Evaluation of Rapid Tests for Recent HIV Infection: Implications for Real-time Surveillance and Epidemic Control

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ABSTRACT

Background: Detection of recent HIV infection is critical for identifying populations with ongoing transmission for epidemic control. Using concept of limiting antigen, we developed a rapid test that can diagnose HIV infection and identify recent infection in a single device. This technology was commercialized by Sedia BioSciences (Portland, OR) as Asante™ Rapid Recency Assay and by Maxim Biomedical (Rockville, MD) as Swift™ Recent Infection Assay. We evaluated the performance of both assays using a panel of well-characterized specimens.

Methods: The specimen panel consisted of 1500 samples that included HIV-1 (n=570, recent =100, long term =470), HIV-2 (n=10) and HIV negatives (n=920) representing subtypes A, B, C, D, and AE from multiple geographic areas. Reference data were generated using Bio-Rad HIV-1-2-O EIA + Western blot algorithm with further serotyping done using Multispot HIV-1/2 assay. LAg-Avidity EIA (Sedia) was used to generate recent infections reference data. Asante and Swift were performed as recommended by the manufacturers and the presence or absence of "Diagnostic Verification/Test line (V or T)" line and "Long term (LT)" on the strips or cassettes were recorded after 20 min. The results were then compared with reference EIA/Western Blot data and LAg data using a cutoff of 2.0.

Results: Two RTRI correctly identified 575 (Asante) and 576 (Swift) of 580 HIV-positive specimens resulting in a sensitivity of 99.14% and 99.31%, respectively (Table 1). Among HIV-negatives, 910/920 and 914/918 specimens were correctly identified by Asante and Swift (2 were invalid), respectively, resulting in a specificity of 98.91% and 99.56%. The agreement between the LAg-Avidity EIA (at ODn cutoff of 2.0) and the LT line of the tests were 91.67% (Asante) and 88.89% (Maxim). Agreement between the two tests in classifying recent or LT infections was >92% with kappa of 0.746.

Conclusion: Both assays have high diagnostic sensitivity (>99%) and specificity (> 98%) that may facilitate regulatory approval of these tests. LT lines from both assays showed good correlations with the LAg-Avidity EIA with estimated MDRI of about 6 months post-seroconversion. Access to these commercial kits should facilitate real-time surveillance of recent infections in routine HIV testing services to identify areas of ongoing transmission and interrupt further transmission as we strive to reach zero new infections.

INTRODUCTION

- Estimation of HIV-1 incidence is important to measure success of HIV program, identify hot-spots and target resources where they are needed most.
- Considerable efforts and resources are devoted to development of laboratory assays to detect recent HIV infections. As a result of these efforts, we developed Limiting-Antigen (LAg) Avidity EIA.
- LAg-Avidity EIA is now widely used in number of surveys including population-based HIV Impact Assessment (PHIA) surveys in several countries.
- We extended the concept of limiting antigen from EIA to rapid test format combining this with routine HIV diagnostic test to simultaneously achieve HIV diagnosis and recency or long-term classification, all in one test.
- AsanteTM Rapid Recency Assay and Maxim SwiftTM Recent Infection Assay (RIA) (Figure 1A and 1B respectively) are commercial tests that combine HIV diagnosis with marker of time since infection.
- This is achieved by antigen striping at limiting antigen concentration to distinguish recent from long-term infection.
- Limiting concentration of antigen ensures binding of only high avidity antibodies present in long-term infections (>12 months).
- Presence of all three lines (C, V/T, and LT) indicate HIV-positive person with long-term infection: presence of only two lines (C and V/T) indicate HIV-positive person with recent infection: presence of only control line (C) indicates seronegative person (Figure 2).
- We evaluated performance of these tests using a large panel of specimens comparing the diagnostic results to reference diagnostic results from EIA/Western Blot algorithm and recency to LAg-Avidity EIA at a cutoff of 2.0 ODn for all HIV-1 positives.

METHODS

All testing were performed according to the manufacturers' instructions and visual and reader-based results were recorded from the test strip (Asante) or cassette (Maxim). The results from both tests were compared to standard EIA/Western Blot algorithm and the LAg Avidity EIA.

Specimen Panel

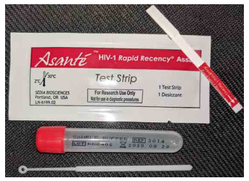
- Well-characterized world-wide panel of specimens
- HIV positive, N = 580; HIV-1 = 570, HIV-2 = 10
- HIV seronegative, N = 920
- Diverse geographic locations: Kenya, Uganda, Cameroon, Ivory Coast, South Africa, Thailand, U.S.A
- Subtype diversity: subtypes A, B, B', C, D, AE
- HIV status determined by EIA followed by confirmatory Western blot testing
- HIV-1 and 2 serotyping done by Multispot, Geenius or in-house peptide-based EIA
- Reference recency testing done by LAg-Avidity EIA for comparison

Purpose of Evaluation

- Performance of diagnostic line (HIV status)
- Performance of incidence line (recent/LT)
- Mean duration of recent infection
- Ease of use
- Ease of interpretation
- Reproducibility
- Lot consistency

Figure 1

(A) Shows components of the Asante™ HIV-1 Rapid Recency Assay Kit that include a sample collection loop, a buffer tube and a test strip. Kit components are available in both 20 sets and 100 sets.



(B) Shows components of the Swift™ HIV Rapid Incidence Assay (RIA) Kit that include a sample collection/delivery pipette, a buffer tube and a test cassette. Kit components are available in 20 sets. Swift kit also includes 20 lancets for finger prick.

Figure 2

Interpretation of rapid tests for recent infection results based on the presence or absence of lines.
The presence of only the control line (C) indicates the client is HIV-seronegative (1), while presence of C and Verification (V) on Asante strip or C and Test (T) lines on Swift cassette indicate HIV-1 positive with recent infection. The presence of all three lines (C, V/T and LT) indicate HIV-positive diagnosis with long-term infection.

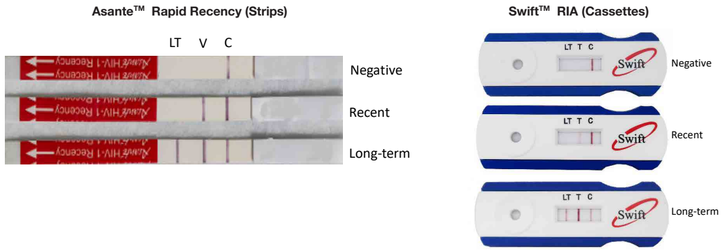


Table 1

A) Comparing the performance of the verification line of the Asante™ assay with results from standard diagnostic algorithm (EIA/Western Blot) and **B)** Comparing the performance of the Test line of the Swift™ assay with standard diagnostic algorithm. n=1500

A) Asante™ Rapid Recency

Asante VL-Visual	Pos	Neg	Total
Pos	575	10	585
Neg	5	910	915
Total	580	920	1500

Sensitivity = 99.14 [98-99.72]
Specificity=98.91 [98.01-99.48]
Overall Agreement with Reference data=99
Kappa=0.979 [0.968-0.99]

B) Swift™ RIA

Swift Test Line-Visual	Pos	Neg	Total
Pos	576	4	580
Neg	4	914	918
Total	580	918	1498*

Sensitivity = 99.31 [98.24-99.81]
Specificity=99.56 [98.89-99.88]
Overall Agreement with Reference data=99.47
Kappa=0.989 [0.981-0.997]
*two specimens were repeatedly invalid

Table 2

Comparing the performance of the Asante LT line (A), and the performance of the Swift LT line (B) with LAg-Avidity EIA at a cutoff of 2.0 ODn (MDRI = 6 months)

A) Asante™ Rapid Recency

Asante Rapid Recency Assay	LAG-Avidity EIA at 2.0 ODn		
	Recent	Long-Term	Total
Recent	80	29	109
Long-Term	18	450	456
Total	98	467	565

% agreement = 91.68%
Kappa = 0.722

B) Swift™ RIA

Swift Recent Infection Assay	LAG-Avidity EIA at 2.0 ODn		
	Recent	Long-Term	Total
Recent	72	35	107
Long-Term	28	432	460
Total	100	467	567

% agreement = 88.9%
Kappa = 0.628

Figure 3

Graphical correlation of visual and strip reader values:
A) Asante Recency Assay, B) Maxim Swift Assay. The results show strip reader values arranged in ascending order and the corresponding visual result on X-axis. Shown is a middle transition zone where visual representation and strip reader results may have occasional different interpretation. As expected, transition zone values are close to the cutoff and range between >2.7 IU to <3.2 IU for Asante (A), and >80.0 LFI to <120 LFI for Swift (B).

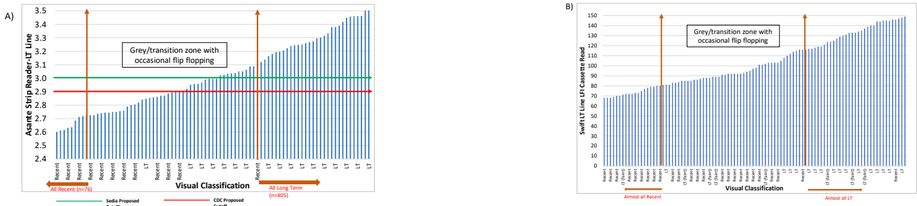


Table 3

LT line agreement between visual results and results from the reader in both Asante (A) and Swift (B) assays. Those with discordant results are close to the cutoff.

A)

Asante LT Line (Reader @3.3 IU)	Asante Long Term Line (Visual)		
	Recent	Long-Term	Total
Recent	103	5	108
Long-Term	6	451	457
Total	109	456	565

% agreement = 98.05
Kappa = 0.937 [0.901-0.974]

B)

Swift LT Line (Reader @100 LFI)	Swift Long Term Line (Visual)		
	Recent	Long-Term	Total
Recent	98	10	108
Long-Term	9	450	459
Total	107	460	567

% agreement = 96.65
Kappa = 0.891 [0.843-0.939]

Table 4

Agreement between AsanteTM and SwiftTM showing visual agreement between the verification line in Asante and the Test line in Swift (A), and the agreement between the LT lines of the two assays (B).

A)

Swift Test Line, Visual	Asante VL Line, Visual		
	Positive	Negative	Total
Positive	575	4	579
Negative	8	911	919
Total	583	915	1498

Kappa = 0.983 [0.974-0.993]
% agreement = 99.20

B)

Swift LT Line, Visual	Asante LT Line, Visual		
	Recent	LT	Total
Recent	88	17	105
LT	21	439	460
Total	109	456	565

Kappa = 0.781 [0.714-0.848]
% agreement = 93.27

CONCLUSIONS

- Both Asante™ and Swift™ kits have diagnostic sensitivity and specificity >99%
- Both tests have similar performance in classifying recent infections
- Visual and reader-based interpretations are similar with some flip-flops near the cutoff boundary, as expected
- Rapid test for recent infection assay is a cross-cutting integration of laboratory, surveillance and prevention
- RTRI can simplify corss-sectional surveys providing information for both prevalence and incidence using a single test
- It can be used to monitor real-time surveillance of new infections when used in routine testing program.
- Facilitates "Detection and Quick Response"
- Contact tracing and partner testing provide opportunity to increase yield as well as interrupt further transmission
- Shift from "monitoring population" to "identifying new infection at the individual level"
- Important tool as we strive to reach zero new infection

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