Estimating HIV incidence in the United States from HIV/AIDS surveillance data and biomarker HIV test results

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SUMMARY

The development of an human immunodeficiency virus (HIV) test that detects recent infection has enabled the U.S. Centers for Disease Control and Prevention (CDC) to estimate annual HIV incidence (number of new infections per year, not per person at risk) in the United States from data on new HIV and acquired immunodeficiency syndrome (AIDS) diagnoses reported to HIV/AIDS surveillance. We developed statistical procedures to estimate the probability that an infected person will be detected as recently infected, accounting for individuals choosing whether and how frequently to seek HIV testing, variation of testing frequency, the reporting of test results only for infected persons, and infected persons who never had an HIV-negative test. The incidence estimate is the number of persons detected as recently infected divided by the estimated probability of detection. We used simulation to show that, under the assumptions we make, our procedures have acceptable bias and correct confidence interval coverage. Because data on the biomarker for recent infection or on testing history were missing for many persons, we used multiple imputation to apply our models to surveillance data. CDC has used these procedures to estimate HIV incidence in the United States. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

Since 1981, the Centers for Disease Control and Prevention (CDC) has collected surveillance data to monitor the status of the acquired immunodeficiency syndrome (AIDS) epidemic in the United States. Data on AIDS cases and deaths are reported from all states, the District of Columbia, and five U.S. dependent areas. Cases of human immunodeficiency virus (HIV) began to be reported in some states in 1985; these are persons with a positive HIV test who do not meet the AIDS case definition. By December 2006, all states, the District of Columbia, and 5 U.S. dependent areas had implemented HIV surveillance; data from 45 states and 5 U.S. dependent areas (those with HIV reporting by name, rather than by a coded identifier) were reported (without names) to CDC [1]. These data allow CDC to estimate AIDS incidence and prevalence in all states and the prevalence of diagnosed HIV in states with a sufficiently long history of HIV reporting by name, but the HIV and AIDS surveillance data provide no direct information on HIV incidence. Estimates of HIV incidence (both numbers of new infections and infection rates) are needed at the national and regional levels to evaluate trends in the epidemic (numbers of new infections, and populations disproportionately affected), to evaluate the success of prevention programs, and for the allocation of HIV prevention resources [2].

Until the mid-1990s, HIV incidence (numbers of new infections per year) could be estimated from AIDS surveillance data using back-calculation [3, 4] based on the incubation period distribution, the probability distribution of the time from HIV infection to AIDS. AIDS incidence has been affected by the change in the AIDS surveillance definition in 1993 to include immunosuppression [5] and the widespread use of highly active antiretroviral therapy (HAART) beginning in 1996. Back-calculation methods are being developed to use all reported HIV diagnoses (Philip Rhodes, CDC; Ping Yan, Health Canada: personal communications).

Incidence rates can be estimated directly from observed seroconversion in cohorts or in persons tested repeatedly at a testing site. Because HIV is an uncommon disease in the United States, such estimates require testing large numbers of persons at risk for infection. In addition, these estimates are not representative of general populations. Brookmeyer and Quinn proposed estimating incidence rates based on the results of the standard HIV test and a test for a biomarker for recent infection [6, 7]. Because their biomarker test is positive before the standard test, their approach would require a biomarker result for all persons who test HIV negative, which is not feasible in the United States. Their test pair also has a very short window period (the time between a positive standard test and a biomarker result indicating recent infection), resulting in imprecise incidence estimates.

Janssen et al. [8] reported the application of an alternative biomarker test designed for estimating HIV incidence in the United States. Their biomarker test distinguishes recent from long-standing infections later in the course of infection than the standard HIV test. The initial HIV biomarker test was a modification of the standard HIV enzyme immunoassay (EIA) [8, 9]. Currently, CDC uses the BED (named for the three HIV subtypes that constitute the polypeptide) capture EIA [10], with a mean window period of 156 days, as the HIV biomarker test. The BED assay cannot be used if a person has AIDS or is taking antiretroviral therapy at the time of HIV diagnosis, as the test can produce false-recent results among such persons even if not recently infected. The combination of the standard EIA test and the BED assay is also known as serologic testing algorithm for recent HIV seroconversion (STARHS); a person who is HIV positive on the diagnostic HIV test and recent on the BED test is classified as BED or STARHS recent; see Figure 1. The critical value for classifying a BED result as recent was chosen so that the probability of having a BED window period longer than 1 year is small [10].
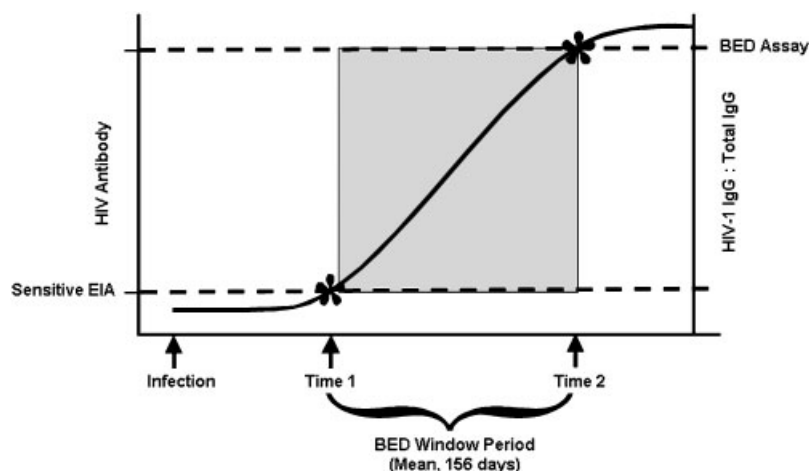
Figure 1. Schematic diagram of serologic testing algorithm for recent HIV seroconversion (STARHS) using the BED assay to determine those recently infected.

When persons are tested after being recruited for a study, rather than choosing when to be tested, the incidence rate of the disease can be estimated from these two test results using the basic epidemiologic relation for disease in a steady state:

$$\text{disease prevalence} \approx \text{incidence rate} \times \text{duration of disease} \qquad (1)$$

Here prevalence is the proportion of recently infected persons among those at risk for detection as being recently infected, and duration is the mean window period [6]. Confidence intervals can be obtained for these incidence estimators [7, 8]. Satten *et al.* extended the estimation procedure to persons tested repeatedly on known test dates [11]. This estimation procedure is a special case of Kaplan and Brookmeyer's snapshot estimator; their target region is what we call the window period [12].

If persons choose when to be tested, the proportion detected as recently infected depends on testing history (whether a person was tested before HIV infection, and if so, testing frequency); hence, (1) is not valid. We develop an incidence estimator that accounts for the effect of testing practices on the number of persons detected as recently infected. The extension of name-based HIV reporting to most states provides information on persons newly diagnosed with HIV from population-based surveillance, including both the information necessary to request a serum aliquot to be tested with the BED assay and information on testing history. Because only HIV-positive test results are reported, our method estimates the number of new infections in the population during a specified time period. To estimate the incidence rate in the population, we can simply divide this number by the total population.

In the next section, we present a very simple model to illustrate our approach. After we summarize the data that are available (stage of disease at diagnosis, HIV testing history, and BED result), we use surveillance data to illustrate how CDC estimates HIV incidence from this model. In the final two sections, we use simulation to evaluate these estimates and discuss potential problems and biases in using this model.

A MODEL FOR ESTIMATING HIV INCIDENCE

We use the following notation. Let I be the incidence rate (number of infections per unit time) and let R be the corresponding rate at which these persons are detected as recently infected by the biomarker (here, the BED test). We use rates because some persons classified as recently infected based on a test in the period of interest were infected before that period, and some persons infected during this period will be detected after this period. Let p be the probability that an infected person will be detected as recently infected based on the biomarker from a sample obtained during the period of interest. Our model is that the number of new infections has a Poisson distribution with rate parameter I, while the number of detected recent infections has a Poisson distribution with rate parameter $R = Ip$. If the observed number of recently detected infections is equal to r, then the maximum likelihood estimator of I is

$$\hat{I} = r/\hat{p} \tag{2}$$

where p is estimated by $\hat{p}$, which depends on the population testing behavior, on the window period distribution, and the availability of a BED result for persons with newly reported HIV infection. Suppose we are interested in a period of length τ. Let p_1 be the probability that an infected person has a first positive HIV test during the τ-year period after infection, p_2 the probability that a newly diagnosed person has a BED result, and p_3 the probability that a person is in the window period at the time of this test conditional on being tested within τ years after infection. Then

$$p = p_1 p_2 p_3 \tag{3}$$

Assume that the date of HIV infection and the testing process are independent and that the BED result is based on a sample obtained at the time of HIV diagnosis. If a person had a negative HIV test, assume that this test was at least τ years earlier and that the infection date had a uniform distribution on the interval between the last negative and first positive test. For persons testing positive for the first time, assume that testing hazard after infection is constant. In addition, assume that the BED assay has τ-specificity 1, in the sense that the window period is always less than τ, and that the assay has sensitivity 1, in the sense that every infected person has a positive window period. We will discuss the plausibility and implications of our assumptions in the Discussion. Under these assumptions, the probability that a person with window period w is classified as recent, given an HIV test within τ years after infection, is w/τ, the proportion of the τ-year period covered by the window period. Therefore, we estimate p_3 by the mean window period divided by τ. The probability p_2 is known from the surveillance data, and the probability p_1 can be estimated from these data, as shown below. Since the number of detected recent infections has a Poisson distribution, the delta method gives

$$\text{Var}(\log(\hat{I})) \approx \frac{1}{r} + \frac{1}{\hat{p}_1^2}\text{Var}(\hat{p}_1) + \frac{1}{\hat{p}_2^2}\text{Var}(\hat{p}_2) + \frac{1}{\hat{p}_3^2}\text{Var}(\hat{p}_3) \tag{4}$$

The parameter p_2 describes a binomial distribution with sample size being the number of persons for whom a BED result would be used had it been obtained. Although this sample size is random, we get an approximately correct variance by assuming that it is fixed. We estimate the standard error of the mean window period estimate to be 0.029 (see the Numerical Results). We could also use the delta method to compute $\text{Var}(\hat{I})$ as a sum of four terms involving $\text{Var}(R)$ and the variances of the $\hat{p}_i$.

We will estimate p_1 separately for persons with and without a negative HIV test. Let $\hat{I}_1$ and $\hat{I}_0$ be the corresponding incidence estimators, with total incidence estimated as $\hat{I} = \hat{I}_1 + \hat{I}_0$. Note that $\hat{I}_1$ and $\hat{I}_0$ are correlated, as both contain $\hat{p}_3$. Therefore,

$$\mathrm{Var}(\log(\hat{I})) \approx \frac{\mathrm{Var}(\hat{I})}{\hat{I}^2}$$

$$= \frac{1}{\hat{I}^2}[\mathrm{Var}(\hat{I}_0) + \mathrm{Var}(\hat{I}_1) + 2\,\mathrm{Cov}(\hat{I}_0, \hat{I}_1)]$$

$$\approx \frac{1}{\hat{I}^2}[\hat{I}_0^2\,\mathrm{Var}(\log(\hat{I}_0)) + \hat{I}_1^2\,\mathrm{Var}(\log(\hat{I}_1)) + 2\hat{I}_0\hat{I}_1\hat{p}_3^2\,\mathrm{Var}(1/\hat{p}_3)] \qquad (5)$$

We estimate $\mathrm{Var}(\log(\hat{I}_0))$ and $\mathrm{Var}(\log(\hat{I}_1))$ using (4). From the delta method,

$$\mathrm{Var}(1/\hat{p}_3) \approx \frac{1}{\hat{p}_3^4}\mathrm{Var}(\hat{p}_3) \qquad (6)$$

Alternatively, we could construct the confidence interval on the original scale with the variance given by $\mathrm{Var}(\hat{I}) = \mathrm{Var}(\hat{I}_0) + \mathrm{Var}(\hat{I}_1) + 2\,\mathrm{Cov}(\hat{I}_0, \hat{I}_1)$.

SUMMARY OF SURVEILLANCE DATA

All 50 U.S. states, the District of Columbia, and 5 U.S. dependent areas have reported AIDS cases to CDC in a uniform format since 1982 [1]. Health departments use name-based case reporting to eliminate duplicates. These political jurisdictions then report cases to CDC after removing names from the database. The data reported include diagnosis date, demographic information (including age, race/ethnicity, sex, and residence), type of testing facility, and transmission category (if known). No information is provided about previous test dates or, for most cases, test results (i.e. most negative test results are not reported).

Newly diagnosed HIV was a reportable disease in all states, the District of Columbia, and five U.S. dependent areas as of January 2004. By December 2006, data from 45 states and 5 U.S. dependent areas (those with HIV reporting by name, rather than by a coded identifier) were reported (the same data elements as for AIDS cases, without names) to CDC [1]. CDC began implementing HIV incidence surveillance in 34 jurisdictions (some are cities) during 2004 as an extension of the existing national population-based HIV/AIDS case surveillance system, using the case surveillance infrastructure to collect the necessary information to estimate HIV incidence [13]. Remnant diagnostic HIV-positive specimens are obtained and tested with the BED assay for persons with a new diagnosis of HIV infection that is reported to the HIV/AIDS surveillance system in these surveillance areas. As part of their routine data collection activities, these surveillance programs also collect supplementary data on testing history and whether the person was taking antiretroviral therapy at the time the positive sample was drawn.

We illustrate our estimators using HIV and AIDS case diagnoses made during 2006 reported before July 2007 from 22 states participating in HIV incidence surveillance. Because testing history and BED result data collection are still being developed, we restricted the data to jurisdictions with a BED result for at least 15 per cent of the diagnoses made during this 1-year period. The numbers of diagnoses by type of diagnosis, BED result, and previous HIV test are shown in Table I.

Table I. Numbers of new HIV diagnoses in 22 states during 2006, reported through June 2007, by type of diagnosis, BED result, and information on a previous negative HIV test obtained from surveillance data.

Diagnosis and BED result	Ever tested HIV			Total
	No	Yes	Unknown	
AIDS diagnosis at HIV diagnosis	1012	1063	4748	6823
AIDS within 6 months of HIV diagnosis	730	833	2410	3973
HIV diagnosis, long-term infection	860	1363	2508	4731
HIV diagnosis, recent infection	298	908	927	2133
HIV diagnosis, no BED test	1563	3437	11 142	16 142
Total	4463	7604	21 735	33 802

ESTIMATING p_1

We wish to estimate incidence during the 1-year period 2006 from the data in Table I. Initially we assume that BED results and testing history information are missing completely at random so that the persons for whom we have this information are a representative sample of all diagnosed cases. We will estimate p_1, the probability of an HIV test within 1 year after infection, separately for persons with and without a previous negative test. Because the time from HIV infection to seroconversion on the EIA assay is usually less than 4 weeks [9], we do not distinguish between HIV infection and HIV seroconversion.

First consider persons with a previous negative test. Assume that the intertest times T follow a renewal process, that this process has reached equilibrium for each person, and that the date of HIV infection has a uniform distribution within the interval ending with the first positive HIV test. Let T^* be the length of this interval. Then the estimate of p_1 for each person is $\min(1, 1/T^*)$, and the average (overall persons diagnosed with HIV, including those with AIDS at the time of their initial diagnosis) of the observed values $\min(1, 1/T^*)$ is an unbiased estimate of p_1. We regard p_1 as estimating a binomial probability; hence, its variance is approximately the variance of the estimate of a binomial probability with fixed sample size (here, the number of persons with a known last negative test date).

Now consider persons whose first HIV test is positive. For these persons, $\hat{p}_1$ should be at least as large as the observed proportion classified as recent. To obtain a point estimate for p_1, we assume that these persons are at risk for an HIV test starting with their date of infection, with a constant hazard for testing equal to $1/\beta$; hence, β is the expected time from infection until the first HIV test for persons whose first HIV test is positive.

Let q be the proportion of these persons with an AIDS diagnosis. Let T_T and T_A be times from infection to HIV test and to AIDS, respectively. As justified below, the AIDS incubation period distribution (the time from HIV infection to AIDS) can be approximated by a gamma distribution. Let the shape and scale parameters for this distribution be α_A and β_A, respectively, and let the density be $\gamma(t)$. Since

$$q = \Pr[T_T > T_A] = \int_0^\infty \exp\left(-\frac{1}{\beta}t\right)\gamma(t)\,\mathrm{d}t = (\beta^*/\beta_A)^{\alpha_A} \qquad (7)$$

where $\beta^* = (1/\beta + 1/\beta_A)^{-1}$,

$$\hat{\beta} = \frac{\beta_A}{q^{-1/\alpha_A} - 1} \tag{8}$$

Since the time to an HIV test has an exponential distribution, we estimate the population parameter p_1 as $1 - \exp(-1/\hat{\beta})$. From the delta method,

$$\begin{aligned}
\text{Var}(\hat{\beta}) &\approx \beta_A^2 (q^{-1/\alpha_A} - 1)^{-4} \alpha_A^{-2} (q^{-1/\alpha_A - 1})^2 \text{Var}(q) \\
\text{Var}(\hat{p}_1) &\approx (1 - \hat{p}_1)^2 \beta^{-4} \text{Var}(\hat{\beta}) \approx (1 - \hat{p}_1)^2 \beta_A^{-2} \alpha_A^{-2} (q^{-1/\alpha_A - 1})^2 \text{Var}(q)
\end{aligned} \tag{9}$$

Because not all cases diagnosed during 2006 were reported by July 2007, we estimate incidence in these 22 states by dividing the incidence estimates from model (2) by the estimated proportion of all cases that had been reported among all cases that will be reported [14, 15]. Finally, assuming that HIV incidence is proportional to AIDS incidence within states, we estimate national incidence by dividing the estimate for these 22 states by the proportion of AIDS cases diagnosed in these areas among all cases that will be reported from the entire United States.

NUMERICAL RESULTS

First we use the data in Table I to estimate incidence in these 22 states based on the data from persons with known testing history under the assumption that testing history data and BED results are missing completely at random. Among 1241 persons who had BED results and had an AIDS diagnosis within 6 months of their HIV diagnosis, 198 (16 per cent) were classified as recently infected, even though the probability of developing AIDS within 1 year after HIV infection is very small. Therefore, we classify all BED results for persons with an AIDS diagnosis within 6 months of their HIV diagnosis as long-standing. Because p_2 is the probability that a person diagnosed with HIV has a BED result that could result in a classification as recent, we estimate p_2 after deleting persons with an initial diagnosis of AIDS or who develop AIDS within 6 months of their HIV diagnosis. The resulting estimates are 0.398 and 0.426 among persons with and without a negative HIV test, respectively. Among 7263 persons with a previous test who reported the month of that test, 34 per cent reported a negative test at most 12 months earlier. We estimate $\hat{p}_1$ to be 0.617 among persons with a previous negative test.

To estimate $\hat{p}_1$ among persons with no previous test, we must choose the parameters for the gamma approximation to the incubation period distribution. This distribution has been modeled as a series of 2–5 stages, each of which has an exponential distribution, e.g. [16, 17]. If these distributions are independent, the incubation period distribution has a gamma distribution, with shape parameter equal to the number of stages [18]. With many stages, the independence assumption is less likely to be true, as a result of the correlation of the lengths of the stages within persons. Therefore, we choose the shape parameter to be 2. With a time scale in years, this implies that the scale parameter is 4 because the product of these parameters must be 8, the mean time from infection to a CD4 count less than 200 cells/μL [17], the primary diagnostic criterion for an AIDS diagnosis. On the basis of the gamma distribution and the observed value of q of 0.227, we have $\hat{\beta} = 3.64$: we estimate that the mean time from infection to first HIV test is 3–4 years for this population.

The assumption that the testing history data and BED results are missing completely at random is false. In a logistic regression model, sex, race/ethnicity, age group, transmission category, the BED result, and the type of facility where HIV was diagnosed were all significant ($p < 0.0001$) predictors of missing testing history information. To reduce the bias resulting from these associations, we assume that the BED result and data on testing history are missing at random (the probability of being missing depends only on covariates we can observe) and use 20-fold imputation to impute testing history and the BED result. We used double imputation, imputing testing history (whether a person had a previous negative test) after imputing missing BED results. We also imputed the number of months since the last negative test for persons who were known or imputed to have such a test. We used the SAS version 9.1 procedure MI (SAS Institute, Cary, NC) with the discriminant function method for monotone missing data within strata defined by sex, with models containing race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, and American Indian/Alaska Native), age, transmission category (five categories for men, three categories for women: men who have sex with men [MSM] without injecting drug use [IDU]; IDU and not MSM; IDU and MSM; heterosexual contact; and other [mostly no reported risk]), facility type (counseling and testing site or sexually transmitted disease clinic, other known facility types, or facility type unknown or missing), AIDS diagnosis at or within 6 months after the HIV diagnosis (excluded from imputing the BED result), ever tested HIV negative (for imputing the BED result), and the BED result (for imputing the testing data). The mean values from these 20 imputations are summarized in Table II. Long-term HIV infection includes persons diagnosed with AIDS within 6 months of an HIV diagnosis. There are no missing BED results for the imputed data; hence, p_2 is 1. Among those with no negative test, the data in Table II give $q = 0.262$; hence, $\hat{\beta} = 4.20$.

The parameter estimates, point estimates for incidence, and confidence intervals for these estimates based on both the original and imputed data are shown in Table III. We estimated the standard error for the mean window period from the variance of the generalized log-logistic distribution approximation to the window period distribution (defined below) and the sample size (94 persons) on which this estimate is based. The resulting standard error estimate is 0.029 years. The confidence intervals for the original data use the variance estimates (4)–(6); those for the imputed data include the variation among the 20 estimates obtained from the multiple imputations [19].

The total incidence estimate based on the original data is the estimate for persons with known testing history divided by the proportion of diagnoses (0.357) with this information. This estimate

Table II. Estimated numbers (mean values based on 20 imputations) of new HIV diagnoses in 22 states during 2006, reported through June 2007, by type of diagnosis, BED result, and information on previous negative HIV test.

	Negative HIV test		
Diagnosis and BED result	No	Yes	Total
AIDS diagnosis	3441	3382	6823
HIV diagnosis, long-term infection	8097	12373	20470
HIV diagnosis, recent infection	1591	4918	6509
Total	13129	20673	33802

Table III. Summary of data, parameter estimates, incidence estimates, and 95 per cent confidence intervals (CIs) for the estimates.

	Original data		Imputed data	
	Repeat testers	New testers	Repeat testers	New testers
BED recent (n)	908	298	4918	1591
$\hat{p}_1$	0.617	0.240	0.614	0.212
p_2	0.398	0.426	1.000	1.000
$\hat{p}_3$	0.427	0.427	0.427	0.427
Incidence estimate (CI)[a]	8660 (7440–10 090)	6820 (5660–8210)	18 800 (16 400–21 500)	17 600 (15 200–20 300)
Stratified estimate (CI)			19 200 (15 000–24 600)	15 700 (11 500–21 400)
Diagnoses	7604	4463	20 673	13 129
Total incidence estimate (CI)[b]	43 400 (34 100–55 200)		36 300 (29 800–44 300)	
Total stratified estimate (CI)			34 900 (30 200–40 300)	

See the text for the stratification used in obtaining the stratified estimates.
[a]Estimates based on the original data are for incidence among persons with known testing history.
[b]Estimates based on the original data are extrapolated to incidence among all persons with a reported diagnosis.

is valid if the testing history data and BED results are missing completely at random. Although we know this assumption to be false, this extrapolated estimate is approximately the same as the estimate based on the imputed data (which assumes only that data are missing at random, with the probability of being missing dependent on the covariates in the models), and both are similar to the number of diagnoses.

Estimates of HIV incidence from the multiple imputation data with or without stratification are also shown in Table III. Strata are defined by sex, race/ethnicity, age group, and transmission category as specified previously. However, due to limited number of cases, cases in the two race groups Asian/Pacific Islander and American Indian/Alaska Native are not stratified by other variables and cases in the transmission category MSM/IDU are not stratified by age. This stratification was done to obtain incidence estimates for each level within a stratum. For repeat testers, the stratified and unstratified estimates based on the imputed data are nearly equal; both are somewhat smaller than the number of diagnoses. For new testers, the stratified estimate is smaller than the unstratified estimate, and both are at least 20 per cent larger than the number of diagnoses. The unstratified and stratified total incidence estimates based on the imputed data are 7 and 3 per cent (respectively) larger than the number of diagnoses.

The confidence intervals for the total incidence estimate using the imputed data are shorter than the intervals based on the original data. The variance of the incidence estimate using the imputed data accounts for both the variance of the individual imputed incidence estimates and the variation among these estimates. The imputed data have many more BED recent diagnoses, which decreases the leading term in the variance formula (4). This compensates for the variation among the imputed estimates. Stratification substantially decreases the length of the confidence interval for the total incidence estimate compared with the unstratified data because it uses the stratum-specific information. The incidence estimates and their confidence interval lengths (rounded to the nearest 100) computed on the original scale using the stratified data differ from the corresponding values in Table III by at most 100 and 200, respectively.

SIMULATION RESULTS

We evaluated the performance of the incidence estimators by simulating the relative bias, coefficient of variation, and confidence interval coverage of an incidence estimate during a time interval [0, 1]. Each simulation had the following steps.

1. *Incident infections*: We assumed that there were 100 new infections during this interval and that infections started 15 years earlier. In the earlier years, we chose the number of infections in each year from a Poisson distribution with a specified mean, either a constant mean, or a specified trend in the mean for years $-1, -2$, and -3 and the same mean as in year -3 for the earlier years.
2. *Seroconversion date*: This date was chosen to be random with a uniform distribution within the specified year.
3. *AIDS date*: The time (in years) from infection to a CD4 count of 200 cells/µL (the criterion for most current AIDS diagnoses) was chosen from a gamma distribution with shape parameter 2 and scale parameter 4.
4. *HIV test dates*: For each infected person with a previous negative test, we assumed that the first HIV test either was at the start of the year 5 years before the year of infection or was chosen from a uniform distribution over the 3 years before the year of infection. Subsequent intertest intervals were randomly chosen from the observed distribution of the time since the last negative HIV test for 219 persons who were BED recent and reported a last negative test date (median, 12 months; first and third quartiles, 5 and 20 months, respectively). This distribution should be approximately the same as the distribution of the renewal process describing intertest intervals for persons who are having tests [20]. In the first case, this process may be approximately at equilibrium at the time of HIV diagnosis (as we assumed in estimating the parameter p_1). For persons with no negative test, the time to the HIV test was chosen from an exponential distribution with a specified mean. Because the development of symptomatic AIDS should stimulate an HIV test, and the time from a CD4 count of 200 cells/µL to symptomatic AIDS has a mean of approximately 2 years, we also defined an HIV test date as the AIDS date plus a random time with a uniform distribution on (0, 2). For persons with no negative test, the test date is the minimum of this date and the test date obtained from the exponential distribution.
5. *BED window period*: A window period was chosen randomly from an estimate of the window period distribution. This estimate is based on data from a series of blood samples obtained from newly infected persons [10]. For the BED test a generalized log-logistic distribution fits this distribution (R. H. Byers, CDC, personal communication). The survival function is $(1+\lambda \exp((\log t - \mu)/\sigma))^{-1/\lambda}$. For the critical value 0.8 of the optical density used to distinguish recent from long-standing infections, for time in days the parameter values are $\lambda = 0.962$, $\mu = 4.898$, and $\sigma = 0.309$, giving a mean value of 155.6 days.

For each scenario, we evaluated the incidence estimator based on 10 000 simulations. On each simulation, we calculated the point estimate, the estimated variance of the logarithm of this estimate, a 95 per cent confidence interval based on this variance, and whether the 95 per cent confidence interval covered the true mean. Our evaluation is based on relative bias (the mean of the point estimates minus 100, divided by 100), variability (CV, the coefficient of variation of the simulated point estimates), and confidence interval coverage. These computations were done using SAS version 9.1 (SAS Institute, Cary, NC).

Table IV. Simulation results for repeat testers, by assumed date of the first HIV test (see the text) and the trend in incidence during the 3 years before the year of interest.

First HIV test date	Trend (per cent per year)	Mean diagnoses (per cent AIDS)	Mean $\hat{p}_1$	Mean relative bias (per cent)	CV (per cent)	95 per cent CI coverage (per cent)
Fixed	−10	115.5 (12.8)	0.463	17.3	18.2	95.0
	0	100.1 (11.4)	0.488	8.3	18.5	98.0
	+10	86.7 (9.9)	0.515	0.4	18.6	99.2
Random	−10	114.0 (11.4)	0.546	11.4	16.9	97.0
	0	99.9 (10.0)	0.573	3.9	17.2	98.8
	+10	87.6 (8.6)	0.601	−3.6	17.4	99.0

Incidence was 100 infections in the year of interest.

Table V. Simulation results for new testers, by mean time of the exponential distribution of the time from infection to the first HIV test and the trend in incidence during the 3 years before the year of interest.

Mean time (years) to HIV test	Trend (per cent per year)	Mean diagnoses (per cent AIDS)	Mean $\hat{\beta}$	Mean $\hat{p}_1$	Mean relative bias (per cent)	CV (per cent)	95 per cent CI coverage (per cent)
3	−10	120.0 (19.7)	3.21	0.273	14.6	28.6	97.8
	0	99.8 (18.3)	3.01	0.290	6.0	29.1	96.8
	+10	82.7 (16.8)	2.80	0.310	−3.3	30.3	94.2
5	−10	123.4 (32.3)	5.31	0.175	11.1	34.6	97.0
	0	99.3 (30.6)	5.01	0.185	3.1	35.4	95.7
	+10	79.2 (28.8)	4.68	0.198	−6.3	36.0	93.1

Incidence was 100 infections in the year of interest.

The results for repeat testers are summarized in Table IV. The bias, CV, and confidence interval coverage are very similar for persons with first HIV test at least 5 years before infection and for persons with that test 0–3 years before infection. Bias is small except for positive bias with a decreasing infection rate. Confidence intervals are conservative. The positive biases with trend 0 are a result of persons (15 per cent in the simulated data for both testing patterns) with a positive HIV test less than 1 year after their last negative test. The observed distributions of the time since the last negative test and the model for the window period distribution yield an estimated value of p_3 of 0.457 for both testing patterns, which would yield mean biases of 1.2 and −3.0 per cent for testing beginning at least 5 years before infection and 0–3 years before infection, respectively. The similarity of the results for these two testing patterns suggests that the estimates from this procedure are not very sensitive to the assumption that the intertest interval renewal process has reached equilibrium before the date of HIV infection.

The results for persons with no negative HIV test are summarized in Table V. Incidence estimates are slightly greater if the mean time from infection to an HIV test is shorter (3 years compared with 5 years). Confidence intervals are correct or conservative. Because the testing hazard is constant, p_3 is approximately equal to the mean window period.

As expected, for both repeat and first-time testers, the number of diagnoses is approximately equal to incidence if there has been no trend in incidence. If incidence has been increasing, our

estimators provide a much less biased estimate of incidence than the number of diagnoses. Our estimator for incidence among first-time testers is much less biased than the number of diagnoses if incidence has been decreasing.

DISCUSSION

If both testing behavior and the number of new HIV infections per year have been constant for a long period of time, and if HIV testing is not motivated by the risky behavior that resulted in infection, we could estimate HIV incidence by the number of diagnosed HIV cases. Incidence can be estimated from surveillance data based on a biomarker for recent infection, but persons reported to surveillance choose when to be tested; hence, such an estimator must account for the effects of frequency of testing on the number classified as recently infected by the biomarker. We have developed and evaluated such incidence estimators. Changes in the testing behavior will have less effect on our estimators (particularly the estimator for repeat testers) than on the number of diagnosed cases. As our simulations show, our estimators will give a less biased estimate of total incidence than the number of diagnosed cases if there was a recent trend in incidence. CDC used these estimators to estimate national HIV incidence in the United States during 2006, including within strata defined by demographic and transmission categories [21].

Quan *et al.* [22] summarized published estimates of HIV incidence in the United States in specific geographic areas for time periods through 1999. Many of the most recent estimates, particularly for men who have sex with men, are based on the estimator (1) for a recruited cohort but with data from counseling and testing sites or sexually transmitted disease clinics. Because this estimator does not take into account testing history, these estimates may be biased.

Our model for estimating HIV incidence is conceptually simple and depends on parameters that can be estimated directly from surveillance data. Many of the assumptions we made to estimate p_1 and p_3 can be evaluated from the surveillance data. Somewhat quantitative estimates of bias can be made if some of the assumptions are false. The complication in using the model is that a majority of persons have no information on testing history or BED result. While completeness is expected to increase as the surveillance systems mature, these data are likely to be incomplete as a result of logistical problems even after HIV surveillance is fully implemented. Because these data are not missing completely at random, multiple imputation should be used to obtain valid incidence estimates not only for the whole population, but also for important population subgroups.

The estimate of p_1 for repeat testers assumes that testing behavior has not changed for at least the last several years so that the testing behavior of the persons diagnosed in the year of interest is representative of the testing behavior of those becoming infected in that year. Because we estimate that more than 60 per cent of repeat testers were tested at most 1 year after HIV infection (Table III), and 34 per cent with a reported test date report a test at most 1 year before diagnosis, our incidence estimate for repeat testers may not be very sensitive to moderate changes in testing behavior.

Constant testing behavior is an important assumption for new testers, as the estimate of p_1 is obtained from the proportions of new HIV diagnoses that are AIDS diagnoses. In addition, the estimate of p_1 for new testers will be biased if there has been a trend in HIV incidence. For example, if incidence has been decreasing, then HIV diagnoses made during the year of interest will tend to have more diagnoses of persons infected relatively long ago compared with what would be observed if incidence has been constant. Thus, the proportion of AIDS diagnoses q would be

larger, which also increases the estimate of β and hence decreases the estimate of p_1, as shown in Table V.

The surveillance data can be used to compute the proportions of initial HIV diagnoses with AIDS and the distributions of the time from an HIV diagnosis before AIDS to an AIDS diagnosis, both by year of initial HIV diagnosis. The current data show only small changes during 2000–2006, suggesting that testing practices may not have changed, but these results would be affected by changes in the use of and the efficacy of therapy. CDC has developed a back-calculation model for HIV and AIDS diagnoses, which includes a time-dependent estimate of risk for an HIV test among persons becoming infected [21]. There is a modest upward trend in the estimated testing risk during 1993–2006 (an overall relative increase of about 10 per cent, with variation among subgroups); a single risk is modeled for 2001–2006; hence, this model cannot detect a recent change in testing practices (Philip Rhodes, CDC, personal communication).

Our estimates of p_1 assume that the testing history information is correct, both whether the person had a negative HIV test and, if so, the date of that test. The BED results suggest that some persons diagnosed with HIV report a last negative test date that is too recent. For example, among persons with BED results, 54 per cent (62/115) and 59 per cent (145/246) of those reporting a last negative test at most 2 and 3–5 months, respectively, before their positive test were BED recent. On the basis of the model for the window period distribution, we would expect approximately 99 and 88 per cent, respectively, to be BED recent. This is likely to be an example of telescoping bias, the tendency of persons to report the date of an appropriate diagnostic test as more recent than the correct date [23–25]. This bias may affect primarily persons with a last negative test at most 1 year before their HIV diagnosis, as the per cent BED recent among those who report a test at least 1 year earlier (23 per cent, 463/2051) is approximately equal to the per cent BED recent (21 per cent) predicted from the reported last negative HIV test dates and the model for the window period distribution. Thus, telescoping bias may have a small effect on the estimate of p_1 for repeat testers.

The estimate of p_3, and of p_1 for persons with no previous test, assumes that the HIV test date is independent of the infection date. For persons with a previous test, we assume that the risk of infection is constant between the last negative and first positive test dates. These assumptions are false if testing was motivated by behavior. We evaluated whether testing was motivated by behavior from responses to a question asking the reason for choosing to be tested. Among persons with a previous negative HIV test and a BED result, the per cent recent was 39 per cent among those who were classified as motivated (176/449) and 38 per cent (591/1553) among those who were not. Among those with a BED result who had not had a negative test, the per cent recent was higher (31 per cent: 99/316) among those classified as motivated than among those classified as not motivated (21 per cent: 225/1054).

We can estimate the potential bias as a result of motivated testing among persons with no previous HIV test. Among those with a BED recent result and motivation information, the proportion of non-motivated testers is 0.694 (225/324). The stratified estimate excluding motivated testers is the estimate in Table III, 15 700, times this proportion, or 10 900. Among persons with motivation information, the proportion motivated is 0.231 (316/1370); hence, the estimated number of motivated testers who were diagnosed with HIV before AIDS (using the data given in Table II) is 9688 times this proportion or 2200. If incidence and testing behavior have been approximately constant, this is also the incidence among motivated testers, giving a total incidence estimate among persons with no previous HIV test of 13 100. This yields an overall incidence estimate of 32 300, which is 7.4 per cent smaller than the estimate of 34 900 infections given in Table III.

Our estimate of p_1 for persons with no previous HIV test assumes that the risk for a test begins with the date of seroconversion and has a constant hazard. This assumption may be false, as it seems likely that the testing hazard would increase later in the course of HIV disease progression as an infected person begins to develop symptoms. However, motivated testing would increase the testing hazard shortly after infection. It is not clear how the estimate of p_1 corresponding to such a U-shaped hazard would compare with our estimate based on a constant hazard. In the future, it may be possible to estimate the form of the testing hazard among persons with no previous HIV test from the distribution of CD4 counts at HIV diagnosis among persons diagnosed before AIDS and the estimated decline in CD4 count as a function of the time since infection.

Because persons with an AIDS diagnosis cannot be classified as BED recent, p_1 is actually the probability of having an HIV test within 1 year after HIV infection and not developing AIDS during that time period. The risk of developing AIDS within 1 year after HIV infection is small [26], and there is not a good estimate of the time-dependence of this risk during that time. Therefore, we ignored the possibility of developing AIDS within 1 year after HIV infection in estimating p_1.

Our estimates assume that all infected persons, but only those infected within 1 year before HIV diagnosis, have a positive probability of being detected BED recent (the BED test has sensitivity and specificity 1). The sensitivity assumption may be false, and the estimate of p_3 would be too large, if the BED assay uses a sample obtained some time after the date of HIV diagnosis.

The specificity of the BED test is less than 1. Based on samples from persons participating in a vaccine trial in the United States and the Netherlands who were followed every 6 months for 3 years [27], CDC found that 5.0 per cent of samples from infected persons who were estimated to have been infected for at least 1 year were classified as recent by the BED test (S. McDougal, CDC, personal communication). It is likely that we have accounted for some of the false-recent effect by classifying infections among persons diagnosed with AIDS within 6 months after their HIV diagnosis as long-standing. The surveillance data show that approximately 4 and 5 per cent of persons with an HIV diagnosis develop AIDS 7–12 and 13–24 months, respectively, after their HIV diagnosis. If the proportion of false-recent results decreases with increasing time from the development of AIDS, false-recent BED results among the small proportion developing AIDS within 7–24 months after HIV diagnosis would have a small effect on specificity. Therefore, the imperfect specificity of the BED assay should lead to a small positive bias in the incidence estimate. If the misclassification rate is 5 per cent, 25 per cent of persons from whom a BED result would be used are classified as recent (plausible, from Table II), and the window period is $\frac{1}{2}$ year, then misclassification increases the number classified as BED recent by approximately 10 per cent.

The estimate of p_3 as the mean window period for repeat testers assumes that none of these persons had a last negative test less than 1 year before their first positive test. In the surveillance data, approximately 29 per cent of repeat testers diagnosed before developing AIDS report a negative test at most 11 months before their positive test so that the correct estimate of p_3 is greater than the mean window period. The correct estimate of p_3 is also greater than the mean window period if testing is motivated by an event resulting in infection, which surveillance data suggest may have been the case for new testers. Therefore, the true value of p_3 is slightly greater than the mean window period for repeat testers.

We can compare the relationships between our incidence estimates and the number of diagnosed cases with the corresponding relationship from the simulations to evaluate whether the assumptions made in the simulations are likely to be valid. For repeat testers, the incidence estimates based on the imputed data in Table III are approximately 7 per cent less than the number of diagnoses. This corresponds to a possible modest decrease in incidence (Table IV). For new testers, the incidence

estimates in Table III based on the imputed data are at least 20 per cent greater than the number of diagnoses, but the estimate adjusted for motivation is approximately equal to the number of diagnoses, suggesting little time trend in incidence.

CDC estimated national incidence from incidence in the 22 states by dividing the estimated incidence within each stratum by the proportion of all AIDS cases in that stratum that were diagnosed in the 22 states. This extrapolation is valid if HIV incidence is proportional to AIDS incidence. Surveillance data show that the distributions of HIV not AIDS diagnoses and AIDS diagnoses in the 22 states within covariates that may be associated with HIV incidence (sex, race/ethnicity, age, transmission category, and urban/rural residence) are similar. AIDS cases diagnosed in the rest of the U.S. during 2006 are somewhat less likely to be female than in the 22 states (22 *versus* 28 per cent) or black (40 *versus* 52 per cent) and more likely to be white (36 *versus* 27 per cent) or from an urban area (population at least 500 000: 86 *versus* 79 per cent). The effect of these differences is likely to be modest and depends on the associations between these covariates and HIV incidence.

CDC adjusts for delays in case reporting. Our incidence estimates assume that all HIV and AIDS diagnoses are eventually reported. There are no recent data on completeness of HIV diagnosis reporting or on the number of HIV-infected persons who die before being diagnosed with HIV. CDC's most recent study of nine reporting areas found that more than 75 per cent of HIV (without AIDS) diagnoses and more than 80 per cent of AIDS diagnoses are reported within 12 months after the diagnosis date [28]. However, we tend to overestimate incidence among persons who will have a reported diagnosis as a result of classifying some persons with long-standing infection as recently infected and using the mean window period as p_3 for repeat testers. This bias will account, at least in part, for infected persons who are never reported to surveillance.

Note that our incidence estimation procedure is similar to estimating the total from a sample survey. The interpretation is that the (unknown) sampling frame is the persons infected during the period of interest, and the sample is those classified as recently infected by the biomarker. Within each stratum, we compute the average probability of being classified as recently infected by averaging over the estimates of $\hat{p}_1$ ($\hat{p}_2$ and $\hat{p}_3$ are constant; in our application, $\hat{p}_1$ is also constant for persons with no previous HIV test). The inverse of this probability is the weight for each person classified as recently infected.

Alternatively, we could compute a weight for each person classified as recently infected who has a previous negative test by estimating the product $\hat{p}_1\hat{p}_3$ from the intertest interval distribution and the window period distribution model. For new testers, we can estimate this product from the estimated distribution of time to an HIV test and the window period distribution model. It would be necessary to stratify the data so that these testing distributions are approximately constant within each stratum. With this procedure, an incidence estimate could be obtained for any group of persons, not just for those defined by the strata used to obtain the weights. The procedure we have proposed is easier to understand and requires only that the estimate of the mean window period be correct. The alternative procedure requires that the model for the window period distribution be correct and is more sensitive to bias in reporting the date of the most recent HIV-negative test for repeat testers (a concern for persons tested within 1 year of diagnosis, as our data show). The alternative estimators will be developed and evaluated in a future communication.

Finally, we emphasize that our procedure can be used with any biomarker for recent infection for which the mean window period (the target region for a snapshot estimator [12]) can be estimated. Alternative biomarker tests for recent HIV infection have been reported [29, 30], but the corresponding window periods have not been estimated.

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REFERENCES

1. Centers for Disease Control and Prevention. *HIV/AIDS Surveillance Report*: *2006*, vol. 18, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, 2008. Available from: http://www.cdc.gov/hiv/topics/surveillance/resources/reports/ (25 March 2008).

2. Ruiz MS, Gable AR, Kaplan EH, Stoto MA, Fineberg HV, Trussell J (eds). *No Time to Lose*: *Getting More from HIV Prevention*. National Academy Press: Washington, DC, 2001.

3. Brookmeyer R. Reconstruction and future trends of the AIDS epidemic in the United States. *Science* 1991; **253**:37–42.

4. Rosenberg PS. Scope of the AIDS epidemic in the United States. *Science* 1995; **270**:1372–1375.

5. Centers for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. *Morbidity and Mortality Weekly Report* 1992; **41**(RR-17).

6. Brookmeyer R, Quinn TC. Estimation of current human immunodeficiency virus incidence rates from a cross-sectional survey using early diagnostic tests. *American Journal of Epidemiology* 1995; **141**:166–172.

7. 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. *American Journal of Epidemiology* 1995; **142**:709–713.

8. Janssen RS, Satten GA, Stramer SL, Rawal BD, O'Brien TR, Weiblen BJ, Hecht FM, Jack N, Cleghorn FR, Kahn JO, Chesney MA, Busch MP. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. *Journal of the American Medical Association* 1998; **280**:42–48.

9. Parekh BS, McDougal JS. New approaches for detecting recent HIV-1 infection. *AIDS Reviews* 2001; **3**:183–193.

10. Parekh BS, Kennedy MS, Dobbs T, Pau C-P, Byers R, Green T, Hu DJ, Vanichseni S, Young NL, Choopanya K, Mastro TD, McDougal JS. Quantitative detection of increasing HIV type I antibodies after seroconversion: a simple assay for detecting recent HIV infection and estimating incidence. *AIDS Research and Human Retroviruses* 2002; **18**:295–307.

11. Satten GA, Janssen R, Busch MP, Datta S. Validating marker-based incidence estimates in repeatedly screened populations. *Biometrics* 1999; **55**:1224–1227.

12. Kaplan EH, Brookmeyer R. Snapshot estimators of recent HIV incidence rates. *Operations Research* 1999; **47**:29–37.

13. Lee LM, McKenna MT. Monitoring the incidence of HIV infection in the United States. *Public Health Reports* 2007; **122**(Supplement 1):72–79.

14. Green TA. Using surveillance data to monitor trends in the AIDS epidemic. *Statistics in Medicine* 1998; **17**:143–154.

15. Song R, Hall HI, Frey R. Uncertainties associated with incidence estimates of HIV/AIDS diagnoses adjusted for reporting delay and risk redistribution. *Statistics in Medicine* 2005; **24**:453–464.

16. Longini Jr IM, Clark WS, Byers RH, Ward JW, Darrow WW, Lemp GF, Hethcote HW. Statistical analysis of the stages of HIV infection using a Markov model. *Statistics in Medicine* 1989; **8**:831–843.

17. Longini Jr IM, Clark WS, Gardner LI, Brundage JF. The dynamics of CD4$^+$ T-lymphocyte decline in HIV-infected individuals: a Markov modeling approach. *Journal of Acquired Immune Deficiency Syndromes* 1991; **4**:1141–1147.

18. Johnson NL, Kotz S. *Distributions in Statistics*: *Continuous Univariate Distributions*, vol. 1. Wiley: New York, 1970.

19. Rubin DB. Multiple imputation after 18+ years. *Journal of the American Statistical Association* 1996; **91**:473–489.

20. Song R, Karon JM, White E, Goldbaum G. Estimating the distribution of a renewal process from times at which events from an independent process are detected. *Biometrics* 2006; **62**:838–846.

21. Hall HI, Song R, Rhodes P, Prejean JG, An Q, Lee LM, Karon J, Brookmeyer R, Kaplan EH, McKenna MT, Janssen RS, the HIV Incidence Surveillance Group. Estimation of HIV incidence in the United States, 2005; submitted for publication.
22. Quan VM, Steketee RW, Valleroy L, Weinstock H, Karon J, Janssen R. HIV incidence in the United States, 1978–1999. *Journal of Acquired Immune Deficiency Syndromes* 2002; **31**:188–201.
23. Zapka JG, Bigelow C, Hurley T, Ford LD, Egelhofer J, Cloud WM, Sachsse E. Mammography use among sociodemographically diverse women: the accuracy of self-report. *American Journal of Public Health* 1996; **86**:1016–1021.
24. Hall HI, Van Den Eeden SK, Tolsma DD, Rardin K, Thompson T, Sinclair AH, Madlon-Kay DJ, Nadel M. Testing for prostate and colorectal cancer: comparison of self-report and medical record audit. *Preventive Medicine* 2004; **39**:27–35.
25. Johnson EO, Schultz L. Forward telescoping bias in reported age of onset: an example from cigarette smoking. *International Journal of Methods in Psychiatric Research* 2005; **14**:119–129.
26. Collaborative Group on AIDS Incubation and HIV Survival Including the CASCADE EU Concerted Action. Time from HIV-1 seroconversion to AIDS and death before widespread use of high-active antiretroviral therapy: a collaborative re-analysis. *Lancet* 2000; **355**:1131–1137.
27. McDougal JS, Parkh BS, Peterson ML, Branson BM, Dobbs T, Ackers M, Gurwith M. Comparison of HIV type 1 incidence observed during longitudinal follow-up with incidence estimated by cross-sectional analysis using the BED capture enzyme immunoassay. *AIDS Research and Human Retroviruses* 2006; **22**:945–952.
28. Hall HI, Song R, Gerstle III JE, Lee LM, on behalf of the HIV/AIDS Reporting System Evaluation Group. Assessing the completeness of reporting of human immunodeficiency virus diagnoses in 2002–2003: capture–recapture methods. *American Journal of Epidemiology* 2006; **164**:391–397.
29. Suligoi B, Galli C, Mass M, Si Sora F, Sciandra M, Pezzotti P, Recchia O, Montella F, Sinicco A, Rezza G. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. *Journal of Clinical Microbiology* 2002; **40**:4015–4020.
30. Barin F, Meyer L, Lancar R, Deveau C, Charib M, Laporte A, Dsenclos J-C, Costagliola D. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. *Journal of Clinical Microbiology* 2005; **43**:4441–4447.