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Short Communication: False Recent Ratio of the Limiting-Antigen Avidity Assay and Viral Load Testing Algorithm Among Cameroonians with Long-Term HIV Infection

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Abstract

Current serological assays that are used for cross-sectional HIV incidence estimation have been shown to misclassify individuals with chronic infection. Limited information exists on the performance of cross-sectional incidence assays in Central Africa. HIV-positive individuals from Cameroon who were infected for at least 1 or 2 years were evaluated to determine the false recent ratio (FRR) of a two-assay algorithm, which includes the Limiting Antigen Avidity (LAg-Avidity) assay (normalized optical density units, ODn <1.5) and HIV viral load (>1000 copies/ml). The subject-level FRR was 5.3% (95% confidence interval [CI], 2.1–10.5) for individuals infected for ≥1 year and 3.9% (95% CI, 0.8–11.0) for individuals infected for ≥2 years. These data suggest that the LAg-Avidity plus viral load incidence algorithm may overestimate HIV incidence rates in Central Africa.

Keywords: HIV, cross-sectional incidence, Cameroon

The rate of new human immunodeficiency virus (HIV) infections in a population can be estimated in a cross-sectional manner using serological-based incidence testing.[1] When validated for a specific population, this cross-sectional biomarker-based approach can be critical for effective surveillance and prevention efforts among populations at high risk for HIV infection.[1] However, concerns about the accuracy of incidence estimates derived from using single tests have arisen, and can be addressed by combining multiple serology assays in a multiassay algorithm.[2] Currently, the United States Centers for Disease Control and others estimate HIV incidence in various populations across the world using a two-test algorithm that includes the Limiting Antigen Avidity (LAg-Avidity) assay and presence of a viral load >1000 copies/ml.[3] The initial test in this algorithm, the LAg-Avidity assay, when used alone can produce false recent ratio (FRR), or the ratio of chronically infected individuals who are misclassified as recently infected, results of <2% in multiple populations, which is below the level of FRR recommended for accurately measuring HIV incidence.[1,4] However, the performance of the LAg-Avidity assay alone can be affected by individual and population-level factors, such as differences in geography, HIV subtype, and prevalence of elite controllers for a given population.[5] A study of Ugandan HIV seroconverters demonstrated that when using the LAg-Avidity assay alone, individuals who were infected with subtype-D misclassified as recently infected at a higher rate than those infected with subtype A.[6] Cameroon and other areas of Central Africa containing all four HIV-1 groups M, O, N, and P have a very broad subtype diversity.[7] This study examined whether the high viral genetic diversity found in Cameroon will result in a higher FRR for the standard LAg-Avidity plus HIV viral load algorithm.

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Table 1. Performance of Limiting Antigen Avidity Assay and Viral Loads Among Individuals in Cameroon

	Infected for ≥1 year				*Infected for ≥2 years*			
	Individuals	*% misclassified*	*Total samples*	*% misclassified*	*Individuals*	*% misclassified*	*Total samples*	*% misclassified*
Total	133	5.3 (7/133)	175	4.6 (8/175)	77	3.9 (3/77)	105	3.8 (4/105)
Gender								
Male	28	3.6 (1/28)	33	3.0 (1/33)	18	5.6 (1/18)	20	5.0 (1/20)
Female	105	5.7 (6/105)	142	4.9 (7/142)	59	3.4 (2/59)	85	3.5 (3/85)
Age[a]								
<30	41	4.9 (2/41)	53	3.8 (2/53)	16	6.3 (1/16)	20	5.0 (1/20)
30–39	57	8.8 (5/57)	75	8.0 (6/75)	36	5.6 (2/36)	50	6.0 (3/50)
≥40	34	0.0 (0/34)	46	0.0 (0/46)	24	0.0 (0/24)	34	0.0 (0/34)
CD4 cell count (cells/mm^3)								
≤200	—	—	24	16.7 (4/24)	—	—	13	23.1 (3/13)
>200	—	—	150	2.7 (4/150)	—	—	92	1.1 (1/92)

[a]Missing one sample in each age category for individuals infected for 1 and 2 years and missing one sample in CD4 cell count category for individuals infected for 1 year.

Plasma samples were obtained from participants from the Medical Diagnostic Center (MDC) cohort in Yaounde, Cameroon, which is a cohort of HIV-positive individuals identified and referred to MDC where they are monitored longitudinally and provided with care.[8] Samples from 2011 to 2015 were collected from individuals known to be HIV infected for >1 year and were collected before antiretroviral therapy initiation.[8] All participants provided informed consent and the study was approved by the Ethics Committee of the Health Science Foundation in Cameroon (IRB #i09-0431). All samples were tested using the LAg-Avidity assay (Sedia Biosciences Corporation, Portland, OR) per manufacturer's recommendations. The proportion of individuals who were misclassified as recent were determined using a predetermined algorithm of an LAg Avidity score of <1.5 normalized optical density units (ODn) and presence of an HIV viral load >1000 copies/ml.[4] Viral loads were measured using the Abbott Real-Time HIV-1 Assay Kit (0.6 ml HIV-1 RNA US). The HIV RNA isolation was done using the m2000sp system and amplified by the m2000rt ThermoCycler (Abbott Molecular, Inc., Des Plains, IL). On a subgroup of individuals, HIV subtype was determined by either standard pol resistance sequencing ($n=39$), next-generation sequencing of reverse transcriptase ($n=3$), or gp41 ($n=1$) as previously described, or both ($n=21$).[9]

The FRR was examined at the sample and subject level. The primary analysis was conducted among individuals who were known to be infected for at least 1 year ($T=1$). A subgroup analysis was then limited to a subset of these same individuals who were known to be infected for at least 2 years ($T=2$). For subject-level analyses, if an individual contributed discordant results (one sample was false-recent whereas the second sample was not false-recent), then that individual contributed a count of 0.5. An overall subject-level FRR and 95% confidence interval (CI) were calculated using a binomial exact test. Statistical analyses were performed using Stata 14 and the ''inctools'' package in R.[10]

A total of 175 samples from 133 individuals were tested by LAg-Avidity assay with 91 individuals providing one time point and 42 individuals providing two time points. Most of the participants were female 78.9% (105/133) and the median age of participants was 34 years (Table 1). Subtypes A, D, F, G, J, and group O were identified in individuals as well as the recombinant form CRF02_AG at 56% (36/64), and other recombinants at 25% (16/64). It should be noted that some of the subtype calls that were based on only small NGS amplicons alone may not reflect the true subtype of the full-length viral sequence.

Overall, the FRR for the LAg Avidity (<1.5 ODn) plus viral loads (>1000 HIV RNA copies/ml) algorithm was 5.3% (7/133; 95% CI, 2.1–10.5) for individuals known to be infected ≥1 year (Table 1). It should be noted that two individuals who misclassified had one sample time point that misclassified and one sample that did not, therefore, each of these individuals contributed 0.5 to the FRR calculation. The FRR was 3.6% (1/28; 95% CI, 0.0–18.3) among males and 5.7% (6/105; 95% CI, 2.1–12.06) among females (Table 1). The individuals whose samples misclassified had a variety of subtypes with four being infected with CRF_02 A/G, three infected with URF_02-22cpx, URF_06cpx_02, or subtype F2, and one sample infected with a group O strain. When limiting the analysis to individuals known to be infected for ≥2 years using the same algorithm resulted in an overall FRR of 3.9% (95% CI, 0.8–11.0; Table 1).

One limitation of this study is that the statistical inference is limited because of the small population size of this cohort. However, there is limited to no data on biomarker-based incidence estimation in the Cameroon and other Central African countries with diverse subtypes. These initial results suggest that there may be potential sources of misclassification in Cameroon using the standard Lag-Avidity plus viral load algorithm, and that it may not be sufficient for incidence estimation in this setting, which contains a high amount of viral subtype diversity. Further investigation of the accuracy of HIV incidence assays using larger cohorts and well-classified seroconversion panels from Central Africa is warranted.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Murphy G, *et al.*: Moving towards a reliable HIV incidence test—Current status, resources available, future directions and challenges ahead. Epidemiol Infect 2017;145:925–941.
2. Guy R, *et al.*: Accuracy of serological assays for detection of recent infection with HIV and estimation of population incidence: A systematic review. Lancet Infect Dis 2009;9:747–759.
3. Kassanjee R, *et al.*: Viral load criteria and threshold optimization to improve HIV incidence assay characteristics. AIDS 2016;30:2361–2371.
4. Duong YT, *et al.*: Detection of recent HIV-1 infection using a new limiting-antigen avidity assay: Potential for HIV-1 incidence estimates and avidity maturation studies. PLoS One 2012;7:e33328.
5. Longosz AF, *et al.*: Incorrect identification of recent HIV infection in adults in the United States using a limiting-antigen avidity assay. AIDS 2014;28:1227.
6. Longosz AF, *et al.*: Comparison of antibody responses to HIV infection in Ugandan women infected with HIV subtypes A and D. AIDS Res Hum Retroviruses 2015;31:421–427.
7. Zhong P, *et al.*: HIV type 1 group M clades infecting subjects from rural villages in equatorial rain forests of Cameroon. J Acquir Immune Defic Syndr 2002;31:495–505.
8. Courtney CR, *et al.*: Monitoring HIV-1 group M subtypes in Yaounde, Cameroon reveals broad genetic diversity and a novel CRF02_AG/F2 infection. AIDS Res Hum Retroviruses 2016;32:381–385.
9. Courtney CR, *et al.*: Contrasting antibody responses to intrasubtype superinfection with CRF02_AG. PLoS One 2017;12:e0173705.
10. Kassanjee R, *et al.*: A new general biomarker-based incidence estimator. Epidemiology 2012;23:721–728.

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