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Original Research Article

Performance characteristics of two new rapid HIV diagnostic assays and use of test band reader

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ABSTRACT

Purpose: Accurate HIV diagnosis is essential for appropriate patient care. This present study evaluated the performance of two different new rapid HIV diagnostic tests; 1) TRUSTline HIV-1/2 Ab rapid test (Athenese-DxPvt. Ltd, Chennai, India)and 2) OnSite HIV 1/2 Ab Plus Combo Rapid Test (CTK Biotech Inc., San Diego, USA) and also validated ALTA Rapid Test Reader (RTR-1) (CTK Biotech Inc., San Diego, USA), the device is a user-friendly and image-analysis based qualitative/semi-quantitative tabletop reader.

Materials and methods: A total of n = 500 characterized specimens were used for this evaluation and the results of the new test kits (TRUSTline and OnSite) were also compared with 4th generation ELISA kit (Genescreen™Ultra HIV Ag-Ab ELISA)and 3 other commercially available rapid tests that were in the market; 1)SD Bioline™ HIV 1/2 3.0, 2) Aspen® HIV 1/2 Rapid Ab Test; and 3) Diagnostic enterprises HIVTRI-DOT. The test band intensities of the TRUSTline and OnSite tests were measured in an ALTA rapid test reader and compared with the naked eye reading.

Results: The sensitivity, specificity, positive predictive value, negative predictive value and efficacy of TRUSTline and OnSite were 100%, 99.6%, 99.5%, 100% and 99.8% and 100%, 100%, 100%, 100% and 100% respectively.

Conclusion: The 'TRUSTline HIV-1/2' and 'OnSite HIV 1/2' kits are suitable to use in the HIV testing algorithm. Use of the ALTA rapid test reader could be user's friendly in the field level testing in resource-limited settings".

1. Introduction

India has the 2nd highest number of people living with HIV in the world even with the low prevalence and has more than 2.3 million estimated HIV infections as per the Government of India official report [1]. Obtaining correct reliable HIV diagnosis and giving test results to the needed individuals in general is critical to fulfilling the UNAIDS 95-95-95 targets [2,3]. Accurate HIV diagnosis is the foundation needed to achieve these ambitious targets and requires a combination of different methods and/or antigenic preparations for the HIV testing algorithm [4–6].Currently, the government of India recommends a minimum of three HIV test kits to be used with an HIV testing algorithm for the diagnosis of the disease in asymptomatic individuals [5]. There are number of HIV diagnostic kits available in the country that have different principles such as immunochromatography, membrane absorption, immune comb, and agglutination. However, while the rapid test device provides immediate test reports, it is very often challenging to read low-intensity bands or dots. Automated reading of band intensity is therefore a prime solution for determining results with very low-intensity bands [4,7,8].

The visual ALTA rapid test reader (RTR-1) manufactured by Athenese-Dx, Chennai, India has been introduced to ease the process of reading and interpreting HIV test results. The ALTA RTR-1 is an image-analysis-based; user-friendly, qualitative/semi-quantitative tabletop rapid test reader intended for point-of-care diagnostic tests. It was designed to measure and interpret lateral-flow immunoassay test results and measure the band intensity of TRUSTline HIV 1/2 Rapid Ab Test (Athenese Dx, Chennai, India) and OnSite HIV 1/2 Ab Combo Rapid Test (CTK Biotech, San Diego, USA) assays. The ALTA RTR-1 can be used with other lateral flow immunochromatographic cards by chang-

Abbreviations: RTR, Rapid Test Reader; VCT, Voluntary Counseling and Testing; IDU, Injecting Drug Users; MSM, Men who have sex with men; HAART, Highly Active Antiretroviral Therapy

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ing the cassette adapter based on the manufacturer's design. In this present study, we evaluated the performance characteristics of 2 new rapid diagnostic assays, TRUSTline and OnSite for the diagnosis of HIV infection and ALTA-RTR 1 reader.

2. Materials and methods

This study was carried out in one of the largest HIV referral centers in Chennai, India, the YRG Centre for AIDS Research and Education (YRG CARE). The center provides care and management of more than 20,000 people living with HIV/AIDS. The study was approved by the YRG CARE institutional review board and the study permitted to use of stored plasma specimens that were collected from clients who visited YRGCARE for HIV tests. Apart from patient specimens, 50 HIV-1 positive and 50 HIV negative proficiency testing (PT) panel survey specimens from the College of American Pathologists, Northfield, IL, USA, and One World Accuracy, Burnaby, Canada were used as characterized specimens. Peer group evaluation results in the summary report of the PT panel survey provider were considered as a gold standard.

The clinical specimens used for this study were characterized as HIV positive or negative based on the National AIDS Control Organization (NACO) HIV testing algorithm [5,6]. The HIV testing algorithm at YRG CARE includes Determine HIV-1/2 test (Alere Medical Co. Ltd., Chiba, Japan), immunochromatography assay as a first test, and the reactive specimens are subjected to further tests and non-reactive specimens are reported as negative. Initially, reactive specimens are tested with two different kits in a parallel manner; immunochromatography assay, First Response HIV 1-2.0 card test (Premier Medical Corp. Ltd., Nani Daman, India), and Signal HIV (Arkray Healthcare Pvt, Ltd., Surat, India), which uses antibody trapping by immobilization. Specimens that are positive in all three assays are reported as HIV-1 positive. The HIV-2 band reactive with the First Response HIV 1-2.0 card test is further tested using the HIV TRIDOT assay (Diagnostic Enterprises, Parwanoo, India), which uses antibody trapping by immobilization. The specimens which are reactive for HIV-2 in First Response and HIV TRIDOT are reported as reactive for HIV-2 suggesting an additional Western blot for confirmation.

A total of 200 HIV1/2 negatives, 150 HIV-1 positives, and 50 HIV-2 positive specimens were used for this study. This set of characterized specimens was run on 5 different HIV diagnostic rapid test kits to evaluate their performance characteristics as per the manufacturer's instructions. The 2 new HIV rapid test kits evaluated were; TRUSTline HIV 1/2 Rapid Ab Test (Athenese Dx, Chennai, India) and OnSite HIV 1/2 Ab Combo Rapid Test (CTK Biotech, San Diego, USA), and 3 other HIV rapid test kits that were available in the market also included in this study to compare the performance characteristics; SD Bioline HIV 1/2 3.0 (Standard Diagnostics, Inc., Giheung-gu, Korea); Aspen® HIV 1/2 Rapid Ab Test (Aspen Laboratories, Delhi, India); and HIV TRIDOT (Diagnostic Enterprises, Parwanoo, India). All the assays were immunochromatographic tests except for HIV TRIDOT, which is a membrane absorption method. All five rapid test kits were capable of differentiating between HIV-1 and HIV-2. Each study specimen was also tested with the 4thgeneration ELISA, Genscreen™ ULTRA HIV Ag-Ab assay (Bio-Rad laboratories, Marnes-La-Coquette, France) to compare their optical density (OD) values to the reading of the ALTA-RTR-1.

The RTR-1 uses an image processing method. During measurement, a lateral flow device test result is captured using a 5-mega pixel camera, and the picture is then analyzed using image processing algorithms for the identification of either the presence or absence of test and control line bands and their intensity levels. The reader converts the image into grayscale and then classifies the intensity levels of the image within the 0 to 100 grayscale range. The image processing algorithm undergoes various levels of filtering and segmentation to yield the correct intensity levels from the image. Based on the preconfigured intensity levels, the reader then classifies the band intensity and reads out the results as either positive or negative, approximately the RTR-1 costs Rs. 35,000 per unit.

Results were visually read and documented by two different personnel. In addition, the band intensity of the TRUSTline and OnSite tests were measured using the reader, ALTA RTR-1 (Fig. 1). This instrument provides scores for HIV-1 and HIV-2 based on the intensity of the bands. The OD obtained from Genscreen™ ULTRA HIV Ag-Ab ELISA was correlated with the ALTA RTR-1 results obtained from the TRUST line and OnSite assays. A minimum RTR cut-off score of 15 was considered indicative of a reactive reaction as per the manufacturer's instructions.

Kit performance characteristics were derived using standard formulas that have been provided elsewhere [6]. A Pearson's correlation coefficient was derived for the Bio-Rad ELISA sample OD/cut-off values and the rapid assay results. Statistical analyses were performed using an online statistics calculator provided by Social Science Statistics (2021 Jeremy Stangroom) [9].

3. Results

A total of 500 specimens (including 100 proficiency testing panel specimens) were evaluated. All 500 characterized specimen results positive, or negative were in agreement with the 4th generation Genscreen ELISA. None of the positive results were indicative of recent infection among the voluntary counseling and testing (VCT) clients by Genscreen ELISA. Diagnostic performance characteristics achieved 100% in detecting both HIV-1 and HIV-2 antibodies for the following kits: OnSite HIV 1/2 Ab Combo Rapid Test and SD Bioline HIV 1/2 3.0. Detailed performance characteristics are provided in Table 1. One hundred percent sensitivity in detecting HIV-1 and HIV-2 was obtained with all 5 test kits except the HIV TRIDOT assay, which did not detect 3HIV-1 positive specimens (thus reducing the sensitivity to 98.5%).

The TRUSTline and Aspen HIV kits had a specificity of 99.6% due to the misidentification of one HIV negative specimen as reactive and found to be the same specimen in both test kits. The performance characteristics used to detect HIV-2 antibodies were more precise when compared to HIV-1, as there were no discrepant results with all the test kits (100% concordance).

There was a strong correlation between the ELISA OD and the ALTA RTR-1 results for the TRUSTline and OnSite test kits, with correlation coefficients of 0.965 and 0.912, respectively (Fig. 2).

Fig. 1. ALTA rapid test reader (RTR-1).

Table 1
Performance characteristics of six diagnostic kits that screen for HIV-1 infection.

		Gold Standard		Sensitiv-ity	95% CI		Specific-ity	95% CI		PPV[a]	NPV[b]	Efficiency
		POS	NEG									
TRUSTline HIV 1/2 Ab (Lot No: F2802181E02)	POS	200	1	100.0	100.0	100.0	99.6	98.7	100.5	99.5	100.0	99.8
	NEG	0	249									
OnSite HIV 1/2 Ab Combo (Lot No: F0315P10F00V)	POS	200	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	NEG	0	250									
SD Bioline HIV 1/2 Ab (Lot No: 03ADB059A)	POS	200	0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	NEG	0	250									
ASPEN HIV 1/2 Ab (Lot No: BT010181)	POS	200	1	100.0	100.0	100.0	99.6	98.7	100.5	99.5	100	99.8
	NEG	0	249									
HIV TRIDOT (Lot No: HTD121745)	POS	197	0	98.5	97.0	100.0	100.0	100.0	100.0	100.0	98.8	99.3
	NEG	3	250									

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.

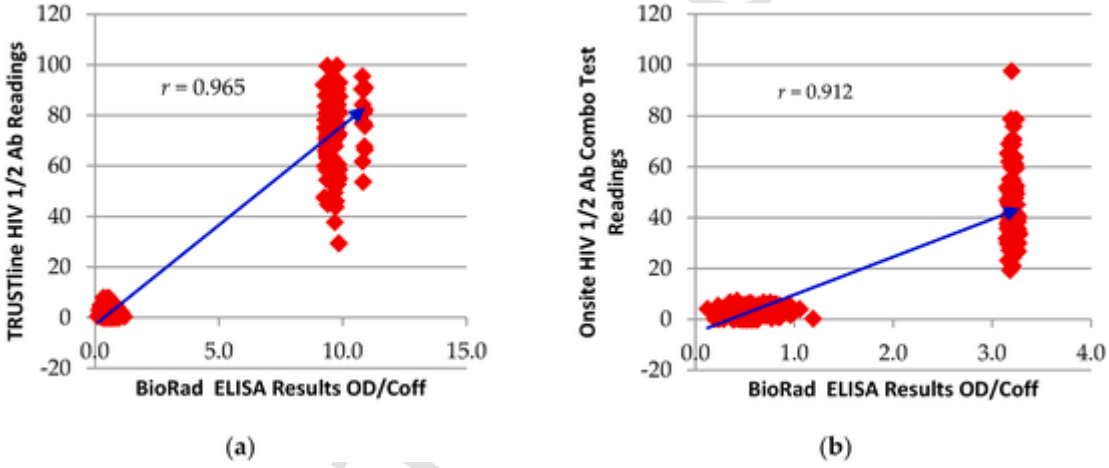

Fig. 2. The correlation between the Bio-Rad ELISA sample/cut-off values and the ALTA rapid test reader measures. a) Readings from the TRUSTline HIV 1/2 assay and b) The Onsite Rapid test assay measurements.

4. Discussion

India has several imported and indigenous HIV test kits. However, there is a need to identify better diagnostic assays given the discrepant results between different assays [4,8,10–12]. This was a large, multi-kit evaluation study that measured the performance characteristics of HIV rapid diagnostics kits that are available in India. To the best of our knowledge, this type of study has not been conducted in India over the past ten years and presents current information that allows for the comparison and selection of the best HIV rapid diagnostic kit available in the country. The performance characteristics of 3rdgeneration assays must equal that of 4thgeneration ones in terms of identifying HIV infection at the earliest possible time point. Therefore, in this study, the performance characteristics of five rapid test assays were measured in comparison to a 4thgeneration ELISA kit and a standard HIV testing algorithm.

There were no discrepant results between the 4thgeneration ELISA and the NACO HIV testing algorithm. However, discrepancies were reported between these gold-standard results and the diagnostic test kits we evaluated. In addition to studying the performance characteristics of the five testing kits, we also compared the results of HIV-negative specimens from the set of characterized specimens with the 4thgeneration ELISA, so as to rule out hidden incident specimens among VCT clients. This screening was necessary as the NACO HIV testing algorithm does not require the confirmation of HIV negative results obtained from the first screening test [5]. Hence, it is always recommended to use a highly sensitive test when screening a specimen for HIV infection.

This study also evaluated the usefulness of the ALTA rapid test reader measurements to estimate the results, and the 15 RTR cut-off score was helpful in determining the results for low-intensity test bands. Our results indicate that HIV ELISA OD was directly proportional to the measurements from the ALTA RTR-1, which helps in determining the line intensity and correctly identifying weak positive reactivity which will also reduce discrepancies in the inter-personnel reading of bands. This in term provides the user with a glimpse of specific antibody quantities available in the patient specimen. As the antibody concentration increases in the circulation from the day of infection, it helps to assess the recency of infection and correlate it with the clinical condition of the patient and information that should be raised during the counseling process [14]. Another aspect of this rapid assay measurement concerned the determination of false-positive results with the HIV testing algorithm (i.e., the correlation of results with other assays used with the algorithm). This user-friendly feature is beneficial for point-of-testing facilities and for staff who lack sufficient laboratory exposure such as nurses, operating room assistants, staff performing HIV testing in the field, and pharmacy assistants. Additionally, these assays can withstand ambient temperatures making field testing feasible. Field testing can also be used in rural areas where people are unable to travel to urban centers due to financial constraints or lack of transportation.

In the diagnostic industry, test kits are expected to perform at high levels of sensitivity and specificity, and some of the kits such as OnSite HIV 1/2 Ab Combo Rapid Test and the SD Bioline HIV 1/2 test are used

in this study demonstrated high-performance characteristics. Our results were in agreement with the characterized specimens and the Bio-Rad 4thgeneration assay, which highlights their accuracy. The other kits also demonstrated acceptable performance characteristics, with the exception of one discrepant specimen for the TRUSTline HIV 1/2 Rapid Ab Test and the Aspen® HIV 1/2 Rapid Ab Test kits.

HIV prevalence is increasing in India, especially among high-risk groups such as IDUs [13] and MSM [12]. Therefore, a rapid diagnostic kit with 100% sensitivity has become a necessity and it will be beneficial in situations where individuals might get the test report on the same day of the visit. Furthermore, false positives may lead to adverse outcomes such as unnecessary HAART initiation and emotional stress. However, false negatives may pose a threat to the community via the spread of the virus, a worsening health condition of the individual, and an underestimation of the HIV burden in the population.

In this study, we have established the performance characteristics (as per the NACO HIV diagnostic algorithm) of 3rdgeneration kits used to capture HIV-specific antibodies. However, there is the possibility that there were no antibodies or a minimal to residual quantity of antibodies during the window period after infection. To overcome this limitation, we tested each specimen with the 4thgeneration Genscreen ULTRA HIV Ag-Ab ELISA assay. Apart from this, even though the ALTA RTR-1 can be used in the field for HIV diagnosis it cannot be used for different spectrum of kits that are available in the market, which can pose challenge for the users to use other kits.

5. Conclusions

Rapid diagnostic tests used in this evaluation study were highly sensitive and specific when determining the appearance of faint or weak bands in HIV rapid immunochromatographic tests. Thus, these kits are suitable for the HIV testing algorithm recommended by NACO.

Ethical approval

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the 'Y.R. Gaitonde Centre for AIDS Research and Education (YRG CARE) institutional review board in Chennai, India (protocol code 316 and 16Feburary2021). (Approval Number: YRG 316) Permission to use leftover specimens from HIV-tested clients was obtained during the pre-test counseling process. This study did not involve any patient interventions.

Informed consent statement

Permission from the IRB was obtained for waiver of informed consent as this study uses only the stored specimen and this study does not involve any patient identification and intervention. Also, permission to use leftover specimens from these HIV test clients was obtained during the pre-test counseling process.

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Declaration of competing interest

The authors declare no conflicts of interest.

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References

[1] National AIDS Control Organization. India HIV estimates 2020 - technical brief [Internet]. NACO, Ministry of health and family welfare government of India; 2021. http://naco.gov.in/sites/default/files/India%20HIV%20Estimates%202020_Web_Version_0.pdf. [Accessed 19 March 2021].

[2] 2025 AIDS targets[Internet]. UNAIDS; 2021. https://aidstargets2025.unaids.org/. [Accessed 19 March 2021].

[3] Heath K, Levi J, Hill A The Joint United Nations Programme on HIV/AIDS 95-95-95 targets: worldwide clinical and cost benefits of generic manufacture. AIDS 2021; 35(Suppl 2):S197–203. https://doi.org/10.1097/QAD.0000000000002983.

[4] Iqbal H, Solomon S, Murugavel K, Solomon S.S, Balakrishnan P Evaluation of two indigenous rapid and two ELISA assays for the diagnosis of HIV infection India. Indian J Med Microbiol 2012;30(4):397–402. https://doi.org/10.4103/0255-0857.103758.

[5] National AIDS Control Organization. National guidelines for HIV testing lab services [Internet]. NACO, Ministry of health and family welfare government of India; 2015. http://naco.gov.in/sites/default/files/National_Guidelines_for_HIV_Testing_21Apr2016.pdf. [Accessed 18 September 2020].

[6] Syed Iqbal H, Balakrishnan P, Murugavel K.G, Suniti S Performance characteristics of a new rapid immunochromatographic test for the detection of antibodies to human immunodeficiency virus (HIV) types 1 and 2. J Clin Lab Anal 2008;22(3):178–85. https://doi.org/10.1002/jcla.20238.

[7] Bwana P, Ochieng L, Mwau M Performance and usability evaluation of the INSTI HIV self-test in Kenya for qualitative detection of antibodies to HIV. PLoS One 2018;13(9):1–17. https://doi.org/10.1371/journal.pone.0202491.

[8] Galiwango R.M, Musoke R, Lubyayi L, Ssekubugu R, Kalibbala S, Ssekweyama V, et al. Evaluation of current rapid HIV test algorithms in Rakai, Uganda. J Virol Methods 2013;192(1–2):25–7. https://doi.org/10.1016/j.jviromet.2013.04.003.

[9] Social science statistics. [Internet]. [cited 2021 Nov 29]. Available from: https://www.socscistatistics.com/.

[10] Huang X, Liu X, Chen J, Bao Y, Hou J, Lu X, et al. Evaluation of blood-based antibody rapid testing for HIV early therapy: a meta-analysis of the evidence. Front Immunol 2018;9(JUN):1–9. https://doi.org/10.3389/fimmu.2018.01458.

[11] Shaw S.Y, Lorway R, Bhattacharjee P, Reza-Paul S, Du Plessis E, McKinnon L, et al. Descriptive epidemiology of factors associated with HIV infections among men and transgender women who have sex with men in south India. LGBT Health 2016; 3(4):292–9. https://doi.org/10.1089/lgbt.2015.0023.

[12] Solomon S.S, Mehta S.H, Srikrishnan A.K, Vasudevan C.K, McFall A.M, Balakrishnan P, et al. High HIV prevalence and incidence among MSM across 12 cities in India. AIDS 2015;29(6):723–31. https://doi.org/10.1097/QAD.0000000000000602.

[13] Solomon S.S, Srikrishnan A.K, McFall A.M, Kumar M.S, Saravanan S, Balakrishnan P, et al. Burden of liver disease among community- based people who inject drugs (PWID) in Chennai, India. PLoS One 2016;11(1):1–15. https://doi.org/10.1371/journal.pone.0147879.

[14] Parekh B.S, Hanson D.L, Hargrove J, Branson B, Green T, Dobbs T, et al. Determination of mean recency period for estimation of HIV type 1 Incidence with the BED-capture EIA in persons infected with diverse subtypes. AIDS Res Hum Retrovir 2011;27(3):265–73. https://doi.org/10.1089/aid.2010.0159.