Laboratory Validation of HIV Rapid Tests for Recent Infection

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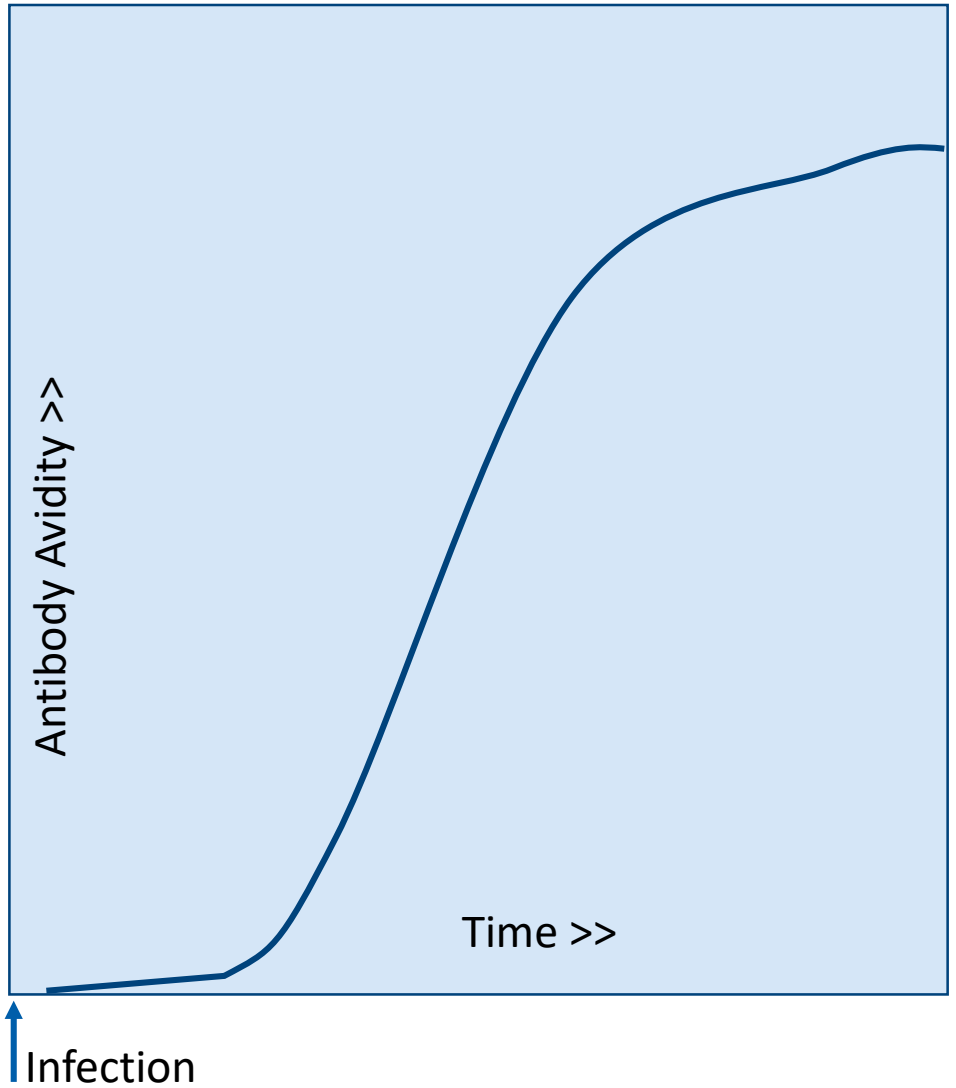
Detecting Recent HIV Infection with Antibody Avidity

- Antibody avidity = binding strength of antibody (how strongly HIV antibodies bind to HIV)
- Functional property of maturing antibodies
- Antibody avidity increases over time after infection
- Surrogate marker of time since infection
- Can be used to detect and distinguish recently infected persons (weak antibodies) from those with long-term infections (strong antibodies)
- Simple method to measure antibody avidity?



From LAg-Avidity EIA to Rapid Test for Recent Infection (RTRI)

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Development of a Novel Rapid HIV Test for Simultaneous Detection of Recent or Long-Term HIV Type 1 Infection Using a Single Testing Device

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Abstract

Laboratory assays for the detection of recent HIV infection for HIV incidence surveillance are essential to HIV prevention efforts worldwide because they can identify populations with a high incidence and allow targeting of resources and monitoring of incidence trends over time. This study describes the development of a novel rapid HIV-1 incidence-prevalence (I-P) test that can be used for the simultaneous detection and discrimination of prevalent (long-term) or incident (recent) HIV infections using a single device. A lateral flow assay was developed that uses a multisubtype recombinant gp41 protein applied at two concentrations of antigen (high and low). Prevalent and incident HIV-1 infections can be distinguished based on differential antibody binding at the two antigen concentrations. High level/high avidity antibodies present in prevalent infections bind to and are detected at both antigen concentrations while low level/low avidity antibodies present in recent HIV infections are detected only at the higher antigen concentration line. A total of 205 HIV-positive specimens with known status (recent=105, long-term=100), including 57 specimens from seroconversion panels, were tested by the

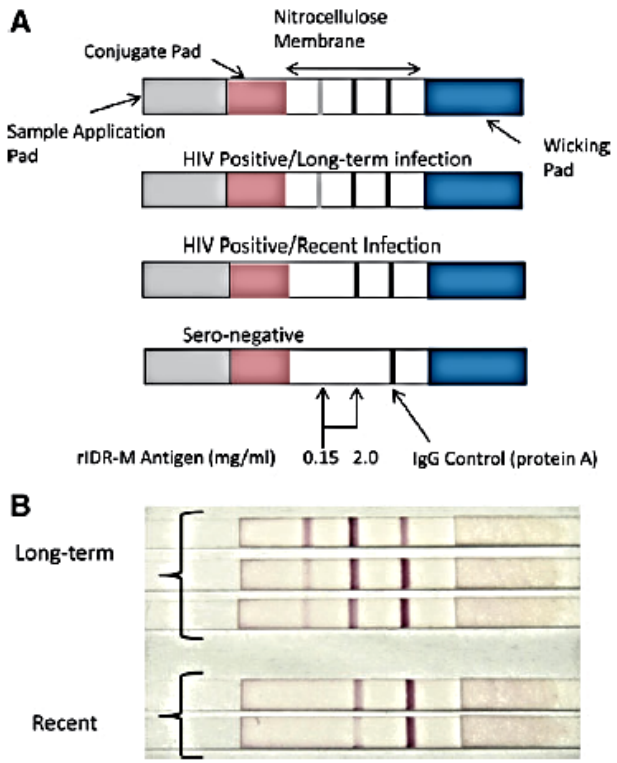


FIG. 2. Schematic diagram of the rapid I-P assay including interpretative criteria. **(A)** Photographs of the rapid I-P assay showing Panel 1 **(B)** including three long-term infections (>1 year) (top); two recent infections (<6 months) (bottom).

Two Commercial Manufacturers of RTRI Assay

Sedia BioSciences

Asante Rapid Recency Assay

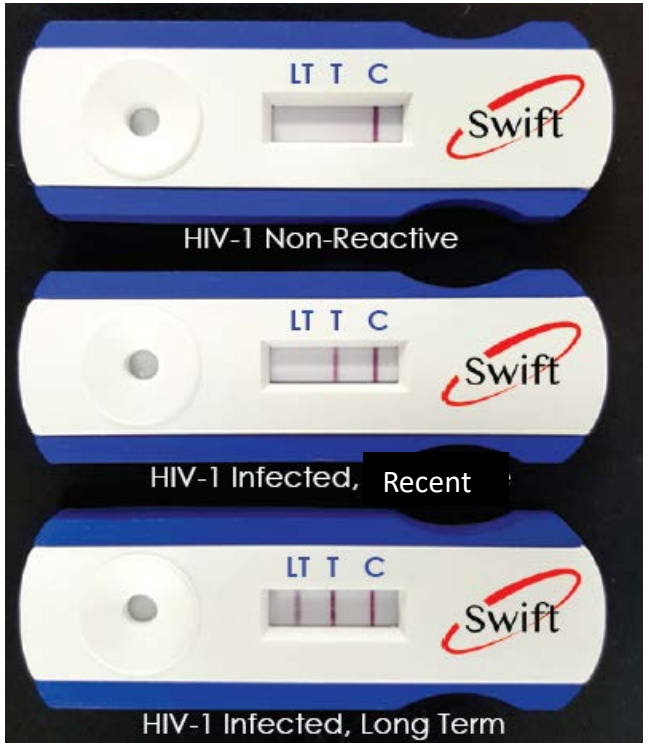
(Dip-stick format)

C= Control line
V= Verification line
LT= Long-term line
T= Test line

Maxim Biomedical Corp

Swift Recent Infection Assay (RIA)

(Cassette format)



Evaluation of RTRI Kits (Commercial Tests)

CDC Specimen Panel

- Well-characterized world-wide panel of specimens, N=1500 (both plasma and serum) (5 different subtypes)
- HIV positive, N=580 (10 HIV-2), HIV negative, N = 920
- Additional testing using longitudinal seroconversion panels

NICD, South Africa Specimen Panel

- Well-characterized panel of specimens, N=745 (plasma or serum)
- HIV positive, N=458 (Subtype C), HIV negative, N = 287

NIHE, Vietnam Specimen Panel

- Well-characterized panel of specimens, N=464 (plasma or serum)
- HIV positive, N=232, HIV negative, N = 232

Note: In all cases, HIV status was determined by EIA or rapid tests followed by confirmatory Western blot testing, while reference recency testing was done by LAg-Avidity EIA for comparison

Parameters Evaluated

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

Asante Performance of Diagnostic Verification Line: CDC Evaluation

Interpretation with a Reader

		EIA/WB Algorithm		
Asante VL (Reader @2.8 IU		HIV pos	HIV neg	Total
	HIV pos	**576**	11	587
	HIV neg	4	**909**	913
	Total	580	920	**1500**

Sensitivity = 99.31%
Specificity = 98.8%
Overall agreement with Reference HIV Testing = 99%
Kappa = 0.979

Visual Interpretation

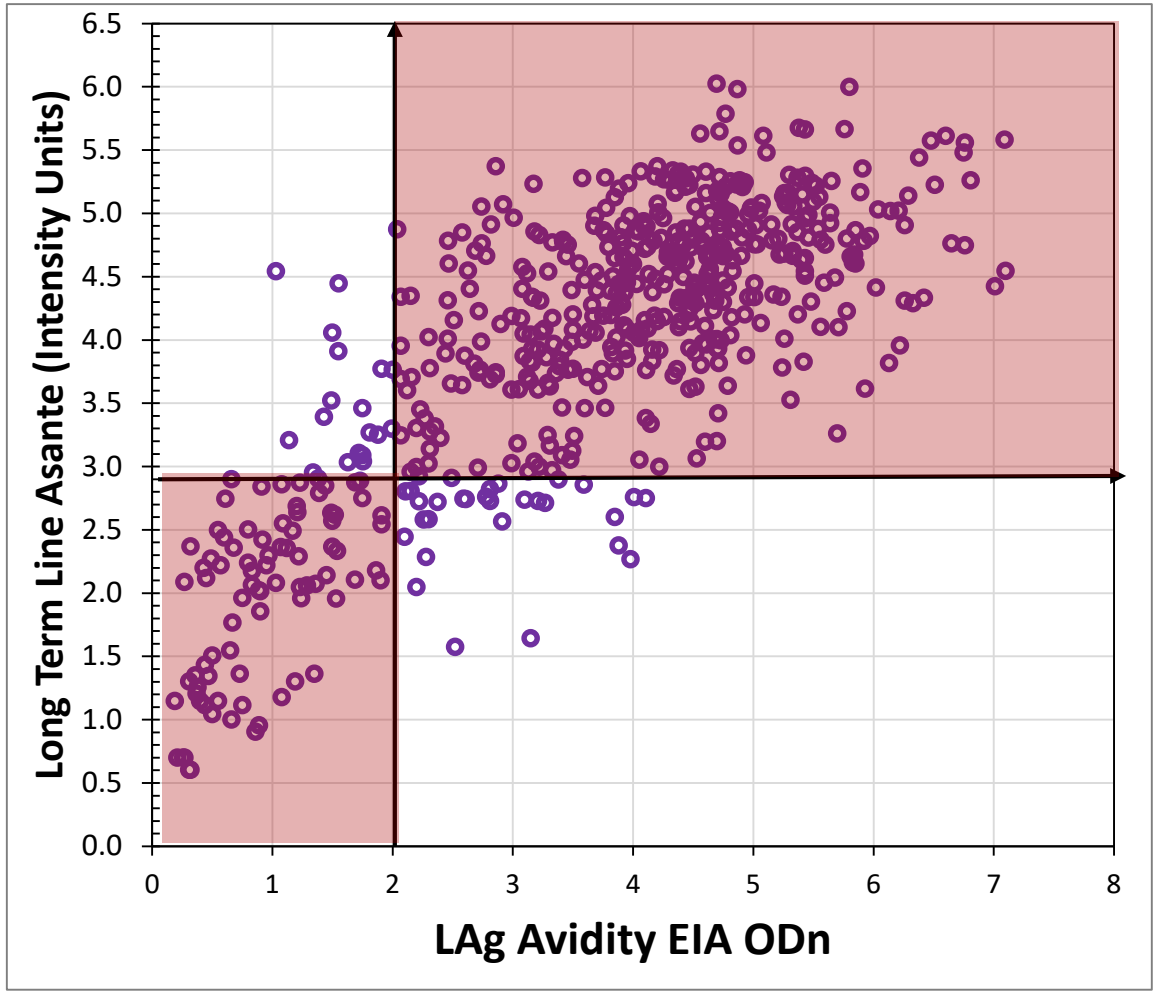
		EIA/WB Algorithm		
Asante VL Visual		HIV pos	HIV neg	Total
	HIV pos	**575**	10	585
	HIV neg	5	**910**	915
	Total	580	920	**1500**

Sensitivity = 99.14%
Specificity = 98.9%
Overall agreement with Reference HIV Testing = 99%
Kappa = 0.976

Acceptable diagnostic performance characteristics for WHO pre-qualification
Sensitivity: =>99%
Specificity: =>98%

Asante Performance of LT Line: CDC Evaluation

Reader Interpretation



% agreement = 91.87%, Kappa = 0.729

Visual Interpretation

		LAg-Avidity EIA		
		Recent	Long-Term	Total
Asante LT Line (Visual)	Recent	**80**	29	109
	Long-Term	18	**438**	456
	Total	98	467	**565**

% agreement = 91.68%, Kappa = 0.722

Both visual interpretation and reader interpretation agree very well with LAg-Avidity EIA

Independent Validation in NICD/South Africa

Asante Validation Results: NICD

Asante Verification Line

EIA + Western Blot

	Pos	Neg	Total
Pos	454	1	455
Neg	4	286	290
Total	458	287	745

Sensitivity = 99.1 [97.8-99.8]
Specificity = 99.7 [98.1-100]
Accuracy = 99.3 [98.4-99.8]
Kappa = 0.986 [0.974-0.998]

Asante LT Line

LAg-Avidity (2.0 ODn)

	Recent	LT	Total
Recent	169	9	178
LT	23	253	276
Total	192	262	454

% agreement = 92.95%
Kappa = 0.854 [0.806-0.903]

Data provided by Dr. Adrian Puren

Independent Validation in NIHE, Vietnam

Asante Validation Results: NIHE

EIA + Western Blot

Asante Verification Line

	Pos	Neg	Total
Pos	231	0	231
Neg	1	232	233
Total	232	232	464

Sensitivity = 99.6 [97.6-100]
Specificity = 100 [98.4-100]
Accuracy = 99.8 [98.8-100]
Kappa = 0.996 [0.987-1.000]

LAg-Avidity (2.0 ODn)

Asante LT Line

	Recent	LT	Total
Recent	27	13	40
LT	15	175	190
Total	42	188	230

% agreement = 87.83%
Kappa = 0.585 [0.446-0.723]
Spearman correlation = 0.704

Data provided by Dr. Hien Bui

Maxim Swift Performance of Test Line: CDC Evaluation

Interpretation with a Reader

		EIA/WB Algorithm		
Swift Test Line (Reader @100 IU		HIV pos	HIV neg	Total
	HIV pos	**575**	4	579
	HIV neg	5	**914**	919
	Total	580	918	**1498**

Sensitivity = 99.14%
Specificity = 99.56%
Overall agreement with Reference HIV Testing = 99.4%
Kappa = 0.987

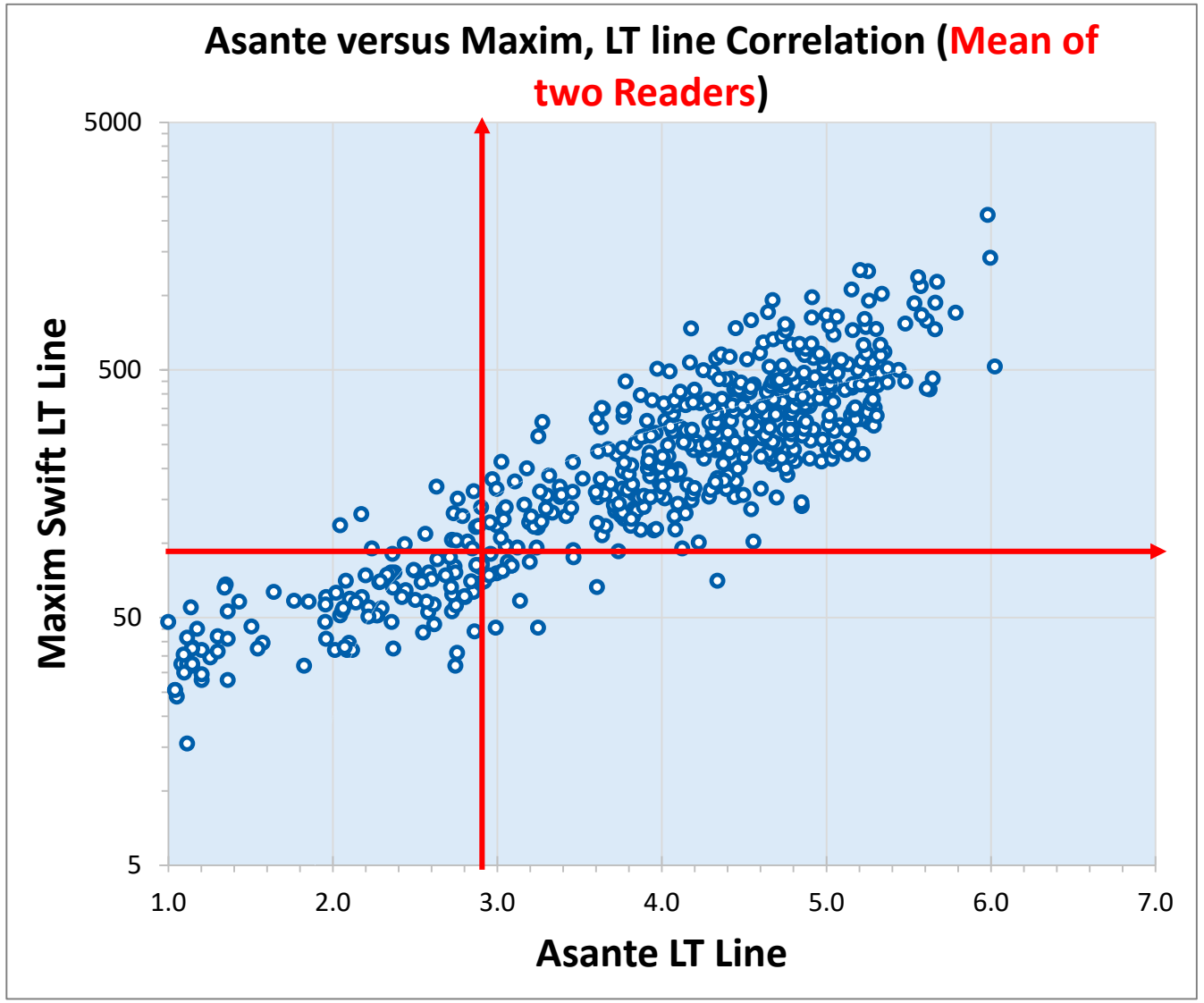
Visual Interpretation

		EIA/WB Algorithm		
Swift Test Line Visual		HIV pos	HIV neg	Total
	HIV pos	**576**	4	580
	HIV neg	4	**914**	918
	Total	580	918	**1498**

Sensitivity = 99.31%
Specificity = 99.56%
Overall agreement with Reference HIV Testing = 99.47%
Kappa = 0.989

Acceptable diagnostic performance characteristics for WHO pre-qualification
Sensitivity: =>99%
Specificity: =>98%

Sedia Asante and Maxim Swift RIA Correlation: CDC Evaluation



Asante LT Line-Reader

Maxim LT Line Reader	Recent	LT	Total
Recent	91	15	106
LT	17	442	459
Total	108	457	565

% Agreement =94.34

Kappa= 0.816 [0.754-0.877]

Asante LT Line-Visual

Maxim LT Line Visual	Recent	LT	Total
Recent	88	17	105
LT	21	439	460
Total	109	456	565

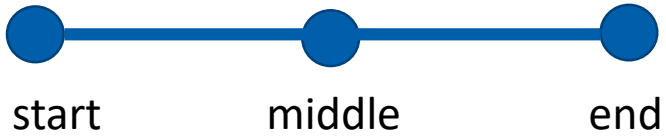
% Agreement = 93.27

Kappa=0.781 [0.714-0.848]

Both visual and reader interpretations agree very well for both tests

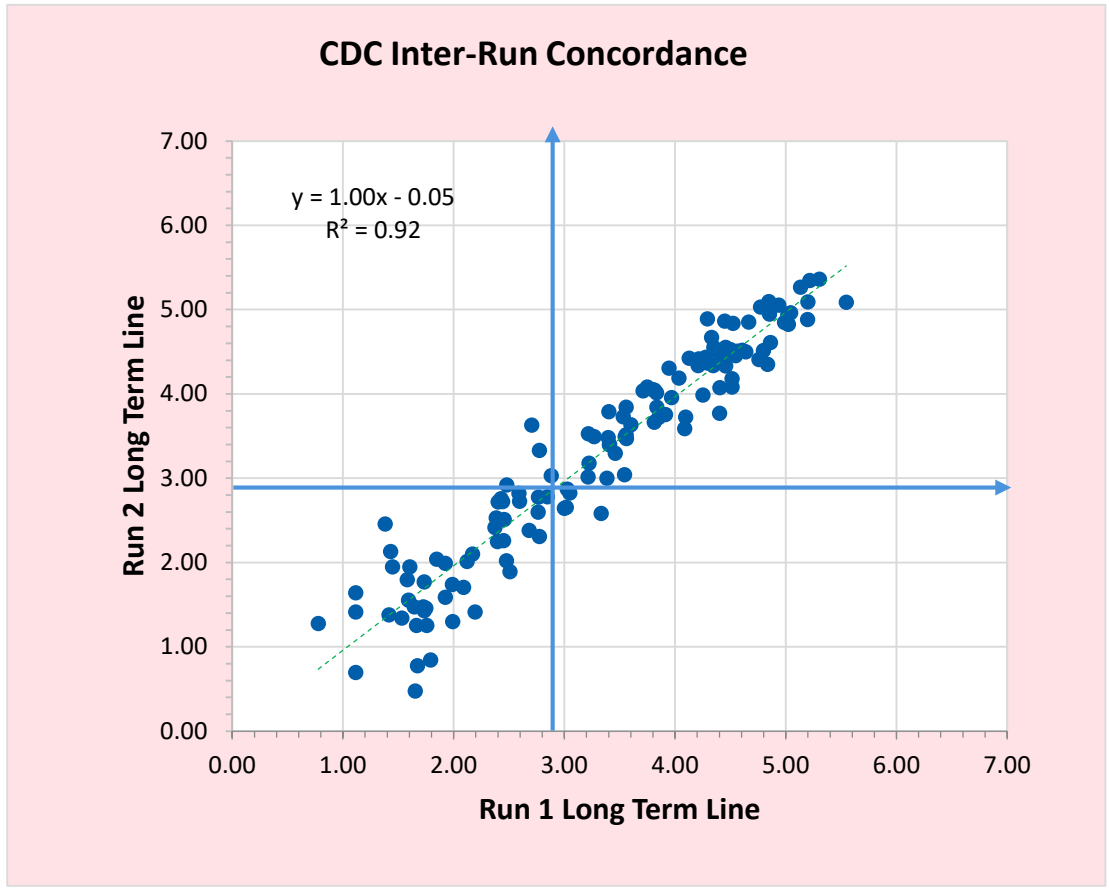
Ensuring Test Quality

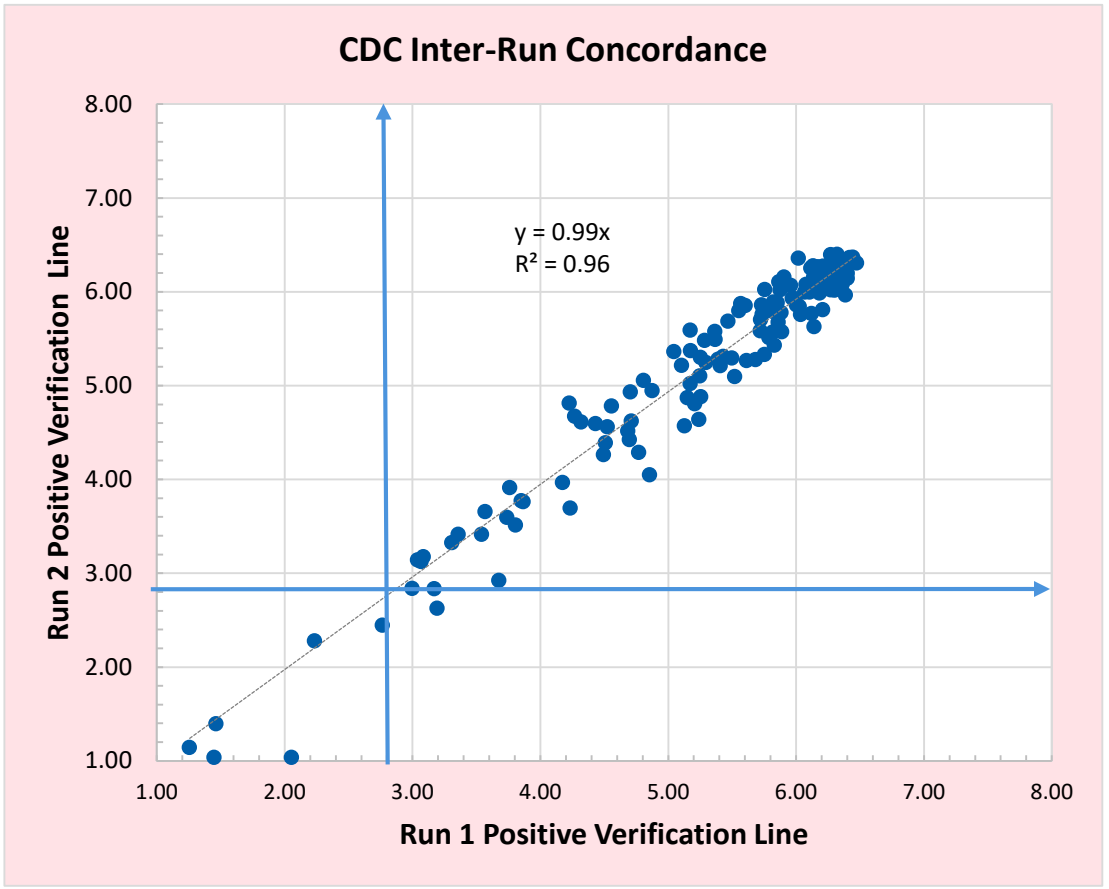
Kit Lot Quality Control (QC)

- Review of QC data from the manufacturer
  - Comparison with expected values: inter-lot consistency
  - Intra-lot consistency (

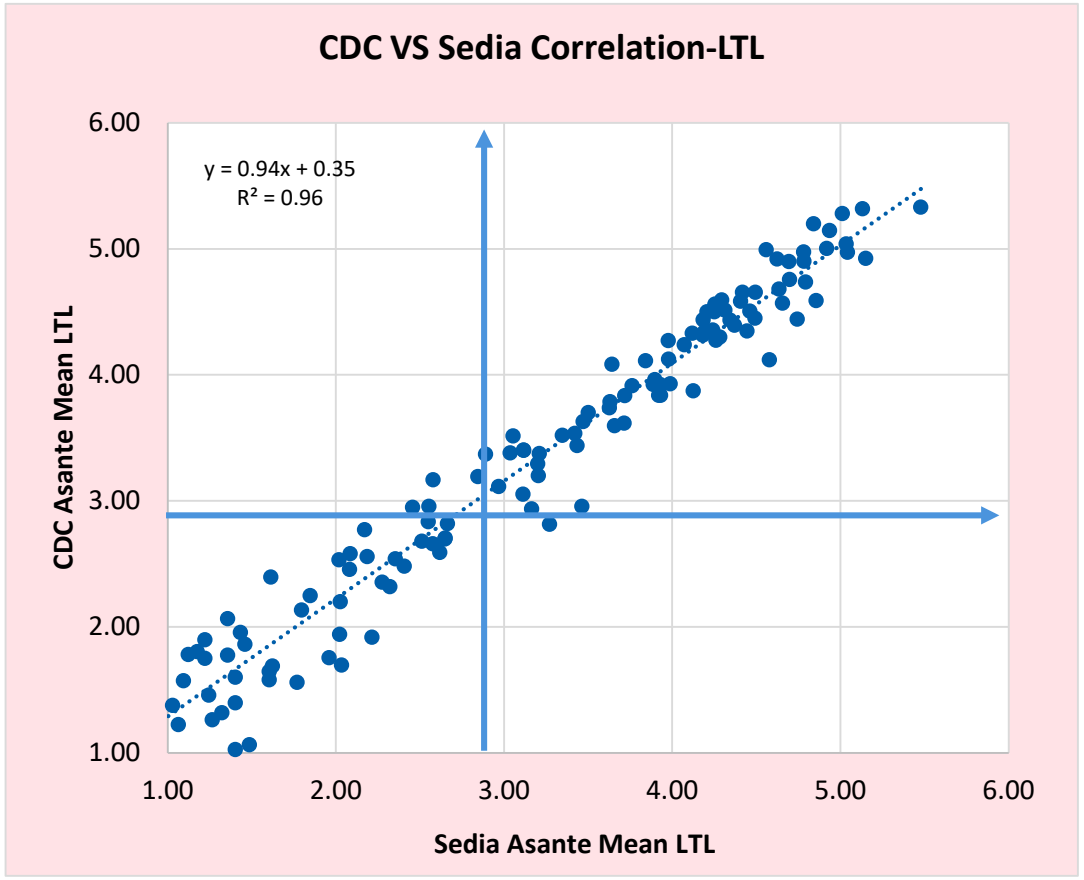
)
- Lot testing in CDC
  - Testing with Lot QC-133 panel
    - Spans the dynamic range
  - Testing by 2 or more operators
  - Comparison with expected values (previously passed lot)
  - Inter-operator reproducibility

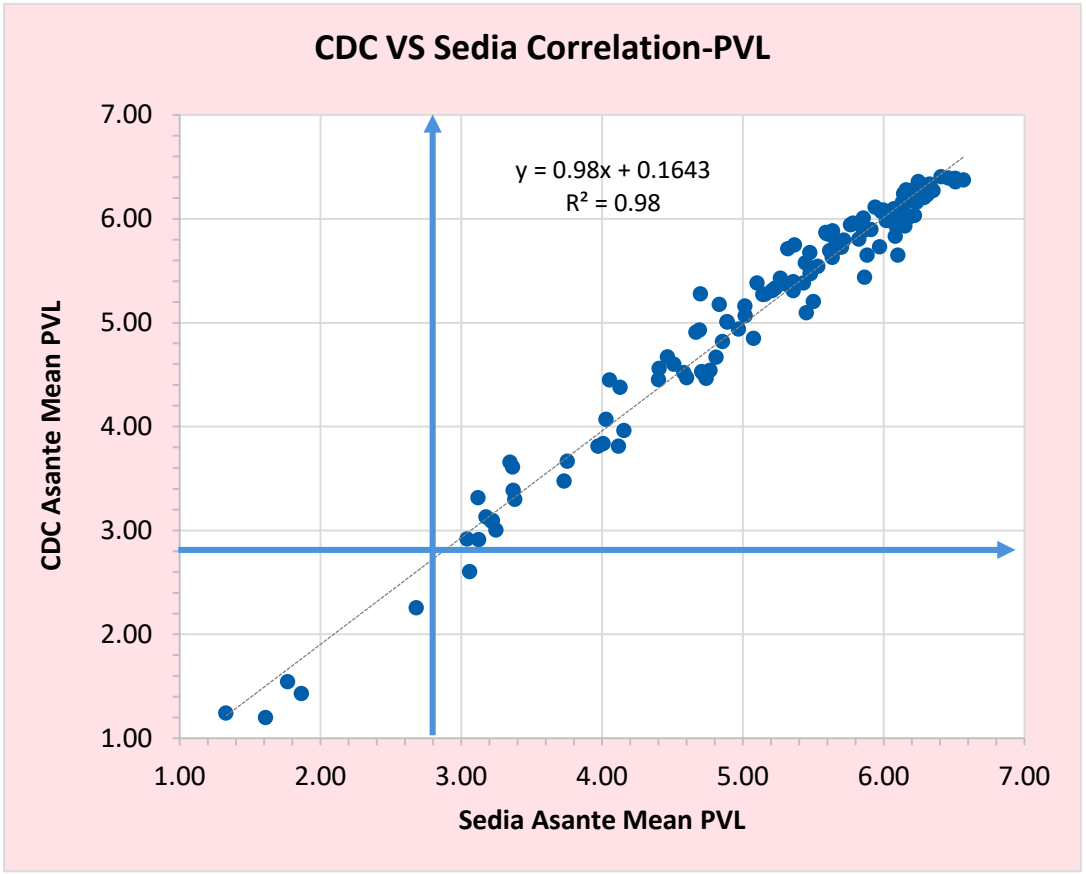
Kit Lot QC: Inter-Operator Reproducibility





Kit Lot QC: Inter-Lot Reproducibility





Conclusions

- Performance of the Asante Assay is good and similar across three independent labs
- For HIV diagnosis, both Asante and Swift RTRI met and exceeded WHO PQ requirements (sensitivity ≥99%, specificity ≥98%)
- Both Asante and Swift recency classification are comparable to LAg-Avidity EIA
- Reader and visual classifications are similar for both tests
- Asante and Swift have high agreement rates of ~93% (visual) and 94% (reader)
- Additional Maxim Swift independent evaluations are underway
- Robust system in place to ensure test quality

Thank You!

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.