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Cross-Sectional HIV Incidence Estimation in HIV Prevention Research

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Abstract

Accurate methods for estimating HIV incidence from cross-sectional samples would have great utility in prevention research. This report describes recent improvements in cross-sectional methods that significantly improve their accuracy. These improvements are based on the use of multiple biomarkers to identify recent HIV infections. These multi-assay algorithms (MAAs) use assays in a hierarchical approach for testing that minimizes the effort and cost of incidence estimation. These MAAs do not require mathematical adjustments for accurate estimation of the incidence rates in study populations in the year prior to sample collection. MAAs provide a practical, accurate, and cost-effective approach for cross-sectional HIV incidence estimation that can be used for HIV prevention research and global epidemic monitoring.

Keywords

HIV; incidence; cross-sectional; multi-assay algorithm

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Protection of Human Subjects

The procedures used in this report were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

1. Introduction

Most HIV prevention research studies rely on HIV incidence as the primary endpoint because of the lack of reliable surrogate endpoints [1, 2]. This presents challenges for observational studies and clinical trials. Many of these challenges arise from methodological issues with the traditional approach for determining HIV incidence: following cohorts of uninfected persons over time and documenting HIV acquisition.

Cohort studies present challenges in HIV prevention research for several reasons. First, if HIV incidence rates are low, large cohorts are required to accumulate sufficient incident infections for accurate determination of incidence rates. Second, it is often difficult to achieve adequate follow-up among high-risk uninfected persons. Third, longitudinal cohort studies are time consuming, often taking years to complete. Fourth, differential loss of follow-up between study arms can bias estimates of intervention effects. Fifth, study participation may modify HIV infection risk for reasons unrelated to the study intervention. For example, provision of risk-reduction counseling during routine follow-up of a biomedical intervention may decrease HIV incidence by decreasing risk behaviors. The Hawthorne effect may also confound prevention studies, since some behavior changes may be related to learning one's HIV status or the awareness of being observed, rather than to the intervention under study [3].

Many challenges of cohort studies can be addressed by assessing HIV incidence using a single, cross-sectional survey. This approach does not require follow-up of cohorts. In this approach, biological samples are collected in a cross-sectional survey, and biomarkers are used to identify recent HIV infections. In early work, individuals were classified as having recent HIV infection if they were acutely infected (HIV p24 antigen positive and HIV antibody negative) [4]. A limitation of that approach is that very large sample sizes are needed to identify recent infections because the duration of acute infection is very short [5]. Subsequently, the criterion for classification as recently infected was a weak anti-HIV antibody response in HIV-seropositive individuals; this was measured using "detuned" serological assays [6] or other serologic assays that measure different characteristics of the immune response to HIV infection [7]. These efforts have been largely unsuccessful because serologic assays classify some individuals with long-standing infections as recent infections [8, 9]. Significant progress has been made using combinations of biomarkers (e.g., multi-assay algorithms, MAAs) for cross-sectional incidence estimation [10–13]. The objective of this paper is to discuss this approach and the role it can play in HIV prevention research. We discuss the conceptual and statistical framework of this approach, limitations of some existing assays, and why and how multiple assays can be effectively combined to overcome these limitations.

2. Use of a biomarker approach for cross-sectional incidence estimation in HIV prevention research

Here, we discuss several applications of cross-sectional HIV incidence estimation that use biomarkers.

Preparatory or feasibility studies are often performed to estimate HIV incidence rates to determine sample sizes for Phase 3 HIV prevention studies. This is challenging, since even small overestimates of HIV incidence rates in sample size calculations can lead to appreciably underpowered studies. Indeed, several HIV prevention trials were stopped early because of lower than expected incidence rates, leading investigators to conclude that the trials would be unable to detect an intervention effect [2, 14–16]. Furthermore, preparatory cohort studies to estimate incidence in target study populations can take years to complete;

in some cases, by the time incidence estimates are obtained, the estimates may be out-of-date (e.g., reflecting temporal trends in the epidemic, demographic changes, or ramp-up of HIV treatment programs). Cross-sectional HIV incidence assessments may provide an alternative, rapid approach to facilitate design of Phase 3 trials.

The cross-sectional approach is especially useful in community-randomized trials of structural interventions. This approach was recently used to evaluate the primary endpoint of a large, multi-national HIV prevention trial, NIMH Project Accept (HPTN 043) [17]. Project Accept evaluated the impact of interventions aimed at increasing the uptake of voluntary counseling and testing, changing community norms, and increasing social support for persons with HIV [18]. A longitudinal cohort study was not suitable to evaluate the impact of the intervention, because HIV testing was part of the study intervention package.

The cross-sectional biomarker approach has also been applied to epidemiological studies of risk factors for HIV acquisition. An early application of this approach was a case-control study of acute HIV infection that used p24 antigen to identify incident HIV infections in HIV-seronegative patients [19]. In later work, the BED capture immunoassay (BED-CEIA) was used to evaluate risk factors for HIV acquisition in a nationally-representative, population-based survey [18]. However, the inability of the BED-CEIA and other serologic incidence assays to accurately distinguish recently occurring from long standing infections limits their utility. Development of accurate methods for identifying recent infection has important applications to epidemiologic studies of risk factors for HIV acquisition. The methods could also have important roles for identification of sexual or other networks with active disease transmission to target prevention efforts [20].

Finally, cross-section incidence estimates could be used to evaluate local and national time trends in HIV incidence. Nationally-representative surveys of HIV prevalence, such as the Demographic and Health Surveys, have been conducted in over 30 countries [21]. Assessment of HIV incidence in samples collected in those surveys (e.g., using a MAA) would incur marginal additional cost and could yield direct estimates of HIV incidence. Furthermore, comparisons of cross-sectional incidence in serial surveys could assess changes in HIV incidence. The approach could circumvent problems with other approaches, such as inferring incidence from changes in HIV prevalence over time, since that approach is known to be very sensitive to assumptions about mortality and migration [22].

3. Conceptual and Statistical Framework

The cross-sectional incidence approach is based on classification algorithms that use assays to classify individuals into one of two states: either MAA positive or MAA negative. An objective of these algorithms is for individuals classified as MAA positive to have shorter durations of infection than individuals classified as MAA negative. The duration of time that persons are classified as MAA positive depends on the specific algorithm that is used. Furthermore, for a given algorithm, the duration of time that individuals are classified as MAA positive varies from person to person. The mean (or average) duration of time individuals are classified as MAA positive for a given algorithm is called μ or the mean window period. From reference samples with known (interval-censored) durations of infection, one can estimate μ . One can also estimate the distribution of durations individuals are MAA positive for a given algorithm, that is, $\phi(t)$ = the proportion of individuals infected for t days who are classified MAA positive.

A fundamental equation in epidemiology describes the relationship between prevalence, incidence, and duration and provides the basis for how to estimate incidence from cross-sectional samples using biomarkers [23]. The estimate of the HIV incidence rate in a population from a representative cross-sectional sample of persons from that population is:

$$I = \frac{w}{n\mu} \quad (1)$$

where w is the number of individuals classified MAA positive and n is the number of individuals who are HIV seronegative [4]. Confidence intervals for the incidence rate that account for uncertainty in μ are obtained using procedures described in previous reports [11, 24, 25].

In general, equation (1) is not estimating incidence at the time of sample collection, but rather at a time before the collection of samples. The question of how far back in time is answered by the concept of the shadow, which is denoted by ψ [26]. Equation 1 is estimating HIV incidence ψ days before collection of the samples [26, 27]. The shadow can be calculated from the curve $\phi(t)$ using numerical integration as described in [27] and [12].

Generally, it is preferable for MAAs to have large mean window periods (μ) and also small shadows (ψ). This is preferable because incidence estimates will have smaller standard errors (and variances) if μ is large, and will also be more current and therefore potentially less biased if ψ is small. While the mean window period (μ) and shadow (ψ) are distinct numbers, they tend to be positively correlated; this presents the classic statistical tradeoff between bias and variance (see Figure 1 in [12]). The question of how to choose optimal MAAs to address this tradeoff is discussed in [12], which describes an approach to identify algorithms that maximize the mean window period subject to the constraint that the shadow is not too large (e.g., <1 year).

HIV prevention interventions in a Phase 3 trial can be compared by the ratio of their incidence rates (i.e., the rate ratio, RR), which is obtained by taking the ratio of equation 1 for the two groups. In the special case where μ is the same for the two groups, the rate ratio for group 1 relative to group 2 is

$$RR = \frac{w_1 n_2}{w_2 n_1} \quad (2)$$

We see that the mean window period, μ , cancels out in equation 2, and, therefore, is not required in the calculation. Two points about equation 2 should be emphasized. First, the equation is estimating the incidence rate ratio ψ days before sample collection, and not on the date of sample collection. This point is important if there is a ramp-up period for an intervention to achieve its maximal effectiveness. Second, the assumption that the mean window periods (μ) cancels out in equation 2 may not hold for all interventions. For example, if an intervention increases uptake of antiretroviral treatment (ART) services, μ may become larger if the MAA is based solely on serologic assays, since the performance of those assays is impacted by viral suppression. MAAs that include assays for viral load or antiretroviral drug exposure can account for this effect by classifying individuals with low viral loads and those on antiretroviral drugs as MAA negative. Therefore, use of equation 2 may be justifiable in some settings using certain testing algorithms.

An important question is how to optimize testing algorithms to maximize the statistical power for detecting an effect in comparative trials. The choice of which assay or MAA to use involves balancing the bias-variance tradeoff. Kulich and colleagues have developed procedures to derive MAAs that are optimized to maximize the power of community-randomized comparative trials [28].

4. Limitations of current serological assays

While reliable methods exist for identifying acute HIV infections, this approach is only useful for surveying very large populations that have high incidence rates because the acute phase of infection is very short [4, 5]. Serologic incidence assays that have much longer mean window periods have been developed that measure different characteristics of the anti-HIV antibody response (e.g., antibody avidity or the proportion of IgG specific for HIV antigens; for review, see [7, 8]). Some serologic incidence assays are based on modifying commercial assays developed for HIV diagnosis (e.g., Abbott detuned assay [6], Vironostika less sensitive assay [29], AxSYM HIV1/2gO avidity [30], the BioRad avidity assay [31]), while others have been based on non-commercial (in-house) assays (e.g., V3 IDE assay [32], Luminex assay [33]). The BED-CEIA [34] and the limited antigen avidity enzyme immunoassay (Lag-Avidity EIA) [35, 36] are the only assays specifically manufactured for HIV incidence testing.

Serologic incidence assays are relatively inexpensive and simple to use. However, if only serological assays are used in a MAA, some individuals remain MAA positive for long periods, while others who become MAA negative may subsequently revert to being MAA positive because of changes in their antibody responses to HIV infection (e.g., due to viral suppression or advanced HIV disease) [29, 37–40]. For these reasons, the distribution of durations that individuals are classified as MAA positive using only serological assays have long right tails [41]. When serologic assays are used exclusively for incidence testing, two factors that commonly cause individuals with long-standing infection to be classified as MAA positive are viral suppression (natural or induced by antiretroviral therapy [ART]) and advanced HIV disease [29, 37–40]. The performance of serologic incidence assays is also impacted by HIV subtype [29, 42, 43]. A systematic evaluation of currently available assays used for cross-sectional HIV incidence estimation is being performed by the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) [44].

5. Use of Multi-assay Algorithms for Cross-sectional Incidence Estimation

The use of multiple assays in combination can increase the accuracy of cross-sectional incidence estimates. The cost of this approach can be reduced by using hierarchical, step-wise testing algorithms (MAAs). In each step, a single assay is used to refine the classification of samples that were provisionally classified as MAA positive at the prior step(s) (Figure 1). If logistically feasible, assays can be performed in order of cost, with less expensive, high-throughput assays in the initial steps, and more expensive or labor-intensive assays performed in the later steps.

A MAA for HIV incidence estimation in subtype B epidemics has been developed that uses two serologic assays (BED-CEIA [measured as a normalized optical density, OD-n] and the BioRad avidity assay [measured as a avidity index, AI]) and two non-serologic biomarkers (CD4 cell count and HIV viral load) as illustrated in Figure 1 (MAA #1) [10]. This MAA was validated using >2,200 validation samples from >1,000 individuals with known duration of HIV infection (range <6 months to >8 years) [10, 12]. The mean window period for this MAA was initially determined to be 141 days (95% confidence interval [CI], 94–150) [10]. The performance of this MAA was evaluated in three longitudinal cohort studies [10, 11, 45]. The cross-sectional estimates of incidence were determined by testing samples collected at the end of the follow-up period of the cohort study. In this manner, the incidence estimates obtained using cross-sectional testing could be compared to those observed in the cohorts based on documentation of HIV acquisition. The MAA and cohort incidence estimates were very similar (Figure 2). In a subsequent study, the same validation data were reanalyzed using multiple imputations to address uncertainty from interval-censored

infection times [12]. Based on that analysis for this MAA, the mean window period was 123 days and the shadow was 146 days [12]. In the same study, the validation data were used to identify an optimal MAA by searching through 11,340 MAAs with different assay cutoffs; the goal was to find the MAA that had the longest mean window period (μ) with the constraint that the shadow (ψ) was less than 1 year [12]. The optimal four-assay MAA had a mean window period of 159 days a shadow of 184 days (Figure 1, MAA #2). The study also evaluated MAAs that included three assays (the BED-CEIA, the avidity assay and viral load, but not CD4 cell count); those MAAs offer advantages in settings where CD4 cell count data cannot be obtained. The optimal three-assay MAA had a mean window period of 101 days and a shadow of 194 days (Figure 1, MAA #3). The three- and four-assay MAAs described above (MAAs #1–3 in Figure 2) did not classify any of 845 samples from individuals who were infected >5 years as MAA positive; the sample set included 512 samples from individuals who were infected >8 years [8].

Kulich et al studied the performance of MAAs for incidence estimation in a Southern African setting using a set of >5,000 subtype A and C validation samples from >3,400 individuals with known durations of infection (range 1 month to >10 years) [28]. The goal of that study was to select the MAA that provided the highest power for detecting a reduction in incidence in intervention communities in the Project Accept trial [18]. The performance of 403 candidate MAAs was evaluated in three simulated epidemic scenarios (emerging, stable, and waning epidemics). Those analyses identified the following optimal MAA for this application: BED-CEIA <1.2 OD-n and AI <90% and CD4 cell count >200 cells/mm³ and viral load >400 copies/mL [28, 46].

In the MAAs described above, viral load testing is used to identify individuals with long-term infection who have low BED-CEIA and avidity assay results because of viral suppression. The precision of MAAs may be further enhanced by also including a criteria of “no ART”, since ART per se may serve as a supplemental, surrogate marker of non-recent infection [47, 48]. If ART is used as a component of an MAA, direct detection of antiretroviral drugs may be more reliable than self-report of antiretroviral drug use [49]. The recent development of a low-cost, high-throughput, multi-drug screening assay makes this kind of screening feasible [51]. This antiretroviral drug screening assay was used in the Project Accept trial as the final step for classifying individuals as MAA positive [46]. It is important to note that some antiretroviral drugs may not be detected in samples because of their short half-lives. Furthermore, because some antiretroviral drugs are used for HIV prevention, some individuals may be classified as MAA negative based on antiretroviral drug detection even though their duration of infection is short (e.g., in recently-infected women receiving nevirapine for prevention of mother-to-child HIV transmission). In addition, antiretroviral drug testing will not identify elite suppressors with long-standing infection, who may be classified as MAA positive based on results from serologic assays; viral load assays are needed to identify those individuals [38].

Viral diversity assays are also being explored for use in HIV incidence testing, either alone or in combination with other assays. The rationale is based on the premise that viral diversity increases with duration of infection, and may serve as a biomarker that is independent of serologic biomarkers. A high resolution melting (HRM) diversity assay has been developed that quantifies the genetic diversity in HIV without sequencing [50]. This assay is relatively inexpensive, easy to perform, and provides quantitative measures of diversity that are highly correlated with diversity measures obtained by deep sequencing [51]. Our recent studies suggest that the HRM diversity assay may offer an alternative to CD4 cell count in MAAs without compromising their performance.

6. DISCUSSION

HIV incidence is a critical outcome in HIV prevention research. Accurate methods for cross-sectional HIV incidence estimation may facilitate HIV prevention research, particularly when evaluating population-level interventions for HIV prevention. Other intermediate outcomes (e.g., self-reported high-risk behaviors, frequency of HIV testing, proportion of eligible HIV-infected persons on ART) have been used as alternative endpoints for assessing the impact of prevention interventions and have provided insights into why some prevention interventions are not effective. However, changes in intermediate endpoints do not necessarily predict changes in HIV incidence. Ultimately, the question that HIV prevention research must address is whether or not an intervention decreases incidence. The cross-sectional biomarker approach for HIV incidence estimation helps address a number of challenges in incidence determination. The main advantage of this approach is that incidence can be determined from samples collected in a single cross-sectional survey, without requiring longitudinal follow-up of uninfected individuals.

Recent improvements in methods for cross-sectional incidence estimation make the approach feasible. To date no single serologic assay has been developed that can accurately estimate incidence. To overcome these limitations, attention has now turned to using multiple biomarkers in combination. Recently, MAAs have been identified where the probability of being classified as MAA positive converges to zero within several years of infection. MAAs that use multiple biomarkers in combination are essentially binary decision trees; results from each assay are used to decide whether or not to test a sample with the next assay in the algorithm. This hierarchical approach to testing reduces costs. Recent research has demonstrated that the multiple biomarker (MAA) approach is extremely powerful and corrects the deficiencies of individual biomarkers.

Statistical methods have been developed for assessing the accuracy of MAAs and for identifying optimal MAAs by calculating mean window periods, shadows, and statistical power for comparative trials. These methods require large validation sample sets from individuals with a broad range of known (interval-censored) durations of infection. The validation samples should represent the full range of durations of infection and include infected persons with the relevant HIV subtypes, with viral suppression, and with advanced HIV disease. Theoretically, the MAAs that we have identified should be capable of estimating incidence with minimal bias regardless of the stage of the epidemic (e.g., mature vs. rapidly changing). The MAAs that we identified characterize individuals with either viral-suppression or advanced HIV disease as MAA negative. Therefore, the mean window period and the shadow of the MAAs that we identified should not be dependent on epidemic stage. Figure 2 shows that one of these MAAs performs well in three very different epidemic settings. Further validation of MAAs in diverse settings is warranted. While the MAAs should yield unbiased estimates of incidence regardless of the epidemic setting, the sample sizes needed to obtain accurate incidence estimates will vary by setting. In general, the sample sizes needed to obtain precise incidence estimates in cross-sectional surveys are greater in lower incidence settings than in higher incidence settings, which is also the case for longitudinal cohort studies.

MAAs have been successfully developed for use in settings with both subtype B and subtype A and C epidemics. These MAAs do not require any further mathematical adjustments for accurate estimation of the incidence rates in study populations in the year prior to sample collection. Work is now ongoing to identify optimal MAAs for other HIV subtypes and for other applications. A robust MAA has been developed that does not include the CD4 cell count. We conclude that the cross-sectional biomarker approach using MAAs

is a practical, accurate, and cost-effective approach for HIV incidence estimation that can be used for HIV prevention research and global epidemic monitoring.

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REFERENCES

1. Lagakos SW, Gable AR. Challenges to HIV prevention--seeking effective measures in the absence of a vaccine. *N Engl J Med*. 2008; 358:1543–1545. [PubMed: 18403764]
2. Institute of Medicine. Methodological challenges in biomedical HIV prevention trials. Washington, DC: The National Academies Press; 2008.
3. De Amici D, Klersy C, Ramajoli F, Brustia L, Politi P. Impact of the Hawthorne effect in a longitudinal clinical study: the case of anesthesia. *Control Clin Trials*. 2000; 21:103–114. [PubMed: 10715508]
4. 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; 141:166–172. [PubMed: 7817972]
5. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. *AIDS*. 2003; 17:1871–1879. [PubMed: 12960819]
6. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *JAMA*. 1998; 280:42–48. [PubMed: 9660362]
7. Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. *Euro Surveill*. 2008; 13
8. Guy R, Gold J, Calleja JM, Kim AA, Parekh B, Busch M, 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. [PubMed: 19926035]
9. 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.
10. Laeyendecker O, Brookmeyer R, Cousins MM, Mullis CE, Konikoff J, Donnell D, et al. HIV incidence determination in the United States: A multiassay approach. *J Infect Dis*. 2013; 207:232–239. [PubMed: 23129760]
11. Eshleman SH, Hughes JP, Laeyendecker O, Wang J, Brookmeyer R, Johnson-Lewis L, et al. Use of a multifaceted approach to analyze HIV incidence in a cohort study of women in the United States: HIV Prevention Trials Network 064 Study. *J Infect Dis*. 2013; 207:223–231. [PubMed: 23129758]
12. Brookmeyer R, Konikoff J, Laeyendecker O, Eshleman SH. HIV incidence estimation using multiple biomarkers. *Amer J Epidemiol*. 2013; 177:264–272. [PubMed: 23302151]
13. Mastro TD. Determining HIV incidence in populations: Moving in the right direction. *J Infect Dis*. 2013; 207:204–206. [PubMed: 23129757]

14. Peterson L, Nanda K, Opoku BK, Ampofo WK, Owusu-Amoako M, Boakye AY, et al. SAVVY (C31G) gel for prevention of HIV infection in women: a Phase 3, double-blind, randomized, placebo-controlled trial in Ghana. *PLoS One*. 2007; 2:e1312. [PubMed: 18091987]
15. Graham SM, Shah PS, Aesch ZC, Beyene J, Bayoumi AM. A systematic review of the quality of trials evaluating biomedical HIV prevention interventions shows that many lack power. *HIV Clin Trials*. 2009; 10:413–431. [PubMed: 20133272]
16. Latkin CA, Donnell D, Metzger D, Sherman S, Aramrattana A, Davis-Vogel A, et al. The efficacy of a network intervention to reduce HIV risk behaviors among drug users and risk partners in Chiang Mai, Thailand and Philadelphia, USA. *Soc Sci Med*. 2009; 68:740–748. [PubMed: 19070413]
17. Sweat M, Morin S, Celentano D, Mulawa M, Singh B, Mbwambo J, et al. Community-based intervention to increase HIV testing and case detection in people aged 16–32 years in Tanzania, Zimbabwe, and Thailand (NIMH Project Accept, HPTN 043): a randomised study. *Lancet Infect Dis*. 2011; 11:525–532. [PubMed: 21546309]
18. Khumalo-Sakutukwa G, Morin SF, Fritz K, Charlebois ED, van Rooyen H, Chingono A, et al. Project Accept (HPTN 043): a community-based intervention to reduce HIV incidence in populations at risk for HIV in sub-Saharan Africa and Thailand. *J Acquir Immune Defic Syndr*. 2008; 49:422–431. [PubMed: 18931624]
19. Bollinger RC, Brookmeyer RS, Mehendale SM, Paranjape RS, Shepherd ME, Gadkari DA, et al. Risk factors and clinical presentation of acute primary HIV infection in India. *JAMA*. 1997; 278:2085–2089. [PubMed: 9403423]
20. Pilcher CD, Eron JJ Jr, Galvin S, Gay C, Cohen MS. Acute HIV revisited: new opportunities for treatment and prevention. *J Clin Invest*. 2004; 113:937–945. [PubMed: 15057296]
21. ICF International. HIV prevalence estimates from the demographic and health surveys. Calverton, Maryland: ICF International; 2012. Available at <http://measuredhs.com>
22. Brookmeyer R, Konikoff J. Statistical considerations in determining HIV incidence from changes in HIV prevalence. *Statistical Communications in Infectious Diseases*. 2011; 3 Article 9.
23. Freeman J, Hutchison GB. Prevalence, incidence and duration. *Am J Epidemiol*. 1980; 112:707–723. [PubMed: 6969024]
24. Cole SR, Chu H, Brookmeyer R. Confidence intervals for biomarker-based human immunodeficiency virus incidence estimates and differences using prevalent data. *Am J Epidemiol*. 2007; 165:94–100. [PubMed: 17056640]
25. Brookmeyer R. Accounting for follow-up bias in estimation of human immunodeficiency virus incidence rates. *J R. Statist. Soc A*. 1997; 160:127–140.
26. Kaplan E, Brookmeyer R. Snapshot estimators of recent HIV incidence rates. *Operations Research*. 1999; 47:29–37.
27. Brookmeyer R. On the statistical accuracy of biomarker assays for HIV incidence. *J Acquir Immune Defic Syndr*. 2010; 54:406–414. [PubMed: 20436353]
28. Kulich M, Laeyendecker O, Eshleman SH, Koma'reka A, Omelkaa M, Donnell D. Evaluation of multi-assay algorithms for cross-sectional HIV incidence estimation. 2013 *Submitted*.
29. 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. [PubMed: 12882657]
30. Suligoi B, Massi M, Galli C, Sciandra M, Di Sora F, Pezzotti P, et al. Identifying recent HIV infections using the avidity index and an automated enzyme immunoassay. *J Acquir Immune Defic Syndr*. 2003; 32:424–428. [PubMed: 12640201]
31. 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 Conf. on Retroviruses and Opportunistic Infections; Feb 16–19, 2010; Abstract 937
32. Barin F, Meyer L, Lancar R, Deveau C, Gharib M, Laporte A, et al. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. *J Clin Microbiol*. 2005; 43:4441–4447. [PubMed: 16145089]

33. Curtis KA, Kennedy MS, Charurat M, Nasidi A, Delaney K, Spira TJ, et al. Development and characterization of a bead-based, multiplex assay for estimation of recent HIV type 1 infection. *AIDS Res Hum Retroviruses*. 2012; 28:188–197. [PubMed: 21585287]
34. 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; 42:2623–2628. [PubMed: 15184443]
35. Wei X, Liu X, Dobbs T, Kuehl D, Nkengasong JN, Hu DJ, et al. Development of two avidity-based assays to detect recent HIV type 1 seroconversion using a multisubtype gp41 recombinant protein. *AIDS Res Hum Retroviruses*. 2010; 26:61–71. [PubMed: 20063992]
36. Duong YT, Qiu M, De AK, Jackson K, Dobbs T, Kim AA, 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. [PubMed: 22479384]
37. Laeyendecker O, Brookmeyer R, Oliver AE, Mullis CE, Eaton KP, Mueller AC, et al. Factors associated with incorrect identification of recent HIV infection using the BED capture immunoassay. *AIDS Res Hum Retroviruses*. 2012; 28:816–822. [PubMed: 22014036]
38. 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. [PubMed: 18520680]
39. Wendel SK, Mullis CE, Eshleman SH, Blankson JN, Moore RD, Keruly JC, et al. Effect of natural and ARV-Induced viral suppression and viral breakthrough on anti-HIV antibody proportion and avidity in Individuals with clade B HIV infection. *PLoS One*. 2013 **In Press**.
40. 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; 24:495–498. [PubMed: 18327979]
41. Mastro TD, Kim AA, Hallett T, Rehle T, Welte A, Laeyendecker O, et al. Estimating HIV incidence in populations using tests for recent infection: Issues, challenges and the way forward. *J HIV AIDS Surveill Epidemiol*. 2010; 2:1–14. [PubMed: 21743821]
42. 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. [PubMed: 20954834]
43. Mullis, C.; Munshwa, S.; Grabowski, M.; Serwadda, D.; Brookmeyer, R.; Eshleman, S., et al. HIV sequence differences are associated with false-recent misclassification with the BED capture enzyme immunoassay in subtype D HIV. 19th Conf. on Retroviruses and Opportunistic Infections; March 5–8, 2012; Seattle, WA. Abstract 541.
44. CEPHIA. [Accessed 1/3/13] Consortium for the Evaluation and Performance of HIV Incidence Assays. <http://www.incidence-estimation.com/page/homepage>
45. Laeyendecker, O.; Wang, L.; Hughes, JP.; Cummings, V.; Piwowar-Manning, E.; Wheeler, D., et al. Use of a multi-assay algorithm for cross-sectional HIV incidence estimation in HPTN 061 (the Brothers Study). 20th Conf. on Retroviruses and Opportunistic Infections; March 3–6, 2013; Atlanta, GA. Abstract X-130.
46. Coates, T.; Eshleman, SH.; Chariyalertsak, S.; Chingono, A.; Gray, G.; Mbwapambo, J., et al. Findings on estimates of community-level HIV incidence in HPTN 043 (Project Accept). 20th Conf. on Retroviruses and Opportunistic Infections; March 3–6, 2013; Atlanta, GA. Abstract #W-173.
47. 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. [PubMed: 20975514]
48. Rehle TM, Hallett TB, Shisana O, Pillay-van Wyk V, Zuma K, Carrara H, 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. [PubMed: 20559425]
49. Kahle, E.; Kashuba, A.; Baeten, J.; Delany, S.; Donnell, D.; John-Stewart, G., et al. Unreported antiretroviral use by HIV-1 infected members of HIV-1 serodiscordant couples enrolling in an

HIV-1 prevention clinical trial. International Microbicides Conference; April 15–18, 2012; Sydney, Australia.

50. 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. [PubMed: 22073290]
51. Cousins MM, Ou SS, Wawer MJ, Munshaw S, Swan D, Magaret CA, et al. Comparison of a high-resolution melting assay to next-generation sequencing for analysis of HIV diversity. *J Clin Microbiol*. 2012; 50:3054–3059. [PubMed: 22785188]
52. Hodder SL, Justman J, Hughes JP, Wang J, Haley DF, Adimora AA, et al. HIV acquisition among women from selected areas of the United States: A cohort study. *Ann Intern Med*. 2013; 158:10–18. [PubMed: 23277896]
53. Celum CL, Buchbinder SP, Donnell D, Douglas JM Jr, Mayer K, Koblin B, 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. [PubMed: 11106536]

Figure 1. Multi-assay algorithms for cross-sectional HIV incidence estimation in subtype B epidemics

Multi-assay algorithms (MAAs) were developed that combine serologic markers (the BED capture immunoassay [BED-CEIA] and an avidity assay) with non-serologic biomarkers (HIV viral load, with or without CD4 cell count) [10, 12]. In each MAA, assays are performed using a hierarchical approach (see text), with an optimal cut-off defined for each assay. CD4 cell count cut-offs are expressed as cells/mm³; BED-CEIA cut-offs are expressed as normalized optical density; avidity results are expressed as avidity index values (%); HIV viral load cut-offs are expressed as HIV RNA copies/mL. For each MAA, samples that meet the criteria for all assays are classified as MAA positive. Three MAAs are shown. MAA #1 is a four-assay MAA described in [10, 12] that was used to estimate incidence in three clinical studies (see Figure 2). MAA #2 is an alternate four-assay MAA described in [12] that maximizes the mean window period subject to the shadow being less than one year. MAA #3 is the three-assay MAA described in [12] that does not require CD4 cell count data and maximizes the mean window period subject to the shadow being less than one year. The mean window periods and shadows for all three of these MAAs were determined in a previous study [12] (see text); the 95% confidence intervals for each mean window period and shadow are shown in parentheses.

Figure 2. Comparison of cross-sectional incidence estimates and incidence observed from longitudinal follow-up of HIV-uninfected cohorts in three clinical studies performed in the United States

HIV incidence was evaluated in three clinical studies: the HIV Prevention Trials Network (HPTN) 064 study [52], the HIV Network for Prevention Trials (HIVNET) 001 Vaccine Preparedness study [53], and the HPTN 061 study [45]. Annual HIV incidence was determined in each study using two methods: longitudinal follow-up of HIV-uninfected individuals (FU, filled symbols) and cross-sectional analysis using a multi-assay algorithm (MAA #1 shown in Figure 1; CS, open symbols) [10, 11, 45]. Note that the mean window period of MAA #1 is based on the analysis reported in [10] using midpoint imputation for seroconversion times; a reanalysis using multiple imputations is reported in [12].