −70 degrees Celsius. Laboratory staff were unaware of all SAMBA II results.

DBS cards were dried, initially stored at −70 degrees Celsius at the PHSKC laboratory, and then shipped under dry ice to the CDC. Three DBS with discordant results between laboratory-based NAT and the SAMBA II Qual test were tested for HIV-1 RNA using a

modification to the DBS protocol on the Abbott m Sample Preparation (m2000sp) RealTi*m*e HIV-1 Viral Load kit.[47,48] Four 6 mm punches, or approximately 50 μL of WB, were used in a laboratory-validated protocol.[49,50] Blood was eluted in 1.3 mL of Lysis buffer in master mix tubes, both provided with the Abbott m2000sp system. Samples were incubated at 55 degrees Celsius for 30 minutes, vortexed, spun, and placed on m2000 for sample extraction and viral load quantification according to the standard HIV-1 RNA quantitative assay 1.0 mL protocol.[51]

Following three discordant results (described below) between the SAMBA II Qual whole blood test and Abbott RealTi*m*e PCR assay, supplemental quality assurance checks were conducted by the manufacturer on unused test kits from the same lot. The test kits were used to test WB samples spiked with HIV Lai at 10 million copies/mL and were compared to another lot of SAMBA II Qual whole blood test kits.

Statistical Analysis

We calculated the overall specificity and sensitivity of the SAMBA II Qual whole blood test across all tested samples and at HIV-1 RNA thresholds of detectable (>40), 200, 400, 1000, 2000, and 3000 copies/mL. To evaluate the SAMBA II Semi-Q whole blood and Semi-Q plasma tests, we calculated the percent concordance of each SAMBA test with the Abbott RealTi*m*e PCR results at SAMBA's approved plasma HIV-1 limit of detection of 1000 copies/mL and within the range of 1000 copies/mL +/− 0.3 log.[41] If no valid SAMBA result was obtained using a specimen, the observation was excluded from the analyses. Sensitivity, specificity, and concordance are presented with 95% confidence intervals (CI) that were calculated using the Wilson Score method.[52] Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC).

Results:

Between June 2018 and September 2019, 280 participants were tested with a SAMBA test during 330 Project DETECT study visits. Participant demographic and visit characteristics are shown in Table 2. The majority (90.7%) of participants were cisgender men. There were 128 visits by 125 HIV-negative participants during the evaluation period; current PrEP use was reported in 34.4% (44/128) of visits. There were 202 total study visits by 155 participants who were HIV-positive during the evaluation. Current ART use was reported at 74.8% (151/202) of visits with PWH.

Table 3 shows the cumulative sensitivity of the SAMBA II Qual whole blood test at increasing HIV-1 RNA thresholds using the Abbott RealTi*m*e PCR assay as the gold standard, stratified by self-reported ART use and specimen type. Among persons with no history of ART use, the sensitivity of the SAMBA II Qual whole blood test at the limit of detection of 400 copies/mL was 91.4% [95% CI: 77.6–97.0%] (32/35) using VP WB and 100% [95% CI: 87.5–100.0%] (27/27) using FS WB (Table 3). At the HIV-1 RNA threshold of 3000 copies/mL, the sensitivity using unprocessed VP WB was 90.6% [95% CI: 75.8–96.8%] (29/32). For comparison, the sensitivity of the Determine HIV-1/2 Ag/Ab Combo test among the same group of persons with no history of ART use was 94.3% (33/35) using the same sample of VP WB.

The SAMBA II Qual whole blood test was used at 128 visits among 125 HIV-negative participants. One visit was excluded due to nonvalid results. The specificity of the SAMBA II Qual whole blood test was 100% [95% CI: 97.1–100%] using unprocessed VP WB (127/127) and 100% [95% CI: 64.6–100%] using FS WB (7/7). For comparison, the Determine HIV-1/2 Ag/Ab Combo POC test also had a 100% specificity using unprocessed VP WB among the same sample of participants.

Table 4 shows the concordance of the SAMBA II Semi-Q whole blood test and Abbott RealTi*m*e PCR at the limit of detection of 1000 copies/mL, among ART-experienced participants. Test concordance was 93.2% [95% CI: 85.9–96.8%] (82/88) using unprocessed VP WB and 96.7% [95% CI: 83.3–99.4%] (29/30) using FS WB. When we consider the range of detection of the SAMBA II Semi-Q test, the concordance using VP and FS WB was 97.7% [95% CI: 92.1–99.4%] (86/88) and 100% [95% CI: 88.7–100%] (30/30), respectively. Among a subset of HIV-positive participants with available plasma specimens, concordance with the Abbott RealTi*m*e PCR assay at the level of 1000 copies/mL of the SAMBA II Semi-Q plasma test was 96.9% [95% CI: 91.2–98.9%] (93/96).

There were three participants with HIV-1 RNA levels greater than 400 copies/mL (the SAMBA II Qual test limit of detection) who had negative SAMBA II Qual results using unprocessed VP WB. The panel of their Project DETECT POC test results are shown in Table 5. Case 1 presented at their first study visit with symptoms consistent with acute HIV infection but did not initiate ART after all POC HIV tests, including the SAMBA II Qual whole blood test using VP WB, were negative. However, SAMBA-II Semi-Q plasma test conducted on previously frozen plasma at a later date was >1000 copies/mL. At the first visit, Case 1 had a reactive laboratory-based antigen-antibody test (HIV-1/HIV-2 Combo EIA) and an HIV-1 RNA level of over 10,000,000 copies/mL. Nine days later at a second visit with Case 1, both SAMBA II Qual whole blood test and Semi-Q plasma test were positive when the HIV-1 RNA level was 2,740,000 copies/mL. Case 2 and 3 had multiple HIV-positive results on FDA-approved POC tests and HIV-1 RNA levels of 59,880 and 696,400 copies/mL, respectively, but tested negative by SAMBA II Qual using VP WB. For all three cases described above, the HIV-1 RNA levels from DBS made with the same VP WB sample used for the initial SAMBA II Qual tests were all positive: >10,000,000 copies/mL for Case 1’s first visit, 25,119 copies/mL for Case 2, and 165,959 copies/mL for Case 3.

Two participants, shown in Table 5 as Case 4 and 5, presented with established HIV infection and had been on ART for 1 and 6 years, respectively. Case 4 had a SAMBA II Semi-Q whole blood test result of >1000 copies/mL using unprocessed VP WB, despite having an undetectable HIV-1 RNA level as measured by Abbott RealTi*m*e PCR. The SAMBA II Semi-Q plasma result from the visit was <1000 copies/mL. Similarly, Case 5 had a result of >1000 copies/mL using the SAMBA II Semi-Q plasma test with an HIV-1 RNA level measured at 53 copies/mL, while the SAMBA II Semi-Q test result using unprocessed VP WB was <1000 copies/mL.

The proportion of initial nonvalid test results among SAMBA II Qual whole blood tests conducted was 6.1% (15/244) using VP WB and 3.4% (2/58) with FS WB. Among Semi-Q

whole blood tests, 3.5% (3/86) and 9.4% (3/32) were initially nonvalid using VP and FS WB, respectively. The proportion of initial nonvalid test results among SAMBA II Semi-Q plasma tests using previously frozen plasma was 16.7% (16/96). When remaining or stored specimens were available, nonvalid results were retested until a valid result was eventually obtained.

Discussion:

In our evaluation, the first of its kind in the US, the SAMBA II Qual and Semi-Q tests had high sensitivity, specificity, and concordance with a laboratory-based NAT when testing unprocessed VP or FS WB and previously-frozen plasma specimens. Our results support additional plans for qualitative POC NAT to be evaluated for its utility in diagnosis of acute HIV infection and supplemental testing and for quantitative and semi-quantitative tests to be evaluated in POC viral load monitoring.

The SAMBA II Qual whole blood test had a sensitivity of 91.4% using VP WB and 100% using FS WB among ART-naïve individuals. The observed sensitivity shows potential for detecting acute HIV infection with a shorter window period compared to other POC HIV tests that are currently approved by the FDA. However, the negative SAMBA II Qual whole blood test results in Cases 1, 2, and 3 in Table 5 raise some concerns about its utility, particularly with Case 1, whose POC tests were all negative. Although we are not able to completely rule out user error or specimen mislabeling, there was only one other participant enrolled on the same day as Case 1. We reduced the likelihood of specimen mix-up by confirming positive HIV-1 RNA levels in the DBS created from the same WB sample as had been tested by SAMBA. Although the DBS confirmed that the specimen had detectable HIV-1 RNA, an inhibitor in the cellular portion of the samples may have affected the HIV-1 signal, causing the SAMBA II Qual whole blood test to yield negative results. No stored specimens remained to test this theory. Device issues are another possible explanation. Cases 1–3 were tested with test kits from the same manufacturer lot. The used test cartridges were discarded after the initial tests were conducted, which did not allow for additional troubleshooting of discordant results seen in our evaluation. However, supplemental quality assurance checks on unused test kits from the same lot returned positive results with slightly reduced signals, though still within an expected range.

The 100% specificity of the SAMBA II Qual whole blood test in this evaluation indicates that it could be useful to rule out false positive results that can occur with other POC HIV tests,[8,45] and it could function as a supplemental test following a single positive or discordant POC test results, depending on the screening strategy.[53,54] Compared to POC HIV diagnostics currently available in the US, one unique quality of the SAMBA II Qual test and other qualitative POC NATs is their ability to detect pro-viral DNA in addition to plasma RNA. Our data show that when using VP WB, the SAMBA II Qual whole blood test had a sensitivity of 85% among ART-experienced HIV-positive participants, including those with undetectable and low-level viremia. Although the SAMBA II Qual whole blood test is not intended to be used in this population, the findings suggest that qualitative POC NAT could help providers distinguish true PrEP failures with undetectable plasma viremia

from HIV-negative persons with false-positive screening test results.[55–58] This quality may become increasingly relevant as more individuals initiate PrEP globally.[59]

Currently available POC NATs are not perfect products. The quantitative POC NAT used most widely is the Cepheid GeneXpert (Cepheid AB, Solna, Sweden),[34] which has a limit of detection of 40 copies/mL but requires centrifugation and testing of 1mL plasma. The SAMBA II Semi-Q whole blood test has a fully-integrated leukodepletion step that eliminates cell-associated viral DNA in order to assess plasma RNA only, but it currently provides a semi-quantitative result at the limit of detection of 1000 copies/mL. This threshold was set across many manufacturers because it was the level determined to be clinically significant by the World Health Organization in 2013.[60] However, the limit of 1000 copies/mL may no longer be sufficient given changes in clinical algorithms and the evidence that persons with an HIV-1 RNA level <200 copies/mL are extremely unlikely to transmit the virus.[61]

One other limitation of many POC NATs is that they take one to two hours to produce results, which may make these devices less useful for testing at POC. There may be settings where this turnaround time is shorter than a standard clinic visit. However, in the US, two hours may be longer than most testing or ART monitoring appointments. Shortening the time to result could increase the number of patients who are able to receive results within an average appointment window or in community-based settings.

There are additional limitations specific to our research study. First, the sample size was small, particularly for unprocessed FS WB specimens. Second, due to limited product availability, the SAMBA II Qual whole blood test was used to test participants with established HIV infection who were ART-experienced, which was an off-label use of the test. However, because the POC NAT results were not used for clinical management, the off-label use did not impact study participants. Third, recruitment for this evaluation was conducted at two clinics within close proximity in Seattle, Washington, and it is likely that only a single HIV-1 subtype was present in the population. Fourth, all testing with unprocessed WB was conducted by one user, so agreement between test operators could not be assessed. Fifth, we were not able to distinguish between operator and machine error when test results were nonvalid. Lastly, plasma specimens from participants with concordant negative POC results were pooled in groups of ten, which may have reduced the sensitivity of the laboratory-based NAT used as our gold standard.

Additional evaluations are needed in order to determine the utility and the cost-effectiveness of POC NAT in different settings within the US, including community clinics and HIV testing sites. Studies using qualitative POC NAT in areas with high HIV incidence are also warranted to determine test sensitivity during acute and early HIV infection. Finally, implementation studies, like the CDC-funded GAIN study (U01PS005196), are needed to determine how qualitative and semi-quantitative or quantitative POC NATs can best be integrated into HIV testing algorithms, PrEP initiation and monitoring visits, and viral load monitoring as part of HIV clinical care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement:

The data generated during and analyzed in the current study are not publicly available but can be available from the corresponding author on reasonable request, pending approval from project lead investigators.

Abbreviations:

HIV	Human immunodeficiency virus
POC	point-of-care
US	United States
FDA	Food and Drug Administration
ART	antiretroviral therapy
NAT	nucleic acid test
WHO	World Health Organization
SAMBA	Simple Amplification Based Assay
DRW	Diagnostics for the Real World
CE	Conformité Européenne
Qual	Qualitative
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
Semi-Q	Semi-quantitative
PCR	polymerase chain reaction
DETECT	Diagnostic Evaluation To Expand Critical Testing Technologies

CDC	Centers for Disease Control and Prevention
VP	venipuncture
WB	whole blood
FS	fingerstick
OF	oral fluid
PHSKC	Public Health – Seattle & King County
DBS	dried blood spot
PrEP	pre-exposure prophylaxis
CI	confidence interval

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Table 1.

Product characteristics for SAMBA II Tests and Methods in Project DETECT, June 2018 – September 2019.

Test [a]	Specimens used	Specimen volume	Time to result	Limit of detection	Targets	Testing population, June 2018 – March 2019	Testing population, April – September 2019
SAMBA II Qual Whole Blood Test	Unprocessed venipuncture and fingerstick whole blood	150 μL	120 minutes	400 copies/mL	HIV RNA & proviral DNA	All participant visits (n=178)	Visits with participants who were HIV-negative or of unknown status (n=51) Visits with HIV-positive participants who were ART-naïve (n=15)
SAMBA II Semi-Q Whole Blood Test	Unprocessed venipuncture and fingerstick whole blood	150 μL	90 minutes	1000 copies/mL (±0.3 log)	HIV RNA	Visits with participants who were HIV-positive, had used or were using ART, and were enrolled in longitudinal arm of study (n=13)	Visits with participants who were HIV-positive and had used or were using ART (n=73) [b]
SAMBA II Semi-Q Plasma Test	Previously frozen plasma	300 μL	90 minutes	1000 copies/mL (±0.3 log)	HIV RNA	Visits with participants who were HIV-positive and had available plasma specimens (n=9).	Visits with participants who were HIV-positive and had available plasma specimens (n=87)

SAMBA: Simple Amplification Based Assay, Qual: Qualitative, Semi-Q: Semi-quantitative, μL: microliters, mL: milliliters, RNA: ribonucleic acid, DNA: deoxyribonucleic acid, ART: antiretroviral therapy.

a. The SAMBA II tests are not approved by the United States Food and Drug Administration (FDA) and were used for investigational purposes in this evaluation.

b. Two SAMBA II Semi-Q whole blood tests used to test a participant who was HIV-negative at separate study visits are excluded from this analysis.

Table 2.

Characteristics of Project DETECT participants and visits, June 2018 – September 2019.

	All participants n (%)	HIV-negative participants n (%)	HIV-positive participants n (%)
	N=280	N=125	N=155
Age (years) – median [IQR]	37 [29–49]	34 [26–42]	42 [32–52]
Race/ethnicity			
American Indian or Alaskan Native	5 (1.8)	0 (0.0)	5 (3.2)
Asian or Pacific Islander	9 (3.2)	6 (4.8)	3 (1.9)
Black or African American	65 (23.2)	17 (13.6)	48 (31.0)
Hispanic or Latinx [a]	45 (16.1)	19 (15.2)	26 (16.8)
Multiracial	11 (3.9)	3 (2.4)	8 (5.2)
White	135 (48.2)	74 (59.2)	61 (39.4)
Unknown/refused	10 (3.6)	6 (4.8)	4 (2.6)
Current gender identity [b]			
Cisgender man	254 (90.7)	122 (97.6)	132 (85.2)
Cisgender woman	18 (6.4)	0 (0.0)	18 (11.6)
Non-binary or genderqueer	4 (1.4)	1 (0.8)	3 (1.9)
Transgender woman	4 (1.4)	2 (1.6)	2 (1.3)
	All visits n (%)	**Visits with HIV-negative participants n (%)**	**Visits with HIV-positive participants n (%)**
	N=330	N=128	N=202
PrEP use at visit			
Currently taking PrEP	N/A	44 (34.4)	N/A
No PrEP use in previous three months	N/A	84 (65.6)	N/A
ART use at visit			
Currently taking ART	N/A	N/A	151 (74.8)
Not currently on or never used ART	N/A	N/A	51 (25.2)
HIV-1 RNA level at visit [c]			
Undetectable HIV-1 RNA	N/A	N/A	66 (32.7)
Detectable but not quantifiable (<40 copies/mL)	N/A	N/A	31 (15.3)
Detectable and quantifiable (>40 copies/mL)	N/A	N/A	105 (52.0)
Median [IQR] (copies/mL)	N/A	N/A	7487 [608–89630]
HIV-1 RNA level at visit [c]			
<1000 copies/mL	N/A	N/A	132 (65.4)
≥1000 copies/mL	N/A	N/A	70 (34.7)

IQR: interquartile range, PrEP: pre-exposure prophylaxis, N/A: not applicable, ART: antiretroviral therapy, RNA: ribonucleic acid

[a] Persons who identified as Hispanic or Latino ethnicity were classified as Hispanic/Latino regardless of race.

[b] Current gender identity is ascertained using a two-step process asking sex assigned at birth and current gender identity.

[c] Abbott RealTime HIV-1 assay.

Table 3.

Cumulative sensitivity of SAMBA II Qual Whole Blood HIV-1 POC NAT results at increasing plasma HIV-1 RNA thresholds by Abbott RealTi*m*e HIV-1 PCR, by specimen type and self-reported participant antiretroviral therapy use.

Plasma HIV-1 RNA threshold (copies/mL) [a]	Participants currently on or with a self-reported history of ART use [b] Sensitivity [95% CI] (n/N)		Participants with no history of ART use Sensitivity [95% CI] (n/N)	
	Venipuncture WB [c]	Fingerstick WB [d]	Venipuncture WB	Fingerstick WB [d,e]
All samples	85.0% [75.6–91.2%] (68/80)	61.9% [40.9–79.3%] (13/21)	91.4% [77.6–97.0%] (32/35)	100.0% [87.5–100.0%] (27/27)
Detectable (≥40 copies) HIV-1 RNA	88.6% [76.0–95.1%] (39/44)	85.7% [60.1–96.0%] (12/14)	91.4% [77.6–97.0%] (32/35)	100.0% [87.5–100.0%] (27/27)
≥200	96.0% [80.5–99.3%] (24/25)	88.9% [56.5–98.0%] (8/9)	91.4% [77.6–97.0%] (32/35)	100.0% [87.5–100.0%] (27/27)
≥400 [f]	95.5% [78.2–99.2%] (21/22)	87.5% [52.9–97.8%] (7/8)	91.4% [77.6–97.0%] (32/35)	100.0% [87.5–100.0%] (27/27)
≥1000	100.0% [80.6–100.0%] (16/16)	100.0% [56.6–100.0%] (5/5)	91.2% [77.0–97.0%] (31/34)	100.0% [87.5–100.0%] (27/27)
≥2000	100.0% [78.5–100.0%] (14/14)	100.0% [51.0–100.0%]] (4/4)	90.6% [75.8–96.8%] (29/32)	100.0% [86.7–100.0%] (25/25)
≥3000	100.0% [77.2–100.0%] (13/13)	100.0% [43.9–100.0%]] (3/3)	90.6% [75.8–96.8%] (29/32)	100.0% [86.7–100.0%] (25/25)

RNA: ribonucleic acid, ART: antiretroviral therapy, CI: confidence interval, WB: whole blood

a. Abbott RealTi*m*e HIV-1 assay.

b. SAMBA II Qual whole blood test was performed on participants who were currently on or had a self-reported history of ART use prior to obtaining the SAMBA II Semi-Q whole blood test. This test is not indicated for use in persons known to be HIV-positive with a history of ART.

c. Excludes one participant who had an HIV-1 RNA level of 7,168 copies/mL and a nonvalid SAMBA II Qual result.

d. Fingerstick whole blood testing was performed on a subset of participants who were newly diagnosed with HIV, with suspected acute infection, and/or enrolled in the longitudinal arm of Project DETECT.

e. Excludes one participant who had a plasma HIV-1 RNA level of 60,110 copies/mL and a nonvalid SAMBA II Qual result.

f. Limit of detection for the SAMBA II HIV-1 Qual whole blood test

Table 4.

Concordance of the SAMBA Semi-Q HIV-1 POC NAT with HIV-1 RNA thresholds by Abbott RealTi*m*e HIV-1 PCR at the limit and within the range of detection among HIV-positive participants, by specimen type.

	Concordance [95% CI] (n/N)		
	Venipuncture WB [a]	**Fingerstick WB** [b]	**Plasma Test** [c]
Total Result Concordance at Limit of Detection (1000 copies/mL)	93.2% [85.9–96.8%] (82/88)	96.7% [83.3–99.4%] (29/30)	96.9% [91.2–98.9%] (93/96)
Total Result Concordance within Range of Detection (1000 copies/mL ±0.3 log)	97.7% [92.1–99.4%] (86/88)	100% [88.7–100%] (30/30)	99.0% [94.3–99.8%] (95/96)

WB: whole blood, CI: confidence interval.

[a] Includes antiretroviral therapy (ART)-experienced HIV-positive participants.

[b] Includes ART-experienced, HIV-positive participants enrolled in the longitudinal arm of the study, those who had suspected acute HIV infection, or who were newly diagnosed with any stage of HIV infection. Excludes two participants who had HIV-1 RNA levels of 314 and 94,720 copies/mL and nonvalid SAMBA II Semi-Q results.

[c] Includes HIV-positive participants with available plasma specimens.

Table 5.

Detailed case reports for result discrepancies between SAMBA POC NAT HIV-1 results and Abbott RealTi*m*e HIV-1 RNA level.

Case	DPP OF [a]	OraQuick VP WB [b]	Determine Ag/Ab VP WB [c]	DPP VP WB [a]	INSTI VP WB [d]	OraQuick OF [b]	Geenius VP WB [e]	SAMBA II Qual VP WB [f]	SAMBA Semi-Q VP WB [g]	SAMBA Semi-Q Plasma [h]	HIV-1 RNA (copies/ mL) [i]	HIV-1 RNA from DBS (copies/ mL) [j]	4th gen EIA [k]	ARTuse [l]
1 visit 1 [m]	NEG	NEG	Ag/Ab NEG	NEG	NEG	NEG	N/A	NEG	N/A	>1000 copies	>10,000,000	>10,000,000	POS	None
1 visit 2 [n]	NEG	POS	Ag/Ab POS	POS	POS	NEG	POS	POS	N/A	>1000 copies	2,740,000	ND	POS	None
2	POS	POS	Ag/Ab NEG	NEG	POS	POS	POS	NEG	N/A	>1000 copies	59,880	25,119	POS	None
3 [o]	POS	POS	Ab POS	POS	POS	POS	POS	NEG	N/A	ND	696,400	165,959	POS	None
4	POS	POS	Ab POS	POS	POS	POS	POS	N/A	>1000 copies	<1000 copies	UNDET	ND	POS	1 year
5	POS	POS	Ab POS	POS	POS	POS	POS	N/A	<1000 copies	>1000 copies	53	ND	POS	6 years

SAMBA: Simple Amplification Based Assay, Semi-Q: semi-quantitative, mL: milliliters, RNA: ribonucleic acid, OF: oral fluid, ART: antiretroviral therapy, VP: venipuncture, WB: whole blood, POS: positive, NEG: negative, ND: not done, N/A: not applicable, Ag: antigen, Ab: antibody, UNDET: undetectable, ART: antiretroviral therapy.

a. Dual Path Platform (DPP) HIV 1/2 Assay (Chembio Diagnostics System, Inc).

b. OraQuick ADVANCE Rapid HIV 1/2 Antibody test (Orasure Technologies, Inc).

c. Determine HIV 1/2 Ag/Ab Combo (Abbott Laboratories).

d. INSTI HIV-1/HIV-2 Rapid Antibody Test (bioLytical Laboratories, Inc).

e. Geenius HIV 1/2 Supplemental Assay (Bio-Rad Laboratories, Inc).

f. SAMBA II Qual whole blood test (Diagnostics for the Real World, Ltd).

g. SAMBA II Semi-Q whole blood test (Diagnostics for the Real World, Ltd).

h. SAMBA II Semi-Q Plasma test (Diagnostics for the Real World, Ltd).

i. RealTi*m*e HIV-1 assay (Abbott Laboratories).

j. Tested using frozen and stored DBS.

k. GS HIV-1/HIV-2 Combo EIA (Bio-Rad Laboratories, Inc).

[l] ART use is self-reported; No participants reported pre-exposure prophylaxis (PrEP) use in the three months before their study visit.

[m] Self-reported last negative test was at some point within 23 days of the study visit. Participant reported symptoms of fever, diarrhea, sore joints and/or muscle pain, and fatigue.

[n] Visit 2 was nine days after visit 1. OraQuick, Determine Ab, DPP, and INSTI performed on fingerstick blood were all positive.

[o] DPP, Determine Ab, INSTI, OraQuick, Geenius, and SAMBA II Qual tests performed on fingerstick blood were all positive.