

HIV Incidence Determination in the United States: A Multiassay Approach

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(See the major article by Eshleman et al, on pages 223–31 and the editorial commentary by Mastro, on pages 204–6.)

Background. Accurate testing algorithms are needed for estimating human immunodeficiency virus (HIV) incidence from cross-sectional surveys.

Methods. We developed a multiassay algorithm (MAA) for HIV incidence that includes the BED capture enzyme immunoassay (BED-CEIA), an antibody avidity assay, HIV load, and CD4⁺ T-cell count. We analyzed 1782 samples from 709 individuals in the United States who had a known duration of HIV infection (range, 0 to >8 years). Logistic regression with cubic splines was used to compare the performance of the MAA to the BED-CEIA and to determine the window period of the MAA. We compared the annual incidence estimated with the MAA to the annual incidence based on HIV seroconversion in a longitudinal cohort.

Results. The MAA had a window period of 141 days (95% confidence interval [CI], 94–150) and a very low false-recent misclassification rate (only 0.4% of 1474 samples from subjects infected for >1 year were misclassified as indicative of recent infection). In a cohort study, annual incidence based on HIV seroconversion was 1.04% (95% CI, .70%–1.55%). The incidence estimate obtained using the MAA was essentially identical: 0.97% (95% CI, .51%–1.71%).

Conclusions. The MAA is as sensitive for detecting recent HIV infection as the BED-CEIA and has a very low rate of false-recent misclassification. It provides a powerful tool for cross-sectional HIV incidence determination.

Keywords. HIV; incidence testing; United States; epidemiology.

Accurate methods to estimate the incidence of human immunodeficiency virus (HIV) infection are needed to monitor the leading edge of the epidemic [1], to identify groups at high risk of infection for targeted prevention interventions, and to evaluate the effectiveness

of prevention interventions [2]. Unfortunately, after >30 years, HIV incidence and prevalence remain high in many settings [3], and practical and accurate methods for estimating HIV incidence remain elusive. Prospective cohort studies that identify HIV seroconverters are expensive and time-consuming, and they may not provide reliable estimates of HIV incidence in the relevant populations because of selection bias, changes in behavior associated with study participation, and loss to follow-up [4]. Biomarker-based methods that identify recently infected persons in cross-sectional samples are a promising alternative approach for estimating HIV incidence. However, concerns have been raised about their accuracy.

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The first biomarker-based method to estimate incidence from cross-sectional samples was proposed by Brookmeyer and Quinn in 1995 [5]. In that study, HIV incidence was assessed by detecting acute HIV infections (ie, measuring the number of HIV-seronegative individuals in a population who were positive for HIV p24 antigen). Unfortunately, because the duration of seronegative HIV infection is very short, this method for determining HIV incidence is only useful for analysis of very large surveys in populations with very high annual incidence [5]. In 1998, Janssen and colleagues developed a “detuned” serologic assay [6] that differentiated between individuals with recent and those with nonrecent HIV infection, which was based on the observation that the HIV antibody titer is usually low during the first few months of infection. Other assays have since been developed for cross-sectional HIV incidence determination that measure different features of the immune response to HIV infection [7–10]. The BED capture enzyme immunoassay (BED-CEIA) is currently the most widely used commercially available assay for detecting recent HIV infection [11, 12]. Other assays measure responses such as antibody avidity and antibody isotype switching [13, 14]. Unfortunately, none of these methods provide the needed accuracy for identifying recent HIV infection in most settings.

A major limitation of serologic assays is the frequent misclassification of individuals with long-standing HIV infection as recently infected (ie, false-recent misclassification) [15]. Two factors that have been associated with false-recent misclassification by the BED-CEIA are low CD4⁺ T-cell count and low HIV load [16]. Some studies have suggested using a mathematical approach to adjust results obtained with these assays, to improve the precision of HIV incidence estimates [17]. However, that approach has limitations, such as requiring precise estimates of additional input adjustment factors [18–21]. Working groups have been assembled to harmonize the terminology used in this field, to facilitate collaboration among investigators, to share the findings of new research, and to focus research efforts [22].

In this report, we describe a multiassay algorithm (MAA) for identifying recent infections that can be used to determine population HIV incidence rates. This approach overcomes limitations of previous approaches developed for estimating HIV incidence that are based on cross-sectional sampling, because all individuals stop appearing to be recently infected and do not regress back to appearing to be recently infected. The MAA combines 2 serological assays with CD4⁺ T-cell count and HIV load. The serological assays are used to cast a wide net to identify individuals who may have recent HIV infection. HIV load and CD4⁺ T-cell count are used to exclude individuals who, because of advanced HIV disease (indicated by low CD4⁺ T-cell count) or natural- or antiretroviral-induced viral suppression (indicated by low HIV load), may be misclassified by serologic assays as recently infected.

We evaluated the performance of the MAA for estimating cross-sectional HIV incidence by using the following approach. First, we tested samples from HIV-infected individuals whose duration of HIV infection was known at the time of sample collection. Then, we determined the probabilities of whether the MAA classifies a person as recently infected and how those probabilities depend on the actual durations of infection. We used those probabilities to calculate the mean duration during which the MAA classifies a person as recently infected, an interval known as the “window period.” We then determined whether the MAA could estimate HIV incidence within 1 year preceding sample collection. Finally, we compared the annual HIV incidence estimate obtained by the MAA with the annual HIV incidence observed in a prospective cohort study (HIV Network for Prevention Trials study 001 [HIVNET 001]).

METHODS

Samples Used for Analysis

Data analyzed in this report were obtained from testing stored plasma or serum from HIV-infected US participants in the AIDS Linked to the Intravenous Experience (ALIVE) study [23], the Multicenter AIDS Cohort Study (MACS) [24], and HIVNET 001 [25] (Table 1). We tested 1782 samples obtained from 709 individuals who had a documented HIV-negative test result with a subsequent documented HIV-positive test result within 18 months. In all 3 studies, samples were assayed for CD4⁺ T-cell count in real time.

Characteristics of Individuals Studied

A total of 1782 samples from 709 individuals with known duration of HIV infection were analyzed (Table 1). These samples were collected between 1987 and 2009 from individuals in HIVNET 001 (808 samples from 103 individuals), the ALIVE study (410 samples from 241 individuals), and the MACS (564 samples from 365 individuals) who acquired HIV infection during study follow-up. Samples from HIVNET 001 were collected near the time of HIV seroconversion and up to 53 months later; samples from the ALIVE study and the MACS were collected between 24 and 100 months after HIV seroconversion. All subjects had provided written informed consent, and the study was approved by the Institutional Review Board of the Johns Hopkins University. Most of the samples (89%) were from men. Overall, 60.1% of the individuals studied had, on the basis of self-report, been exposed to antiretroviral therapy when 1 or more of the samples was collected (44.5% of the samples included in the analysis were collected from individuals exposed to antiretroviral therapy).

Laboratory Methods

The BED-CEIA was performed according to the manufacturer’s instructions (Calypse Biomedical, Lake Oswego, OR) [8],

Table 1. Characteristics of Study Subjects, by Cohort

Characteristic	Cohort		
	HIVNET 001	ALIVE Study	MACS
No. of samples	808	410	564
No. of unique individuals	103	241	365
Risk factor(s) for HIV acquisition	Various ^a	Injection drug use	MSM
Time between last negative and first positive HIV test result, d, median (range) ^b	180 (0–376)	187 (0–539)	182 (0–547)
Duration of HIV infection, mo ^c	1–53	25–71	33–100
No. of samples per subject	1–13	1–2	1–2
Age, y, mean±SD	36.2±7.6	40.1±6.8	41.3±8.6
Male sex, % of subjects	88.5	75.6	100.0
Collection date, no. (%) of samples			
1987–1989	0	0	110 (19.5)
1990–1994	0	155 (37.8)	208 (36.9)
1995–1999	808 (100.0)	188 (45.9)	145 (25.7)
2000–2004	0	58 (14.1)	60 (10.6)
2005–2009	0	9 (2.2)	41 (7.3)
CD4 ⁺ T-cell count in cells/mm ³ , no. (%) of samples			
>500	490 (60.6)	108 (26.3)	236 (41.8)
201–500	295 (36.5)	199 (48.5)	233 (41.3)
51–200	22 (2.7)	79 (19.3)	65 (11.5)
≤50	1 (0.1)	24 (5.9)	30 (5.3)
HIV load in copies/mL, no. (%) of samples			
>50 000	160 (19.8)	114 (27.8)	163 (28.9)
10 001–50 000	204 (25.3)	135 (32.9)	147 (26.1)
401–10 000	214 (26.5)	116 (28.3)	140 (24.8)
≤400	230 (28.5)	45 (11.0)	114 (20.2)
No. (%) of samples from individuals receiving ART ^d	343 (42.5)	166 (40.5)	284 (50.4)

Abbreviations: ALIVE, AIDS Linked to the Intravenous Experience (1987–2009); ART, antiretroviral therapy; HIVNET 001, HIV Network for Prevention Trials study 001 (1995–1999); MACS, Multicenter AIDS Cohort Study (1990–2009); MSM, men who have sex with men.

^a MSM, 78% (80 of 103); men who use injection drugs, 4% (4 of 103); women who use injection drugs, 6% (6 of 103); women who have sex with men and use injection drugs, 7% (7 of 103).

^b If participants were HIV RNA positive at their last seronegative visit (documented acute infection), the value 0 was used; the number of such participants in HIVNET 001, the ALIVE study, and MACS were 4, 18, and 50, respectively.

^c Calculated as the time between the estimated date of HIV seroconversion and the date of sample collection.

^d Samples collected in the era before highly active antiretroviral therapy were from individuals who were receiving less potent antiretroviral drug regimens; many of those individuals did not have viral suppression at the time of sample collection.

with the following modification. All samples were run in duplicate, and the average normalized optical density (OD-n) was used for analysis. Antibody avidity was measured using a modified version of the Genetic Systems 1/2 + O enzyme-linked immunosorbent assay (Bio-Rad, Hercules, CA) [26]. Briefly, samples were diluted 1:10 in duplicate and were incubated at 4°C for 30 minutes (initial antibody-binding step). Samples were then incubated for 30 minutes at 37°C with or without the chaotropic agent diethylamine (antibody-disassociation step). For each sample, the avidity index was calculated as follows: [(optical density of the diethylamine-treated well)/(optical density of the nontreated well)] × 100.

In the MAA, a cutoff OD-n of 1.0 (rather than the standard assay cutoff of 0.8 OD-n) was used for the BED-CEIA, and a cutoff of 80% (rather than the standard assay cutoff of 40%) was used for the avidity assay; these higher assay cutoffs were used to increase the sensitivity for identifying individuals who were likely to have been recently infected at the time of sample collection; viral load and CD4⁺ T-cell count were used to exclude samples that falsely categorized infection as recent from that enlarged pool. Samples with the following test results yielded by the MAA were considered to be from recently infected individuals: a CD4⁺ T-cell count of >200 cells/mm³, a BED-CEIA of <1.0 OD-n, an avidity index of <80%, and an HIV load of

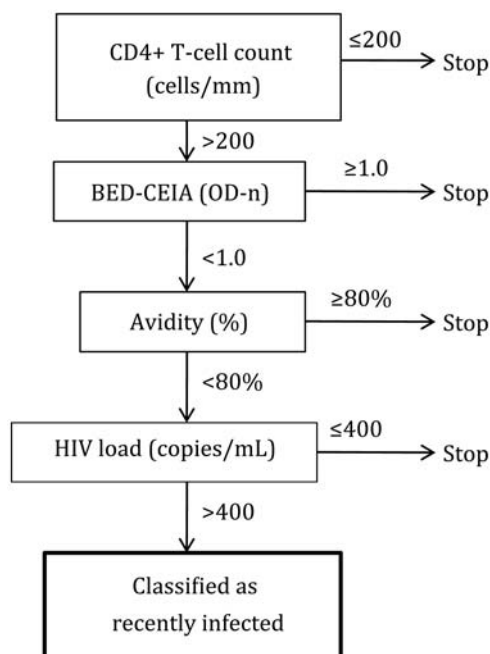


Figure 1. Multiassay algorithm for cross-sectional human immunodeficiency virus (HIV) incidence determination. Individuals with all of the following results are classified as recently infected: CD4⁺ T-cell count of >200 cells/mm³, a BED capture enzyme immunoassay (BED-CEIA) finding of <1.0 optical density units (OD-n), an avidity index of <80%, and a viral load of >400 copies/mL (see Methods). The term “stop” indicates nonrecent HIV infection.

>400 copies/mL (Figure 1). When the BED-CEIA was used alone, a cutoff of <0.8 OD-n was used to indicate recent infection.

Statistical Methods

All samples were evaluated using both the MAA (Figure 1) and the BED-CEIA alone (see the Laboratory Methods subsection). The proportion of individuals classified as recently

infected with each method was calculated by duration of infection (stratified into intervals, Table 2). The date of HIV seroconversion was estimated for each individual as the midpoint between the last HIV-negative test result and the first HIV-positive test result except if acute infection was documented (ie, a sample was HIV RNA positive and HIV antibody negative), in which case the date of HIV seroconversion was estimated as 2 weeks after the study visit in which acute infection was documented. Uncertainty in seroconversion dates was accounted for as described further below. The logit of the probability of samples being classified as recent was modeled as a function of the duration of infection, using cubic splines (with a knot at 2 years). The cubic spline allows the probability curves to be flexible and imposes minimal assumptions on the shape of the curves. The approach does not assume that the probabilities must decrease with the duration of infection or that the probabilities are initially 1 at the time of occurrence of infection. We calculated the mean window period by computing the area under the modeled probability curves, using numerical integration [27, 28]. We also calculated the shadow, which is a statistical measure of how far back into the past (from the point that the samples were collected) HIV incidence can be estimated by the MAA (eg, a shadow of 1 year means that the MAA has the potential to measure HIV incidence up to 1 year prior to the time the samples were collected) [27, 28]. Another way of interpreting the shadow is that it is the expected duration that a person who is classified by the MAA as recently infected has actually been living with HIV infection. The shadow can be calculated directly from the probability curves modeled by the cubic splines, as described above [27]. We used bootstrapping (blocked on individual and stratified by study) to calculate confidence intervals (CIs) for the proportions classified as indicative of recent infection, the mean window duration, and the shadow [29]. We bootstrapped by resampling with replacement individuals (to

Table 2. Proportion of Samples Classified by the BED Capture Enzyme Immunoassay (BED-CEIA) Alone and the Multiassay Algorithm (MAA) as Indicative of Recent Human Immunodeficiency Virus Infection, by Estimated Infection Duration

Infection Duration (y)	Samples Tested (no.)	BED-CEIA		MAA	
		Samples Classified as Recent (no.)	Samples Classified as Recent (%; 95% CI)	Samples Classified as Recent (no.)	Samples Classified as Recent (%; 95% CI)
0.0 to 0.49 ^a	142	80	56.3 (43.7–65.9)	68	47.9 (34.5–57.3)
0.50 to 0.99 ^a	166	61	36.7 (27.4–48.1)	15	9.0 (6.3–19.3)
1.00 to 1.99 ^a	263	65	24.7 (17.0–34.1)	2	0.8 (0.0–2.4)
2.00 to 2.99	301	62	20.6 (14.0–28.3)	2	0.7 (0.0–1.9)
3.00 to 3.99	440	64	14.5 (10.4–18.4)	2	0.5 (0.0–1.5)
4.00 to 4.99	125	15	12.0 (7.5–20.2)	0	0.0 (0.0–1.4)
≥5.00	345	47	13.6 (10.2–17.2)	0	0.0 (0.0–1.1)

Abbreviation: CI, confidence interval.

^aAll samples from individuals infected for <2 years were from the HIV Network for Prevention Trials study 001 cohort.

account for multiple samples per individual) from each of the 3 studies (the MACS, the ALIVE study, and HIVNET 001). Seroconversion dates were sampled uniformly from the intervals in which seroconversion occurred within the bootstrap, to account for uncertainty in those dates. All calculations were done using the *R* statistical programming language.

We used the MAA to derive a cross-sectional annual HIV incidence estimate for the HIVNET 001 clinical cohort, using the following equation: $[(\text{number of samples classified as indicative of recent infection}) \times (100)] / [(\text{number of HIV-negative samples}) \times (\text{mean window period in years})]$. The CIs calculated for HIV incidence based on the MAA accounted for uncertainty in the mean window duration [30].

RESULTS

Performance of the BED-CEIA and the MAA

Overall, 22.1% of the samples were classified as indicative of recent infections by use of the BED-CEIA alone, compared with 5.0% by use of the MAA. Table 2 shows the proportions of samples classified as indicative of recent infection, stratified by duration of infection. Among samples collected from individuals infected for <6 months, the proportions classified by the BED-CEIA alone and the MAA as indicative of recent infection were 56.3% (95% CI, 43.7%–65.9%) and 47.9% (95% CI, 34.5%–57.3%), respectively. Among samples collected from individuals infected for >6 months, the proportions of samples classified by the BED-CEIA as indicative of recent infection were higher at all intervals, compared with the proportions classified as such by the MAA (Table 2). Among samples collected from individuals infected for >5 years, the proportions of samples classified by the BED-CEIA alone and the MAA as indicative of recent infection were 13.6% (95% CI, 10.2%–17.2%) and 0.0% (95% CI, .0%–1.1%), respectively.

We calculated the durations of times that the 2 methods classified infections as recent. Figure 2 shows the modeled probability curves of samples being classified using the BED-CEIA alone and the MAA as indicative of recent infection. The curves indicate that the MAA is as sensitive for detecting recent infection as the BED-CEIA but has a lower false-recent misclassification rate. The mean window period during which the MAA classified samples as recent was 141 days (95% CI, 94–150 days). The shadow of the MAA was 128 days (95% CI, 115 to 202 days). The finding that the upper confidence limit of the shadow (202 days) was <1 year indicates the MAA is capable of estimating HIV incidence within 1 year of sample collection.

The probability that the BED-CEIA alone classified a sample as indicate of recent infection decreased for the first few years after HIV infection but then rose (Figure 2). By year 8, approximately 15% of samples were still classified as indicative of recent infections. As such, it was not possible to reliably

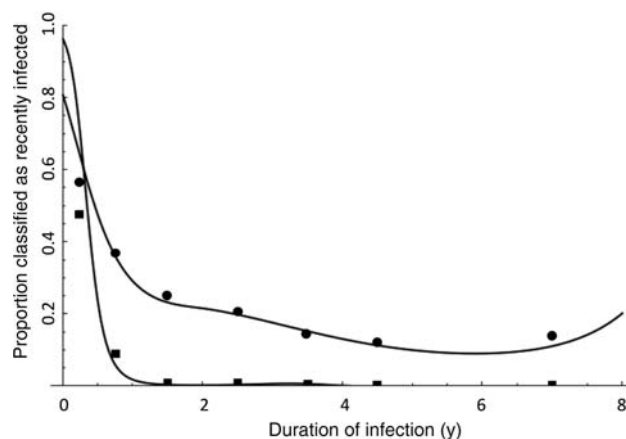


Figure 2. Modeled probabilities of an individual being classified as recently infected as a function of duration of infection. Figure shows modeled probability curves of samples being classified as recent using the BED capture enzyme immunoassay (BED-CEIA) alone and the multi-assay algorithm from fitting a cubic spline to the logit of the probability. Circles and squares show observed proportions by the BED-CEIA alone and the multiassay algorithm, respectively, when samples are stratified into intervals (Table 1).

calculate either the mean window period or the shadow by means of the BED-CEIA alone because those quantities depend on the tails of the probability curves, which did not converge to 0. Nevertheless, extrapolation of the cubic spline suggests that the mean window and shadow determined using the BED-CEIA alone are both at least 5.0 years and could be considerably greater.

Comparison of HIV Incidence Observed From Assessment of HIV Seroconversion and Derived From Cross-Sectional Assessment Using the MAA

In HIVNET 001, 90 individuals acquired HIV infection by the 18-month visit. Samples corresponding to the 18-month study visit were available for 81 of those individuals (9 individuals who received a diagnosis of HIV infection at earlier study visits chose not to enroll in the follow-up study or were lost to follow-up). Of the 81 samples, 30 were identified using the BED-CEIA as being from recently infected individuals, and 14 were identified using the MAA as being from recently infected individuals. The annual HIV incidence during longitudinal follow-up of this cohort, calculated on the basis of HIV seroconversion between the 12- and 18-month visits, was 1.04% (95% CI, .70%–1.55%) [31]. The annual incidence estimated using the MAA was 0.97% (95% CI, .51%–1.71%); this estimate was based on analysis of samples from 4175 HIV-uninfected individuals in HIVNET 001 that were collected at the 18-month visit and was adjusted for the 9 participants who did not have MAA results. The estimated annual incidence obtained with the BED-CEIA alone was 1.80%, using a

previously published mean window period [32]. While we recognize that the cross-sectional sample set that was used to estimate incidence with the MAA did not include individuals with long-term, chronic HIV infection (ie, infection of >18 months duration), only 0.3% (1 of 361) of all HIVNET 001 samples from individuals who were infected for >18 months and none of the samples from individuals who were infected for >3 years were classified by the MAA as indicative of recent infection.

DISCUSSION

In this report, we describe the performance of a MAA for identifying recent HIV infections that can be used to estimate HIV incidence. We found that none of the samples collected >5 years after HIV seroconversion were classified as recent by the MAA. This corrects a significant source of error with the BED-CEIA, which is currently in widespread use. Furthermore, the shadow of the MAA indicates that those samples classified as recent by the MAA were from individuals who, on average, had actually been infected for <1 year. The concordance between the MAA estimate of incidence and the observed incidence based on direct longitudinal follow-up for HIV seroconversion provides further validation of the MAA. Our analyses indicate that the MAA has the potential to estimate accurately HIV incidence levels within 1 year of sample collection. This study included samples from both men and women from across the United States, with different races and ethnic backgrounds and with different risk factors for HIV acquisition (eg, heterosexual sex, male-male sex, and injection drug use). This increases the relevance of the study findings.

An advantage of the MAA is that it uses a hierarchical approach for sample testing. Many of the samples from individuals with long-standing infection will be identified using the BED-CEIA. This assay is commercially available and is relatively inexpensive. The next step of sample testing uses the avidity assay. This assay requires a minor modification of a commercially available EIA used for HIV diagnosis and is also relatively inexpensive. Relatively few samples require HIV load testing (ie, only those with CD4⁺ T-cell counts of >200 cells/mm³, BED-CEIA results of <1.0 OD-n, and an avidity index of <80%); in this study, only 169 (9.5%) of 1782 samples required viral load testing. Furthermore, the order in which the BED-CEIA and avidity assays are performed can be interchanged, which may increase the cost-effectiveness of the algorithm in some settings.

It is important to note that the MAA described in this report does not require exclusion of individuals who are receiving antiretroviral therapy. The World Health Organization Working Group on HIV Incidence Assays does note that the presence of antiretroviral drugs in specimens can be used as a biomarker of long-term infection [33], and this approach has

been used for cross-sectional incidence estimation in South Africa [34]. This is based on the assumption that individuals who have been infected for <1 year are unlikely to be receiving antiretroviral treatment. However, self-report of antiretroviral use may be unreliable, and laboratory tests may fail to detect antiretroviral drugs if sample collection was not timed to drug dosing. Furthermore, some recently infected individuals may be exposed to antiretroviral drugs (eg, women who receive antiretroviral drugs for prevention of mother-to-child transmission) [35]. Our previous studies show an association between antiretroviral use and misclassification by the BED-CEIA [16, 36]. However, in multivariate logistic regression models, this association is fully attenuated when HIV load is included in the model [36]. In contrast, low HIV load remains strongly associated with misclassification. Furthermore, low HIV load is associated with misclassification by the BED-CEIA and other cross-sectional incidence assays in the absence of antiretroviral drug use (eg, in elite suppressors) [37, 38]. These data fit with a conceptual model in which the anti-HIV antibody response is downregulated when the amount of replicating virus is low, regardless of the cause of viral suppression [39]. This is addressed in our MAA by excluding individuals who have a low viral load.

One limitation of the MAA is that it requires CD4⁺ T-cell count data. The other components of the MAA (BED-CEIA, avidity, and HIV load) can be obtained using stored plasma or serum samples; we have previously shown that BED-CEIA and avidity assay results are not significantly affected by sample storage conditions or freeze-thaw cycles [40]. In contrast, CD4⁺ T-cell counts must be obtained either in real time from all HIV-infected individuals or retrospectively by using cryopreserved viable samples, both of which are costly. We are exploring whether CD4⁺ T-cell count and HIV load testing can be replaced in the MAA with a high-resolution melting (HRM) assay that measures the level of genetic diversity of HIV in a plasma or serum sample [41]. An advantage of the HRM diversity assay over CD4⁺ T-cell count testing is that the HRM assay can be performed retrospectively on stored serum or plasma samples; with this approach, testing with the HRM assay would be limited to the subset of samples that are classified by the less expensive BED-CEIA and avidity tests as recent infections.

HIV surveillance by the BED-CEIA was the basis for recent estimates of HIV incidence in the United States that were prepared by the Centers for Disease Control and Prevention (CDC) [11, 12]. To address the issue of false-recent misclassification, the CDC recommended excluding persons with AIDS and persons receiving antiretroviral treatment from being counted as having recent infection, regardless of their BED-CEIA test results. The proposed MAA is consistent with that recommendation, in that it uses HIV load and CD4⁺ T-cell count to classify individuals with advanced HIV disease or

viral suppression as not recently infected. Previous studies have shown that HIV subtype influences the results obtained using BED-CEIA and avidity assays [32, 42]. The results presented in this report are based on analysis of samples collected in the United States, which are assumed to be predominantly from individuals with subtype B HIV infection [43]. All of the HIV strains from the cohorts studied in the report that have been sequenced are subtype B [25, 44–46]. Further work is needed to evaluate the performance of the MAA in other populations with clade B epidemics and in populations with non-clade B epidemics. Further studies are also needed to compare HIV incidence estimates obtained with the MAA to those obtained from longitudinal follow-up in other cohorts. In summary, our results indicate that the MAA can be an accurate and practical approach to estimating HIV incidence in the United States, provided that CD4⁺ T-cell counts are available and that the surveys are representative of the population studied.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

- Brookmeyer R. Measuring the HIV/AIDS epidemic: approaches and challenges. *Epidemiol Rev* 2010; 32:26–37.
- Fiamma A, Lissouba P, Amy OE, et al. Can HIV incidence testing be used for evaluating HIV intervention programs? A reanalysis of the Orange Farm male circumcision trial (ANRS-1265). *BMC Infect Dis* 2010; 10:137.
- UNAIDS. Global report: UNAIDS report on the global AIDS epidemic 2010. Geneva, Switzerland: UNAIDS, 2010.
- Brookmeyer R, Quinn T, Shepherd M, Mehendale S, Rodrigues J, Bollinger R. The AIDS epidemic in India: a new method for estimating current human immunodeficiency virus (HIV) incidence rates. *Am J Epidemiol* 1995; 142:709–13.
- Brookmeyer R, Quinn TC. Estimation of current human immunodeficiency virus incidence rates from a cross-sectional survey using early diagnostic tests. *Am J Epidemiol* 1995; 141:166–72.
- Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA* 1998; 280:42–8.
- Rawal BD, Degula A, Lebedeva L, et al. Development of a new less-sensitive enzyme immunoassay for detection of early HIV-1 infection. *J Acquir Immune Defic Syndr* 2003; 33:349–55.
- Dobbs T, Kennedy S, Pau CP, McDougal JS, 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; 42:2623–8.
- Suligoi B, Butto S, Galli C, et al. Detection of recent HIV infections in African individuals infected by HIV-1 non-B subtypes using HIV antibody avidity. *J Clin Virol* 2008; 41:288–92.
- Li H, Ketema F, Sill AM, Kreisel KM, Cleghorn FR, Constantine NT. A simple and inexpensive particle agglutination test to distinguish recent from established HIV-1 infection. *Int J Infect Dis* 2007; 11: 459–65.
- Hall HI, Song R, Rhodes P, et al. Estimation of HIV incidence in the United States. *JAMA* 2008; 300:520–9.
- Prejean J, Song R, Hernandez A, et al. Estimated HIV incidence in the United States, 2006–2009. *PLoS One* 2011; 6:e17502.
- Guy R, Gold J, Calleja JM, 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; 9:747–59.
- Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. *Euro Surveill* 2008; 13:pii18966.
- Mastro TD, Kim A, Hallett T, et al. Estimating HIV incidence in populations using tests for recent infection: issues, challenges and the way forward. *J HIV/AIDS Surveill Epidemiol* 2010; 2:1–7.
- Laeyendecker O, Brookmeyer R, Oliver AE, et al. Factors associated with incorrect identification of recent HIV infection using the BED capture immunoassay. *AIDS Res Hum Retroviruses* 2011; 28:816–22.
- McDougal JS. BED estimates of HIV incidence must be adjusted. *AIDS* 2009; 23:2064–5; author reply 2066–8.
- Hargrove JW, Humphrey JH, Mutasa K, et al. Improved HIV-1 incidence estimates using the BED capture enzyme immunoassay. *AIDS* 2008; 22:511–8.
- Brookmeyer R. Should biomarker estimates of HIV incidence be adjusted? *AIDS* 2009; 23:485–91.
- Welte A, McWalter TA, Barnighausen T. Reply to ‘Should biomarker estimates of HIV incidence be adjusted?’ *AIDS* 2009; 23:2062–3; author reply 2066–8.
- Wang R, Lagakos SW. On the use of adjusted cross-sectional estimators of HIV incidence. *J Acquir Immune Defic Syndr* 2009; 52: 538–47.
- Incidence Assay Critical Path Working Group. More and better information to tackle HIV epidemics: towards improved HIV incidence assays. *PLoS Med* 2011; 8:e1001045.
- Vlahov D, Anthony JC, Munoz A, et al. The ALIVE study, a longitudinal study of HIV-1 infection in intravenous drug users: description of methods and characteristics of participants. *NIDA Res Monogr* 1991; 109:75–100.
- Kaslow RA, Ostrow DG, Detels R, Phair JP, Polk BF, Rinaldo CR Jr. The Multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. *Am J Epidemiol* 1987; 126:310–8.
- Celum CL, Buchbinder SP, Donnell D, et al. Early human immunodeficiency virus (HIV) infection in the HIV Network for Prevention Trials Vaccine Preparedness Cohort: risk behaviors, symptoms, and early plasma and genital tract virus load. *J Infect Dis* 2001; 183:23–35.

26. Masciotra S, Dobbs T, Candal D, et al. Antibody avidity-based assay for identifying recent HIV-1 infections based on Genetic Systems 1/2 plus O EIA [abstract 937]. Presented at: 17th Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 16–19 February 2010.
27. Brookmeyer R. On the statistical accuracy of biomarker assays for HIV incidence. *J Acquir Immune Defic Syndr* 2010; 54:406–14.
28. Kaplan EH, Brookmeyer R. Snapshot estimators of recent HIV incidence rates. *Operations Res* 1999; 47:29–37.
29. Efron B, Tibshirani RJ. An introduction to the bootstrap. Monographs on Statistics and Applied Probability. Vol. 57. Boca Raton, FL: Chapman & Hall/CRC, 1993.
30. Brookmeyer R. Accounting for follow-up bias in estimation of human immunodeficiency virus incidence rates. *J R Stat Soc* 1997; 160:127–40.
31. Seage GR 3rd, Holte SE, Metzger D, et al. Are US populations appropriate for trials of human immunodeficiency virus vaccine? The HIVNET Vaccine Preparedness Study. *Am J Epidemiol* 2001; 153:619–27.
32. Parekh BS, Hanson DL, Hargrove J, 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; 27:265–73.
33. Busch MP, Pilcher CD, Mastro TD, et al. Beyond detuning: 10 years of progress and new challenges in the development and application of assays for HIV incidence estimation. *AIDS* 2010; 24:2763–71.
34. Rehle TM, Hallett TB, Shisana O, et al. A decline in new HIV infections in South Africa: estimating HIV incidence from three national HIV surveys in 2002, 2005 and 2008. *PLoS One* 2010; 5:e11094.
35. World Health Organization (WHO). Programmatic update: use of antiretroviral drugs for treating pregnant women and preventing HIV infection in infants. Geneva, Switzerland: WHO, 2012.
36. Laeyendecker O, Brookmeyer R, Mullis C, et al. Specificity of four laboratory approaches for cross-sectional HIV incidence determination: analysis of samples from adults with known non-recent HIV infection from five African countries. *AIDS Res Hum Retroviruses* 2012. In Press.
37. Laeyendecker O, Rothman RE, Henson C, 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; 48:211–5.
38. Hayashida T, Gatanaga H, Tanuma J, Oka S. Effects of low HIV type 1 load and antiretroviral treatment on IgG-capture BED-enzyme immunoassay. *AIDS Res Hum Retroviruses* 2008; 24:495–8.
39. Trkola A, Kuster H, Leemann C, et al. Humoral immunity to HIV-1: kinetics of antibody responses in chronic infection reflects capacity of immune system to improve viral set point. *Blood* 2004; 104:1784–92.
40. Laeyendecker O, Latimore A, Eshleman SH, et al. The Effect of sample handling on cross sectional HIV incidence testing results. *PLoS One* 2011; 6:e25899.
41. Cousins MM, Laeyendecker O, Beauchamp G, 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.
42. Mullis CE, Munshaw S, Grabowski MK, et al. Differential misclassification of HIV-1 cross-sectional incidence assays by subtype in Rakai, Uganda [abstract 541]. Paper presented at: 18th Conference on Retroviruses and Opportunistic Infections, Seattle, Washington, 2012.
43. Hemelaar J, Gouws E, Ghys PD, Osmanov S. Global trends in molecular epidemiology of HIV-1 during 2000–2007. *AIDS* 2011; 25: 679–89.
44. Gottlieb GS, Heath L, Nickle DC, et al. HIV-1 variation before seroconversion in men who have sex with men: analysis of acute/early HIV infection in the multicenter AIDS cohort study. *J Infect Dis* 2008; 197:1011–5.
45. Rinaldo CR Jr, Gupta P, Huang XL, et al. Anti-HIV type 1 memory cytotoxic T lymphocyte responses associated with changes in CD4+ T cell numbers in progression of HIV type 1 infection. *AIDS Res Hum Retroviruses* 1998; 14:1423–33.
46. Markham RB, Wang WC, Weisstein AE, et al. Patterns of HIV-1 evolution in individuals with differing rates of CD4 T cell decline. *Proc Natl Acad Sci U S A* 1998; 95:12568–73.