Multicountry Validation of SAMBA - A Novel Molecular Point-of-Care Test for HIV-1 Detection in Resource-Limited Setting

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Introduction: Early diagnosis of HIV-1 infection and the prompt initiation of antiretroviral therapy are critical to achieving a reduction in the morbidity and mortality of infected infants. The Simple AMplification-Based Assay (SAMBA) HIV-1 Qual Whole Blood Test was developed specifically for early infant diagnosis and prevention of mother-to-child transmission programs implemented at the point-of-care in resource-limited settings.

Methods: We have evaluated the performance of this test run on the SAMBA I semiautomated platform with fresh whole blood specimens collected from 202 adults and 745 infants in Kenya, Uganda, and Zimbabwe. Results were compared with those obtained with the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 assay as performed with fresh whole blood or dried blood spots of the same subjects, and discrepancies were resolved with alternative assays.

Results: The performance of the SAMBA and CAP/CTM assays evaluated at 5 laboratories in the 3 countries was similar for both adult and infant samples. The clinical sensitivity, specificity, positive predictive value, and negative predictive value for the SAMBA test were 100%, 99.2%, 98.7%, and 100%, respectively, with adult samples, and 98.5%, 99.8%, 99.7%, and 98.8%, respectively, with infant samples.

Discussion: Our data suggest that the SAMBA HIV-1 Qual Whole Blood Test would be effective for early diagnosis of HIV-1 infection in infants at point-of-care settings in sub-Saharan Africa.

Key Words: HIV-1, point-of-care, early infant diagnosis, nucleic acid test

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INTRODUCTION

The UNAIDS 2014 Gap Report estimated that there were 3.2 million children infected with HIV worldwide, and 91% of whom were living in sub-Saharan Africa.[1] New infections in children had fallen by 50% between 2010 and 2015,[2] indicating that substantial progress is being made in the prevention of mother-to-child transmission. In key high-burden countries in Africa, however, early infant diagnosis (EID) coverage was estimated to range from as high as 42% in Kenya to as low as 4% in Chad and Nigeria, so that challenges remain to the goal of reducing childhood morbidity and mortality.[1] Early diagnosis of HIV-1 infection in infants followed by prompt initiation of antiretroviral therapy (ART) is critical to prevent progression of disease and to reduce mortality, given that about one-third of infected children die by their first birthday and half die by their second.[3] WHO guidelines therefore recommend that ART be initiated without delay in infants with an initial positive virological test result.[4]

In contrast to adults, for whom rapid antibody tests are readily available and affordable, accurate serological HIV-1 diagnosis in children younger than 18 months is not possible because of the passive transfer of maternal antibodies during pregnancy. Diagnosis of HIV-1 infection in infants therefore

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requires sophisticated and specialized laboratory diagnostic tests to detect viral nucleic acid or viral products such as the p24 antigen.[5–8] The expense, complexity, and infrastructure requirements of these technologies mean that access to timely testing services is highly limited for HIV-exposed infants in many resource-limited settings. Low EID rates contribute substantially to the poor or nonexistent status of links between prevention of mother-to-child transmission and pediatric treatment programs, with only 49% of HIV-infected infants in low-and middle-income countries worldwide receiving treatment by the end of 2015.[2] The current practice for EID in many resource-limited settings is based on the collection of dried blood spot (DBS) samples on filter paper from infants attending peripheral clinics and the forwarding of these specimens to centralized laboratories for molecular testing to detect HIV-1 genome. Although testing of samples may take only a few hours to a day, the time taken in their collection, storage, transportation, processing, and forwarding of results can result in delays of up to 2 months before results are provided to the patient.[9] As a result of this long turnaround time, loss to follow-up has become one of the major problems in the provision of effective pediatric treatment and care for HIV-1–infected children.[10]

Point-of-care (POC) nucleic acid tests could shorten the turnaround time for test results from weeks to hours and thereby greatly reduce loss to follow-up. The utility of POC tests will depend on their simplicity of use, affordability, and stability at room temperature as well as on their ability to deliver results in field conditions with an accuracy and precision similar to those for standard quantitative or qualitative diagnostic tests performed in centralized facilities.[11–14]

The Simple AMplification-Based Assay (SAMBA) HIV-1 Qual Whole Blood Test was designed for the qualitative detection of both HIV-1 proviral DNA and RNA in whole blood of infected adults and infants, with the result provided through a visual readout on a dipstick.[15] The test was also specifically designed for use in POC settings, having a total assay time of approximately 2 hours. We now present the results of a cross-sectional field evaluation of the SAMBA HIV-1 Qual Whole Blood Test performed with clinical samples obtained from adults and infants in 3 countries in sub-Saharan Africa with a high prevalence of HIV-1 infection.

METHODS

Research Ethics and Informed Consent

The study was performed in accordance with the Declaration of Helsinki and participating countries' research and ethical regulations. Ethical approval was obtained from the Scientific Steering and Ethical Review Committee of the Kenya Medical Research Institute–Center for Disease Control (KEMRI-CDC) in Kisumu, Kenya; from the Uganda National Council for Science and Technology for Arua District Hospital, Uganda; from the Joint Clinical Research Centre Ethics Committee, the Uganda National Council for Science and Technology, and the Institutional Review Board of Baylor College of Medicine for the Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration and Baylor College of Medicine Children's Foundation (Baylor-Uganda), Kampala; and from the Institutional Review Board of Harare Central Hospital and the Medical Research Council of Zimbabwe for the National Microbiology Reference Laboratory, Harare, Zimbabwe. All adult subjects provided written informed consent for study participation, with the parents or guardians of infant subjects also providing such consent.

Sample Collection and Preparation

Whole blood was collected either by venepuncture into BD Vacutainer K2-EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) or, in the case of Kenyan infants, by heel or finger pricks. Specimens from adults were initially tested for the presence of antibodies to HIV-1 at the health care setting as per the standard of care. To limit patient burden on sample collection, venepuncture sample collection was carried out. EDTA samples collected for CD4 count were used in some cases. Laboratory technicians prepared DBS specimens in the testing laboratory by pipetting 70 μL of K2-EDTA whole blood onto each 4 circles of 2 Whatman 903 cards, which were then dried overnight and packed into individual ziplock foil bags with desiccant. One card was maintained at ambient temperature before transportation to the testing laboratories within 72 hours or was stored at −20°C for up to 2 weeks until transfer. The second card was retained frozen for quality control purposes or discrepant analysis. At sites using the London laboratories for discrepant analysis, 2 additional 200 μL samples of whole blood were frozen onsite at −20°C and subsequently shipped on dry ice to the Virology laboratories at the Royal London Hospital or Public Health England, both in London, United Kingdom.

Evaluation Protocol and Recruitment

In Kenya, 335 infants, including 135 exposed and 200 known HIV-1 positive, were recruited to the study between November 2013 and April 2014 (Table 1). The infants were recruited from support and health facilities for HIV-infected patients in Kisumu, Siaya, Kisiii, Migori, Nyamira, and Homa Bay. Whole blood samples (150 μL) were tested within 24 hours of collection both with the SAMBA HIV-1 Qual Whole Blood Test (Diagnostics for the Real World) and with the Roche COBAS AmpliPrep/COBAS TaqMan (CAP/CTM) HIV-1 Qualitative test as performed by local trained technicians at KEMRI-CDC. Discrepant results were resolved by testing of whole blood specimens with the Abbott HIV-1 RealTime Assay as performed on the Abbott m2000 system, also at KEMRI-CDC (Table 1).

In Arua, Uganda, whole blood and DBS samples were collected from 102 adults recruited from Médecins sans Frontières (MSF) programs at Arua Regional Referral Hospital in September 2011. The subjects included 22 ART-naive, HIV-1–positive patients and 80 individuals of previously unknown HIV status from the voluntary testing and counseling clinic. Whole blood samples were tested with the SAMBA assay as performed by a trained MSF technician onsite within 4 hours of collection. DBS samples were sent to

TABLE 1. Sites for Sample Collection and Testing

Country	Sample Collection Site	SAMBA Testing Laboratory (Sample Type)	CAP/CTM Testing Laboratory (Sample Type)	Discrepant Testing Laboratory (Test, Sample Type)	Age of Subjects (months or years)	HIV-1 Status	No. of Samples
Kenya	Kisumu, Siaya, Kisii, Migori, Nyamira, Homa Bay	KEMRI-CDC (whole blood)	KEMRI-CDC (whole blood)	KEMRI-CDC (Abbott, whole blood)	≤18 m	Exposed	135
						Infected	200
Uganda	Mulago Hospital PNC	Mulago CL (whole blood)	CPHL (DBS)	PHE (proviral DNA PCR, whole blood)	≤12 m	Exposed	175
						Infected	45
	Baylor-Uganda PIDC				≤12 m	Infected	29
	MU-JHU				≤12 m	Exposed	26
	Mildmay Uganda Clinic				≤12 m	Infected	36
	Arua District Hospital	MSF lab (whole blood)	CPHL (DBS)	RLH (proviral DNA PCR, whole blood)	≥18 yrs	Unknown	80
						Infected	22
Zimbabwe	Harare Central Hospital, VCT	NMRL (whole blood)	NMRL (DBS)	CIMAS-PVT (Abbott, DBS)	≥18 yrs	Unknown	100
	Harare Central Hospital, pediatric ward				≤18 m	Exposed	99

CIMAS-PVT, commercial and industrial medical aid society in Zimbabwe; CL, core laboratory; PHE, Public Health England; RLH, Royal London Hospital; VCT, voluntary counseling and testing.

the Central Public Health Laboratory (CPHL) in Kampala for testing with the CAP/CTM assay. Discrepant analysis was performed at the Royal London Hospital using at the Royal London Hospital, using a HIV-1 proviral DNA PCR targeted against 4 different regions of the viral genome (Table 1).

In Kampala, Uganda, whole blood and DBS specimens were collected between January and September 2014 from a total of 311 infants, including 201 vertically exposed infants from MU-JHU or the postnatal clinic at Mulago Hospital and 110 known HIV-1–positive infants recruited from the pediatric infectious disease clinic at Baylor-Uganda, the Mildmay Uganda Clinic, or the postnatal clinic at Mulago Hospital. These infants were also enrolled in an ongoing clinical research study. Whole blood samples were tested with the SAMBA assay at the Mulago Core Laboratory by local trained technicians within 1–2 hours of collection. DBS samples were sent to CPHL within 3 days of preparation for testing with the CAP/CTM assay. Frozen whole blood for all discordant samples and 10% of randomly selected concordant samples was sent to Public Health England for testing with the 4-gene proviral DNA assay (Table 1).

In Zimbabwe, whole blood and DBS samples were collected from 100 adult patients of previously unknown HIV status and from 99 exposed infants recruited from Harare Central Hospital between July and August 2014. Whole blood and DBS samples were tested within 6 hours of collection with the SAMBA and CAP/CTM assays, respectively, as performed by local trained technicians at the National Microbiology Reference Laboratory (NMRL), Ministry of Health and Child Care, in Harare. Discrepant results were resolved by testing of DBS samples with the Abbott HIV-1 RealTime Assay as performed on the Abbott m2000 system at the Cimas Private Laboratory of the Cimas Medical Aid Society in Harare (Table 1).

The SAMBA HIV-1 Qual Whole Blood Test was performed on the semiautomated SAMBA I system, consisting of SAMBAprep and SAMBAamp instruments,[16] at all sites with the exception of Arua, Uganda, where a semiautomated SAMBA system consisting of prototype sample preparation and amplification–detection instruments was used. Sample preparation takes 45 minutes and amplification 60 minutes. The limit of detection is 400 HIV-1 RNA copies/mL. Results were read by the user through a visual readout on a lateral flow test strip,[15–17] with the presence of HIV and control lines indicating a positive result, a control line only indicating a negative result, and no lines indicating an invalid result.

All testing with the CAP/CTM and Abbott assays was performed according to the manufacturers' instructions. The four-gene proviral DNA assay was performed according to a modified protocol described previously.[18,19] Samples consisting of 200 or 350 μL of whole blood were subjected to extraction manually with the use of either the QIAamp DNA Blood Mini Kit (Qiagen, Crawley, United Kingdom) or the Qiagen EZ1 automated extraction system (EZ1 DNA program), respectively. In addition to the discordant samples, 10%–20% (depending on the site) of the total concordant positive or negative samples were tested in a blinded manner to prevent bias.

All SAMBA and CAP/CTM testing was also performed in a blinded manner, and results for the 2 assays were then compared to determine concordance. Tie-breaker results for discrepant samples and results for randomly selected concordant positive or negative samples were also sent to the study coordinator to determine the true status of the discordant specimens. No SAMBA test results were shared with parents or guardians of infant subjects. However, if a final resolved result differed from the previously determined HIV-1 status of the subject at baseline, the result was shared with the clinicians for follow-up.

Statistical Analysis

Sensitivity and specificity for the resolved data for the SAMBA HIV-1 Qual Whole Blood Test and CAP/CTM were calculated together with the corresponding 95% confidence intervals (CIs). The calculations were performed with the use of MedCalc software (https://www.medcalc.org/calc/diagnostic_test.php).

TABLE 2. Detailed Results Obtained With the SAMBA HIV-1 Qual Whole Blood Test and CAP/CTM Assay

		Concordant Samples		Discordant Samples		Confirmatory Assay		
Country	Population	Positive	Negative	SAMBA$^+$ CAP/CTM$^-$	SAMBA$^-$ CAP/CTM$^+$	Abbott HIV-1 RealTime	HIV-1 DNA PCR	Final Result
Uganda	Adult	32	68	2			Pos	2 CAP/CTM false negatives
Zimbabwe	Adult	43	56	1		Neg		1 SAMBA false positive
Kenya	Infant	200	131	1		Neg		1 SAMBA false positive
					3	Neg		3 CAP/CTM false positives
Uganda	Infant	100	203	3			Pos	3 CAP/CTM false negatives
					5		Pos	5 SAMBA false negatives
Zimbabwe	Infant	23	75		1	Neg		1 CAP/CTM false positive

RESULTS

Performance of the SAMBA HIV-1 Qual Whole Blood Test With Adult Samples

Sites in Uganda and Zimbabwe, respectively, tested 102 and 100 venous samples from adults (Table 1). In Uganda, there were 32 concordant positive and 68 concordant negative results with the SAMBA and CAP/CTM assays, whereas there were 43 concordant positive and 56 concordant negative results in Zimbabwe (Table 2). Three discrepant results, 2 in Uganda and one in Zimbabwe, were positive with the SAMBA test and negative by CAP/CTM. Frozen whole blood for the 2 discrepant samples from Uganda was sent to the Royal London Hospital together with 2 concordant positive samples and 2 concordant negative samples. The results of the concordant samples were confirmed, and the 2 discrepant samples were found to be positive, in agreement with the SAMBA test results. The discordant sample from Zimbabwe was confirmed negative by DBS testing with the Abbott HIV-1 RealTime Assay at Cimas in Harare, consistent with the result of the CAP/CTM assay. The resolved sensitivity and specificity of the SAMBA HIV-1 Qual Whole Blood Test for the 202 adult samples were thus 100% (95% CI: 95.3 to 100) and 99.2% (95% CI: 95.6 to 99.9), respectively (Table 3). The resolved sensitivity and specificity of the CAP/CTM assay were 97.4% (95% CI: 90.9 to 99.7) and 100% (95% CI: 97.1 to 100), respectively.

Performance of the SAMBA HIV-1 Qual Whole Blood Test With Infant Samples

In Kenya, a total of 335 infants were enrolled in the study (Table 1). Infant samples were collected through heel or finger prick, as per the standard of care at the various sites, and fresh whole blood was used for testing with both SAMBA and CAP/CTM assays. There were 200 concordant positive and 131 concordant negative results for the 2 tests (Table 2). Whole blood for the 4 discordant samples, including 3 that were SAMBA negative but CAP/CTM positive and one that was SAMBA positive but CAP/CTM negative, was tested with the Abbott HIV-1 RealTime Assay at KEMRI-CDC. The 3 samples that were SAMBA negative were shown to be true negatives by the Abbott test, whereas the discordant SAMBA-positive sample was found to be a false positive. The resolved sensitivity and specificity of the SAMBA HIV-1 Qual Whole Blood Test for this Kenyan cohort of 335 infant whole blood samples were thus 100% (95% CI: 98.2 to 100), 99.3% (95% CI: 95.9 to 99.9), 99.5% (95% CI: 97.3 to 99.9), and 100% (95% CI: 97.3 to 100), respectively (Table 4). The sensitivity and specificity of

TABLE 3. Resolved Performance of the SAMBA HIV-1 Qual Whole Blood Test and CAP/CTM Assay With 202 Adult Samples

			Confirmed Results						
Assay	Country	Sample Type	TP	TN	FP	FN	Total	Sensitivity (95% CI)	Specificity (95% CI)
SAMBA	Uganda	Venous FWB	34	68	0	0	102	100% (89.7 to 100)	100% (94.7 to 100)
SAMBA	Zimbabwe	Venous FWB	43	56	1	0	100	100% (91.8 to 100)	98.3% (90.6 to 99.9)
SAMBA	Total		77	124	1	0	202	100% (95.3 to 100)	99.2% (95.6 to 99.9)
CAP/CTM	Uganda	Venous DBS	32	68	0	2	102	94.1% (80.3 to 99.3)	100% (94.7 to 100)
CAP/CTM	Zimbabwe	Venous DBS	43	56	0	0	100	100% (91.8 to 100)	100% (93.7 to 100)
CAP/CTM	Total		75	125	0	2	202	97.4% (90.9 to 99.7)	100% (97.1 to 100)

FN, false negative; FP, false positive; FWB, fresh whole blood; TN, true negative; TP, true positive.

TABLE 4. Resolved Performance for the SAMBA HIV-1 Qual Whole Blood Test and CAP/CTM Assay With 745 Infant Samples

			Confirmed Results						
Assay	**Country**	**Sample Type**	**TP**	**TN**	**FP**	**FN**	**Total**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
SAMBA	Kenya	Capillary FWB	200	134	1	0	335	100% (98.2 to 100)	99.3% (95.9 to 99.9)
SAMBA	Uganda	Venous FWB	103	203	0	5	311	95.4% (89.5 to 98.5)	100% (98.2 to 100)
SAMBA	Zimbabwe	Venous FWB	23	76	0	0	99	100% (85.2 to 100)	100% (95.3 to 100)
SAMBA	Total		326	413	1	5	745	98.5% (96.5 to 99.5)	99.8% (98.7 to 99.9)
CAP/CTM	Kenya	Capillary FWB	200	132	3	0	335	100% (98.2 to 100)	97.8% (93.6 to 99.5)
CAP/CTM	Uganda	Venous DBS	105	203	0	3	311	97.2% (92.1 to 99.4)	100% (98.2 to 100)
CAP/CTM	Zimbabwe	Venous DBS	23	75	1	0	99	100% (85.2 to 100)	98.7% (92.9 to 99.9)
CAP/CTM	Total		328	410	4	3	745	99.1% (97.4 to 99.8)	99.0% (97.5 to 99.7)

FN, false negative; FP, false positive; FWB, fresh whole blood; TN, true negative; TP, true positive.

CAP/CTM for the same cohort were 100% (95% CI: 98.2 to 100), 97.8% (95% CI: 93.6 to 99.5), 98.5% (95% CI: 95.7 to 99.7), and 100% (95% CI: 97.2 to 100), respectively.

In Uganda, samples from 311 infants were tested (Table 1). There were 100 concordant positive and 203 concordant negative results for the 2 assays (Table 2). Five samples were positive by CAP/CTM but negative by SAMBA, and 3 samples were positive by SAMBA but negative by CAP/CTM. The 8 discordant samples, together with 60 randomly selected concordant samples (positives and negatives), were tested for proviral DNA at Public Health England. The results from all of the randomly selected concordant samples demonstrated consistency after testing at VRD-PHE. The 5 samples that were negative by SAMBA but positive by CAP/CTM were confirmed positive (SAMBA false negative), and the 3 samples that were positive by SAMBA but negative by CAP/CTM were also confirmed positive (CAP/CTM false negative). The resolved sensitivities of the SAMBA test with whole blood and of the CAP/CTM assay with DBS samples for these 311 Ugandan infants were thus 95.4% (95% CI: 89.5 to 98.5) and 97.2% (95% CI: 92.1 to 99.4), respectively (Table 4). The specificities were 100% for both assays.

In Zimbabwe, 99 infants were tested (Table 1). There were 23 concordant positive and 75 concordant negative results for the 2 assays (Table 2). The one discordant sample, which was positive by CAP/CTM but negative by SAMBA, was found to be negative by testing with the Abbott HIV-1 RealTime Assay at Cimas (CAP/CTM false positive). The resolved sensitivity and specificity of the SAMBA test for this cohort of Zimbabwean infants were thus all 100% (Table 4). The resolved sensitivity and specificity of CAP/CTM were 100% (95% CI: 85.2 to 100), 98.7% (95% CI: 92.9 to 99.9), 95.8% (95% CI: 78.9 to 99.9), and 100% (95% CI: 95.2 to 100), respectively.

Overall, the results for the 745 infants tested in the 3 African countries showed the sensitivity of the SAMBA Qual Whole Blood Test to be 98.5% (95% CI: 96.5 to 99.5) and that for CAP/CTM to be 99.1% (95% CI: 97.4 to 99.8), with corresponding specificity values of 99.8% (95% CI: 98.7 to 99.9) and 99.0% (95% CI: 97.5 to 99.7), respectively (Table 4). In addition, the 202 adults tested showed a sensitivity of 100% for SAMBA and that for CAP/CTM to be 97.4%, with corresponding specificity values of 99.2% and 100.0% (Table 3). Combination of the results for the infant samples with those for the 202 adult samples yielded a sensitivity of 98.8% (95% CI: 97.2 to 99.6) for both SAMBA and CAP/CTM and specificities of 99.6% (95% CI: 98.7 to 99.9) and 99.3% (95% CI: 98.1 to 99.8), respectively.

DISCUSSION

There is an urgent need for highly accurate, affordable, robust, and easy-to-use HIV-1 tests that can be applied to EID in POC settings. The development of such tests with performance characteristics similar to those of PCR-based assays currently used for testing of DBS samples in centralized laboratories should increase access to testing, whereas the ability to provide test results at the same clinic visit as sample collection should reduce loss to follow-up, facilitate timely initiation of treatment, contributing to an overall reduction in morbidity and mortality for infected infants.

Roche CAP/CTM as performed with DBS samples is currently the most widely used assay for EID of HIV-1 infection in sub-Saharan Africa. However, centralized system has an associated high equipment cost, requires considerable bench space, a controlled environment, and frozen reagents; the assay is technically complex, and it requires highly trained personnel in a centralized facility. The SAMBA I system is a considerably simpler instrument with a smaller footprint, it does not require a temperature-controlled environment, and it relies on freeze-dried reagents that can be stored at room temperature.[15] It is therefore suitable for operation in small laboratories in district hospitals such as those in Chiradzulu, Malawi, and Arua, Uganda, where it has been in routine use since September 2013.

In this study, the SAMBA HIV-1 Qual Whole Blood Test showed a sensitivity and specificity similar to those of the CAP/CTM assay,[5] the current gold standard for EID in resource-limited settings. However, CAP/CTM can only be used in few centralized laboratories, and thus incurs the delays and attrition that preclude a truly effective EID program. The 2 assays performed similarly at sites in Kenya, Uganda, and Zimbabwe, suggesting that the performance of the SAMBA test is unaffected by differences in HIV-1 subtype frequency (predominantly subtype A in Kenya,[20,21] subtypes A and D in Uganda,[21] and subtype C in Zimbabwe[21]) or in geographical

settings. A previous study also indicated that the SAMBA assay is highly effective with a broad coverage HIV-1 subtypes, including circulating recombinant forms.[15]

Our field evaluations showed that the SAMBA HIV-1 Qual Whole Blood Test performed similarly to CAP/CTM when adult and infant data were examined separately or combined. With adult or infant samples from the same individuals, the assay sensitivity was >98% for SAMBA and >97% for CAP/CTM, and the specificity was at least 99% for both assays. The deficit in sensitivity of both assays was essentially due to 8 discordant infant and 2 discordant adult samples in Uganda (5 for CAP/CTM and 5 for SAMBA) that were confirmed positive in the discrepant analysis (Table 2). Unfortunately, sample volume collected was not sufficient to allow discrepant testing with both the Abbott and proviral DNA assays.

There are some limitations to this study. First, the median age of infants was 6.9 months at Mulago Hospital and Mildmay Uganda Clinic in Uganda, 8.7 months in Kenya, and 12 months in Zimbabwe. Given that current WHO guidelines stipulate that EID should be performed at 4–6 weeks of age,[4] it will be important to determine the performance of the SAMBA HIV-1 Qual Whole Blood Test in younger infants. Second, the DBS samples were prepared for this study by pipetting anticoagulated venous blood on the filter paper, providing samples for the CAP/CTM that were possibly of better quality than usually possible to obtain in practice in optimal conditions, which are not always encountered in the field. The SAMBA I instrument system is a near POC designed for use in resource-limited settings, such as district level hospitals and health centers, where space is restricted, throughput is low, facilities are basic, and training of staff is limited. This SAMBA I system including 2 separate instruments and a minimum amount of hands-on steps is adapted to the needs of small- and medium-size health setting processing between 16 and 48 samples/day. Another variant of the SAMBA technology-SAMBA II is a simple "sample in-result out" true point-of-care system better adapted to next to patient, mobile clinics, or even home POC. Further implementation of the SAMBA HIV-1 Qual Whole Blood Test will be undertaken in the near future.

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