Recent HIV-1 Infection Detection: Comparison of Incidence Estimates Derived by Laboratory Assays and Repeat Testing Data

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Introduction: Advances in laboratory methods capable of detecting recent HIV infection offer the promise of quickly and efficiently measuring HIV incidence in cross-sectional surveys, thereby greatly expanding the capabilities of surveillance programs. We compared HIV-1 incidence estimates derived from 3 different methods: Vironostika-less sensitive, BED capture enzyme immuno assay (BED-CEIA), and repeat testing history.

Methods: A cross-sectional analysis was performed using HIV testing data from the population of all men who have sex with men presenting for serological HIV voluntary counseling and testing at the largest testing programs in San Francisco from 2000 to 2004 (n = 15,010). Specimens were evaluated for recent HIV-1 infection using Vironostika-LS and BED-CEIA. Concordance between the 2 assays was assessed using the Kappa statistic.

Results: The BED-CEIA and Vironostika-LS concurred in 90% of specimen classifications (Kappa = 0.77; "good" strength of agreement). Predictors of recent HIV-1 infection common to both methods were unprotected receptive anal intercourse ($P < 0.001$), sex with a known HIV-positive partner ($P < 0.001$), and amphetamine use ($P < 0.01$). Temporal trends in HIV-1 incidence were also consistent and stable.

Conclusions: There was good concordance in the classification of recent HIV-1 infection between BED-CEIA and Vironostika-LS and in the correlates of acquisition of infection. The findings suggest that these incidence assays can be used for the basic epidemiological purposes of measuring HIV-1 incidence, identifying populations at risk for infection, and tracking the leading edge of the epidemic over time.

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INTRODUCTION

Despite the great need to estimate the rate of new HIV infections to track the leading edge of the epidemic, target prevention activities, and assess the impact of interventions, there has been a paucity of direct measures of HIV incidence due to the costs, complexities, and potential biases of cohort studies. Advances in laboratory methods capable of detecting recent HIV infection offer the promise of quickly and efficiently measuring HIV incidence in cross-sectional surveys, thereby greatly expanding the capabilities of surveillance programs.[1,2]

The basic biological principle for the distinction between recent versus long-standing infections are the immunological changes in HIV antibody levels during the first few months after initial infection. For example, as seroconversion progresses, there is an increase in HIV-specific antibody titers and affinity. One approach termed "serological testing algorithm for recent HIV seroconversion" distinguishes recent from longer standing infection by using 2 enzyme-linked immunoassays (EIAs): a standard assay (Vironostika HIV-1) that is sensitive to low levels of HIV antibody and a less sensitive one (Vironostika-LS) that classifies recent infection using a 170-day window period.[3,4] A second laboratory method, the BED capture enzyme immunoassay (BED-CEIA), is calibrated against the proportion of HIV-1–specific immunoglobulin G in total immunoglobulin G present in the blood after 155 days postseroconversion.[5,6]

However, concerns about these assays have been raised with regards to their use for estimating HIV incidence. In particular, the Joint United Nations Programme on HIV/AIDS Reference Group on Estimates Modeling and Projections issued a statement of caution on the use of BED-CEIA, as estimates of HIV-1 incidence seemed to be higher than would be expected based on observed prevalences and other methods.[7] Examination of longitudinal seroconverter panels suggested that BED-CEIA was misclassifying persons with long-standing infections as recent not only in the early period

after seroconversion but also in a subpopulation of persons who never evolved higher levels of HIV-specific antibody even after many years of infection. This latter phenomenon of long-term misclassification would result in an overestimation of HIV-1 incidence especially in high prevalence populations that include many persons with undiagnosed HIV infections. As a result of these concerns, formulas correcting for these types of misclassification were developed.

In the present study, we compared HIV-1 incidence estimates derived by 3 methods: BED-CEIA, Vironostika-LS, and repeat testing history. The study population of men who have sex with men (MSM) presenting at HIV testing facilities in San Francisco is one with a high frequency of HIV testing and a low level of undiagnosed infections.

METHODS

All MSM presenting for serological HIV voluntary counseling and testing at anonymous testing sites (ATS) in San Francisco from 2000 to 2003 and the municipal sexually transmitted diseases (STDs) clinic from 2000 to 2004 were included in the analysis. The study sites represent the largest testing programs in the city. Of note, a recent survey found that 97% of MSM in San Francisco reported having been ever tested for HIV and 34% reported having been tested in the past 6 months.[8]

Blood specimens from the ATS (n = 5828) and the STDs clinic (n = 9182) were screened for the presence of HIV-1 antibodies using a standard EIA (Vironostika HIV-1 Microelisa; bioMérieux, Durham, NC). Specimens testing HIV-1 antibody positive by standard EIA were further evaluated for recent HIV-1 infection (n = 658; 219 from ATS; 439 from STDs clinic) using Vironostika-LS and BED-CEIA according to standard Centers for Disease Control and Prevention protocols.[4–6]

HIV-1 incidence estimates using Vironostika-LS results were calculated by dividing the number of persons with recent infection by persons at risk (recently infected plus uninfected) and then annualized using the following formula: crude incidence × [(365 days/170 days) × 100%].[9] HIV-1 incidence estimates using BED-CEIA results were calculated using a revised formula that adjusts for the misclassification of long term and recent cases and for HIV-positive samples not available for recent infection testing.[10] Concordance between Vironostika-LS and BED-CEIA results for recent infection identification was evaluated using the Kappa statistic to quantify the degree of agreement between the 2 methods. Multivariate logistic regression analysis was performed to assess associations between recent HIV-1 infection and data on demographic characteristics and risk behavior during the past 12 months that were collected through client intake forms administered by counselors at the sites during routine pretest counseling. As a further comparison, HIV-1 incidence was calculated based on self-reported HIV testing history, which represents an overlapping but not a complete subset of the entire testing population. The sum of the time between the last negative test and subsequent positive test was used to approximate the time interval at risk, as previously described.[11]

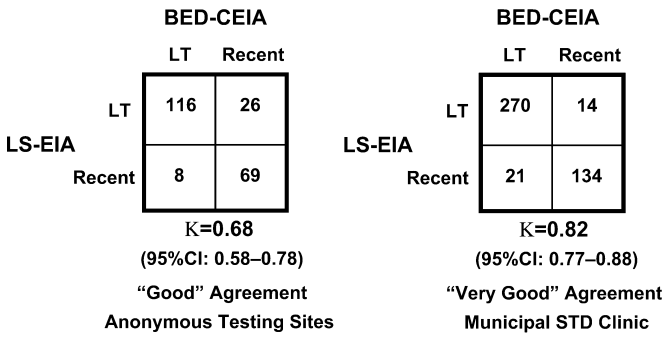
RESULTS

The MSM testing population at ATS was comprised of 70% whites, 12% Asians/Pacific Islanders, 11% Latinos, and 3% African Americans. Twenty-six percent of MSM testers were under 25 years old, 34% were 25–34 years old, and 40% were 35 years and older. At the municipal STDs clinic, 60% of MSM testers were whites, 19% Latinos, 11% Asians/Pacific Islanders, and 7% African Americans. Fifteen percent of MSM testers were under 25 years old, 44% were 25–34 years old, and 41% were 35 years and older. Demographic characteristics of the 2 testing populations remained stable during the analysis period. HIV testing was the primary reason for the ATS visits as compared with STDs care at the municipal STDs clinic.

At the STDs clinic, Vironostika-LS classified 155 specimens as recent infections compared with 148 specimens by BED-CEIA (Fig. 1). At the ATS, 77 specimens were classified as recent infections by Vironostika-LS and 95 by BED-CEIA. Together, the 2 assays concurred in the classification of 90% of ATS and STDs clinic specimens, yielding a Kappa score of 0.77 (95% confidence interval: 0.72 to 0.82), which is considered "good" strength of agreement.

HIV-1 incidence estimates derived from BED-CEIA, Vironostika-LS, and repeat testing history for the ATS and the municipal STDs clinic are shown in Figure 2. At the ATS, the high and low incidence estimates ranged from 4.46% in 2002 to 2.26% in 2003 by BED; 3.66% in 2000 to 2.07% in 2003 by Vironostika-LS; and 2.57% in 2001 to 1.78% in 2003 by repeat testing. At the STDs clinic, the high and low incidence estimates ranged from 4.01% in 2004 to 2.36% in 2001 by BED; 4.17% in 2002 to 2.71% in 2001 by Vironostika-LS; and 4.11% in 2004 to 2.78% in 2003 by repeat testing. Estimates from BED-CEIA tended to be higher than Vironostika-LS and repeat testing estimates, though none showed a significant temporal trend.

Several predictors of recent HIV-1 infection were common to all 3 methods. Recent HIV-1 infection classification by BED-CEIA was associated with unprotected receptive anal intercourse [$P < 0.001$; odds ratio (OR) = 2.67], sex with a known HIV-positive partner ($P < 0.001$; OR = 2.01), having more than 10 sexual partners ($P = 0.009$; OR = 1.54), Asian



K=Kappa score

FIGURE 1. Comparison of recent versus LT HIV-1 infection classification by BED-CEIA and Vironostika-LS (LS-EIA) of testers from ATS (2000–2003) and the municipal STDs clinic (2000–2004). LT, long term.

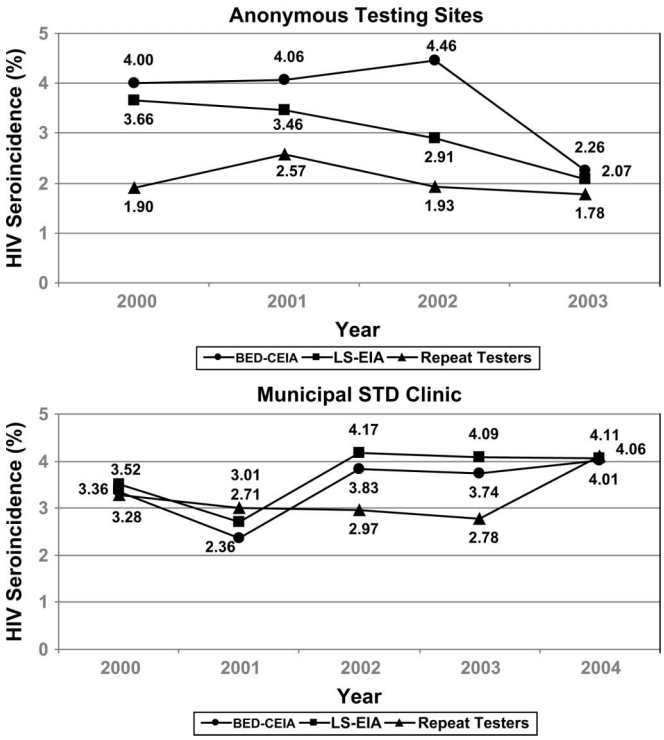


FIGURE 2. HIV-1 incidence estimates derived from BED-CEIA, Vironostika-LS, and repeat testing history for ATS (2000–2003) and municipal STDs clinic (2000–2004) in San Francisco.

ethnicity ($P = 0.014$; OR = 1.73), and amphetamine use ($P = 0.012$; OR = 1.75). Recent HIV-1 infection classification by Vironostika-LS was associated with unprotected receptive anal intercourse ($P < 0.001$; OR = 2.42), sex with a known HIV-positive partner ($P < 0.001$; OR = 2.16), having exchange sex ($P = 0.042$; OR = 2.05), and amphetamine use ($P = 0.002$; OR = 1.98). Predictors of seroconversion among repeat testers were unprotected receptive anal intercourse ($P < 0.001$; OR = 2.35), sex with a known HIV-positive partner ($P < 0.001$; OR = 1.35), Latino ethnicity ($P < 0.001$; OR = 1.86), African American ethnicity ($P < 0.001$; OR = 2.18), injection drug use ($P < 0.001$; OR = 1.87), and amphetamine use ($P < 0.001$; OR = 2.06).

DISCUSSION

We observed good concordance between BED-CEIA and Vironostika-LS in the classification of recent HIV-1 infection, with Kappa scores indicating good to very good agreement. The point estimates for HIV-1 incidence observed in our study population were comparable to a recent national review of estimates for free-standing testing sites and STDs clinics.[12] Temporal trends of the 3 different HIV-1 incidence estimation methods tracked each other fairly well and are consistent with epidemiological trend data on new HIV diagnoses, STDs, and risk behaviors.[13] Moreover, key predictors of recent HIV-1 infection were consistent with each other and with known correlates of acquisition of infection in other studies.[14,15] The findings suggest that these incidence assays can be used for the basic epidemiological purposes of measuring HIV-1 incidence, identifying populations at risk for infection, and tracking the leading edge of the epidemic over time. Our data therefore support the new Centers for Disease Control and Prevention–coordinated HIV-1 incidence surveillance approach that incorporates the BED-CEIA.[16,17]

The Joint United Nations Programme on HIV/AIDS has remarked that these incidence assays, particularly BED-CEIA, overestimate incidence compared with model-based estimates such as the Estimation and Projection Package and Spectrum.[18] The overestimation bias is based on the misclassification of a substantial proportion of persons with long-standing infections as recent. In our study, use of the adjustment formula only slightly reduced the HIV-1 incidence estimates. The adjustment formula corrects for the sensitivity and dual-parameter specificity, which takes into account misclassifications as a result of low antibody levels during very early infection and late-stage disease and persons with long-standing infections who never evolve high antibody levels. The corrections are more substantial in high prevalence populations with low levels of diagnosis.[19] The fact that the corrected estimates in this study were only slightly lower than the uncorrected estimates is consistent with the very high level and frequency of testing in San Francisco, such that there were few long-standing undiagnosed infections in our study population. We also acknowledge that point estimates can be biased by the effect of persons seeking HIV-1 testing for reasons related to recent seroconversion, such symptoms, or recent exposures.[20] Differences in symptoms or test-seeking behavior may account for the different concordances of the assays observed between the ATS and STDs sites.

Although our testing population cannot be generalized to all MSM populations, the application of BED-CEIA to this readily accessible population, along with the additional validation against repeat testing data, may provide a rapid and cost-effective snapshot of the epidemic. Our findings suggest that application of BED-CEIA in settings with the ability to identify persons with known long-standing infections, for example, in populations with high frequencies of HIV testing and low levels of undiagnosed infections, can generate acceptable HIV-1 incidence estimates. In populations with many late-diagnosed infections, the assay may be more prone to misclassification errors. Application of BED-CEIA could be widened to a more representative sampling design to provide efficient incidence estimates in other studies. An implication of our results is that use of BED-CEIA to estimate incidence at voluntary counseling and testing sites with a high proportion of regular testers decreases the apparent misclassification error.

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