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RESEARCH ARTICLE

VISITECT® CD4 advanced disease assay in routine use: Diagnostic accuracy and usability, Ethiopia and Indonesia

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Abstract

We assessed the diagnostic accuracy and usability of the rapid semiquantitative point of care VISITECT® CD4 Advanced Disease Assay, compared to routinely obtained CD4 counts among people living with HIV in Ethiopia and Indonesia. We consecutively enrolled people living with HIV aged ≥10 years who were newly diagnosed, had interrupted antiretroviral treatment for ≥3 months, or were feeling unwell. Venous blood was drawn to obtain CD4 counts on routinely available test instruments and a VISITECT® CD4 Advanced Disease Assay result. The sensitivity of VISITECT® CD4 Advanced Disease Assay was high (97.8%, 95% confidence interval [CI], 94.4–99.4%), but the specificity was low (32.7%, 95%CI, 27.9–37.8%). It significantly increased to 42.5% (95%CI, 33.2–52.1%) after retraining of laboratory staff, but remained low. The low specificity of VISITECT® CD4 Advanced Disease Assay was due to poor capacity in test reading by routine staff. Reading the test strip is error-prone and requires intensive and regular training. Reading aids may improve the usability of this essential point of care test.

KEYWORDS

CD4, diagnostic utility, people living with HIV, point of care (POC), VISITECT-CD4-AD

BACKGROUND

Next to viral load, an essential parameter to evaluate immunosuppression and monitor antiretroviral treatment (ART) response in people living with HIV (PLHIV) is the cluster of differentiation 4 (CD4) cell count. Advanced HIV disease (AHD, defined as a CD4 cell count of <200 cells/mL or meeting the criteria for WHO Stage 3 or 4 [1]), may lead to a range of opportunistic infections, including tuberculosis (TB) [2]. Quick identification of AHD, follow-up testing for opportunistic infections, and provision of prophylaxis for some of these is important to reduce mortality in this vulnerable group of patients [2]. To support rapid TB diagnosis among PLHIV, WHO recommends the use of a simple instrument-free bedside urine-based lateral flow (LF) mycobacterial lipoarabinomannan (LAM) antigen test (Alere Determine TB LAM Ag, Abbott, Palatine, IL) [3]. However, this LF assay provides higher sensitivity and specificity at lower CD4 counts [4, 5], creating the need for a quick estimate of the CD4count to ensure accurate diagnosis.

Flow cytometry is regarded as the "reference standard" for enumeration of CD4 cells but is usually only available at referral level. This means that blood samples need to be transported for analysis, and the resulting blood counts are not readily available to the clinician [6, 7]. Until recently, several near point of care (POC) analysers for the estimation of CD4 counts were available, such as Alere Pima (Abbott, Illinois, USA) [8–10] and FACSPresto (BD, NJ) [11]. Unfortunately, for commercial reasons, Pima was discontinued in 2022 and FACSPresto in 2023, leaving Omega Diagnostics' (UK) VISITECT® CD4 Advanced

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Disease assay (VISITECT-CD4-AD) as the only option currently for obtaining an estimate of a PLHIV's CD4 count at POC [12]. This semi-quantitative rapid LF assay, now called AccuBio VISITECT® CD4 Advanced Disease (AccuBio Ltd., UK), uses capillary or whole blood to estimate whether the CD4 count is above, or below and equal to 200 cells/μL. It is regarded as an instrument-free technology [13, 14] and can be used at the primary health care level and even at the patient's bedside. The VISITECT-CD4-AD assay also is used to triage patients for LAM testing in resource-limited settings as it has demonstrated high sensitivity but a somewhat lower specificity [14, 15].

In this study, nested in a project assessing the utility of FujiLAM lateral flow assays in the diagnosis of TB, we assessed the diagnostic accuracy and usability of VISITECT-CD4-AD when routinely implemented as a POC test to detect CD4 counts below and equal to or above 200 cells/μL in comparison with routinely used analysers for CD4 count among PLHIV presenting at primary, secondary, and tertiary health facilities in Ethiopia and Indonesia.

METHODS

Study design

Prospective diagnostic accuracy study following the Standards for Reporting Diagnostic accuracy studies (StaRD) [16] (Table S2).

Study population

Between 14 August 2020 to 31 July 2021, we consecutively enrolled outpatient and inpatient PLHIV aged 10 years and above from in- and outpatient wards of health facilities serving large cohorts of PLHIV: three hospitals and four health centres in Addis Ababa, Ethiopia, and four hospitals in Jakarta, Indonesia. Eligible participants were either newly diagnosed with HIV or had interrupted ART for at least 3 months at the time of enrollment, or presented with any symptoms or signs suggestive of TB per local guidelines, or were seeking care because they reported feeling unwell. After confirming eligibility and providing information about the study, written informed consent was obtained from all participants. For participants younger than 18 years, written parental consent was required; for children aged 12–17 years, in addition, we obtained their written assent. Demographic information (e.g., date of HIV diagnosis, and information on ART, HIV clinical staging [1], clinical complaints and hospital admission) was collected on standardised forms.

Training

Prior to study start, an in-person one-day training on VISITECT-CD4-AD was provided to laboratory technicians from all sites by trainers who had received training from the manufacturer. The training included theory, test result interpretation using a panel of 40 dummy strips from blood samples with different CD4 reference counts that had been provided by the manufacturer for training purposes and a practical assignment overseen by the expert trainer. Shortly before the start of the study, a refresher training, including practice, was given by local study staff who had been trained as a trainer.

In May 2021, after noticing discrepancies between VISITECT-CD4-AD results and reference CD4 counts, the investigators contacted the manufacturer who then offered an online self-assessment to assess their competency in strip reading. Next, the manufacturer's experts, by sharing a checklist to tackle the possible causes of observed discrepancies and discussions, remotely checked if blood samples had been correctly drawn and stored, if the VISITECT-CD4-AD test was conducted according to the instructions, and if there were any quality issues with the instruments for the CD4 reference counts. After all potential causes for the discrepancies were excluded, the manufacturer's staff provided comprehensive retraining including online theory and practical video-taped exercises on VISITECT-CD4-AD result interpretation to all laboratory technicians in both countries. At the end of the training, all trainees were certified by the manufacturer.

VISITECT-CD4-AD and reference CD4 count testing

From each participant, venous blood was drawn into 4 mL EDTA-containing tubes. In Indonesia, two tubes of blood were collected per participant: one for VISITECT-CD4-AD and one for the reference CD4 count, and the blood was tested within 2 h of collection. In Ethiopia, one tube was collected for both tests; blood was kept at room temperature during transport and storage and was tested within 24 h of collection. VISITECT-CD4-AD was conducted according to the manufacturer's instructions [17]. In short, 30 μL of whole blood was transferred to well A of the test device and after 3 min, a drop of running buffer was added to the same well. After 17 min, three drops of sample buffer were added to well B. After 20 min, the test result was read as >200 cells/μL if the intensity of the test (T) line was higher than that of the reference (200 line), and as ≤200 cells/μL if the test line's intensity was equal to or lower than that of the reference line, following the manufacturer's instructions. Tests missing a control (C) or 200-line were considered invalid and were repeated once using the remaining whole blood. The final test result was used for data analysis.

Reference CD4 counts were obtained using the available platform on-site: FACSPresto (Becton Dickinson and Company, BD Biosciences, San Jose, California, USA; in most Ethiopian sites), FACSCalibur (BD Biosciences, San Jose, California, USA; one Ethiopian site), Pima™ (Abbott Rapid Diagnostics, Jena, Germany; two Indonesian sites) or FACSVia (BD Biosciences, San Jose, CA; two Indonesian sites).

Laboratory staff conducting VISITECT-CD4-AD were blinded to the results of flow cytometry and vice versa. VISITECT-CD4-AD is commercially available and was purchased for the purposes of the study. The manufacturer, nor the funder of this study, were involved in data analysis and interpretation of the study results.

Quality control

In total, four lot numbers of VISITECT-CD4-AD were purchased and validated for quality by the company prior to shipment, as verified by Certificates of Analysis. Upon arrival, the local laboratory staff checked the kits for any irregularities as well as the expiry date and performed an entry validation. The manufacturer does not provide validated internal control samples. However, it is recommended that a sample greater than 200 CD4+ T cells/μL (determined by flow cytometry) with all three lines visible is run on a regular basis. Each cassette contains a control and a 200-reference line to report whether the result was valid or not. If any of these lines do not appear, the test is invalid and should be repeated according to the instructions of the manufacturer.

Data analysis

Data analysis was conducted in Stata/SE 15.1 for Windows (www.stata.com). A p-value of <0.05 was considered statistically significant. We estimated the diagnostic accuracy (sensitivity and specificity) of VISITECT-CD4-AD to detect CD4 counts of ≤200 cells/μL against conventional CD4 counts obtained by FACS or Pima. We divided the CD4 reference counts into counts of ≤200 cells/μL and of >200 μL. Counts of ≤200 cells/μL were seen as 'abnormal'/test-positive, counts of >200 μL as 'normal'/test-negative, and invalid test results were treated as missing values. Binomial confidence intervals were calculated around the diagnostic accuracy estimates. Diagnostic accuracy was calculated by country. Post-hoc, because of the issues observed with the low specificity of the test, we added additional sub-group

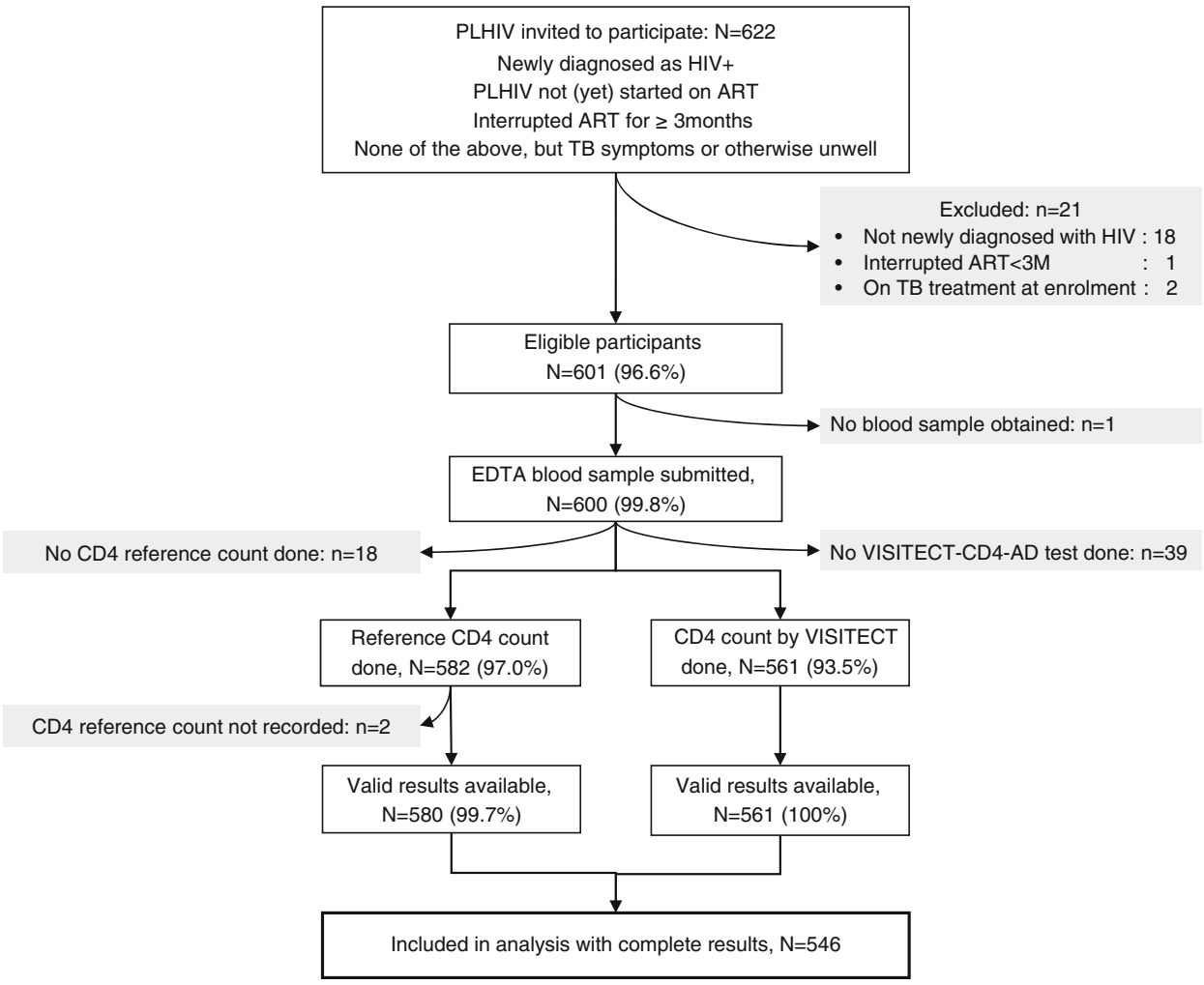

FIGURE 1 Study flow chart.

analyses, including training received, VISITECT-CD4-AD lot number, health facility and participant characteristics.

Ethical clearance

The study protocol was approved by the Ethiopian Public Health Institute (reference number EPHI 6.13/679), the University of Indonesia (reference number KET-52/UN2. F1/ETIK/PPM.00.02/2020) and the National Institute for Health Research and Development in Indonesia (reference number LB.02.01/2/KE.113/2020). Amendments were approved to prolong study enrollment beyond the original study end dates due to the COVID-19 pandemic. Written (parental) informed consent and, when applicable, assent were obtained prior to participation from all participants. Electronic data forms did not contain personal identifying information.

TABLE 1 Characteristics of the study population, by country.

Characteristic	Ethiopia $N = 485$	Indonesia $N = 61$	p
Age, mean (SD)	40.4 (12.3)	32.8 (9.3)	<0.0001
Female, *N* (%)	285 (58.8)	14 (23.0)	<0.0001
Ever received ART, *N* (%)	334 (68.9)	22 (36.1)	<0.0001
Among those, ART interrupted, *N* (%)	53 (15.9)	17 (77.3)	<0.0001
WHO clinical staging for HIV, *N* (%)			<0.0001
1	329 (67.8)	18 (29.5)	
2	49 (10.1)	9 (14.8)	
3	85 (17.5)	22 (36.1)	
4	22 (4.5)	12 (19.7)	
Advanced HIV disease, *N* (%)	107 (22.1)	34 (55.7)	<0.0001
Admitted to hospital, *N* (%)	31 (6.4)	21 (34.4)	<0.0001
Body mass index[a], mean (SD)	22.0 (4.4)	21.2 (4.3)	0.19
CD4 reference count, median (IQR)	395 (184–595)	69 (28–239)	<0.0001

[a]Body mass index was available for 480 participants from Ethiopia and for 57 from Indonesia.

RESULTS

Of 601 eligible PLHIV, 600 had EDTA blood drawn, of whom 546 (91.0%) had both a reference CD4 count and a VISITECT-CD4-AD result (Figure 1). These were included in the analysis. Ethiopian sites contributed 485 (88.8%) participants. Compared to Indonesian participants, Ethiopian participants were slightly older, more often female, and more often reported a history of ART, which they less often had interrupted prior to enrolment (Table 1). Indonesian participants generally had more advanced HIV disease (55.7% vs. 22.1% being in HIV-stage 3 or 4, $p < 0.001$) and were more often admitted to hospital than Ethiopian participants (34.4% vs. 6.4%, $p < 0.001$) (Table 1).

Reference CD4-counts were obtained on FACSPresto (83.9%), Pima (7.1%), FACSCalibur (4.9%) and FACSVia (4.0%). Indonesian participants had statistically significantly lower CD4 reference counts compared to Ethiopian participants (median counts: 69 vs. 395 cells/μL, $p < 0.001$). Low CD4 counts were associated with HIV staging, presence of TB symptoms, and VISITECT-CD4-AD result ($p < 0.001$); see Figure 2

Most VISITECT-CD4-AD test results were read as ≤200 cells/μL (74.3% in Ethiopia, 100% in Indonesia). Only three (0.6%) of the initial VISITECT-CD4-AD test results were

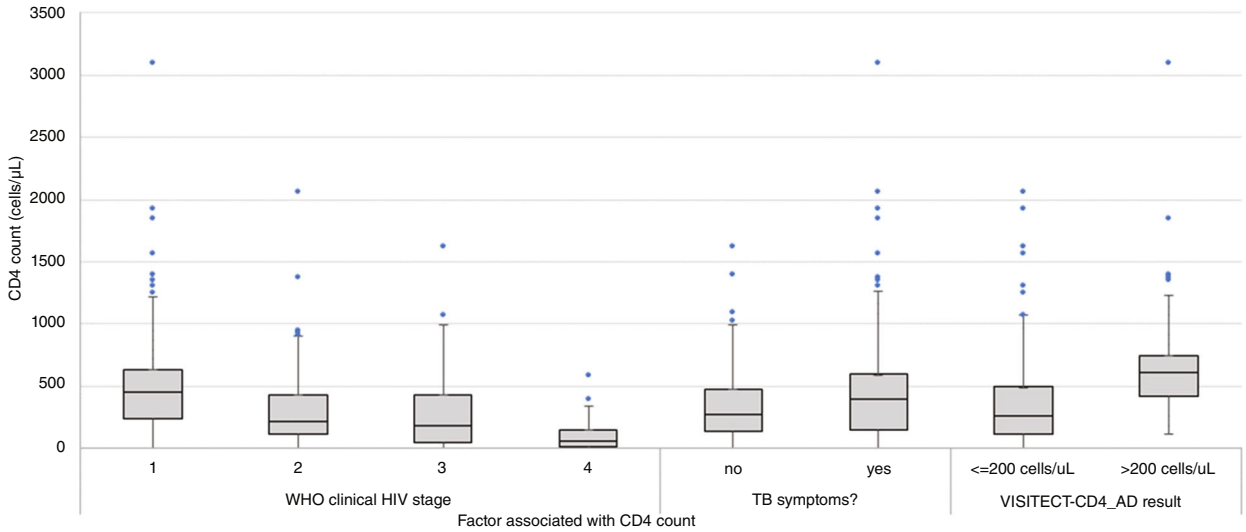

FIGURE 2 Box- and whisker plots showing variations in reference CD4 counts with WHO clinical HIV stage, presence or absence of TB suggestive symptoms, and VISITECT® CD4 Advanced Disease (VISITECT-CD4-AD).

TABLE 2 VISITECT® CD4 Advanced Disease (VISITECT-CD4-AD) assay results against reference CD4 counts: median, distribution and diagnostic accuracy by country and period.

	Overall									
	Total study period				**Ethiopia**				**Indonesia**	
	VISITECT-CD4-AD result				**VISITECT-CD4-AD result**				**VISITECT-CD4-AD result**	
CD4 reference count (cells/μL)	**≤200 *N* = 422**	**>200 *N* = 124**	**Total *N* = 546**	**p**	**≤200 *N* = 361**	**>200 *N* = 124**	**Total *N* = 546**	***p***	**≤200 *N* = 61**	**>200 *N* = 0**
Median (25th–75th percentile)	258 (112–494)	607.5 (422–747)	338.5 (141–573)	<0.001	301 (134–522)	607.5 (422–747)	395 (184–595)	<0.001	69 (28–239)	NA†
≤100, *N* (column %)	102 (99.0)[a]	1 (1.0)[a]	102 (18.9)	<0.001	68 (98.6)[a]	1 (1.5)[a]	69 (14.2)	<0.001	34 (55.7)	0
101–200, *N* (column %)	73 (96.1)[a]	3 (4.0)[a]	77 (13.9)		62 (95.4)[a]	3 (4.6)[a]	65 (13.4)		11 (18.0)	0
201–300, *N* (column %)	56 (84.9)[a]	10 (15.2)[a]	66 (12.1)		50 (83.3)[a]	10 (16.7)[a]	60 (12.4)		6 (9.8)	0
301–400, *N* (column %)	47 (81.0)[a]	11 (19.0)[a]	58 (10.6)		41 (78.9)[a]	11 (21.2)[a]	52 (10.7)		6 (9.8)	0
>400, *N* (column %)	144 (59.3)[a]	99 (40.7)[a]	243 (44.5)		140 (58.6)[a]	99 (41.4)[a]	239 (49.3)		4 (6.6)	0
Sensitivity (95% CI)	97.8% (94.4–99.4%)				97.0% (92.5–99.2%)				100% (92.1–100%)	
Specificity (95% CI)	32.7% (27.9–37.8%)				34.2% (29.2–39.4%)				0% (0–20.6%)	
	Before retraining (May 2021)									
	VISITECT-CD4-AD result				**VISITECT-CD4-AD result**				**VISITECT-CD4-AD result**	
CD4 reference count (cells/μL)	**≤200 *N* = 292**	**>200 *N* = 74**	**Total *N* = 366**	***p***	**≤200 *N* = 250**	**>200 *N* = 74**	**Total *N* = 324**	***p***	**≤200 *N* = 42**	**>200 *N* = 0**
Median (25th–75th percentile)	269 (127–508)	626.5 (457–743)	343.5 (165–570)	<0.001	307.5 (164–535)	626.5 (457–743)	396.5 (199.5–596)	<0.001	97.5 (29–265)	NA†
<100, *N* (column %)	62 (21.2)	0 (0)	62 (16.9)		41 (16.4)	0 (0)	41 (12.7)		21 (50.0)	0
100–200, *N* (column %)	48 (16.4)	2 (2.7)	50 (13.7)		40 (16.0)	2 (2.7)	42 (13.0)		8 (19.1)	0
201–400, *N* (column %)	80 (27.4)	9 (12.2)	89 (24.3)		71 (28.4)	9 (12.2)	80 (24.7)		9 (21.4)	0
>400, *N* (column %)	102 (34.9)	63 (85.1)	165 (45.1)		98 (39.2)	63 (85.1)	161 (49.7)		4 (9.5)	0
Sensitivity (95% CI)	98.2% (93.7–99.8%)				97.6% (91.6–99.7%)				100% (88.1–100.0%)	
Specificity (95% CI)	28.3% (22.9–34.3%)*				29.9% (24.2–36.1%)**				0.0% (0.0–24.7%)	
	After retraining (May 2021)									
	VISITECT-CD4-AD result				**VISITECT-CD4-AD result**				**VISITECT-CD4-AD result**	
CD4 reference count (cells/μL)	**≤200 *N* = 130**	**>200 *N* = 50**	**Total *N* = 180**	***p***	**≤200 *N* = 111**	**>200 *N* = 50**	**Total *N* = 161**	***p***	**≤200 *N* = 19**	**>200 *N* = 0**
Median (25th–75th percentile)	194.5 (80–482)	596 (379–759)	327.5 (116–573.5)	<0.001	271 (101–511)	596 (379–759)	381 (141–594)	<0.001	45 (26–130)	NA†
<100, *N* (column %)	39 (30.0)	1 (2.0)	40 (22.2)	<0.001	27 (24.3)	1 (2.0)	28 (17.4)	<0.001	12 (63.2)	0
100–200, *N* (column %)	26 (20.0)	1 (2.0)	27 (15.0)		22 (19.8)	1 (2.0)	23 (14.3)		4 (21.1)	0
201–400, *N* (column %)	23 (17.7)	12 (24.0)	35 (19.4)		20 (18.0)	12 (24.0)	32 (19.9)		3 (15.8)	0
>400, *N* (column %)	42 (32.3)	36 (72.0)	78 (43.3)		42 (37.8)	36 (72.0)	78 (48.4)		0 (0)	0

(Continues)

TABLE 2 (Continued)

	After retraining (May 2021)							
	VISITECT-CD4-AD result				VISITECT-CD4-AD result			
CD4 reference count (cells/μL)	**≤200 $N = 130$**	**>200 $N = 50$**	**Total $N = 180$**	***p***	**≤200 $N = 111$**	**>200 $N = 50$**	**Total $N = 161$**	***p***	**VISITECT-CD4-AD result**	
									≤200 $N = 19$	**>200 $N = 0$**
Sensitivity (95% CI)	97.0% (89.6–99.6%)				96.1% (86.5–99.5%)				100.0% (79.4–100.0%)	
Specificity (95% CI)	42.5% (33.2–52.1%)				43.6% (34.2–53.4%)				0.0% (0.0–70.8%)	
Difference in diagnostic accuracy, after vs. before retraining:										
Sensitivity (95%CI)	−1.2% (−6.0% to 3.6%)				−1.5% (−7.8% to 4.8%)				NA†	
Specificity (95%CI)	14.1% (3.5–24.8%)*				13.8% (2.8–24.7%)**				NA†	

Note: *One-sided $p = 0.008$; **One-sided $p = 0.012$; † Not applicable.
[a]Row percentages are shown instead of column percentages.

invalid (no 200 line); all three tests were repeated using the same blood sample and provided a valid test result on retesting. Test (T) and reference lines (200) were often faint, especially when compared to the control (C) line.

Participants with a score of >200 cells/μL on VISITECT-CD4-AD had significantly higher reference CD4 counts than those whose score was ≤200 cells/μL (Table 2). On the other hand, even at CD4 reference counts >400 cells/μL, 59% of the paired VISITECT-CD4-AD test results indicated counts of ≤200 cells/μL (Table 2).

The corresponding sensitivity of the VISITECT-CD4-AD test was very high (97.8%, 95% CI, 94.4–99.4%), but the corresponding specificity was low (32.7%, 95% CI, 27.9–37.8%). This means that VISITECT-CD4-AD correctly identified almost all CD4 cell counts of ≤200 cells/μl but only 33% of cell counts of >200 cells/μL. There was no difference in diagnostic performance of VISITECT-CD4-AD with regards to age (children vs. adults), hospital admission (yes/no), and having ever received ART (yes/no). Although some lot variability was observed, there were no significant differences in sensitivity or specificity (Table S1). However, among Ethiopian health facilities, three scored markedly higher in terms of specificity (55% and above) than the other three (28% and below) (Figure 3a). The online retraining did help to significantly improve the VISITECT-CD4-AD's specificity, while not importantly decreasing its sensitivity. However, the specificity of VISITECT-CD4-AD for ruling out CD4 counts of 200 cells/μL or lower remained low at 43% (Table 2). When analysed by site, among the Ethiopian health facilities, two experienced a marked increase in specificity of VISITECT-CD4-AD after retraining, while in three facilities, the specificity remained similar (two of these already scored higher than 50% before retraining), and one site experienced a slight decrease in specificity (Figure 3b).

DISCUSSION

Routine laboratory-based implementation of VISITECT-CD4-AD after a basic training resulted in a very high sensitivity (98%) of VISITECT-CD4-AD to detect CD4 counts ≤200 cells/μL, but a low specificity (33%). The majority of participants with a VISITECT-CD4-AD result of ≤200 cells/μL had reference counts of >200 cells/μL; even at reference counts of >400 cells/μL, 144/243 (59%) participants had a score of ≤200 cells/μL using VISITECT-CD4-AD (Table 2). While the sensitivity of the VISITECT-CD4-AD was 100% in all sites but one, the specificity differed markedly across study sites.

This study reports a significantly lower specificity than other studies [14, 15, 18–20]. Although the laboratory technicians were trained to follow the manufacturer's instructions to compare the 200-reference line to the test line, not to the control line, in reality, the higher intensity of the control line compared to the 200-reference and test lines may have biased the readers towards scoring strips as ≤200 cells/

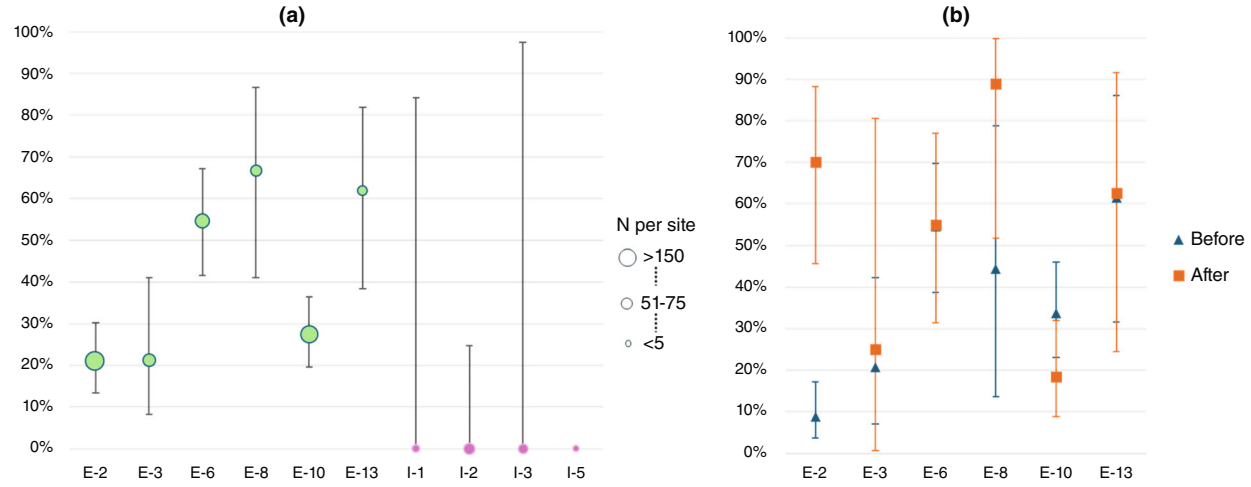

FIGURE 3 Specificity of VISITECT-CD4-AD with 95% confidence intervals, by study site: (a) Site-specific specificity over total study period in all 10 health facilities. Size of marker indicates number of participants enrolled. (b) Specificity in Ethiopian health facilities before and after retraining.

µL [21]. An online competency self-assessment showed that all laboratory technicians had difficulties with result interpretation, with some technicians (especially in Indonesian sites) obtaining very low scores. After retraining by Omega staff, the specificity increased significantly (from 28% to 43%, Table 1), but remained too low for use of VISITECT-CD4-AD as an efficient rule-in test for further diagnostic workup and treatment interventions in these routine settings [17], as it means that the majority of PLHIV tested would erroneously be identified as patients with critically low CD4 counts. While follow-up testing of these patients would likely do no harm, it does involve costs for the health care system and may include additional burden in terms of time and costs spent, and samples collected and treatments received, to the patient.

In our study, technicians from high-throughput laboratories conducted the test after receiving a one-day training from persons trained by the manufacturer's staff, which was followed by short refresher trainings given by local staff shortly before starting enrolment. The online refresher training was provided by experts from Omega, but this did not include supervised testing and reading of test results, nor one-on-one sessions. Others provided comprehensive in-person training directly delivered by Omega staff [13, 18, 19]. Some included multiple repeat training [15, 18]; in one of these, testers with low performance were retrained and on average received five trainings over a one-year period [18]. Another study had only two readers (who both scored all strips), one of whom was part of the study team [14]. The lowest overall specificity (61.4% (95%CI, 58.4–64.3%)) was reported for a multi-country diagnostic accuracy study which offered various types of trainings (online and on-site) by different trainers (local or expert trainer), depending on the country [20]. There was considerable variation in country-specific specificity, ranging from 44.8% (95%CI,31.7–58.5%) in Thailand to 82.9% (75.3–89.0%) in Vietnam [20]. It is unknown if this variation was partly due to the type of training that the testers had received.

Gils and colleagues found that the specificity was lower for lower CD4 counts [20]. This may also explain the lower specificity reported for Indonesia than Ethiopia in our study, with median CD4 counts of 69 (interquartile range (IQR), 28–239) and 301 (IQR, 134–522), respectively. Like Gils et al. [20], we observed some variation in specificity by lot number, but this did not reach statistical significance.

Comprehensive practical training and regular supervision help to improve the test's specificity [18], but offering such a comprehensive training and re-training package may not be feasible in routine low-resource settings, where staff turnover tends to be high, handover is suboptimal, and regular retraining does not occur [22, 23]. Nonetheless, all studies but one [19] investigating the feasibility and acceptability of the VISITECT-CD4-AD test reported issues with test interpretation [13, 14, 18, 24], leading to a lack of confidence in test interpretation [14].

Apart from the issues with interpretation, the performance of the test is challenging, as it requires multiple incubation steps with critical timing [13, 14, 21, 19, 24], and incorrect timing can influence the test's diagnostic performance. For example, taking <3 minutes for the first incubation step may lead to fainter test lines [14].

The limitations of our study were that due to COVID-19, no on-site monitoring by the international study team was possible. In Indonesia, only inpatients could be included as outpatient services were temporarily stopped, which led to the inclusion of fewer and generally sicker patients than intended. Therefore, the results for Indonesia may not be generalisable to the key population that requires VISITECT-CD4-AD testing. Also, the test was conducted and interpreted by laboratory staff, who may be less experienced in reading LF POC test strips than clinical workers, although the only study reporting about this did not find evidence for

a lower specificity among laboratory staff compared to non-laboratory staff [19].

In conclusion, while VISITECT-CD4-AD is a true POC test in the sense that it requires no electricity, equipment (apart from a timer) or consumables other than those available in the test package, our study, as well as other studies [20, 24], indicates that misinterpretation of the test result may occur. This may depend on the amount of training received and the experience of the reader with reading test strips [20, 24]. Reading aids which automatically score colour intensity may be of help to enable unbiased strip reading. As on-site CD4 testing is much needed, and VISITECT-CD4-AD is currently the only LF POC test available, investments to further improve the usability for routine use of this assay should be prioritised.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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REFERENCES

1. WHO. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva: World Health Organization; 2007. https://iris.who.int/bitstream/handle/10665/43699/9789241595629_eng.pdf?sequence=1&isAllowed=y
2. Kwan CK, Ernst JD. HIV and tuberculosis: a deadly human syndemic. Clin Microbiol Rev. 2011;24(2):351–76.
3. WHO. Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update 2019. Geneva, Switzerland: World Health Organization; 2019. https://iris.who.int/bitstream/handle/10665/329479/9789241550604-eng.pdf?sequence=1
4. Lawn SD, Kerkhoff AD, Vogt M, Wood R. Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study. Lancet Infect Dis. 2012;12(3):201–9.
5. Bjerrum S, Schiller I, Dendukuri N, Kohli M, Nathavitharana RR, Zwerling AA, et al. Lateral flow urine lipoarabinomannan assay for detecting active tuberculosis in people living with HIV. Cochrane Database Syst Rev. 2019;10:CD011420.
6. Spooner E, Reddy T, McHunu N, Reddy S, Daniels B, Ngomane N, et al. Point-of-care CD4 testing: differentiated care for the most vulnerable. J Glob Health. 2022;12:4004.
7. Larson BA, Brennan A, McNamara L, Long L, Rosen S, Sanne I, et al. Lost opportunities to complete CD4+ lymphocyte testing among patients who tested positive for HIV in South Africa. Bull World Health Organ. 2010;88(9):675–80.
8. Namuniina A, Lutwama F, Biribawa VM, Kizza D, Kabuubi BR, Kitandwe PK, et al. Field performance of PIMA point-of-care machine for CD4 enumeration under a mobile HIV counseling and testing program in remote fishing communities of Lake Victoria, Uganda. AIDS Res Hum Retroviruses. 2019;35(4):382–7. https://doi.org/10.1089/AID.2018.0223
9. Wade D, Daneau G, Aboud S, Vercauteren GH, Urassa WS, Kestens L. WHO multicenter evaluation of FACSCount CD4 and Pima CD4 T-cell count systems: instrument performance and misclassification of HIV-infected patients. J Acquir Immune Defic Syndr. 2014;66(5):e98–e107.
10. Pham MD, Agius PA, Romero L, McGlynn P, Anderson D, Crowe SM, et al. Performance of point-of-care CD4 testing technologies in resource-constrained settings: a systematic review and meta-analysis. BMC Infect Dis. 2016;16(1):592.
11. Makadzange AT, Bogezi C, Boyd K, Gumbo A, Mukura D, Matubu A, et al. Evaluation of the FACSPresto, a new point of care device for the enumeration of CD4% and absolute CD4+ T cell counts in HIV infection. PLoS One. 2016;11(7):e0157546.
12. Hurpy MXH, Meehan T. Supply chain implications of the latest CD4 market changes. Navigating the changing CD4 landscape to ensure HIV diagnostic service continuity. Arlington, VA: The Partnership for Supply Chain Management; 2023. https://pfscm.org/wp-content/uploads/2023/10/Supply-chain-implications-of-the-latest-CD4-market-changes-2023.pdf
13. Scorgie F, Mohamed Y, Anderson D, Crowe SM, Luchters S, Chersich MF. Qualitative assessment of south African healthcare worker perspectives on an instrument-free rapid CD4 test. BMC Health Serv Res. 2019;19(1):123.
14. Ndlovu Z, Massaquoi L, Bangwen NE, Batumba JN, Bora RU, Mbuaya J, et al. Diagnostic performance and usability of the VISITECT CD4 semi-quantitative test for advanced HIV disease screening. PLoS One. 2020;15(4):e0230453.
15. Lechiile K, Leeme TB, Tenforde MW, Bapabi M, Magwenzi J, Maithamako O, et al. Laboratory evaluation of the VISITECT advanced disease Semiquantitative point-of-care CD4 test. J Acquir Immune Defic Syndr. 2022;91(5):502–7.
16. Cohen JF, Korevaar DA, Altman DG, Bruns DE, Gatsonis CA, Hooft L, et al. STARD 2015 guidelines for reporting diagnostic accuracy studies: explanation and elaboration. BMJ Open. 2016;6(11):e012799.
17. Luchters S, Technau K, Mohamed Y, Chersich MF, Agius PA, Pham MD, et al. Field performance and diagnostic accuracy of a low-cost instrument-free point-of-care CD4 test (Visitect CD4) performed by different health worker cadres among pregnant women. J Clin Microbiol. 2019;57(2):e01277-18.
18. Nalintya E, Sekar P, Namakula OL, Tadeo KK, Kwizera R, Apeduno L, et al. The diagnostic performance of the Visitect advanced disease point-of-care CD4 platform: a pragmatic mixed-methods multisite validation, costing, and qualitative analysis. J Acquir Immune Defic Syndr. 2024;97:387–96.
19. Haraka F, Moore A, Odinga G, Bailey R, Songoro J, Haule D, et al. Accuracy, use and acceptability of the VISITECT CD4 semi-quantitative test to advance HIV disease screening in routine programmatic settings in Tanzania. Clin Epidemiol Glob Health. 2024;27(2024):101616. https://doi.org/10.1016/j.cegh.2024.101616
20. Gils T, Hella J, Jacobs BKM, Sossen B, Mukoka M, Muyoyeta M, et al. A prospective evaluation of the diagnostic accuracy of the point-of-care VISITECT CD4 advanced disease test in seven countries. J Infect Dis. 2025;231(1):e82–90. https://doi.org/10.1093/infdis/jiae374
21. WHO. WHO prequalification of in vitro diagnostics. Public Report. Product: VISITECT CD4_Advanced Disease. WHO reference number: PQDx 0384–077-00. WHO, Geneva: July 2023, version 5. 2023.

https://extranet.who.int/prequal/sites/default/files/whopr_files/PQDx_0384-077-00_VISTECT-CD4_AdvancedDisease_v5.0.pdf
22. Noya FC, Carr SE, Thompson SC. Attracting, recruiting, and retaining medical workforce: a case study in a Remote Province of Indonesia. Int J Environ Res Public Health. 2023;20(2):1435. https://doi.org/10.3390/ijerph20021435
23. Alebachew A, Waddington C. Improving health system efficiency. Ethiopia: human resources for health reforms. Geneva: World Health Organization; 2015 Report No.: WHO/HIS/HGF/CaseStudy/15.6 Contract No.: WHO/HIS/HGF/CaseStudy/15.6.
24. Gils T, Kamele M, Madonsela T, Bosman S, Ngubane T, Joseph P, et al. Implementation of the advanced HIV disease care package with point-of-care CD4 testing during tuberculosis case finding: a mixed-methods evaluation. PLoS One. 2023;18(12):e0296197.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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