Specificity of Four Laboratory Approaches for Cross-Sectional HIV Incidence Determination: Analysis of Samples from Adults with Known Nonrecent HIV Infection from Five African Countries

AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 28, Number 10, 2012

DOI: 10.1089/aid.2011.0341

Specificity of Four Laboratory Approaches for Cross-Sectional HIV Incidence Determination: Analysis of Samples from Adults with Known Nonrecent HIV Infection from Five African Countries

Oliver Laeyendecker,[1,2] Ron Brookmeyer,[3] Caroline E. Mullis,[2] Deborah Donnell,[4,5] Jairam Lingappa,[4,6,7] Connie Celum,[4,7,8] Jared M. Baeten,[4,7] Mary S. Campbell,[7] Max Essex,[9] Guy de Bruyn,[10] Carey Farquhar,[4,7,8] Thomas C. Quinn,[1,2] and Susan H. Eshleman,[11] for the Partners in Prevention HSV/HIV Transmission Study Team

Abstract

Assays to determine cross-sectional HIV incidence misclassify some individuals with nonrecent HIV infection as recently infected, overestimating HIV incidence. We analyzed factors associated with false-recent misclassification in five African countries. Samples from 2197 adults from Botswana, Kenya, South Africa, Tanzania, and Uganda who were HIV infected >12 months were tested using the (1) BED capture enzyme immunoassay (BED), (2) avidity assay, (3) BED and avidity assays with higher assay cutoffs (BED + avidity screen), and (4) multiassay algorithm (MAA) that includes the BED + avidity screen, CD4 cell count, and HIV viral load. Logistic regression identified factors associated with misclassification. False-recent misclassification rates and 95% confidence intervals were BED alone: 7.6% (6.6, 8.8); avidity assay alone: 3.5% (2.7, 4.3); BED + avidity screen: 2.2% (1.7, 2.9); and MAA: 1.2% (0.8, 1.8). The misclassification rate for the MAA was significantly lower than the rates for the other three methods (each $p < 0.05$). Misclassification rates were lower when the analysis was limited to subtype C-endemic countries, with the lowest rate obtained for the MAA [0.8% (0.2, 1.9)]. Factors associated with misclassification were for BED alone: country of origin, antiretroviral treatment (ART), viral load, and CD4 cell count; for avidity assay alone: country of origin; for BED + avidity screen: country of origin and ART. No factors were associated with misclassification using the MAA. In a multivariate model, these associations remained significant with one exception: the association of ART with misclassification was completely attenuated. A MAA that included CD4 cell count and viral load had lower false-recent misclassification than the BED or avidity assays (alone or in combination). Studies are underway to compare the sensitivity of these methods for detection of recent HIV infection.

Introduction

Accurate methods for cross-sectional HIV incidence determination are needed to monitor the HIV/AIDS epidemic and to evaluate the effectiveness of interventions for HIV prevention.[1] These methods would also enable use of cross-sectional surveys to estimate HIV incidence for prevention studies in populations at high risk of HIV acquisition. Most laboratory tests that are currently used to estimate HIV incidence are based on analysis of anti-HIV antibodies.[2,3] One

[1]Laboratory of Immunoregulation, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.
[2]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.
[3]Department of Biostatistics, School of Public Health, University of California at Los Angeles, Los Angeles, California.
[4]Department of Global Health, University of Washington, Seattle, Washington.
[5]Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, Washington.
[6]Department of Pediatrics, University of Washington, Seattle, Washington.
[7]Department of Medicine, University of Washington, Seattle, Washington.
[8]Department of Epidemiology, University of Washington, Seattle, Washington.
[9]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts.
[10]Perinatal HIV Research Unit, University of Witwatersrand, Johannesburg, South Africa.
[11]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

widely used method is the BED capture enzyme immunoassay (BED-CEIA), which measures the proportion of all IgG antibodies that bind to an HIV peptide.[4] Recently, an avidity assay has been developed for HIV incidence determination that is based on the BioRad 1/2+O ELISA test.[5] The capacities of these assays to identify recently infected individuals are described elsewhere.[5,6]

A critical limitation of the BED-CEIA for HIV incidence determination is that individuals with long-standing HIV infection are often misclassified as recently infected. This type of misclassification can lead to a significant overestimation of HIV incidence rates, and has prompted the Joint United Nations Programme on HIV/AIDS (UNAIDS) to discourage the use of the BED-CEIA for HIV incidence determination.[7] Factors previously associated with misclassification of the BED-CEIA in African populations include low HIV viral load, low CD4 cell count, and long-term antiretroviral therapy (ART).[8–12] However, none of those studies has compared false-recent misclassification among demographically similar populations in different African countries, and little is known about the frequency and nature of false-recent misclassification using the avidity assay, especially in an African setting.

The Incidence Assay Critical Path Working Group has recently recommended using a testing algorithm for HIV incidence that combines results from two different assays to reduce misclassification.[13] This approach was used in a recent study from Rwanda, which demonstrated that combining the BED-CEIA with an avidity assay based on the AxSYM limited false-recent misclassification while maintaining adequate sensitivity for detection of recently infected individuals.[14] While it is important to limit misclassification of individuals with long-standing infection as recently infected, it is also desirable to identify recently infected individuals, and to maximize the length of time where laboratory assays reliably identify infections as recent (the window period for recent infection). The goals of this study were to compare the false-recent misclassification rates of four different laboratory approaches for HIV incidence determination, and to identify factors associated with false-recent misclassification using each of these testing approaches: (1) the BED-CEIA, (2) an avidity assay, (3) the BED-CEIA and the avidity assay used jointly with elevated assay cutoffs (BED+avidity screens), and (4) a multiassay algorithm that included the BED+avidity screens, as well as CD4 cell count and HIV viral load. The frequency of misclassification and factors associated with misclassification were assessed by testing samples obtained from a cohort of men and women from Botswana, Kenya, South Africa, Tanzania, and Uganda who were known to have been infected with HIV for at least 12 months.

Materials and Methods

Samples used for analysis

Samples were obtained from 2197 HIV-infected participants in the Partners in Prevention HSV/HIV Transmission Study,[15] a clinical trial that enrolled stable, HIV-serodiscordant couples (one partner HIV infected and one partner HIV uninfected) from sub-Saharan Africa to investigate the impact of acyclovir treatment on HIV transmission. We tested samples that were collected from HIV-infected participants a median of 21 months after enrollment (range 12–24 months). The duration of infection was calculated based on the participants' report of their first positive test date: using this approach, participants were infected a median of 25 months (interquartile range 20–34, range 12–253). Samples were collected from participants from Botswana ($N=199$), Kenya ($N=902$), South Africa ($N=330$), Tanzania ($N=138$), and Uganda ($N=628$); some samples from Uganda were obtained from an ancillary study.[16] Epidemiologic and laboratory data, including HIV viral load and CD4 cell count, were obtained during the trial and were included in the analysis. The use of ART was based on self-report.

Laboratory testing

The BED-CEIA was performed according to the manufacturer's instructions (Calypte Biomedical Corporation, Lake Oswego, OR). The BED-CEIA measures the proportion of total IgG that binds to a branched synthetic tripeptide that contains three 18-amino acid components derived from an immunodominant region of gp41 (regions corresponding to positions 590–607 of HXB2 gp160 in HIV subtypes B, E, and D).[17] Results from the BED-CEIA are reported as normalized optical density units (OD-n). A standard assay cut-off of <0.8 OD-n was used to define recent HIV infection when the BED-CEIA was used alone.[4] The avidity assay was performed using a modified Genetic Systems HIV-1/HIV-2+O EIA (enzyme linked immunoassay, Bio-Rad Laboratories, Redmond, WA) with diethylamine (DEA) as the chaotropic agent.[5] An avidity index (AI) was calculated by dividing the optical density of the DEA-treated well by the optical density of the nontreated well for the same sample, and multiplying by 100. A standard cut-off of $<40\%$ was used to define recent infection when the avidity assay was used alone. When the BED-CEIA and avidity assays were used in combination, we used cut-offs of <1.0 OD-n (BED screen) and $<80\%$ (avidity screen), respectively. These higher cut-off values were also used in the multiassay algorithm (MAA); in the MAA, recent infection was defined as BED-CEIA <1.0 OD-n, avidity index $<80\%$, HIV viral load of >400 copies/ml, and CD4 cell count >200 cells/mm^3. The cut-offs used in the MAA were selected by analyzing data obtained by testing samples from individuals in other cohorts and clinical studies who had known durations of HIV infection.

Statistical analysis

The misclassification rates with exact 95% confidence intervals (95% CI) were calculated using BED-CEIA alone, avidity assay alone, BED-CIEA and avidity in combination (BED+avidity screens), and the MAA; these frequencies were compared using Fisher's exact test or chi-square test. The association of the misclassification rates of each test method was assessed for age, viral load, CD4 cell count, country of origin, and ART using logistic regression.

Additionally, categories were generated based on the circulating subtypes in each country (subtype C endemic: Botswana and South Africa; subtype A and D endemic: Kenya and Uganda; Tanzania was not included in either group because of the heterogeneity of subtypes in that country). All factors associated in the univariate analysis based on logistic regression ($p<0.1$) were included in the multivariate analysis. Multivariate logistic regression analysis was performed to identify factors associated with misclassification with each method after adjustment for other factors. All statistical

analyses were performed using STATA v11 (StataCorp, College Station, TX).

Human subjects

All work was conducted in accordance with the Declaration of Helsinki. Written consent was provided from each participant for participation in the Partners in Prevention Study. Experiments were conducted with the approval by the appropriate institutional review boards.

Results

We analyzed samples from 763 men and 1434 women enrolled in the Partners in Prevention HSV/HIV Transmission Study; one sample was analyzed for each of the 2197 participants. Each participant was known to have been HIV infected for at least 12 months at the time of sample collection. Though the ages were similar between countries, the proportion of female, virally suppressed, taking ART, and mean viral load was different by country (see Table 1). Of the participants with viral load data, 17% (488/2193) were virally suppressed (viral load <400 copies/ml). Among 1916 participants who indicated that that they were not on ART, 275 (14%) were virally suppressed. Only 6% (142/2197) of participants had a CD4 cell count <200 cells/mm^3, and only 0.3% (7/2197) had a CD4 cell count <50 cells/mm^3. Nineteen participants (0.8%) had a viral load <400 copies/ml and a CD4 cell count <200 cells/mm^3.

Table 2 presents the frequency of false-recent misclassification by the BED-CEIA (BED <0.8 OD-n), the avidity assay (AI $<40\%$), the BED + avidity screens (BED <1.0 and AI $<80\%$), and the MAA (see Materials and Methods). The percent misclassified for the BED-CEIA was 7.6% (95% CI: 6.6%, 8.8%). Country, HIV viral load, CD4 cell count, and ART were significantly associated with misclassification by the BED-CEIA ($p<0.02$ for all comparisons). The avidity assay misclassified 3.5% (95% CI: 2.7%, 4.3%) of the samples as recently infected. Country was the only factor significantly associated with misclassification ($p<0.001$). Using the BED + avidity screens, 2.2% (95% CI: 1.7%, 2.9%) of the samples were misclassified as recently infected. With this assay combination, country of origin and ART were significantly associated with misclassification. Using the MAA, 1.2% (95% CI: 0.8%, 1.8%) of the samples were misclassified. Neither ART nor country of origin was associated with misclassification using the MAA ($p>0.37$ and $p>0.41$, respectively). The subtype C endemic areas (South Africa and Botswana) had significantly lower rates of false-recent misclassification then the subtype A and D endemic areas (Uganda and Kenya) for the BED-CEIA: 4.9% (95% CI: 3.2%, 7.1%) vs. 8.7% (95% CI: 7.3, 10.2%), $p=0.004$, the avidity assay: 1.0% (95% CI: 0.3%, 2.2%) vs. 4.6% (95% CI: 3.6%, 5.7%), $p<0.001$, and the combined BED-CEIA and avidity screens: 1.0% (0.3% to 2.2%) vs. 2.8% (2.0% to 3.7%), $p=0.017$. The misclassification rate of the MAA was also lower in the subtype C than the A and D endemic areas; this difference was not statistically significant: 0.75% (95% CI: 0.21%, 1.92%) vs. 1.43% (95% CI: 0.90%, 2.17%), $p=0.267$.

Multivariate logistic regression was performed to compare the odds of false-recent misclassification by BED-CEIA alone, the avidity assay alone, and the BED + avidity screens (Table 3); because no factors were associated with misclassification using the MAA ($p>0.37$ for all associations), the MAA results are not shown. For the BED-CEIA, three factors were independently associated with misclassification: country, viral load, and CD4 cell count. The adjusted odds ratio for misclassification was two times higher in Uganda and Kenya than in South Africa. For the avidity assay, the only factor associated with misclassification in the multivariate model was country. The adjusted odds ratio for misclassification was eight times higher in Uganda than in South Africa, and three times higher in Kenya than in South Africa. For the BED + avidity screens, the only factor associated with misclassification in the multivariate model was country of origin, where, for example, the adjusted odds ratio for misclassification was approximately four times higher in Uganda than in South Africa. It is noteworthy that the strength of association for all variables in the univariate analysis was similar to that in the multivariate analysis apart from ART use. ART use was not associated with misclassification by the BED-CEIA, the avidity assay, or the BED + avidity screens when the analysis was adjusted for other variables.

Discussion

In this study, we compared false-recent misclassification using four different laboratory approaches for HIV incidence determination. This study was based on analysis of samples collected from five countries in East and Southern Africa. A potential limitation of the study was that criteria used for study enrollment may have introduced bias into the cohort. All participants were HSV infected and in a stable HIV discordant relationship; most of the participants were

Table 1. Population Characteristics by Country of Origin

	Botswana	*Kenya*	*South Africa*	*Tanzania*	*Uganda*
Number of subjects	199	902	330	138	628
Mean age (SD)	35.7 (8.3)	34.2 (8.9)	33.8 (8.6)	34.8 (7.8)	34.6 (8.5)
Gender (% female)	63.82%	69.85%	74.55%	82.61%	50.48%
Pregnant	5.26%	8.22%	8.46%	8.11%	10.44%
Mean $\log_{10}$ viral load (SD)	3.92 (0.91)	3.80 (0.96)	3.90 (0.92)	3.95 (1.08)	4.04 (1.00)
Virally suppressed	15.58%	23.39%	15.45%	26.09%	18.95%
Taking ART	10.05%	13.41%	6.36%	5.07%	17.86%
Non-ART virally suppressed	7.26%	14.98%	13.27%	22.90%	14.34%
Mean CD4 (SD)	427 (210)	481 (240)	491 (243)	552 (285)	446 (244)

SD, standard deviation; virally suppressed, having a viral load <400 copies/ml; ART, antiretroviral treatment; CD4, CD4 cell count (cells/mm^3).

Table 2. Factors Associated with False Recent Misclassification by the BED-CEIA, an Avidity Assay, a Combined BED/Avidity Screen, and a Multiassay Algorithm (Partners in Prevention Trial, Africa, 2007–2009)

	BED-CEIA (OD-n <0.8)		*Avidity assay (AI <40%)*		*Combined BED/avidity screen (OD-n <1.0 and AI <80%)*		*MAA (OD-n <1.0, AI <80%, CD4 >200, VL >400)*	
	% Misclassified	*OR (95% CI)*	*% Misclassified*	*OR (95% CI)*	*% Misclassified*	*OR (95% CI)*	*% Misclassified*	*OR*
All	7.6% (168/2197)		3.5% (76/2197)		2.2% (49/2197)		1.2% (27/2196)	
Clade C endemic	4.9% (26/529)		1.0% (5/529)		1.0% (5/529)		0.8% (4/529)	
Clade A and D endemic	8.7% (133/1530)		4.6% (70/1530)		2.8% (42/1530)		1.4% (22/1530)	
Age								
19–28	5.9% (34/576)	1	2.4% (14/576)	1	1.7% (10/576)	1	0.9% (5/571)	1
29–33	8.7% (48/552)	*1.52 (0.96–2.39)*	3.6% (20/552)	1.51 (0.75–3.02)	2.9% (16/552)	1.69 (0.76–3.76)	1.6% (9/552)	1.89 (0.63–5.68)
34–39	8.6% (46/533)	*1.51 (0.95–2.38)*	3.8% (20/533)	1.57 (0.78–3.13)	2.6% (14/533)	1.53 (0.67–3.47)	0.9% (5/533)	1.08 (0.31–3.76)
40–70	7.5% (40/536)	1.29 (0.80–2.06)	4.1% (22/536)	1.72 (0.87–3.39)	1.7% (9/536)	0.97 (0.39–2.40)	1.5% (8/536)	1.73 (0.56–5.32)
Gender								
Female	8.2% (117/1434)	1	3.2% (46/1434)	1	2.3% (33/1434)	1	1.2% (17/1434)	1
Male	6.7% (51/763)	0.81 (0.57–1.13)	3.9% (30/763)	1.23 (0.77–1.97)	2.1% (16/763)	0.91 (0.50–1.66)	1.3% (10/763)	1.11 (0.50–2.42)
Pregnant								
No	8.7% (68/783)	1	3.6% (28/783)	1	2.4% (19/783)	1	1.3% (10/783)	1
Yes	4.1% (3/74)	0.44 (0.14–1.45)	4.1% (3/74)	1.14 (0.34–3.84)	1.4% (1/74)	0.55 (0.07–4.17)	1.4% (1/74)	1.06 (0.13–8.39)
Country								
South Africa	4.6% (15/330)	1	0.9% (3/330)	1	0.9% (3/330)	1	0.9% (3/330)	1
Botswana	5.5% (11/199)	1.23 (0.55–2.73)	1.0% (2/199)	1.11 (0.18–6.68)	1.0% (2/199)	1.11 (0.18–6.68)	0.5% (1/199)	0.55 (0.06–5.33)
Tanzania	6.5% (9/138)	1.47 (0.63–3.43)	0.7% (1/138)	0.80 (0.08–7.72)	1.5% (2/138)	1.60 (0.26–9.70)	0.7% (1/138)	0.80 (0.08–7.72)
Kenya	8.8% (79/902)	***2.02 (1.14–3.55)****	2.8% (25/902)	*3.11 (0.93–10.4)*	1.9% (17/902)	2.09 (0.61–7.19)	1.1% (10/902)	1.22 (0.33–4.47)
Uganda	8.6% (54/628)	***1.98 (1.11–3.56)****	7.2% (45/628)	***8.41 (2.59–27.3)****	4.0% (25/628)	***4.52 (1.35–15.1)****	1.9% (12/628)	2.12 (0.59–7.58)
Viral load								
>50,000	4.7% (25/536)	1	4.3% (23/536)	1	2.1% (11/536)	1	1.7% (9/536)	1
50,000 to 10,000	4.7% (25/529)	1.01 (0.57–1.79)	3.2% (17/529)	0.74 (0.39–1.40)	1.7% (9/529)	0.83 (0.34–2.01)	1.3% (7/539)	0.79 (0.29–2.12)
10,000 to 400	6.1% (42/684)	1.33 (0.80–2.22)	2.5% (17/684)	0.57 (0.30–1.08)	1.6% (11/684)	0.78 (0.34–1.81)	1.6% (11/684)	0.96 (0.39–2.32)
<400	17.0% (76/448)	***4.18 (2.61–6.70)****	4.2% (19/448)	0.99 (0.53–1.84)	4.0% (18/448)	*2.00 (0.93–4.28)*	—	
CD4								
>500	10.3% (84/816)	1	3.3% (27/816)	1	2.1% (17/816)	1	1.2% (10/816)	1
500–201	6.1% (75/1239)	***0.56 (0.41–0.78)****	3.5% (43/1239)	1.05 (0.64–1.71)	2.2% (27/1239)	1.05 (0.57–1.93)	1.4% (17/1239)	1.12 (0.52–2.46)
<200	6.3% (9/142)	0.60 (0.31–1.20)	4.2% (6/142)	1.28 (0.52–3.18)	3.5% (5/142)	1.71 (062–4.73)	—	—
Taking ART								
No	7.0% (133/1915)	1	3.2% (61/1915)	1	2.0% (38/1915)	1	1.2% (22/1893)	1
Yes	12.5% (35/281)	***1.91 (1.28–2.83)****	5.3% (15/281)	*1.71 (0.96–3.06)*	3.9% (11/281)	***2.01 (1.01–3.98)****	1.8% (5/281)	1.56 (0.59–4.15)

*p value < 0.05.
†p value < 0.01.
MAA, multiassay algorithm; AI, avidity index; VL, viral load; OR, odds ratio; CI, confidence intervals; CD4, CD4 cell count (cells/mm^3); viral load (copies/ml); ART, antiretroviral therapy. Statistically significant values are shown in bold text. Values with $p < 0.1$ (trends) are in italics.

Table 3. Adjusted Odds of Misclassification for the BED-CEIA, an Avidity Assay, and a Combined BED/Avidity Screen (Partners in Prevention Trial, Africa, 2007–2009)

	BED-CEIA (OD-n <0.8) aOR (95% CI)	*Avidity assay (AI <40%) aOR (95% CI)*	*BED/avidity screen (OD-n <1.0 and AI <80%) aOR (95% CI)*
Age			
19–28	1	—	—
29–33	1.44 (0.90–2.29)	—	—
34–39	1.44 (0.90–2.32)	—	—
40–70	1.21 (0.75–1.98)	—	—
Country			
South Africa	1	1	1
Botswana	1.28 (0.57–2.89)	1.07 (0.18–6.60)	1.08 (0.18–6.58)
Tanzania	1.18 (0.57–2.81)	0.80 (0.08–7.75)	1.44 (0.24–8.76)
Kenya	***1.87 (1.05–3.34)****	*3.03 (0.91–10.1)*	1.90 (0.55–6.59)
Uganda	***2.00 (1.10–3.65)****	***8.11 (2.49–26.4)***†	***4.26 (1.27–14.3)****
Viral load			
>50,000	1	—	1
50,000 to 10,000	0.95 (0.54–1.70)	—	0.85 (0.35–2.09)
10,000 to 400	1.20 (0.71–2.03)	—	0.90 (0.38–2.10)
<400	***3.49 (2.10–5.82)***†	—	*2.02 (0.91–4.48)*
CD4			
>500	1		
500–201	***0.60 (0.42–0.85)***†	—	—
<200	0.68 (0.31–1.20)	—	—
Taking ART			
No	1	1	1
Yes	1.09 (0.69–1.72)	1.36 (0.76–2.45)	1.12 (0.52–2.43)

*p value <0.05.
†p value <0.01.
AI, avidity index; aOR, adjusted odds ratio; CI, confidence interval; CD4, CD4 cell count (cells/mm^3); viral load (copies/ml); ART, antiretroviral therapy. Statistically significant values ($p<0.05$) are shown in bold text. Values with $p<0.1$ (trends) are in italics.

relatively healthy, and a substantial proportion (17%) was virally suppressed. Additionally, the length of time that individuals were infected was not known, although all individuals were known to have been infected for at least 1 year. In the cohort studied, the frequency of false-recent misclassification was 7.6% the BED-CEIA alone, 3.5% using the avidity assay alone, 2.2% using a BED screen and an avidity screen combined (i.e., using both assays with higher assay cutoffs), and 1.2% using a MAA that combined the BED screen, and avidity screen, CD4 cell count, and HIV viral load. In subtype C endemic areas, the misclassification frequency of the MAA was 0.8%.

In univariate models, several factors were significantly associated with false recent misclassification using the BED-CEIA alone, an avidity assay alone, or the BED+avidity screens. These factors included country (for all three methods), HIV viral load (for BED-CEIA), CD4 cell count (for BED-CEIA), and ART use (for BED-CEIA and the BED+avidity screens). In contrast, we did not observe any statistically significant associations between any of the factors examined and false recent misclassification using the MAA. In a multivariate model, the only statistically significant associations observed were for country (for BED and avidity either alone or in combination), viral load (BED-CEIA only), and CD4 cell count (BED-CEIA only). The association that we observed for the BED-CEIA between misclassification and high CD4 cell count (>500 cells/mm^3) was surprising, although a previous study from Uganda also demonstrated a similar finding among individuals on ART.[11] In previous studies, individuals with advanced HIV disease (e.g., CD4 cell counts <200 cells/mm^3) were more likely to be misclassified as recently infected than those with higher CD4 cell counts. That association was presumed to reflect immunologic decline, with impaired antibody production. The frequency of BED-CEIA misclassification that we observed in Uganda (8.6%) was lower than the misclassification frequency reported in a previous study in Uganda (14.9%)[18]; this difference may reflect the fact that individuals in the cohort studied in this report were less likely to have advanced HIV disease.

In these analyses, associations between participants' country of residence and false recent misclassification are likely to reflect differences in the prevalent HIV subtypes, although other factors may also have influenced assay performance among the countries studied. Misclassification rates were higher for Kenya and Uganda (East African countries where subtypes A and D are prevalent) than for South Africa and Botswana (Southern African countries where subtype C is prevalent). The frequency of misclassification was two times higher in the subtype A and D endemic countries using the BED-CEIA, and was four to five times higher in those countries using the avidity assay. Previous studies have shown that HIV subtype can impact the performance of cross-sectional incidence assays.[6,19] In South Africa and Botswana almost all infections are subtype C, while in Kenya and Uganda, the most common subtypes are A and D, with some infections caused by subtype C and A–D recombinants. In Tanzania,

subtypes A, C, and D and intersubtype recombinant strains are prevalent.[20]

This report reveals significant differences in the rate of false-recent misclassification using different laboratory methods developed for cross-sectional HIV incidence determination. The lowest misclassification rates were observed with the MAA (1.2% overall, 0.8% for subtype C endemic areas). A key feature of the MAA is inclusion of two independent serologic assays (the BED-CEIA and the avidity assay); this increases the specificity of the MAA for detection of recent HIV infection. For example, when samples analyzed in this report were tested using a similar algorithm that did not include the BED-CEIA (with recent infection defined as avidity $<80\%$ + CD4 cell count >200 cells/mm^3 + HIV viral load >400 copies/ml), the false-recent misclassification rate was four times higher that the rate obtained using the four-assay MAA. One advantage of using the BED-CEIA and the avidity assay for HIV incidence testing is that these assays can be performed using commercially available kits (with a minor modification in testing procedures for the avidity assay). Neither of these assays requires use of large, specialized equipment, which is an advantage in resource-limited settings. The avidity assay is based on the BioRad 1/2+O ELISA kit, which was designed for diagnosis of HIV infection. The target antigens in this kit are large polypeptides (p24 and gp160) that include multiple antigens that are recognized by anti-HIV antibodies from individuals infected with diverse HIV strains.[21] This assay is used globally for diagnosis of HIV infection and performs well across all subtypes for the detection on anti-HIV antibodies. Our results demonstrate that the avidity assay based on the BioRad 1/2+O ELISA has a relatively low rate of false-recent misclassification when samples are analyzed from African individuals who are likely to be infected with different HIV subtypes.

A disadvantage of the MAA is the requirement for CD4 cell count data. Storage of cryopreserved samples that can be used for retrospective CD4 testing is costly and not feasible in many settings. Therefore, in most settings, CD4 cell count data must be obtained at the time of sample collection. We are currently evaluating whether CD4 testing can be replaced in the MAA by a high resolution melting (HRM) assay that measures HIV diversity without sequencing,[22] which can be performed using stored serum or plasma samples. In the MAA, viral load testing is needed only for a small subset of samples (i.e., those with BED-CEIA <1.0 OD-n + avidity $<80\%$ + CD4 >200 cells/mm^3). This is an advantage, since HIV viral load tests are the most expensive component of the MAA. We feel it is important to include a direct measurement of HIV viral load in the MAA, rather than relying on self-report of antiretroviral drug (ARV) use as a surrogate of viral suppression. Self-reports of ARV use may be unreliable, and some individuals on ARV therapy may not be virally suppressed. Furthermore, our previous studies have shown a high rate of false-recent misclassification among HIV-infected elite suppressors who have low or undetectable viral loads in the absence of ARV use.[23] In this study, ARV use was not associated with false-recent misclassification in multivariate models that also included HIV viral load.

This report is focused on the specificity of laboratory methods for HIV incidence determination. The specificity of HIV incidence algorithms substantially impacts their performance, since the number of prevalent infections is usually much greater than the number of incident infections. We recognize, however, that the sensitivity for detecting recent infections is also an important indicator of test performance. Furthermore, our nonrecent samples included only those samples with duration of infection >12 months. The operating characteristics (i.e., sensitivity and specificity) of the MAA are expected to vary with different definitional criteria for recent versus nonrecent infections based on duration of infection. Further studies are needed to assess the sensitivity of the MAA in detecting recent HIV infection, the window length for differentiating recent versus nonrecent infection, and whether sensitivity varies by subtype.

Acknowledgments

The authors would like to thank the Partners in Prevention study teams and participants, S. Michelle Owen (United States Centers for Disease Control and Prevention), Erin Kahle (University of Washington), Harald S. Haugen (University of Washington), and Amy Mueller (Johns Hopkins University).

This work was supported by (1) the HIV Prevention Trials Network (HPTN) sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Mental Health (NIMH), the National Institute on Drug Abuse (NIDA), Office of AIDS Research, of the National Institutes of Health (NIH), Department of Health and Human Services (DHHS) (Grants U01-AI46745, U01-AI48054, U01-AI068613, and UM1-AI068613), and the International Maternal Pediatric and Adolescent AIDS Clinical Trials (IMPAACT) Network (U01-AI068632), (2) the Division of Intramural Research, NIAID, (3) the NIAID (Grant 1R01-AI095068), and (4) the Bill and Melinda Gates Foundation (Grant 26469).

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the National Institutes of Health. Use of trade names is for identification purposes only and does not constitute endorsement by the National Institutes of Health and Prevention or the Department of Health and Human Services.

Author Disclosure Statement

No competing financial interests exist.

References

1. Brookmeyer R: Measuring the HIV/AIDS epidemic: Approaches and challenges. Epidemiol Rev 2010;32:26–37.
2. Murphy G and Parry JV: Assays for the detection of recent infections with human immunodeficiency virus type 1. Euro Surveill 2008;13:pii18966.
3. Busch MP, Pilcher CD, Mastro TD, Kaldor J, Vercauteren G, Rodriguez W, *et al.:* Beyond detuning: 10 years of progress and new challenges in the development and application of assays for HIV incidence estimation. AIDS 2010;24:2763–2771.
4. Dobbs T, Kennedy S, Pau CP, McDougal JS, and Parekh BS: Performance characteristics of the immunoglobulin G-capture BED-enzyme immunoassay, an assay to detect recent human immunodeficiency virus type 1 seroconversion. J Clin Microbiol 2004;42:2623–2628.
5. Masciotra S, Dobbs T, Candal D, Hanson D, Delaney K, Rudolph D, *et al.:* Antibody avidity-based assay for identifying recent HIV-1 infections based on Genetic Systems TM 1/2 plus O EIA. 17th Conference on Retroviruses and

Opportunistic Infections, Abstract 937. San Francisco, CA, February 16–19, 2010.
6. Parekh BS, Hanson DL, Hargrove J, Branson B, Green T, Dobbs T, *et al.:* Determination of mean recency period for estimation of HIV type 1 incidence with the BED-Capture EIA in persons infected with diverse subtypes. AIDS Res Hum Retroviruses 2011;27:265–273.
7. UNAIDS Reference Group on Estimates, Modeling, and Projections: Statement on the use of the BED assay for estimation of HIV-1 incidence or epidemic monitoring. Weekly Epidemiol Rec 2006;81:33–40.
8. Barnighausen T, Wallrauch C, Welte A, McWalter TA, Mbizana N, Viljoen J, *et al.:* HIV incidence in rural South Africa: Comparison of estimates from longitudinal surveillance and cross-sectional cBED assay testing. PLoS One 2008;3:e3640.
9. Karita E, Price M, Hunter E, Chomba E, Allen S, Fei L, *et al.:* Investigating the utility of the HIV-1 BED capture enzyme immunoassay using cross-sectional and longitudinal seroconverter specimens from Africa. AIDS 2007;21: 403–408.
10. Marinda ET, Hargrove J, Preiser W, Slabbert H, van Zyl G, Levin J, *et al.:* Significantly diminished long-term specificity of the BED capture enzyme immunoassay among patients with HIV-1 with very low CD4 counts and those on antiretroviral therapy. J Acquir Immune Defic Syndr 2010;53: 496–499.
11. Hladik W, Olara D, Mermin J, Moore D, Were W, Alexander L, and Downing R: Effect of CD4+ T-cell count and antiretroviral treatment on two serological HIV incidence assays. AIDS Res Hum Retroviruses 2012;28(1):5–99.
12. Hayashida T, Gatanaga H, Tanuma J, and Oka S: Effects of low HIV type 1 load and antiretroviral treatment on IgG-capture BED-enzyme immunoassay. AIDS Res Hum Retroviruses 2008;24:495–498.
13. Incidence Assay Critical Path Working Group: More and better information to tackle HIV epidemics: Towards improved HIV incidence assays. PLoS Med 2011;8:e1001045.
14. Braunstein SL, Nash D, Kim AA, Ford K, Mwambarangwe L, Ingabire CM, *et al.:* Dual testing algorithm of BED-CEIA and AxSYM Avidity Index assays performs best in identifying recent HIV infection in a sample of Rwandan sex workers. PLoS One 2011;6:e18402.
15. Celum C, Wald A, Lingappa JR, Magaret AS, Wang RS, Mugo N, *et al.:* Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. N Engl J Med 2010;362:427–439.
16. Heffron R, Donnell D, Rees H, Celum C, Mugo N, Were E, *et al.:* Use of hormonal contraceptives and risk of HIV-1 transmission: A prospective cohort study. Lancet Infect Dis 2012;12:19–26.
17. Parekh BS, Kennedy MS, Dobbs T, Pau CP, Byers R, Green T, *et al.:* Quantitative detection of increasing HIV type 1 antibodies after seroconversion: A simple assay for detecting recent HIV infection and estimating incidence. AIDS Res Hum Retroviruses 2002;18:295–307.
18. Kim AA, Hallett T, Stover J, Gouws E, Musinguzi J, Mureithi PK, *et al.:* Estimating HIV incidence among adults in Kenya and Uganda: A systematic comparison of multiple methods. PLoS One 2011;6:e17535.
19. Young CL, Hu DJ, Byers R, Vanichseni S, Young NL, Nelson R, *et al.:* Evaluation of a sensitive/less sensitive testing algorithm using the bioMerieux Vironostika-LS assay for detecting recent HIV-1 subtype B′ or E infection in Thailand. AIDS Res Hum Retroviruses 2003;19:481–486.
20. Hemelaar J, Gouws E, Ghys PD, and Osmanov S: Global trends in molecular epidemiology of HIV-1 during 2000–2007. AIDS 2011;25:679–689.
21. Owen SM, Yang C, Spira T, Ou CY, Pau CP, Parekh BS, *et al.:* Alternative algorithms for human immunodeficiency virus infection diagnosis using tests that are licensed in the United States. J Clin Microbiol 2008;46:1588–1595.
22. Cousins MM, Laeyendecker O, Beauchamp G, Brookmeyer R, Towler WI, Hudelson SE, *et al.:* Use of a high resolution melting (HRM) assay to compare gag, pol, and env diversity in adults with different stages of HIV infection. PLoS One 2011;6:e27211.
23. Laeyendecker O, Rothman RE, Henson C, Horne BJ, Ketlogetswe KS, Kraus CK, *et al.:* The effect of viral suppression on cross-sectional incidence testing in the Johns Hopkins Hospital Emergency Department. J Acquir Immune Defic Syndr 2008;48:211–215.

Address correspondence to:
Oliver Laeyendecker
Senior Research Associate, LIR/NIAID/NIH
855 North Wolfe Street, Room 538A
Baltimore, Maryland 21205

E-mail: olaeyen1@jhmi.edu