Short Communication: Low False Recent Rate of Limiting-Antigen Avidity Assay Among Long-Term Infected Subjects from Guangxi, China

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Abstract

Assays used for HIV cross-sectional incidence testing can misclassify some individuals with nonrecent HIV infection as recently infected, overestimating HIV incidence. We analyzed the frequency and factors associated with false-recent misclassification on subjects from Guangxi, China known to have long-term infection using the limited antigen-avidity assay (LAg-Avidity). Stored samples from treatment-naïve individuals from Guangxi, China were tested using the LAg-Avidity. A total of 362 samples from individuals known to be infected 2 to 13.5 years were tested and the false-recent rate (FRR), the frequency of samples with a positive result, was determined at different cutoff values of the assay. Additionally, factors associated with misclassification were determined. The FRR of the LAg-Avidity was 1.1% (4/362) using a cutoff of 1.5 normalized optical density units (OD-n). All four samples had viral loads >1,000 copies/ml. Using a cutoff of 3.0 OD-n the FRR was 5.5% (20/362), with four samples having viral loads <1,000 copies/ml. Factors associated with a higher odds of misclassification were female gender (OR 7.7, 95% CI 1.0–56.4) and being a female sex worker (OR 31.3, 95% CI 4.0–242). At the higher cutoff, being of Zhuang descent, relative to Han, had higher odds of misclassification (OR 6.2, 95% CI 1.99–19.0). The LAg-Avidity assay had a low FRR in this Chinese population. Further investigations of the higher frequency of low LAg-Avidity results seen in female sex workers and individuals of Zhuang descent should be explored in a larger study.

ACCURATE METHODS OF ESTIMATING population level incidence are essential in determining the impact of prevention efforts and identifying high-risk groups for targeted intervention campaigns.^{1,2} Previous research has shown that serologic testing methods can be used to identify individuals who have been recently infected.³ One such serologic assay is the Limiting-Antigen Avidity assay (LAg-Avidity).⁴ Specifically, the test developers recommends a cutoff of a 1.5 normalized optical density (OD-n) and subsequent viral load confirmation (viral load >1,000 copies/ml).^{5,6} An additional recommendation is the generation of a local false-recent rate (FRR), which is the fraction of long-term infected (>2 years) who test positive for the testing algorithm.^{5,7,8}

In this report, we present LAg-Avidity assay results from individuals in Guangxi province of China who have been infected a median of 5.4 years (range 2.0 to 13.5 years), based on prior HIV antibody testing. A total of 362 specimens from

341 individuals were tested; 320 individuals provided one time point, 19 individuals provided two time points, and 2 individuals provided three time points. All subjects tested were treatment naïve, based on the national HIV/AIDS information system. All subjects provided written informed consent and the study was approved by the Guangxi Internal Review Board. The LAg-Avidity assay was performed by the manufacture's protocol, with the following modification. All samples were tested in duplicate and the average of the duplicate value was used in the analysis. Viral load data were obtained using the Easy Q system v2.0 (Merieux), for all samples with a LAg-Avidity value <3.0 OD-n. Statistical analysis was performed using STATA v.12 (College Station, TX).

For this population, the overall mean LAg-Avidity OD-n was 5.04 (SD 1.03), range 0.80 to 6.99. For a given individual, the variation in LAg-Avidity OD-n between multiple time points was 0.47 OD-n (SD 0.29). Overall, the

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TABLE 1. FACTORS ASSOCIATED WITH LOW LIMITING-ANTIGEN AVIDITY RESULTS AMONG INDIVIDUALS FROM GUANGXI, CHINA INFECTED MORE THAN 2 YEARS

	LAg-Avidity (<1.5 OD-n)		LAg-Avidity (<3.0 OD-n)	
	% Misclassified	OR (95% CI)	% Misclassified	OR (95% CI)
All	1.1% (4/362)	—	5.5% (20/362)	—
Age (years)				
23–30	1.5% (1/67)	1	5.6% (4/67)	1
31–35	0.0% (0/99)	—	5.1% (5/99)	0.84 (0.22–3.24)
36–40	0.9% (1/106)	0.63 (0.4–10.2)	5.7% (6/106)	0.95 (0.26–3.48)
41–60	2.2% (2/90)	1.50 (0.13–16.9)	5.6% (5/90)	0.92 (0.24–3.59)
Duration of infection (years)				
2.0–3.8	0.0% (0/90)	1	5.6% (5/90)	1
3.9–5.4	4.4% (4/93)	—	9.7% (9/93)	1.82 (0.58–5.67)
5.5–6.9	0.0% (0/86)	—	3.5% (3/86)	0.61 (0.14–2.65)
7.0–13.5	0.0% (0/93)	—	3.2% (3/93)	0.57 (0.13–2.44)
Gender				
Male	0.6% (2/319)	1	4.7% (15/319)	1
Female	4.3% (2/43)	7.73 (1.06–56.4)	11.6% (5/43)	2.67 (0.92–7.75)
Race				
Han	0.9% (3/324)	1	4.3% (14/324)	1
Zhang	4.4% (1/23)	4.86 (0.49–48.7)	30.8% (5/23)	6.15 (1.99–19.0)
Unkown	0.0% (0/15)	—	6.7% (1/15%)	1.58 (0.19–12.9)
Risk factor				
PWID	0.6% (2/346)	1	4.6% (16/346)	1
FSW	15.4% (2/13)	31.3 (4.0–242)	30.8% (4/13)	9.2 (2.55–33.0)
MSM	0.0% (0/1)	—	0.0% (0/1)	—
PRG	0.0% (0/2)	—	0.0% (0/2)	—
CD4 (cells/ μ l)				
>500	1.8% (2/112)	1	4.5% (5/112)	1
500–200	0.0% (0/1239)	—	3.8% (7/184)	0.85 (0.26–2.73)
<200	3.0% (2/66)	1.72 (0.24–12.5)	12.1% (8/66)	2.95 (0.92–9.43)

Odds ratios with *p*-values <0.05 are in **bold**.

LAg, limiting-antigen; OD-n, normalized optical density; OR, odds ratio; PWID, people who inject drugs; FSW, female sex worker; MSM, men who have sex with men; PRG, pregnancy screen; CD4, CD4 T cell count.

LAg-Avidity OD-n values did not vary by gender (mean 4.81, SD 1.38 for women, and men 5.08, SD 0.98; *p*=0.22), though women did have higher odds of having a low LAg-Avidity value (see Table 1). Interestingly, the LAg-Avidity values of individuals representing the Zhuang descent were lower than those of the Han descent (mean 4.49, SD 1.35 vs. 5.09, SD 1.00; *p*=0.052). Though female sex workers (FSW) did not have significantly lower LAg-Avidity values than persons who inject drugs (PWID) (mean 4.20, SD 1.99 vs. mean 5.08, SD 0.98; *p*=0.14), they did have a higher odds of having a sample with a value <1.5OD-n (see Table 1).

Overall, the FRR was 1.1% (95% CI 0.3–2.8%, 4/362) for a LAg-Avidity using 1.5 OD-n, which was unchanged with the addition of a viral load exclusion (at 1,000 copies/ml). However, two subjects with LAg OD-n <1.5 also had a CD4 count <100 cells/ μ l. If the cutoff was increased to 3.0 OD-n, the FRR result was 5.5% (95% CI 3.4–8.4%, 20/362), which was reduced to 4.4% (95% CI 2.5–7.1%, 16/362) with a viral exclusion of 400 copies/ml. Increasing the viral load exclusion to 1,000 copies/ml did not decrease the FRR. The statistical power was limited by the small sample sizes in these subgroups. These results should be seen as potential sources of misclassification that warrant further investigation.

There are limited data on LAg-Avidity FRR for individuals from Southeast Asia.⁶ However, the results in this study

were similar to the newly published study, 1.6%,⁶ though the recently published study by the CDC did not stratify the FRR results by infecting subtype. A previous study from Vietnam showed a very low FRR for the BED Capture EIA (BED-CEIA),⁷ and though the results of the BED-CEIA and LAg-Avidity assay are highly correlated, it is notable that the BED-EIA has a higher FRR than the LAg-Avidity. This low FRR seen in our study may be a reflection of an anti-retroviral (ARV)-naïve population and the specific subtypes infecting these individuals. The subtype distribution in Guangxi is approximately 75% CRF01AE and 25% B or BC recombinants.^{8–10} Previous research in Thailand estimated that the FRR was 0% using a cutoff of 1.0 OD-n for 128 samples from CRF01_AE-infected subjects.⁴ Additionally, individuals infected with subtype B and C historically have a low FRR.⁷ Further investigations of the higher frequency of low LAg-Avidity results seen in female sex workers and individuals of Zhuang descent should be explored in a larger study.

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Author Disclosure Statement

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