Laboratory Evaluation of the VISITECT Advanced Disease Semiquantitative Point-of-Care CD4 Test

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Background: Advanced HIV disease (AHD; CD4 counts <200 cells/μL) remains common in many low- and middle-income settings. An instrument-free point-of-care test to rapidly identify patients with AHD would facilitate implementation of the World Health Organization (WHO) recommended package of care. We performed a laboratory-based validation study to evaluate the performance of the VISITECT CD4 Advanced Disease assay in Botswana.

Setting: A laboratory validation study.

Methods: Venous blood samples from people living with HIV having baseline CD4 testing in Gaborone, Botswana, underwent routine testing using flow cytometry, followed by testing with the VISITECT CD4 Advanced Disease assay by a laboratory scientist blinded to the flow cytometry result with a visual read to determine whether the CD4 count was below 200 cells/μL. A second independent investigator conducted a visual read blinded to the results of flow cytometry and the initial visual read. The sensitivity and specificity of the VISITECT for detection of AHD were determined using flow cytometry as a reference standard, and interrater agreement in VISITECT visual reads assessed.

Results: One thousand fifty-three samples were included in the analysis. The VISITECT test correctly identified 112/119 samples as having a CD4 count <200 cells/μL, giving a sensitivity of 94.1% (95% confidence interval: 88.3% to 97.6%) and specificity of 85.9% (95% confidence interval: 83.5% to 88.0%) compared with flow cytometry. Interrater agreement between the 2 independent readers was 97.5%, Kappa 0.92 ($P < 0.001$).

Conclusions: The VISITECT CD4 advanced disease reliably identified individuals with low CD4 counts and could facilitate implementation of the WHO recommended package of interventions for AHD.

Key Words: HIV, AIDS, CD4 count, point-of-care, advanced HIV disease

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INTRODUCTION

CD4 cell count testing continues to have an important role in the care of people living with HIV.[1,2] The removal of the CD4-based antiretroviral therapy (ART) initiation criteria[3] and the scaling up of viral load testing in line with World Health Organization (WHO) recommendations[4] has led to a scaling back of CD4 testing at treatment initiation and for monitoring.[5] However, CD4 count measurement remains essential for the identification of patients living with advanced HIV disease (AHD),[6] defined as a CD4 cell count <200 cells/μL, and is required to determine whether individuals need screening and prophylaxis for opportunistic infections and enhanced monitoring.[7] CD4 testing is the gateway to the WHO package of evidence-based interventions for AHD,[8] which includes cryptococcal antigen (CrAg) screening, tuberculosis screening, and cotrimoxazole prophylaxis.

Recent data show that baseline CD4 count testing is declining in many high HIV-prevalence settings in low- and middle-income countries (LMICs),[9–13] as funding and support for CD4 testing infrastructure is withdrawn.[12] This reduction in baseline CD4 testing makes effective implementation of the WHO-recommended package of AHD interventions challenging;

clinical staging has extremely low sensitivity for identification of AHD,[14] and failure to identify vulnerable individuals with low CD4 counts is likely to have negative implications for overall HIV care in many LMICs, particularly in sub-Saharan Africa, where the burden of AHD and incidence of opportunistic infections remains high,[15–17] and are associated with ongoing HIV-related mortality.[18] The proportions of individuals presenting with advanced HIV disease is still reported to be in the range of 20%–35% in recent studies conducted in the southern and east African region,[12,15–18] and the overall number of individuals with very low CD4 counts has remained stable for the past decade.[17,19]

A further challenge to effective implementation of the WHO AHD package has been the widespread uptake of rapid or same-day ART initiation.[20] With rapid ART initiation, even if baseline CD4 tests are sent, the current turn-around times with flow-cytometry[21] testing in centralized laboratories, which remains the mainstay of CD4 testing in most LMICs, means that ART initiation occurs in most cases before the availability of CD4 results. Because there is a relatively short window of opportunity in which to implement key life-saving interventions such as CrAg screening,[22] tuberculosis screening,[23] and cotrimoxazole prophylaxis,[24] many opportunities to reduce morbidity and mortality are missed.[9]

Low-cost rapid CD4 tests that can be performed at or near the point-of-care (POC) by any health care worker, and accurately identify individuals with AHD, would help overcome the barriers currently posed to implementation of WHO-recommended package of AHD care by the scaling back of centralized CD4 testing and adoption of rapid ART initiation guidelines. The VISITECT CD4 Advanced Disease test is an instrument-free semiquantitative diagnostic lateral flow assay detecting the CD4 protein on the surface of CD4$^+$ T lymphocytes designed to be used at point of care or primary health care level using capillary blood or venous blood, providing a dichotomous "high" or "low" result if CD4 count is higher or lower than 200 cells/μL within 40 minutes (Fig. 1A).[6] We performed a laboratory-based validation study to evaluate the performance of the VISITECT CD4 Advanced Disease against the FACSCalibur and AQUIOS flow cytometry platforms in Botswana using venous blood collected from HIV-positive individuals, assessing diagnostic accuracy and interrater agreement in interpretation of the lateral flow assay.

METHODS

Participants

Between November 2020 and April 2021, we screened sequential venous EDTA blood samples sent for CD4 testing at the Botswana-Harvard HIV Reference Laboratory (BHHRL) for study inclusion. The BHHRL performs nearly all CD4 measurements for patients attending clinics offering HIV care in greater Gaborone, with a total catchment population of approximately 300,000. We restricted our study to samples from individuals who were having "baseline" CD4 tests and had no record of previous CD4 count measurement in the electronic national HIV database dating back to 2004 using a unique laboratory identification number to avoid testing large numbers of CD4 samples with high CD4 counts from individuals established on ART undergoing annual CD4 monitoring. Samples from individuals below 5 years old were

FIGURE 1. A, Omega diagnostics VISITECT CD4 advanced disease test kit–supplied with foil pouches containing cassettes and desiccants, buffer solution, sterile retractable lancets, sampling devices, and alcohol swabs. B, VISITECT CD4 technical guidance 16 batch testing guide—Batch Testing Method 1 (maximum 6 samples). C, Results interpretation of VISITECTCD4 test. full color online

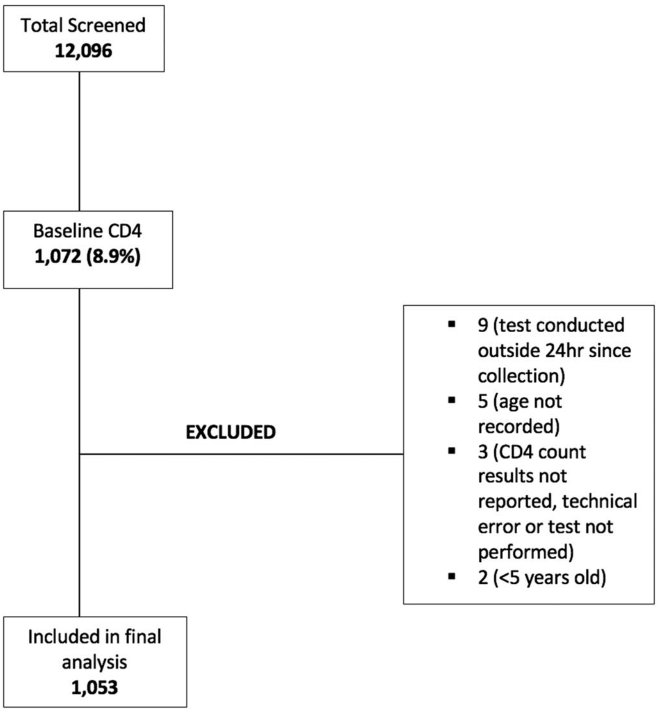

FIGURE 2. Participants schema.

excluded, as were samples that had been collected more than 24 hours before screening by the study team. Anonymized age and sex data were recorded for each sample.

Testing

After routine CD4 testing at BHHRL using the FACSCalibur and AQUIOS flow cytometry instruments, patient samples were tested with VISITECT CD4 Advanced Disease kit by a laboratory scientist (K.L.) blinded to the flow cytometry result. The EDTA blood samples were inverted until homogenization took place before testing, and 30 μL of sample was used for each test. Testing was batched, with a maximum of 6 tests performed at a time according to method 1 of the manufacturer's instructions (Fig. 1B). A visual read was conducted by the testing laboratory scientist (K.L.) to determine whether the sample had a CD4 count above or below 200 cells/μL and results were recorded. A test line that appeared darker than the reference line indicated a CD4 count above range, whereas test line that appeared the same intensity or lighter than the reference line or no test line visible indicated a CD4 count below range (Fig. 1C). The absence of a control line and/or reference line indicated an invalid run and the test was repeated. For the last 4 months of the study, a second independent investigator (TBL) conducted an additional visual read blinded to the results of flow cytometry results and the initial visual read.

Data Management and Statistical Analysis

Data from the VISITECT CD4 Advanced Disease test were recorded by investigators in a laboratory testing log. Routine CD4 count results from the flow cytometry platforms, along with age and sex data, were subsequently extracted from the electronic medical records system and transcribed into the laboratory log. Data were then double entered into a CVS file and imported into STATA 14 (StataCorp, College Station, TX) for cleaning and analysis. In all analyses the VISITECT visual reads of the first reader (K.L.) were used as the definitive VISITECT CD4 result for consistency across all samples. The baseline characteristics of the cohort were summarized using descriptive statistics. Routine CD4 results from the flow cytometry testing were summarized according to VISITECT CD4 result (above range or below range) and visualized using a scatter plot. We calculated sensitivity, specificity, and negative and positive predictive values of the VISITECT CD4 Advanced Disease assay against a reference standard of flow cytometry for a CD4 count at or below or above 200 cells/μL. To determine interrater reliability, we calculated percent agreement between the 2 readers and Cohen kappa statistic.

Ethics Statement

This research project was approved by institutional review boards at the Botswana Ministry of Health and Wellness (the Health Research and Development Committee) and University of Botswana. A waiver of individual consent was granted because the study used only anonymized residual laboratory samples and anonymized patient data.

RESULTS

We screened 12,096 sequential patient samples during the study period to determine whether they received baseline CD4 testing at the BHHRL. Of these, 1072 (8.9%) were baseline CD4 tests with no record of previous CD4 testing post-2004 in the national database. We excluded 19 samples from the analysis, 9 because VISITECT testing could not be conducted within 24 hours from the time of sample collection, 5 because no age was recorded, 3 because no CD4 result was available because of technical errors or the tests not being performed, and 2 because they were from individuals below 5 years old (Fig. 2). Of the 1053 samples included in the final analysis, 654 (62.2%) were from females, and the median age of the cohort was 38 years [interquartile range (IQR) 31–46 years]. One hundred and nineteen (11.3%) of the patients receiving baseline CD4 testing had advanced HIV disease (CD4 < 200 cells/μL) based on the reference flow cytometry testing. Individuals presenting with AHD were more likely to be male than those without AHD (57% compared with 35%,

TABLE 1. Diagnostic Performance of the VISITECT Versus Flow Cytometry Results for Sensitivity and Specificity

Flow cytometry result	VISITECT®			Value, % (95% CI)	
	<200 Cells/μL	≥200 Cells/μL	Total	Sensitivity	Specificity
<200	112	7	119	94.1 (88.3 to 97.6)	85.9 (83.5 to 88.0)
≥200	132	802	934		
Total	244	809	1053		

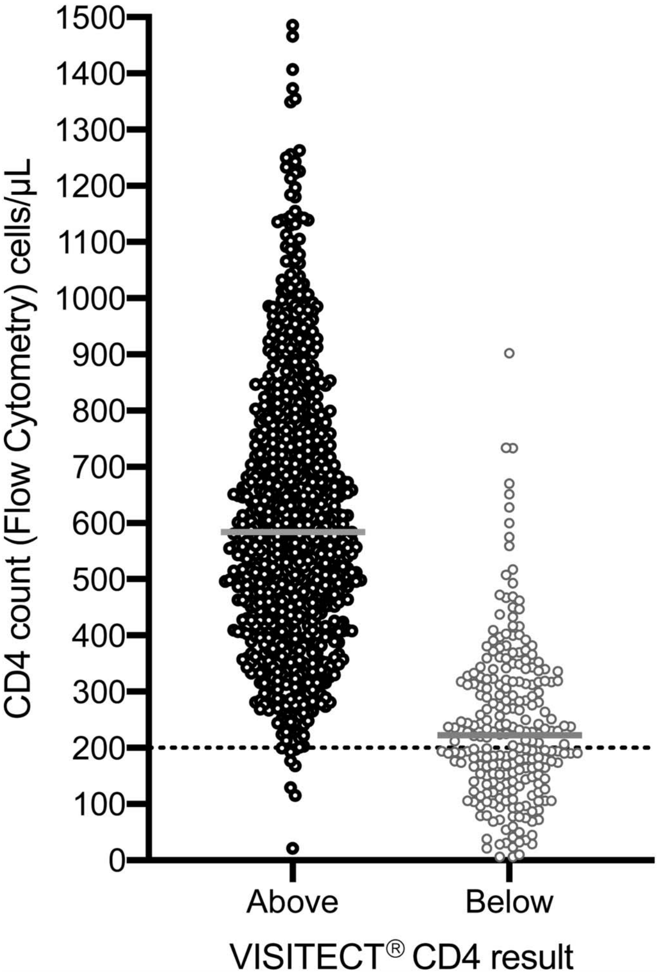

FIGURE 3. Distribution of CD4 cell counts obtained by flow-cytometry classified by the VISITECT CD4 advanced disease test. The gray bars indicate the median CD4 count in each group.

$P < 0.001$), and were older with a median age of 40 years (IQR 34–46) compared with 38 years (IQR 31–46) in those without AHD ($P = 0.04$).

Diagnostic Performance of the VISITECT CD4 Advanced Disease Assay

The VISITECT CD4 Advanced Disease assay was above range in 809/1053 (76.8%) and below range in 244/1053 (23.2%) samples tested. The VISITECT test correctly identified 112/119 samples as having a CD4 count below 200 cells/µL, giving a sensitivity of 94.1% [95% confidence interval (CI): 88.3% to 97.6%] compared with flow cytometry, with an 85.9% (95% CI: 83.5% to 88.0%) specificity (Table 1). The median CD4 count was 584 (IQR 442–765) in the above range category and 223 (IQR 147–319) in the below range category (Fig. 3). In our cohort with a relatively low overall prevalence of AHD (11.3%), the positive predictive value of the VISITECT test for a CD4 cell count <200 cells/µL was 45.9% (95% CI: 39.5% to 52.4%) with 112 of 244 samples classified as below range having a CD4 <200 cells/µL. The negative predictive value was 99.1% (95% CI: 98.2% to 99.7%) with 802 of 809 samples classified as above range having a CD4 >200 cells/µL. Of the 132 samples with CD4 counts >200 cells/µL misclassified by the VISITECT test as being below range, most (89/132, 67%) had CD4 counts below 350 cells/µL. Interrater agreement was assessed through paired readings of 472 samples (Table 2). Overall agreement was excellent, with 97.5% agreement between the 2 independent readers giving a Kappa statistic of 0.92 ($P < 0.001$) (Table 3).

DISCUSSION

The VISITECT CD4 Advanced Disease had excellent sensitivity and good specificity for detection of advanced HIV disease when compared with the gold standard of flow cytometry CD4 count testing on venous blood. Interrater agreement in the reading of results between the 2 independent investigators was excellent.

Comparable performance characteristics were observed in the only other reported validation study of the VISITECT CD4 Advanced Disease assay, using a smaller sample of 708 venous blood samples in Malawi, Zimbabwe and Democratic Republic of Congo, which found a sensitivity of 95% and a specificity of 81.9%.[25] This previous study also examined the performance of the VISITECT assay on 433 finger-prick blood, reporting similar sensitivity and specificity results to those obtained using venous blood.[25]

In many regions of sub-Saharan Africa, where AHD is still common despite wide roll out of ART[7,15,20,26,27] and data show that the lack of support for CD4 infrastructure is resulting in the scaling back of centralized flow-cytometry CD4 testing,[5] the VISITECT Advanced Disease test could provide an affordable and rapidly implementable means of effectively identifying individuals with AHD, enabling stratification of high risk individuals into appropriate differentiated service delivery streams. Our study implemented the VISITECT Advanced Disease test in a central laboratory. Although instrument-free CD4 tests that do not require any specialized external reagents or electricity potentially have an important role in very low-resource and/or rural laboratories where flow cytometry is not easily accessible, a key benefit of the low-cost, instrument-free, VISITECT POC assay is that can potentially be performed by any health care worker in the primary health care setting. Limited data suggest that VISITECT semiquantitative CD4 testing can be reliably performed by clinicians or lay staff at the point-of-care,[28]

TABLE 2. Diagnostic Performance of VISITECT for Interrater Agreement

	Scorer A		
Scorer B	**Below Average**	**Above Average**	**Total**
Below average	83	3	86
Above average	9	377	386
Total	92	380	472

TABLE 3. Interrater Agreement

Interrater Reliability	% Agreement				
	Expected	Observed	Cohen's kappa	Standard Error	*P*
	69.4%	97.5%	0.9169	0.0460	<0.0001

with users generally rating the testing process easy to perform and results easy to read.[1] However, the testing process requires a series of timed steps and accurate application of sample and buffers, requiring user focus and precision,[1] and in the previous validation study, some nonlaboratory-based health care workers reported concerns about their ability to conduct VISITECT CD4 Advanced Disease testing while multitasking in their other work roles. Further research is needed to determine how the VISITECT CD4 assay can be best incorporated into CD4 testing algorithms; it is likely that differing approaches will be required depending on testing context, with POC testing by clinical or lay staff in low through put rural settings, and batched testing by dedicated lay or laboratory staff in higher volume clinics.

A further important consideration in implementation of the VISITECT CD4 assay is the prevalence of AHD in the targeted population. A lower than anticipated proportion of our study sample had AHD, with 11.3% of samples tested having a CD4 cell count <200 cells/μL. Although the VISITECT test had an excellent negative predictive value for AHD, the positive predictive value (PPV) in our study population was below 50%. The PPV would be higher in populations with higher AHD prevalence (eg, applying our observed test specificity to an equivalent population with a 25% AHD prevalence would yield a PPV of 69%), but even in this context a substantial proportion of identified as having AHD on the VISITECT assay would have CD4 counts above 200 cells/μL. Our data demonstrate that most of these misclassified individuals have CD4 counts between 200 and 350 cells/μL; screening such individuals for opportunistic infections or stratifying them into more intensive clinical follow-up would be very unlikely to cause adverse patient outcomes, but would have implications for resource use that need to be considered by program managers.

In conclusion, our study demonstrates that the VISITECT CD4 Advanced Disease assay can rapidly and effectively identify individuals with AHD. This low-cost, instrument-free, POC test provides a valuable tool to facilitate the effective identification and management of individuals presenting with AHD in resource-limited settings.

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