

Classification of recent and long-term infection among high-risk individuals using HIV-1 rapid recency testing

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ARTICLE INFO

Article history:

Received 27 November 2022

Accepted as revised 10 March 2023

Available online 12 March 2023

Keywords:

Recent HIV infection, long-term HIV infection, MSM, transgender women.

ABSTRACT

Background: HIV prevention with a significant reduction of new infections is important for epidemic control strategies. Antibody-based tests for recent infection (TRI) that can help distinguish recent from long-term HIV infection have been used since the mid-1990s to estimate population-level HIV incidence. Because the tests are done centrally, test results are not available to providers immediately. Point of care rapid testing for recent HIV infection (RTRI) was used for distinguishing recent from long-term status and estimated HIV incidence for preventing HIV infection. The usefulness of RTRI for recent HIV infection detection can be accelerated prevention of HIV infection transmission.

Objectives: To demonstrate RTRI assay utility to distinguish recent from long-term HIV infection among high-risk individuals.

Materials and methods: Between September and October 2022, HIV seropositive plasma samples of a high-risk group (N=90) and HIV-negative group (N=90) were collected from the outpatient department of Khon Kaen Hospital. All Specimens were tested by Asante™ Rapid Recency Assay (ARRA) and classified as negative, recent and long-term infection based on the presence or absence of specific lines (control line [CL], Positive verification line [PVL] and Long-term line [TL]) by visual reading.

Results: Among 90 HIV seropositive plasma samples, 14.4% (13/90) were recent HIV infection and 84.4% (76/90) were long-term HIV infection. There were no significant differences between recent and long-term infections among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers. Most of recently infected HIV patients were the MSM/TG group (53.9%, 7/13) and patients aged between 18 and 25 years old (76.9%, 10/13). CD4 count among recent cases was 396.7 ± 278.4 cells/mm³. ARRA performance has resulted in a sensitivity of 98.89% (95% CI: 93.96-99.97) and specificity of 100.00% (95% CI: 95.98-100.00).

Conclusion: ARRA performance showed excellent agreement with high sensitivity and specificity to comparing with a standard algorithm for HIV diagnosis. Its ability to classify new infections is crucial in HIV intervention and prevention strategies.

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doi: 10.12982/JAMS.2023.040

E-ISSN: 2539-6056

Introduction

Detection and identification of HIV transmission for early treatment with prioritized prevention strategies for high-incidence populations are important for driving epidemic control.^{1,2} The prevalence of HIV has been used to assess epidemic patterns and trends. The measurement of incidence can elucidate the transmission dynamic of

new infections. Laboratory assays for the detection of recent HIV infection for HIV incidence surveillance are essential to HIV prevention worldwide because they can identify populations with a high incidence and allow the targeting of resources and monitoring of incidence trends over time. Detecting recent HIV infections can help prioritize prevention and early treatment for those at high risk of transmission. Tests that can detect recently infected individuals were the achieved goal of epidemic strategies.^{3,4} Laboratory assays for the estimation of recent HIV infection were described as early as 1998, and initially were modifications of commercially available HIV antibody tests.⁵ These assays indirectly measured antibody titers that are low in early infected individuals and high in chronically infected persons. However, the use of such modified commercial assays for incidence testing may not provide consistent identification of HIV incident infections in populations with diverse HIV-1 circulating subtypes since almost all commercial products use HIV-1 subtype B-derived antigens.^{6,7}

For the routine diagnosis of HIV infection, the most used tests are rapid spot tests or ELISA that detect HIV antibodies; these cannot necessarily discriminate between early and chronic infections. These platforms require sophisticated laboratory infrastructure and a trained workforce.

Antibody-based tests for recent infection (TRI) that can help distinguish recent from long-term HIV infection have been used since the mid-1990s to estimate population-level HIV incidence and to evaluate the impact of large-scale HIV interventions in preventing HIV infection.^{8,9} Testing for recent infection among newly diagnosed HIV-positive persons has been integrated into routine HIV surveillance worldwide.¹⁰ TRI assays are done centrally; test results are not available to providers immediately and cannot be used to identify persons most likely to transmit the virus. Alternative methods that can expedite the results of recent infection testing through point-of-care testing (POCT) are needed.

The rapid test for recent infection (RTRI) can improve our understanding of the HIV epidemic by demonstrating shifting dynamics in the HIV epidemic over time, such as changes in the proportion of recent infections allowing public health officials and providers to trace active HIV transmission networks and deploying targeted prevention strategies.¹¹ While results from laboratory-based recent infection tests may take weeks to months to provide results due to sample transportation, laboratory processing, and turnaround time from the testing laboratory to the referring clinic, the results of the rapid test for recent infection can be available within minutes, facilitating more efficient and targeted responses for maximum public health impact.

Asante™ Rapid Recency Assay (ARRA) is a single test device that can simultaneously diagnose HIV infection and differentiate between recent and long-term HIV-1 infection that has been developed and described previously by the CDC¹². The performance of ARRA has been evaluated using a well-characterized panel of cross-sectional specimens

with known HIV serology status and recent or long-term status based on comparative Limiting Antigen (LAG) Avidity Enzyme immunoassay (EIA) results, which has been validated to detect recent infections.¹³

The development of a rapid test for recent infection allows one to perform HIV recency testing in a routine HIV management in real time and utilize data for targeted prevention. ARRA was the rapid antibody-based test that followed the lateral flow immunoassay principle for recent infection identification. ARRA is a strip test that combines HIV diagnosis with a time indicator since infection as previously described.¹² This is accomplished by including an additional line where the antigen is striped at a limiting antigen concentration to distinguish recent from long-term infection.

Current study conducted the performance characteristics of ARRA among men who have sex with men (MSM), transgender women (TG), heterosexual people, and sex workers, demonstrating a positive verification line (PVL) of the Asante strips compared with the standard HIV diagnostic algorithm and separating recent from long-term HIV infection.

Materials and methods

Blood specimens

Leftover specimens were collected between September and October 2022 from MSM/TG and sex workers. All specimens were recruited from the outpatient department of Khon Kaen Hospital. All samples were examined in accordance with the Thailand National Guidelines on HIV/AIDS Treatment and Prevention 2021/2022. The specimens were diagnosed with HIV antibodies by the 4th generation Electrochemiluminescence Immunoassay (ECLIA) (Cobas e801, Roche Diagnostics), Alere™ HIV combo (Alere Medical Co., Ltd, Japan) and SD BIOLINE HIV-1/2 3.0 (Standard Diagnostics, INC, Korea). The panel consisted of HIV seropositive plasma samples of high-risk individuals (N=90) and HIV-negative patients (N=90). Following that, ARRA was used to examine all specimens.

ARRA procedure

The ARRA was conducted following the manufacturer's instructions (Sedia Biosciences Corporation, Portland, USA).¹⁴ Serum or plasma samples were warmed to room temperature before an assay. Using the collecting loop included in the test set, 5 µL of sample was transferred into and mixed in a 0.5 mL buffer tube. A test strip was inserted into the tube after the specimen collection loop was removed and incubated for 20 minutes. After 20 minutes, the strips were taken out and wiped onto filter paper to remove any excess buffer before being visually categorized as recent, long-term, or negative. To prevent false positive, the visual reading should be performed immediately. The results were recorded in a worksheet file.

Data collection

Clinical data, including age, sex, sexual behavior, and

route of transmission, were collected from OPD cards and laboratory data; CD4 count, hepatitis C antibody (HCV Ab), hepatitis B antigen (HBsAg), and rapid plasma reagin (RPR) were recorded from the laboratory information system. ARRA results were recorded from the presence of all control lines (CL), positive verification lines (PVL) and long-

term lines (LTL) by visual reading. The presence of all three lines (CL, PVL and LTL) indicated an HIV-positive diagnosis with long-term infection. The presence of CL and PVL indicated an HIV-positive diagnosis with recent infection while the presence of only the control line (CL) indicated an HIV-seronegative sample (Figure 1).

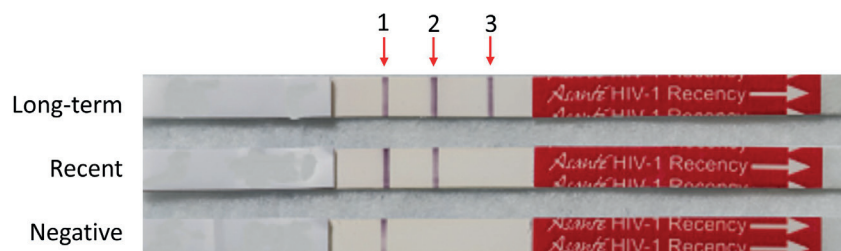


Figure 1 Interpretation of the Asante Rapid Recency Assay results is based on the presence or absence of specific lines. Arrows 1, 2 and 3 represent control line (CL), positive verification line (PVL) and long-term line (LTL), respectively, by visual reading.

Statistical analysis

Performance evaluation of ARRA was calculated by diagnostic statistics for sensitivity and specificity with the standard seroconversion algorithm.¹⁵ Percentage means and standard deviation were performed using STATA version 14.0. ANOVA and chi-square were used to compare statistical significance between demographic data and recent infection or long-term infection.

Ethical consideration

The protocol was approved by the Ethical Committee of Khon Kaen Hospital, Ministry of Public Health (KEX65044).

Results

Among 90 HIV seropositive samples, 14.4% (13/90) was recent HIV infection and 84.4% (76/90) was long-term HIV infections. Demographic data of this population demonstrated that most of recent HIV infection were MSM/TG (53.9%, 7/13) and patients aged between 18 and 25 years old (76.9%, 10/13). There were no significant differences in age, sex, sexual orientation, and route of transmission between recent and long-term infections among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers ($p>0.05$). Unprotected anal activity was a higher-risk route of infection transmission compare with vaginal sex. The absolute CD4 count of recent infection cases was 396.7 ± 278.4 cells/cu.mm. The prevalence of low CD4 count (lower than 200 cells/mm³) was 28.6% (2/7) in recent infection and 16.7% (8/48) in long-term infection cases. HIV viral load among long-term infection cases demonstrated viral suppression at 73.9% (17/23), while recent infection showed only 33.3% (2/6). Other sexual transmission infection (STI) markers, including RPR, HBsAg and HCV Ab, were not different between recent and long-term HIV infection (Table 1).

The performance of ARRA was calculated by comparing

the visual results with the standard HIV algorithm. The ARRA assay showed 89 HIV-positive results and 1 negative result with a sensitivity of 98.89% (95% CI=93.96-99.97), specificity of 100.00% (95% CI=95.98-100.00), agreement 99.44% (95% CI=96.94-99.99) and Kappa 0.989 (95% CI=0.967-1.000) as shown in Table 2.

Discussion

Diagnosis of HIV infection has spread globally due to rapid diagnostic testing, ease of performance and low cost. Recent infection identification is important for epidemic control since it has been suggested that a high proportion of new infections originates from recently infected individuals.¹⁶ Identifying recent infections was difficult for point-of-care testing due to technical complications, specimen quality, process, and storage before testing was performed.^{17,18}

The study was done to find the incidence of newly diagnosed HIV case among high-risk individuals using ARRA and to explore the demographic data in recently and long-term infected patients. Our study assessed the utility of the Asante Rapid Recency Assay to classify recent and long-term infections among high-risk seropositive cases as recommended by WHO-PQ/FDA¹⁹ and associated results to HIV seropositive from a standard algorithm with high sensitivity and specificity (98.89% and 100.00%, respectively) (Table 2). Our study results in the classification of recent and long-term infections related to the previous study.^{12,20} ARRA qualification feature has impacted recent infection surveillance surveys of recruiting new infection cases for prevention.²¹⁻²³ The advantage of this assay serves to confirm HIV seropositive status and classify recent and long-term individuals. Early identification of recent infection is important for HIV treatment and breaking off HIV transmission in the early stage, which can accelerate epidemic control. Although our data showed high sensitivity and specificity of the ARRA, this test is

Table 1 Patient characteristics and HIV status among men who have sex with men (MSM), transgender women (TG), heterosexuals and sex workers.

	Recent infection		Long-term infection		Total
	N	%	N	%	
Age, years					
18-25	10	76.9	41	53.9	51
26-35	2	15.4	22	28.9	24
>36	1	7.7	13	17.2	14
Sex					
Male	12	92.3	66	86.8	78
Female	1	7.7	10	13.2	11
Sexual orientation					
MSM/TG	7	53.9	38	50.0	45
Heterosexual	5	38.4	33	43.4	38
Sex worker	1	7.7	5	6.6	6
Route of transmission					
Anal sex	7	53.9	40	52.6	47
Vaginal sex	6	46.1	36	47.4	42
CD4 count (cell/mm³)					
<200	2	28.6	8	16.7	10
200-500	2	28.6	25	52.1	27
>500	3	42.8	15	31.2	18
HIV viral load copies/mL					
Suppressed (<1,000)	2	33.3	17	73.9	19
Unsuppressed (≥1,000)	4	66.7	6	26.1	10
RPR					
Reactive	3	42.9	9	18.7	12
Non-reactive	4	57.1	39	81.3	43
HBsAg					
Positive	1	14.2	1	2.1	2
Negative	6	85.8	47	97.9	53
HCV Ab					
Positive	0	0.0	3	6.3	3
Negative	7	100.0	45	93.7	52

Table 2 Two by two comparison of HIV seropositive results from the standard algorithm with visual interpretation for the positive verification line (PVL) of the Asante Rapid Recency Assay (N=180).

Asante PVL visual		HIV seropositive from standard algorithm		
		HIV-positive	HIV-negative	Total
	HIV-positive	89	0	89
	HIV-negative	1	90	91
	Total	90	90	180

not currently used in surveillance surveys for recent HIV infection detection in the national testing algorithm of Thailand because of lack of approval. A further study of rapid tests for recent infection combined with a routine HIV diagnosis will be beneficial for recent infection surveillance. However, ARRA has not been evaluated using a large number of specimens. We describe here the utility of this test kit on classification of recent and long-term infection in patients' specimens that were examined by routine standard laboratory protocols. The purpose of this evaluation was to compare the performance of Asante strips with the standard HIV diagnostic algorithm.

However, there were some limitations to be concerned. Firstly, HIV recency testing was offered to individuals with a new HIV diagnosis. As only consenting individuals were tested for recency, our study sample did not include all individuals with a new HIV diagnosis. Secondly, some recent infections classified by ARRA may have to be reclassified as long-term infection. We could not exclude patients with CD4 counts <200 cells/mm³ out of the recent infection group and cannot reclassify the recent infection group of patients who have viral loads ≤ 1000 copies/mL to minimize the impact of false recent infections. Because specimens for viral load detection (HIV VL) were collected after 3 months of antiretroviral therapy, which was complicated and expensive. Moreover, many patients diagnosed with HIV, especially in groups that received service in issuing proactive units, refused to enter the treatment process, resulting in a lack of CD4, HIV VL, and other baseline laboratory test results. Lastly, this study screened newly infected people in small number of samples due to short expiration date of the test kit (short study time) and fewer people used the service in the hospital during the COVID-19 outbreak.

Conclusion

ARRA performance showed excellent agreement with high sensitivity and specificity to comparing with a standard algorithm for HIV diagnosis. Its ability to classify new infections is crucial in HIV intervention and prevention strategies.

Conflicts of interest

In this study, the author declared no conflicts of interest in the research.

Acknowledgments

We would like to thank Dr. Kriengsak Vacharanukulkiet for providing and supporting this work. We would also like to thank Dr. Suthin Chanabun for his assistance in statistical analysis and Dr. Manthana Mitchai for her kind suggestions and correction of the manuscript.

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