Performance Evaluation of Asante™ Rapid Recency Assay for HIV Diagnosis and Detection of Recent Infection: Potential for Surveillance and Prevention

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Abstract

Background: Detection of recent infection is critical for incidence estimates from surveys and can also help in targeted prevention. We previously described development of a rapid test that can diagnose HIV infection and detect recency of infection in one device. This technology was successfully transferred to a commercial partner as AsanteTM Rapid Recency Assay developed by Sedia BioSciences (Portland, OR). We evaluated performance of this assay in laboratory using a well-characterized panel of specimens.

Methods: Specimen panel consisted of 1500 sera or plasma from 580 HIV positive (570 HIV-1, 10 HIV-2), and 920 seronegative individuals 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 was used to generate recency information. Asante assay was performed as recommended by the manufacturer and line intensities for diagnostic and incidence lines were read using a hand-held reader. Ability to diagnose HIV and detect recent infections were determined using a reader cut-off values of 1000 for each and results were compared to reference results. Reproducibility of the assay was measured with multiple measurements.

Results: AsanteTM rapid recency assay detected 575/580 HIV positive specimens correctly resulting in a sensitivity of 99.1% (95% CI 98.0-99.6) while specificity of Asante assay was 98.7% (908/920) (95% CI 97.7-99.3). There was high correlation (Spearman rank correlation r=0.785) between ODn of LAg-Avidity EIA and incidence line intensity of the Asante test for 570 HIV-1 specimens with cutoff of 1000 matching with LAg ODn of 2.0 (See Figure) corresponding to mean duration of recent infection of about 180 days. Recency assay classified 98 specimens as recent infections compared to 103 by LAg-Avidity EIA. The assay had high reproducibility with %CV of <10% in the dynamic range.

Conclusion: AsanteTM assay meets requirements of a diagnostic assay with sensitivity and specificity close to 99%, while identifying recent infections with a mean window period of about 6 months post-seroconversion. This point-of-care assay has implications for enhancing surveillance and targeted prevention in high incidence population.

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 but is difficult to accomplish in traditional cross-sectional surveys.
- 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 that has been well characterized, commercialized and field validated, when used in an algorithm with viral load, to estimate HIV-1 incidence.
- This assay 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.
- Asante Rapid Recency Assay is a commercial test that combines 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 (>6 months) [Figure 1]
- Presence of all three lines (C, D, and LT) indicates HIV-positive person with long-term infection (>6 months); presence of only two lines (C and D) indicates HIV-positive person with recent infection (<6 months); presence of only control line (C) indicates seronegative person.
- We further evaluated performance of this test in a large panel of specimens comparing it to reference diagnostic results and recent/LT classification compared to LAg-Avidity EIA.

Methods

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, Sierra Leone, South Africa, Thailand, U.S.
- 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: Kit components of the Asante™ Rapid Recency Assay. (A) Shows components of the Asante™ Rapid Recency Assay Kit that include the Specimen Collection Loop, Sample Buffer Tube and Test Strip that are individually sealed in foil pouches. Kit components are available for 100 test sets (B) An example of a test strip displaying the control, diagnostic and incidence lines.

Figure 2: Schematic Interpretation of the Asante Rapid Recency Assay based on the presence or absence of test lines. The test can be interpreted both qualitatively and quantitatively. The presence of only the control line (C) indicates the client is HIV-seronegative, while presence of C and diagnostic (D) lines only indicates HIV-positive diagnosis with recent infection. The presence of all three lines (C, D and LT) indicate HIV-positive diagnosis with long-term infection. Quantitative results (intensity units) can also be obtained with a strip reader as shown in Figure 2.

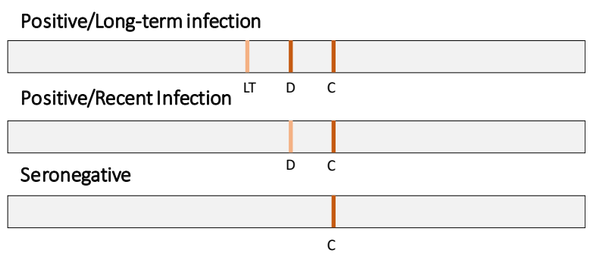
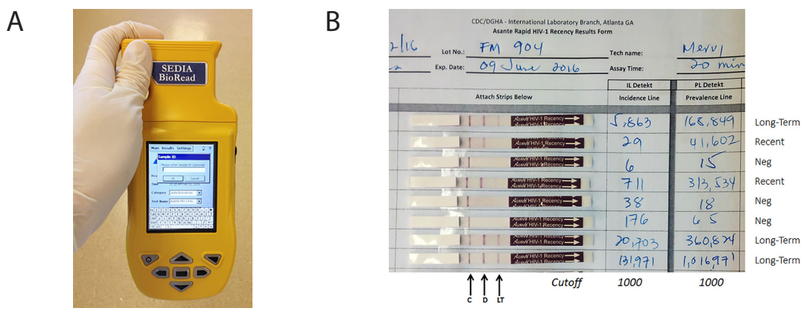


Figure 3. Asante™ Rapid Recency Assay Strip reader. (A) Shows the Asante assay Sedia strip reader instrument and (B) Examples of developed test strips on the Asante result form showing line intensity for the incidence and diagnostic lines obtained from the strip reader. The final classification is shown based on the cut offs of 1000 intensity units for both lines. The arrows indicate the positions of the control, diagnostic and incidence lines



Results

Figure 4: Relationship between incidence line and diagnostic line presented as X-Y plot for 1500 specimens using cut offs of 1000 for both diagnostic and incidence lines. The horizontal arrow shows the cut off of the diagnostic line at 1000 intensity units, while the vertical line shows the cut off of the incidence line at 1000 intensity units.

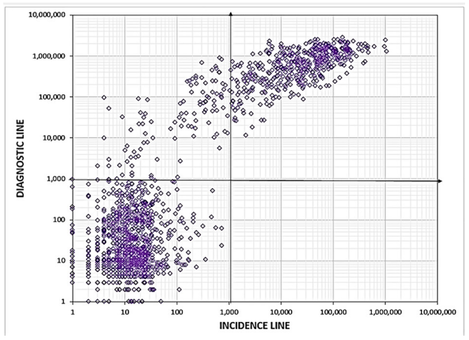


Table 1: A) Comparing the performance of the diagnostic component of the Asante assay with results from standard diagnostic algorithm –EIA/Western Blot and B) Comparing the performance of the incidence component of the Asante assay with LAg-Avidity EIA at a cutoff of 2.0 ODn. N=1500

A

Asante Diagnostic Line	EIA/WB algorithm		
	HIV pos	HIV neg	Total
HIV pos	**575**	12	587
HIV neg	5	**908**	913
Total	580	920	**1500**

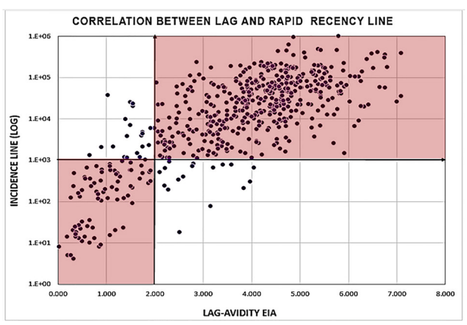
Sensitivity = 99.14%
Specificity = 98.7%
Overall agreement with Reference HIV Testing = 98.9%
Kappa = 0.976 (CI 0.965-0.987)

B

Asante Rapid Recency Assay	LAG-Avidity EIA		
	Recent	Long-Term	Total
Recent	**81**	17	98
Long-Term	22	**450**	472
Total	103	467	**570**

% agreement = 93.2%
Kappa = 0.764 (0.694-0.835)

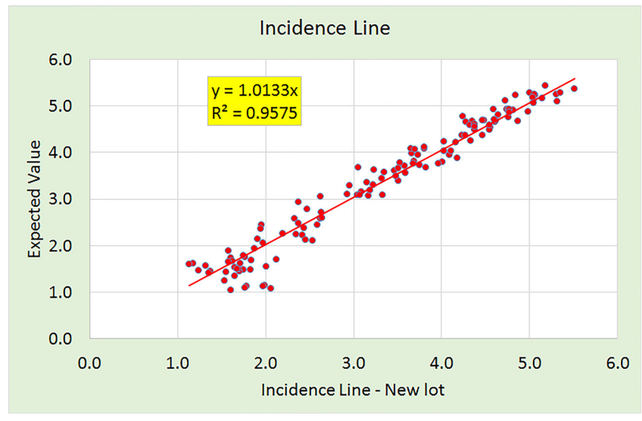
Figure 5: Correlation of Asante Rapid Recency Test with *LAg-Avidity EIA: LAg ODn versus Incidence Line intensity.* The horizontal arrow shows the cut off of the Asante incidence line at 1000 units, while the vertical line shows the cut off of the LAg assay ODn at 2.0. The areas of the graph represented in purple contain specimens that have the same classifications by both Asante and LAg assays.



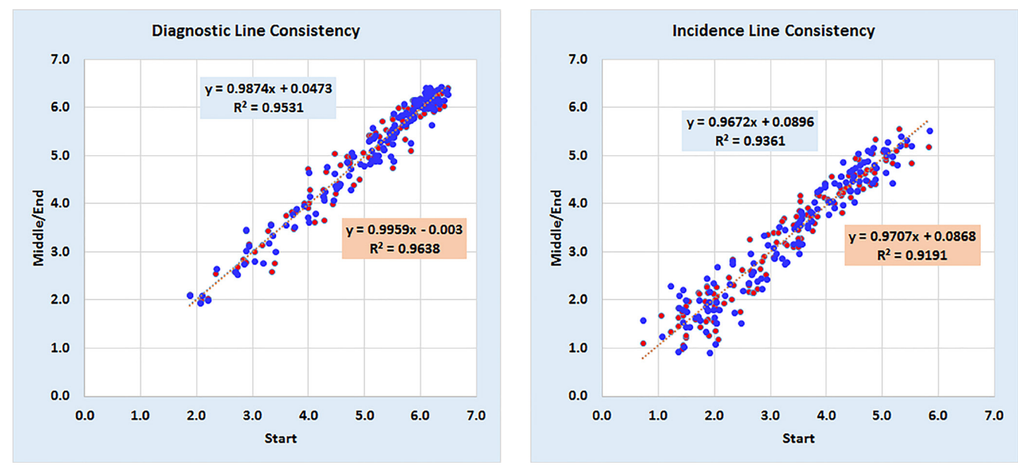
- The Asante has an estimated mean window period of about 6 months but actual time period is not that critical when used for prevention
- Subtype differences, if any, do not impact in a significant way
- Accuracy of even 80% to 90% can have major impact on prevention
- Targeted use in newly diagnosed persons can further improve PPV

Figure 6: Asante assay reproducibility. (A) New lot QC – comparison with expected data, (B) Kit lot consistency evaluation of both the diagnostic line and the incidence line during manufacturing: comparing kits from within the same lot but at different times during manufacturing.

A



B



Conclusions

- Asante Rapid Recency assay is a cross-cutting integration of laboratory, surveillance and prevention
- Can simplify survey providing information for both prevalence and incidence using a single test
- Can be used to monitor declining # of recent infections over time in ***real time*** (M&E tool)
- 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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