Independent assessment of the Sedia Asanté HIV-1 Rapid Recency Assay

Eduard Grebe,[1,2] Shelley N. Facente,[1,3,4] Rachel Owen,[1] Dylan Hampton,[1] Celine Cheng,[1] Usha Sharma,[5] Christopher D. Pilcher,[3] Gary Murphy,[6] Alex Welte,[2] Michael P. Busch[1,3] on behalf of the *Consortium for the Evaluation and Performance of HIV Incidence Assays*

[1]Vitalant Research Institute, San Francisco, CA; [2]DST-NRF Centre of Excellence in Epidemiological Modelling and Analysis, Stellenbosch University; [3]University of California San Francisco; [4]Facente Consulting; [5]National Institute of Allergy and Infectious Diseases, National Institutes of Health; [6]Public Health England

Atlanta, March 28, 2019.

Thanks to Mars Stone, Inderdeep Singh and Honey Dave for additional testing

vitalant Research Institute

Disclosures

All authors receive grant support and/or have consulting agreements with Sedia Biosciences Corporation.

The company had no role in data generation, interpretation or analysis.

THESE DATA AND RESULTS ARE PRELIMINARY AND SUBJECT TO CHANGE.

Background

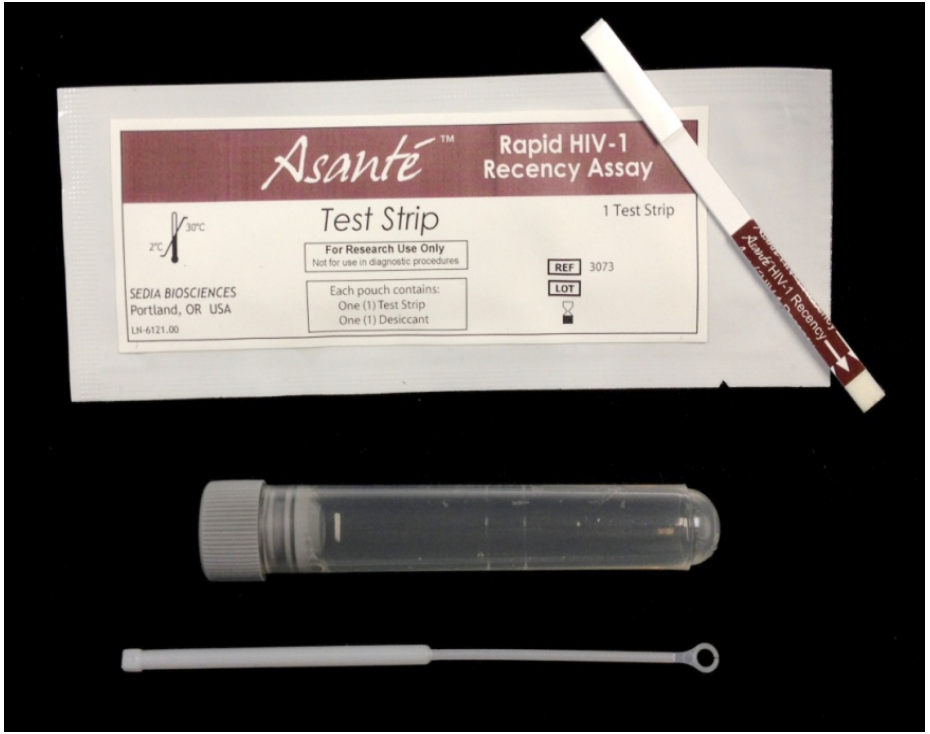
- CEPHIA conducted an independent evaluation of the Sedia™ Asanté HIV-1 Rapid Recency Assay
  - PoC lateral flow assay with Control (CTRL), Verification (VER) and Long-term/Recent (LT/R) lines
  - Evaluation focused on reproducibility and the HIV incidence surveillance use case
- We tested the
  - CEPHIA Qualification Panel
    - 250 specimens
    - including 10 replicates each of 5 blinded reproducibility control specimens
    - each specimen tested 6 times
  - CEPHIA Evaluation Panel
    - 2,500 specimens spanning range of times since infection for robust estimation of mean duration of recent infection (MDRI) and false-recent rate (FRR)
    - 25 replicates each of three blinded reproducibility controls
    - tested according to the SOP/package insert

vitalant Research Institute

Procedures

- Strips can be interpreted visually or using an electronic reader
- Visual interpretation:
  - line intensities scored on an ordinal scale (0-4)
  - primarily absence/presence of CTRL, VER and LT/R lines (i.e. 0 or ≥1)
  - negative verification line triggers serological confirmation and re-testing
  - unverified specimens are not given a recency interpretation
- Reader interpretation:
  - line intensities recorded under standard lighting conditions
  - negative VER triggers serological confirmation, borderline VER results trigger confirmatory testing (final result – median of three)
  - borderline LT/R results trigger confirmatory testing (median of three)

Assay format

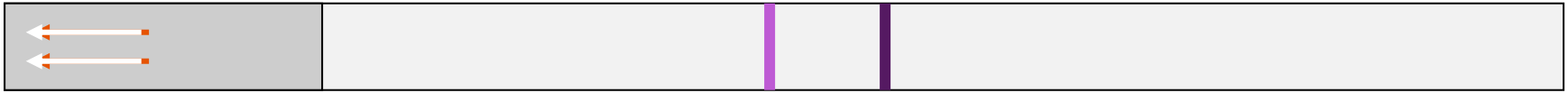


vitalant Research Institute

Interpretation

Positive/Long-term infection

Positive/Recent Infection



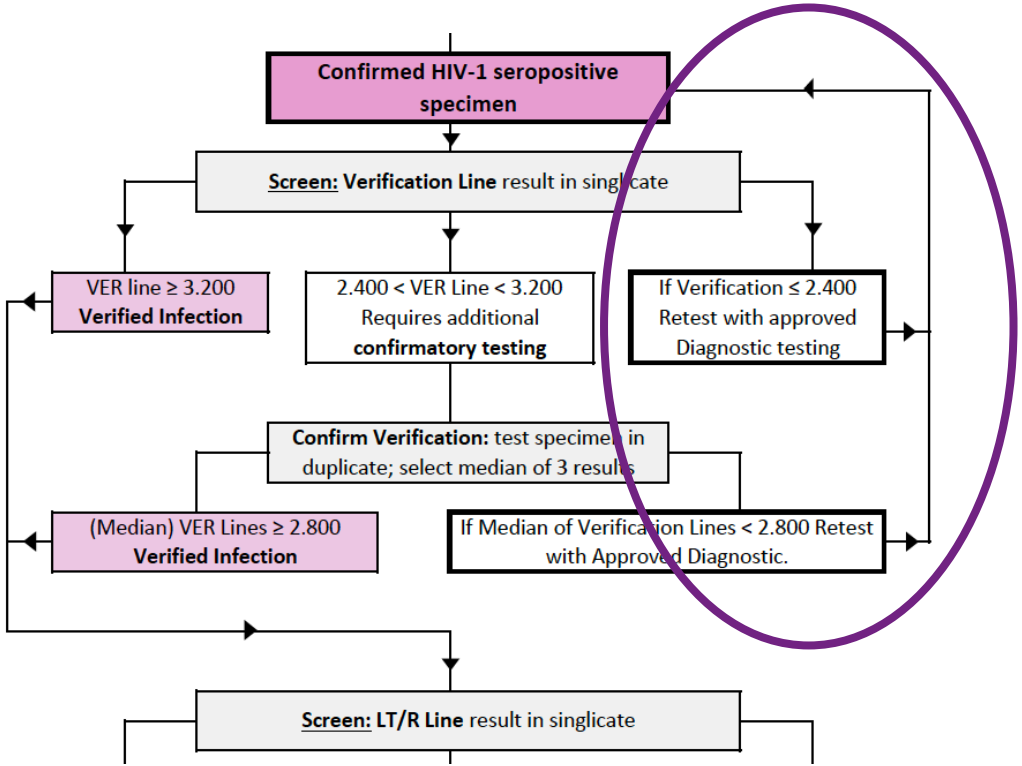
Seronegative

vitalant Research Institute

Test strip reader

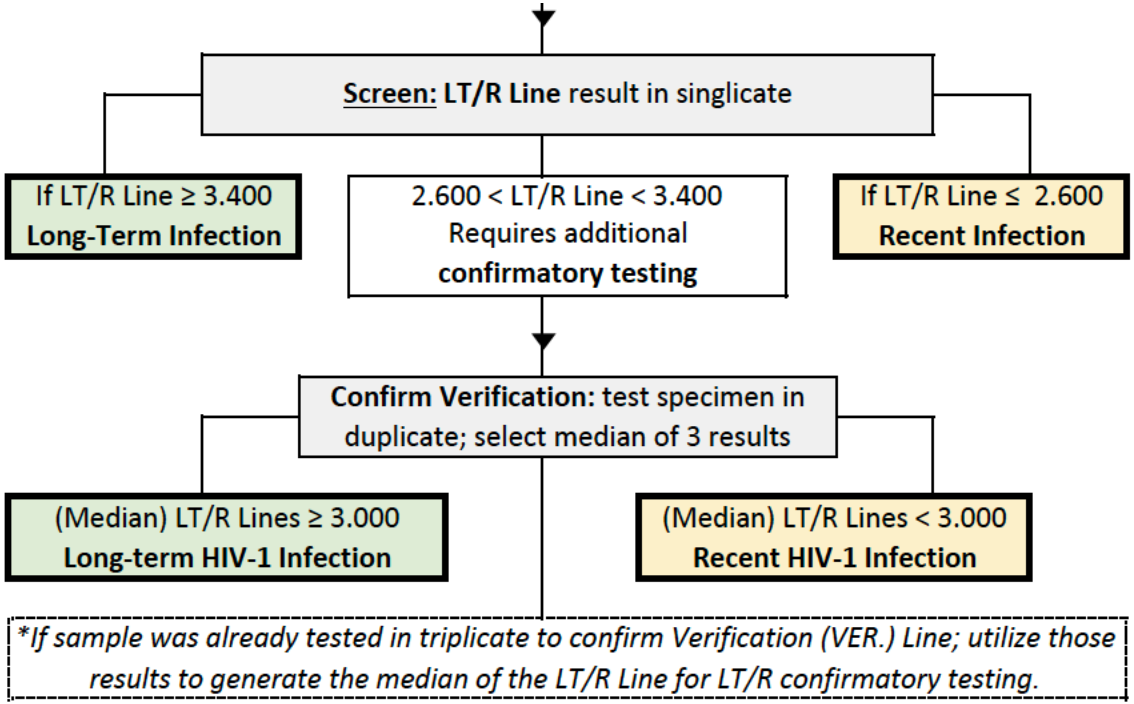
vitalant Research Institute

Reader verification algorithm



vitalant Research Institute

Reader recency algorithm



vitalant Research Institute

Reproducibility

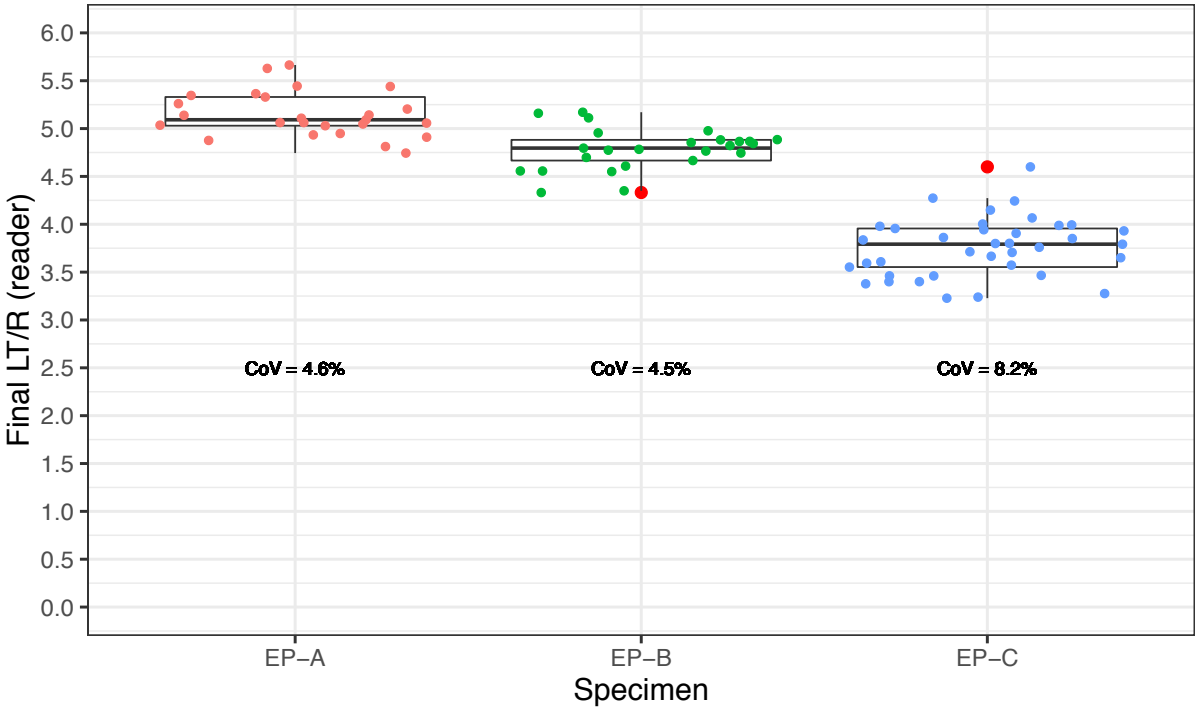
vitalant Research Institute

Repeat-tested specimens: Reader

Specimen	Replicates	Control line		Verification line		LT/R line	
		Mean	CV	Mean	CV	Mean	CV
EP-A	25	5.69	5.6%	6.29	1.5%	5.15	4.6%
EP-B	25	5.78	8.4%	6.16	2.2%	4.78	4.5%
EP-C	37	6.02	5.9%	6.03	2.9%	3.76	8.2%
QP-A	40	5.73	4.4%	5.27	4.4%	2.68	11.3%
QP-B	31	5.77	7.9%	5.59	4.3%	3.49	8.2%
QP-C	31	5.99	5.6%	5.13	5.2%	3.21	9.5%
QP-D	30	6.01	6.2%	4.63	4.6%	1.74	31.2%
QP-E	30	5.80	4.1%	5.82	3.1%	4.87	6.2%
QP-F	31	5.85	5.1%	6.05	2.5%	3.41	7.3%
QP-G	31	6.01	5.2%	4.71	5.9%	2.23	18.3%
QP-H	30	6.01	4.8%	4.94	3.7%	3.76	5.6%
QP-I	34	5.91	6.7%	5.36	4.7%	3.69	6.8%
QP-J	30	6.15	4.6%	5.83	4.6%	3.60	9.4%

vitalant Research Institute

EP Blinded replicate specimens: Reader



vitalant Research Institute

Blinded replicate specimens (Evaluation Panel): visual

Specimen	Replicates	Control line		Verification line		LT/R line			
		Valid	Invalid	Verified	Unverified	Mean	Recent	Long-term	Recent (%)
EP-A	25	25	0	25	0	3.88	0	25	0.0%
EP-B	25	25	0	25	0	3.20	0	25	0.0%
EP-C	37	37	0	37	0	2.08	0	37	0.0%
QP-A	40	40	0	40	0	0.68	14	26	35.0%
QP-B	31	31	0	31	0	1.26	1	30	3.2%
QP-C	31	31	0	31	0	1.16	5	26	16.1%
QP-D	30	30	0	30	0	0.20	24	6	80.0%
QP-E	30	30	0	30	0	2.67	0	30	0.0%
QP-F	31	31	0	31	0	1.16	2	29	6.5%
QP-G	31	31	0	31	0	0.52	16	15	51.6%
QP-H	30	30	0	30	0	1.53	0	30	0.0%
QP-I	34	34	0	34	0	1.47	1	33	2.9%
QP-J	30	30	0	30	0	1.30	4	26	13.3%

vitalant Research Institute

Sensitivity of verification line

vitalant Research Institute

This is NOT a clinical sensitivity study!

vitalant Research Institute

Initial verification line results (reader)

Initial results	ARCHITECT +	ARCHITECT -	Total	Geenius +	Geenius Indet	Geenius -	Total
Verified Infection	2320	0	**2320**	2320	5	0	**2325**
Requires confirmatory testing	116	0	**116**	113	13	0	**126**
Requires serological confirmation	55	3	**58**	35	3	10	**48**
	2491	**3**	**2494**	**2468**	**21**	**10**	**2499**
Proportion verified	**93.1%**	0.0%		**94.0%**	23.8%	0.0%	
Proportion requiring further verification	**6.9%**	100.0%		**6.0%**	76.2%	100.0%	

vitalant Research Institute

Initial verification line results (visual)

Initial results	ARCHITECT +	ARCHITECT -	Total	Geenius +	Geenius Indet	Geenius -	Total
Verified Infection	2338	0	**2338**	2338	4	0	2342
Requires confirmatory testing	101	1	**102**	96	6	1	103
Requires serological confirmation	52	2	**54**	34	11	9	54
	2491	**3**	**2494**	**2468**	**21**	**10**	2499
Proportion verified	**93.9%**	0.0%		**94.7%**	19.0%	0.0%	
Proportion requiring further verification	**6.1%**	100.0%		**5.3%**	81.0%	100.0%	

vitalant Research Institute

Final sensitivity of verification line: Treatment-naïve, non-EC

- Analysis restricted to unique treatment-naïve, non-elite controller specimens in the CEPHIA Evaluation Panel
- Reference test: Geenius HIV 1/2 Supplemental Assay confirmed +
- Non-reactive specimens repeat-tested according to SOP

Reader

Sensitivity = 98.73% (98.25%-99.20%)

Asanté Verification Line	Bio-Rad Geenius		
	Confirmed +	Confirmed -	*Total*
Verified Infection	2092	0	2092
Repeat Non-reactive	27	0	27
Total	2119	0	2119

Visual

Sensitivity = 99.62% (99.36%-99.88%)

Asanté Verification Line	Bio-Rad Geenius		
	Confirmed +	Confirmed –	*Total*
Verified Infection	2111	0	2111
Repeat Non-reactive	8	0	8
Total	2119	0	2119

vitalant Research Institute

Final sensitivity of verification line: Bio-Rad Geenius

- Analysis restricted to unique specimens in the CEPHIA Evaluation Panel
- Reference test: Geenius HIV 1/2 Supplemental Assay confirmed +
- Non-reactive specimens repeat-tested according to SOP

Reader

Sensitivity = 96.84% (96.15%-97.53%)

Asanté Verification Line	Bio-Rad Geenius		
	Confirmed +	Confirmed -	*Total*
Verified Infection	2390	0	*2390*
Repeat Non-reactive	78	0	*78*
Total	*2468*	*0*	*2468*

Visual

Sensitivity = 98.18% (97.60%-98.71%)

Asanté Verification Line	Bio-Rad Geenius		
	Confirmed +	Confirmed –	*Total*
Verified Infection	2423	0	*2423*
Repeat Non-reactive	45	0	*45*
Total	*2468*	*0*	*2468*

vitalant Research Institute

Final sensitivity of verification line: Abbott ARCHITECT

- Analysis restricted to unique specimens in the CEPHIA Evaluation Panel
- Reference test: Abbott ARCHITECT HIV Ag/Ab Combo confirmed +
- Non-reactive specimens repeat-tested according to SOP

Reader

Sensitivity = 95.84% (95.04%-96.64%)

Asanté Verification Line	Abbott ARCHITECT		
	Confirmed +	Confirmed -	*Total*
Verified Infection	2305	0	*2305*
Repeat Non-reactive	100	0	*100*
Total	*2405*	*0*	*2405*

Visual

Sensitivity = 97.21% (96.56%-97.87%)

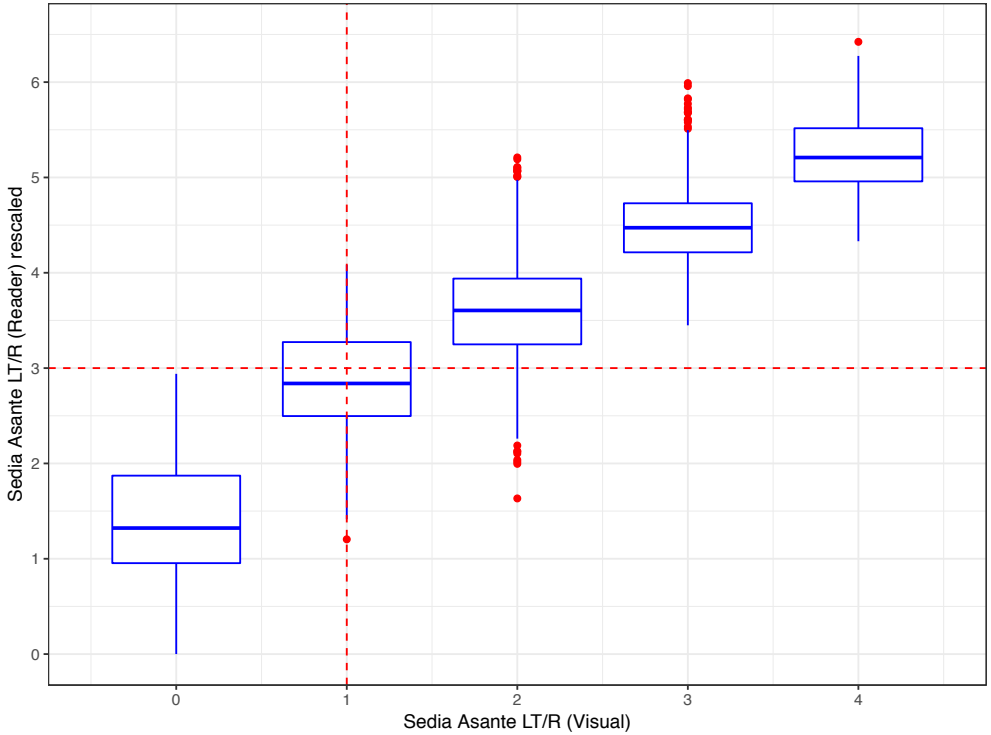
Asanté Verification Line	Abbott ARCHITECT		
	Confirmed +	Confirmed –	*Total*
Verified Infection	2338	0	*2338*
Repeat Non-reactive	67	0	*67*
Total	*2405*	*0*	*2405*

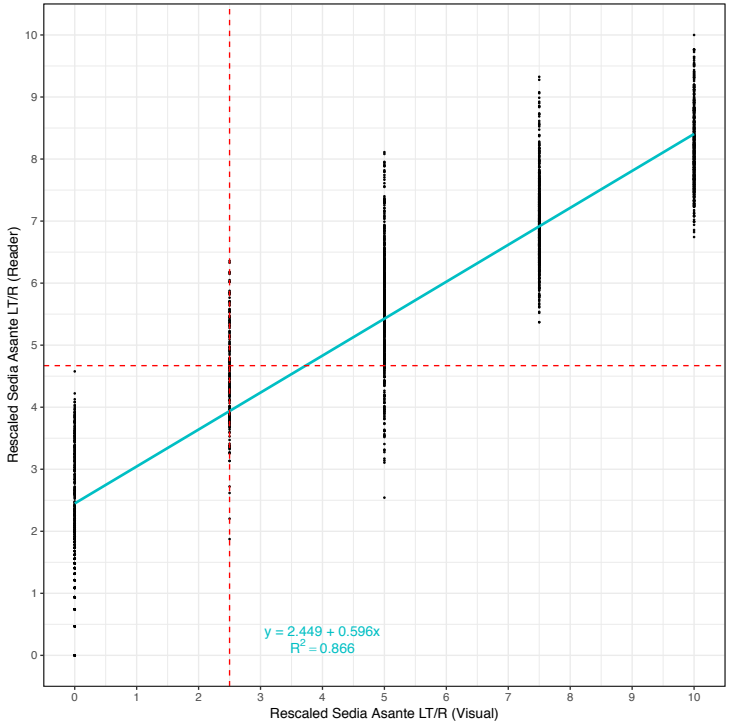
vitalant Research Institute

Correspondence

vitalant Research Institute

LT/R reader vs. LT/R visual





vitalant Research Institute

Reader and visual recency classification agreement

Treatment-naïve, non-EC specimens

$\kappa = 0.74$ (95% CI: 0.71-0.78)

Visual	Reader		
	Recent	Long-term	*Total*
Recent	322	0	*322*
Long-term	175	1860	*2035*
Total	*497*	*1860*	*2357*

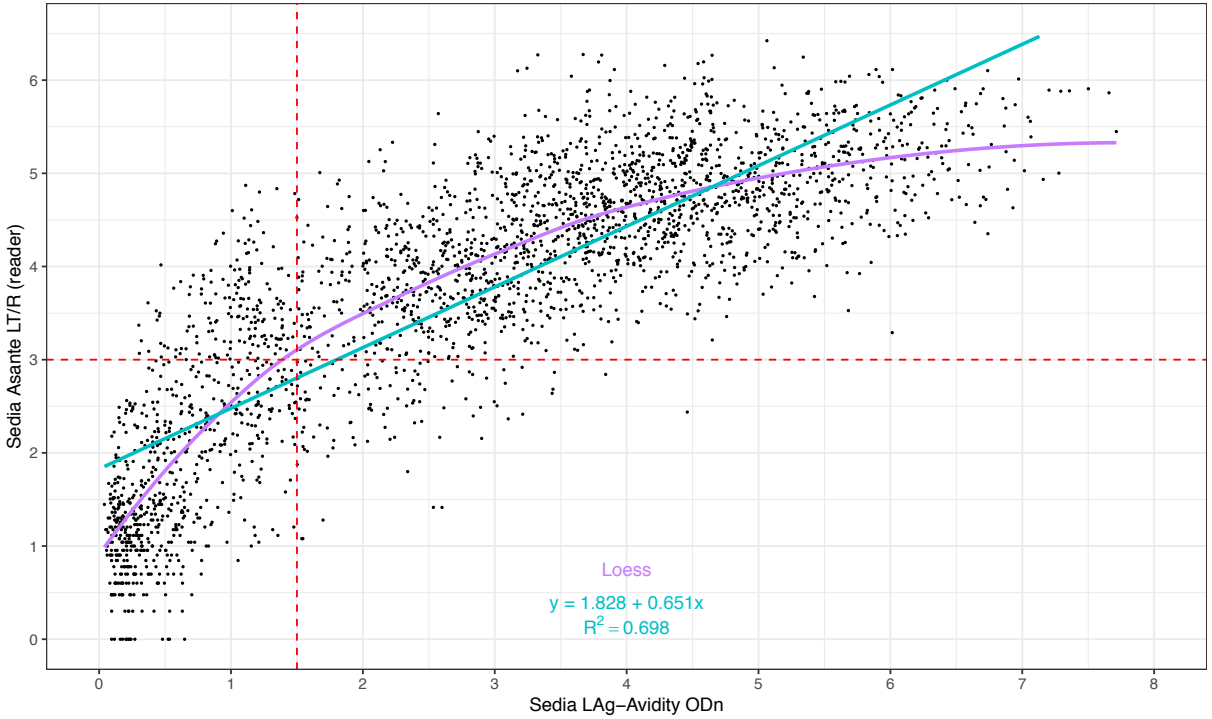
All specimens

$\kappa = 0.80$ (0.77-0.82)

Visual	Reader		
	Recent	Long-term	*Total*
Recent	539	0	*539*
Long-term	202	2008	*2210*
Total	*741*	*2008*	*2749*

vitalant Research Institute

Sedia Asanté LT/R vs. Sedia LAg-Avidity ODn



vitalant Research Institute

Asanté (reader) and LAg-Avidity recency classification agreement

LAg-Avidity ODn ≤ 1.5

$\kappa = 0.75$ (0.72-0.78)

Asanté	Sedia LAg-Avidity		
	Recent	Long-term	*Total*
Recent	637	104	741
Long-term	174	1834	2008
Total	811	1938	2749

LAg-Avidity ODn ≤ 2.0

$\kappa = 0.74$ (0.71-0.76)

Asanté	Sedia LAg-Avidity		
	Recent	Long-term	*Total*
Recent	678	63	741
Long-term	245	1763	2008
Total	923	1826	2749

vitalant Research Institute

Performance for incidence surveillance

vitalant Research Institute

Methods

- We use CEPHIA Evaluation Panel data to estimate mean duration of recent infection (MDRI) and false-recent rate/proportion false-recent (FRR)
  - MDRI: Average time post-infection a subject spends exhibiting the ‘recent’ marker
  - FRR: Proportion of long-infected subjects who nevertheless exhibit the ‘recent’ marker
- For the primary analysis we ignore verification status of specimens
  - this is favorable to the assay, since we are obtaining recency interpretations where current SOP does not allow an interpretation
  - we recommend that valid results from confirmed seropositive specimens that fail to verified be interpreted as recent
- We evaluate performance (precision of incidence estimates) in three epidemiological/surveillance scenarios

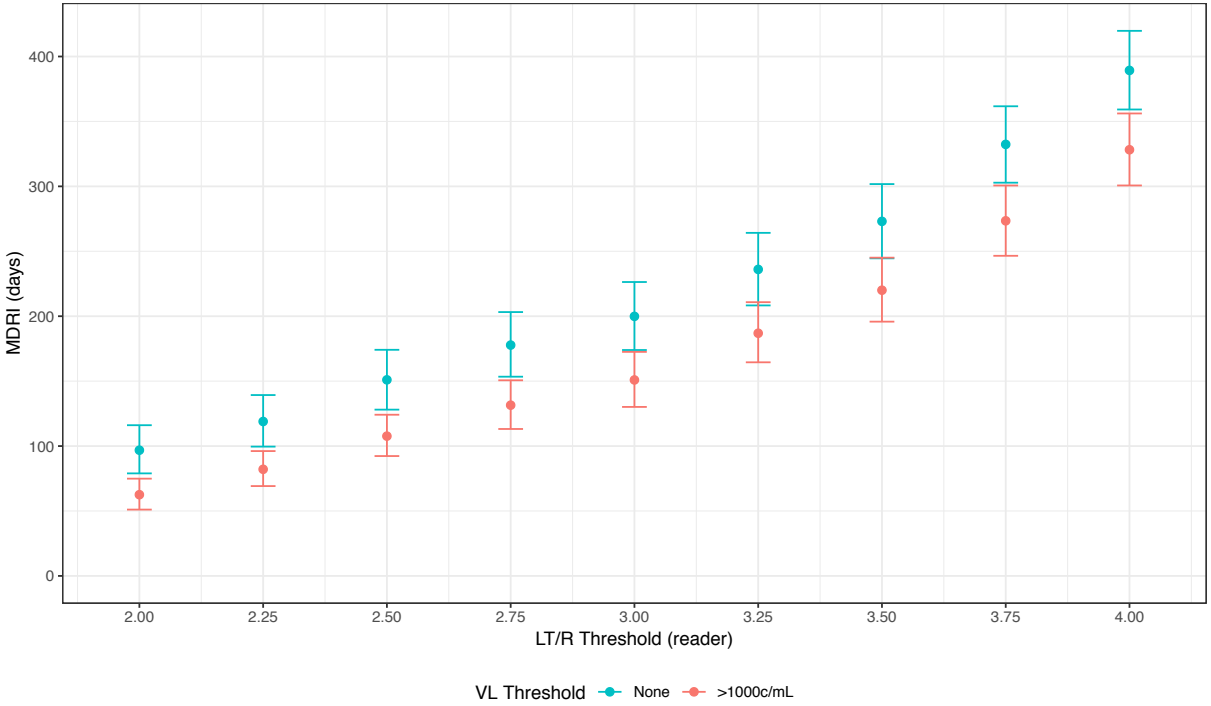
Results: ‘standard’ recency discrimination thresholds

Algorithm	MDRI (Model A) *PE (95% CI)*	MDRI (Model B) *PE (95% CI)*	MDRI (WB) *PE*	MDRI (4thgen RT) *PE*	MDRI (4thgen lab) *PE*	FRR (naïve) *PE (95% CI)*	FRR (suppressed) *PE (95% CI)*
Asanté alone Reader (LT/R < 3)	200 (173-227)	207 (181-234)	170	187	189	3.8% (1.5%-7.7%)	62.5% (53.5%-70.9%)
Asanté alone Visual (LT/R < 1)	128 (108-149)	137 (118-157)	99	116	117	2.7% (0.9%-6.2%)	54.7% (45.7%-63.5%)
Asanté & VL Reader (LT/R < 3, VL > 1000)	151 (130-172)	159 (138-180)	121	138	140	1.7% (0.4%-4.9%)	0.0% (0.0%-2.8%)
Asanté & VL Visual (LT/R < 3, VL > 1000)	91 (77-107)	102 (88-117)	62	79	81	1.7% (0.4%-4.9%)	0.0% (0.0%-2.8%)
Sedia LAg alone (ODn ≤ 1.5)*	210 (188-233)	215 (192-237)	180	197	199	2.2% (0.6%-5.5%)	56.3% (47.2%-65.0%)
Sedia LAg & VL (ODn ≤ 1.5, VL > 1000)*	167 (149-186)	173 (155-191)	137	154	156	1.1% (0.1%-4.0%)	0.0% (0.0%-2.8%)
Sedia LAg & VL (ODn ≤ 2.0, VL > 1000)	199 (179-221)	205 (185-226)	170	187	189	1.7% (0.4%-4.9%)	0.0% (0.0%-2.8%)

* Correction (April 10, 2019): The original slides presented contained incorrect FRR estimates for these algorithms.

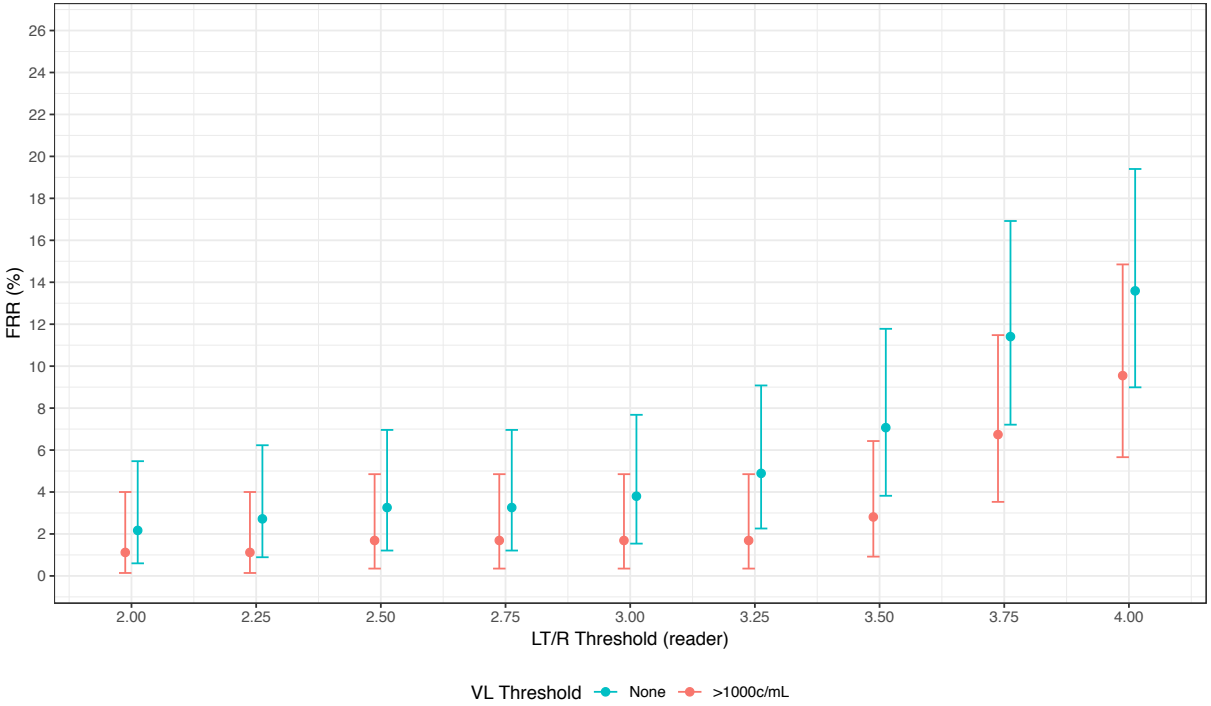
vitalant Research Institute

Results: MDRI vs. LT/R threshold (reader)



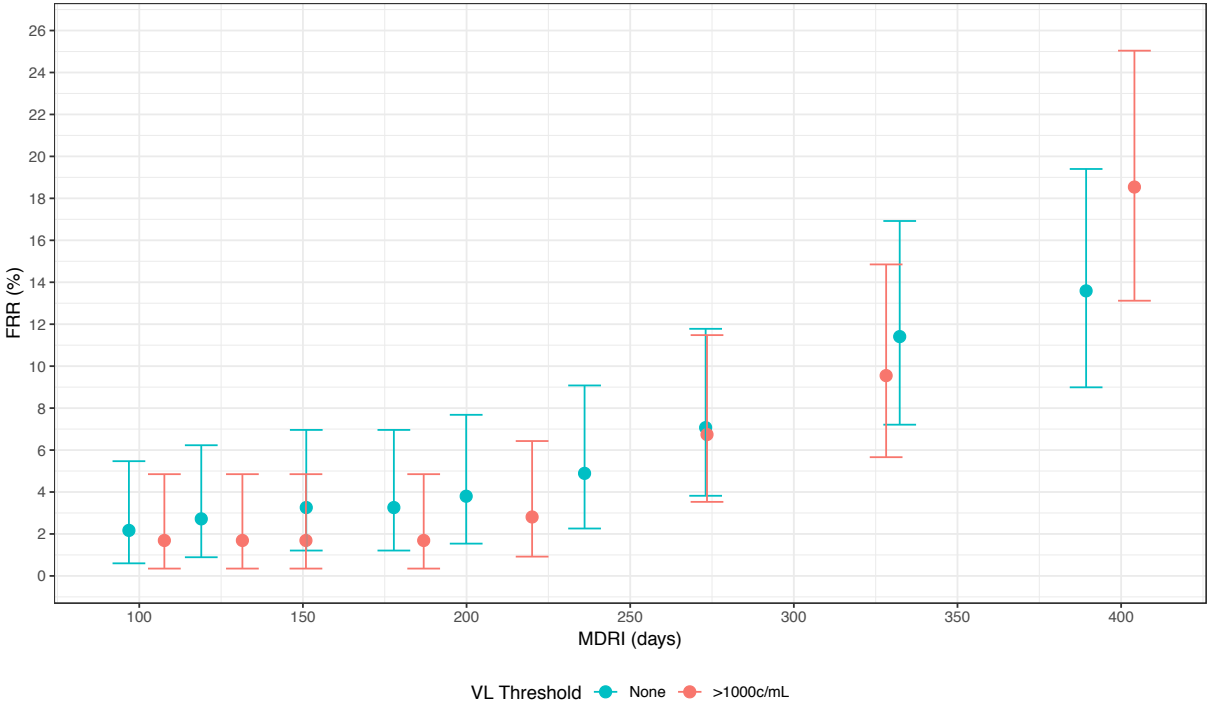
vitalant Research Institute

Results: FRR vs. LT/R threshold (reader)



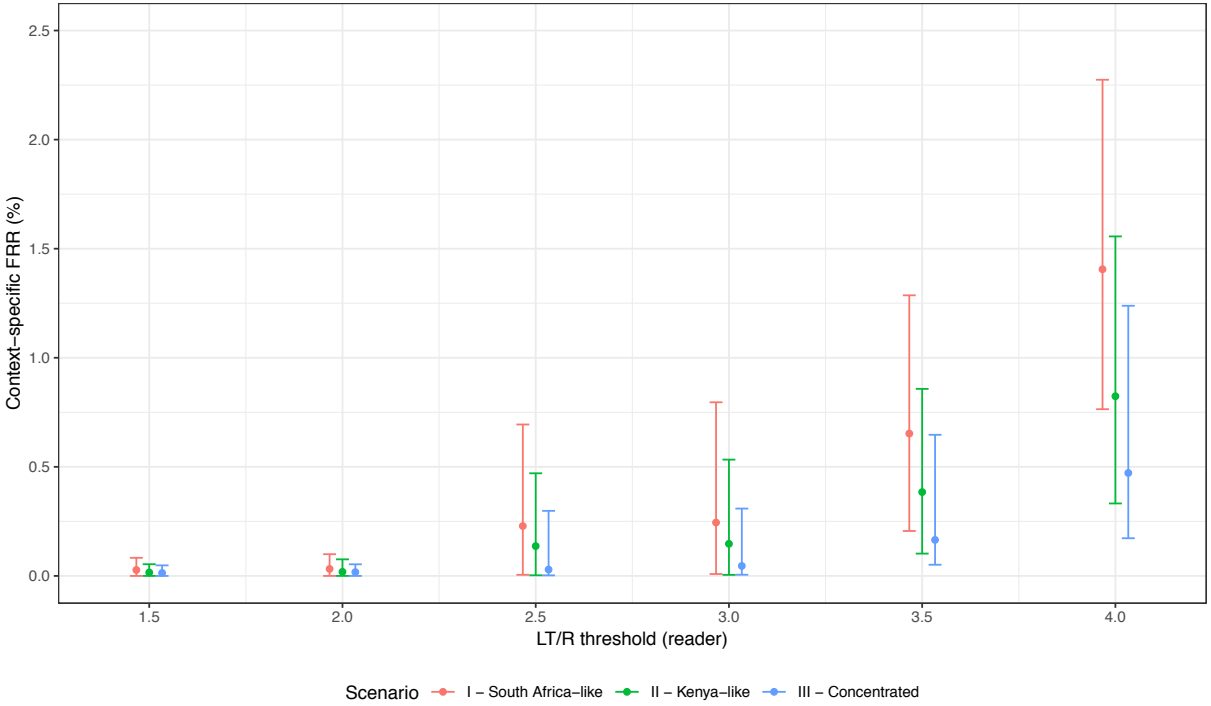
vitalant Research Institute

FRR (treatment naïve, non-EC) vs. MDRI



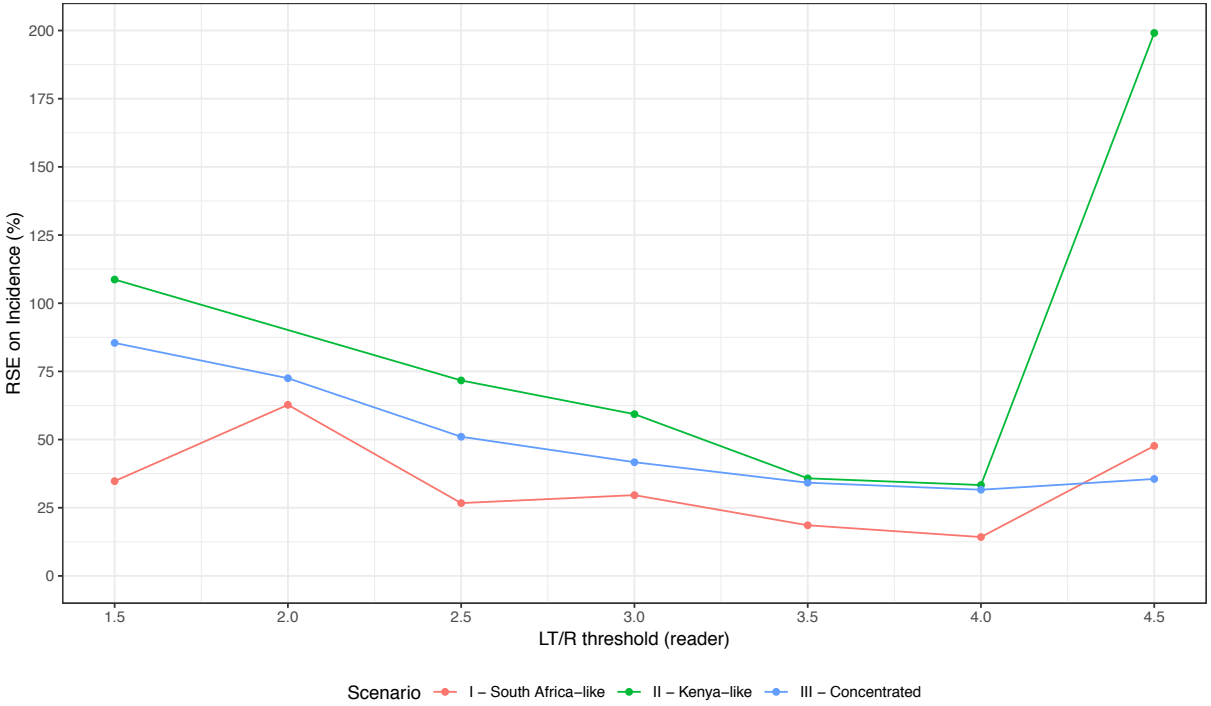
vitalant Research Institute

Context-specific FRR vs. LT/R threshold (reader)



vitalant Research Institute

Precision of incidence estimates vs. LT/R threshold (reader)



vitalant Research Institute

Conclusions

- Sensitivity of the verification line is good on strongly antibody-positive, untreated specimens specimens, but
  - sensitivity is lower in when very recently-infected and virally suppressed specimens are included
  - exclusion of confirmed positive but verification line non-reactive specimens from the recency interpretation would reduce precision of incidence estimates
- The Sedia Asanté HIV-1 Rapid Recency assay's performance is acceptable for use in population-level incidence surveillance, but must be combined with a supplemental viral load threshold to achieve acceptable FRR
  - MDRI is substantially lower when visually interpreting the LT/R line
- Alternative (higher) LT/R thresholds should be considered when using the test strip reader to increase MDRI

Thank you