Avidity Assay for Cross-Sectional Incidence based on a 4th Generation Combo Ag/Ab EIA

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Background

- Accurate methods for cross-sectional incidence estimation are needed for HIV surveillance and HIV prevention research.
- 3rd generation HIV screening tests, such as Genetic Systems (GS) HIV 1/2 +O ELISA (BioRad), have been modified for use as incidence assays, but are not available in some settings or may be phased out.
- We developed an HIV avidity assay based on the 4th generation GS HIV Combo Ag/Ab EIA (BioRad Combo assay) and evaluated its performance.

Methods

- 1,587 samples (446 individuals) from 4 cohorts with known duration of infection were analyzed (Table 1).
- The BioRad Combo assay was modified by diluting samples 1:10 and incubating them for 30 minutes at 4°C, with and without 0.025 M diethylamine (DEA).
- The avidity index (AI) was calculated as the optical density ratio of the DEA-treated to the untreated well.
- Assay performance was assessed using the modified BioRad Combo assay alone, or with a viral load (VL) assay, using a cutoff of <400 c/ml to classify samples as non-recent.
- Mean duration of recent infection (MDRI) was estimated using a time window of 2 years for AI cut-offs of 20-90%[1].
- False recent rate (FRR, fraction of samples misclassified as recent), was calculated with samples from individuals infected >2 years using an AI cutoff of 80% (Table 2).
- Factors associated with misclassification were examined in individuals from the ALIVE cohort using logistic regression, with general estimating equations and a robust variance estimator, to calculate odds ratios (OR). A multivariate analysis was performed with factors shown to have an association (p<0.1) in the univariate analysis (Table 3).

Table 1. Samples Per Cohort Tested

	T ≤ 2 years		T > 2 years	
Cohort	No. Individuals	No. Samples	No. Individuals	No. Samples
HIVNET 001	103	580	65	236
ALIVE	-	-	283	488
JHH Clinical Cohort (JHHCC)	-	-	38	245
Elite Suppressors (ES)	-	-	22	38

Results

Figure 1. Avidity Index by Years from Seroconversion in HIVNET 001 Cohort

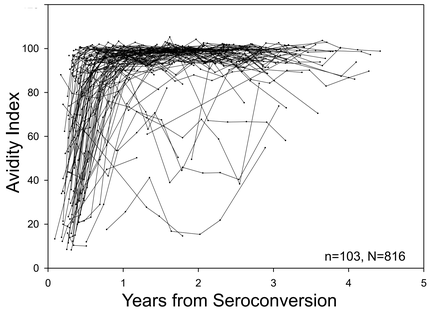


Figure 2. BioRad Avidity (3rd Gen) Assay vs. BioRad Combo Avidity (4th Gen) Assay

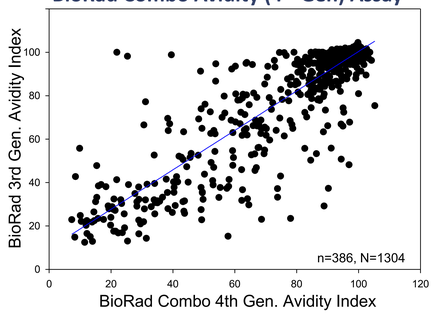


Figure 3. Individual Example of Affect of Viral Suppression and Breakthrough on Avidity Index

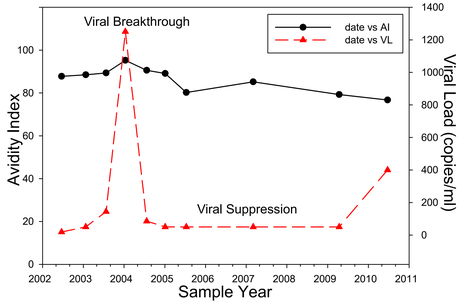


Figure 4. Change in Avidity Index in Paired Time Points by Viral Load Status in JHHCC Samples with Viral Breakthrough

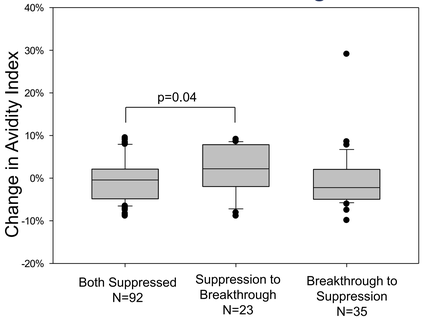


Figure 5. Avidity Index by Years on HAART in Virally Suppressed JHHCC Samples

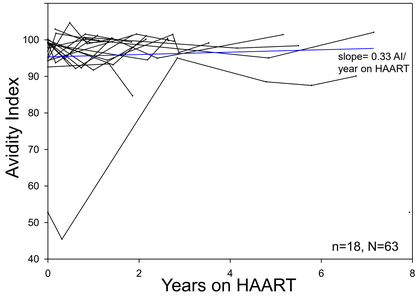


Figure 6. Avidity Index for Individuals Known to be Infected >2 Years

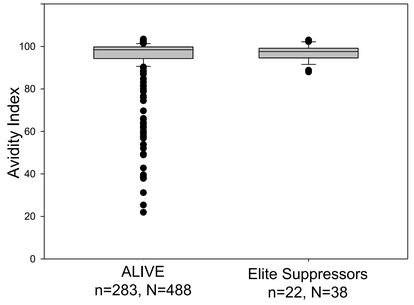


Table 2. Performance of the BioRad Combo Avidity Assay

	BioRad Combo Avidity		BioRad Combo Avidity + HIV VL>400 c/mL	
Avidity Index	**MDRI (95% CI)**	**FRR % (95% CI)**	**MDRI (95% CI)**	**FRR % (95% CI)**
Cohort	HIVNET 001 N=801	ALIVE + JHHCC + ES N=1007	HIVNET 001 N=801	ALIVE +JHHCC + ES N=976
20%	69 (46, 87)	0.20 (0.02-0.72)	65 (35, 82)	0.00 (0-0.38)
30%	86 (63, 102)	0.70 (0.28-1.43)	79 (57, 94)	0.31 (0.06-0.90)
40%	105 (87, 120)	1.49 (0.84-2.44)	95 (75, 108)	0.61 (0.23-1.33)
50%	123 (105, 136)	2.28 (1.45-3.41)	109 (91, 122)	0.82 (0.35-1.61)
60%	137 (122, 150)	3.48 (2.43-4.80)	119 (103, 131)	1.54 (0.86-2.52)
70%	159 (146, 171)	5.16 (3.88-6.72)	136 (123, 147)	2.25 (1.42-3.40)
80%	178 (165, 190)	6.26 (4.84-7.93)	152 (140, 162)	2.46 (1.58-3.64)
90%	221 (208, 235)	10.43 (8.61-12.48)	186 (170, 199)	3.69 (2.60-5.10)

Table 3. Factors Associated with Misclassification

	Avidity Index < 80%		
	% Misclassified (n/N)	**OR (95% CI)**	**aOR* (95% CI)**
Sex			
Female	3.15 (4/127)	1	-
Male	8.59 (31/361)	0.37 (0.10-1.33)	-
Age (years)			
18-35	1.33 (2/150)	1	1
36-45	9.87 (23/233)	**3.82 (1.68-8.69)**	**3.31 (1.22-8.98)**
> 45	9.52 (10/105)	**3.31 (1.10-9.96)**	2.74 (0.81-9.25)
Race			
Black	7.33 (33/450)	1	-
Non-black	5.26 (2/38)	0.59 (0.08-4.60)	-
HIV viral load (copies/ml)			
> 50 000	8.00 (10/125)	1	1
10 001-50 000	3.33 (5/150)	0.70 (0.34-1.46)	0.66 (0.30-1.45)
401-10 000	3.62 (5/138)	0.72 (0.33-1.60)	0.68 (0.28-1.67)
≤ 400	25.49 (13/51)	**2.59 (1.12-6.00)**	**2.68 (1.18-6.08)**
CD4+ cell count (cells/µl)			
> 400	5.77 (12/208)	1	-
201-400	4.43 (7/158)	0.78 (0.42-1.45)	-
51-200	8.51 (8/94)	1.58 (0.80-3.12)	-
≤ 50	28.57 (8/28)	1.41 (0.23-8.65)	-
BioRad 3rd Gen Avidiy Assay <80%			
No	3.62 (17/469)	1	-
Yes	94.74 (18/19)	**28.4 (4.70-171.73)**	-
Total	7.17 (35/453)		

Abb: OR=odds ratio, aOR=adjusted odds ratio
Bolded font signifies OR p<0.05.
*Controlled for age and HIV VL as categorical variables.

Summary & Conclusions

- The performance characteristics of this assay suggest that it may be useful as one component of a multi-assay algorithm (MAA) for HIV incidence estimation.
- When combined with a VL exclusion the FRR was halved but MDRI estimates were reduced by approximately 10%.
- This may provide a useful tool for HIV incidence estimation in the future, if 3rd generation HIV screening tests are replaced with 4th generation tests.
- Further research is needed to evaluate the modified BioRad Combo assay in combination with other assays to accurately estimate HIV incidence based on 4th generation HIV tests.

References

1. Kassanjee R, McWalter TA, Bärnighausen T, Welte A. A New General Biomarker-Based Incidence Estimator. *Epidemiology (Cambridge, Mass.)* 2012;23(5):721-728. doi:10.1097/EDE.0b013e3182576c07.