Improving Biomarker-based HIV Incidence Estimation in the Treatment Era

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Background: Estimating HIV-1 incidence using biomarker assays in cross-sectional surveys is important for understanding the HIV pandemic. However, the utility of these estimates has been limited by uncertainty about what input parameters to use for false recency rate (FRR) and mean duration of recent infection (MDRI) after applying a recent infection testing algorithm (RITA).

Methods: This article shows how testing and diagnosis reduce both FRR and mean duration of recent infection compared to a treatment-naive population. A new method is proposed for calculating appropriate context-specific estimates of FRR and mean duration of recent infection. The result of this is a new formula for incidence that depends only on reference FRR and mean duration of recent infection parameters derived in an undiagnosed, treatment-naive, nonelite controller, non-AIDS-progressed population.

Results: Applying the methodology to eleven cross-sectional surveys in Africa results in good agreement with previous incidence estimates, except in 2 countries with very high reported testing rates.

Conclusions: Incidence estimation equations can be adapted to account for the dynamics of treatment and recent infection testing algorithms. This provides a rigorous mathematical foundation for the application of HIV recency assays in cross-sectional surveys.

Keywords: HIV, Incidence; LAg-Avidity; Recency; RITA

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Understanding incidence, the rate at which susceptible individuals in a population become infected, is necessary to effectively monitor and track the spread of an infectious disease and evaluate programs and interventions designed to stop spread.

Cross-sectional surveys combined with assays that can distinguish between recent and long-term infections have been a mainstay for monitoring HIV incidence since early in the pandemic. The basic analytic approach dates back to Brookmeyer and Quinn, who proposed detection of p24 in the absence of antibodies as a recency indicator (acute infection). Following this, many other indicators were proposed and explored, including less sensitive enzyme immunosorbant assays, CD4 count progression, BED capture EIA, avidity index, and most recently, the limiting-antigen (LAG) avidity assay.

The analysis framework used to generate incidence from the assay results has changed little since the method was originally proposed. Kassanjee and colleagues provided the derivations and formulations most often used today. The formula requires three parameters. The first is a cutoff value for time since seroconversion defining the difference between a recent and a long-term infection. The second parameter is the proportion of long-term infections that test recent on the assay (false recency rate [FRR]) and the third is the average time a recent individual remains classified as recent by the assay (mean duration of recent infection [MDRI]). Many studies have been performed to estimate these parameters in untreated reference populations.

New challenges using recency assays to measure HIV incidence have arisen as the response to the HIV pandemic has progressed to universal antiretroviral (ARV) therapy eligibility...
for all people with HIV. The recency rate among virally suppressed, ARV-treated and AIDS-progressed individuals is greatly elevated,10,12 and the mean duration of a recent infection is also affected by treatment. There is, therefore, a divergence between the parameter values, calculated in untreated reference populations, and the recency rates and durations in the study population.

To mitigate the effect of these false recent classifications, The Joint United Nations Programme on AIDS (UNAIDS)/World Health Organization Working Group on Global HIV/AIDS and STI Surveillance issued a recommendation to treat virally suppressed individuals as nonrecent, regardless of their test results.14 Subsequent guidance suggested that additionally screening out individuals with ARV biomarkers would further avoid elevated recency rates, though this screening has not been formalized into a recommendation.15 This approach of combining recency assays with additional biomarker tests for viral load and antiretrovirals has been the standard approach for estimating incidence in Population HIV Impact Assessment surveys,16 the South Africa National HIV Prevalence, Incidence, Behavior and Communication Surveys17; and other national household surveys that measure HIV incidence.

For clarity, in this article, reference FRR and MDRI will refer to the parameters calculated in a reference population. Residual FRR and context-specific MDRI will be used to refer to the values in the study population after a screening process is employed.

The introduction of an additional screening step complicates the analysis of recency assays. While residual FRR is reduced, there will still be some misclassification and it is unclear how to adjust the observed proportion classified as recent obtained from a long-term treatment-naïve population to get a residual FRR for a combined assay plus screening test. Additionally, because the transition from recent to nonrecent classification by the algorithm can either occur as a result of a nonrecent result on the assay test or the initiation of ARV treatment, the duration of recency is a competing risk process, which shortens the context-specific MDRI compared to a treatment-naïve population.

This article extends the mathematical framework for incidence estimation from cross-sectional recency assays to the case where individuals are screened out of recency due to treatment initiation. This framing guides us to compute residual FRR and context-specific MDRI values that are applicable to the population under investigation, accounting for the competing-hazards screening process. We show reference FRR and MDRI parameters estimated in treatment-naïve, nonelite controller populations to be directly applicable to incidence estimation using a new incidence formula that only requires these as external parameters. Finally, the new methodology is applied to 11 cross-sectional nationally representative surveys conducted in sub-Saharan Africa from 2015 through 2018.

METHODS

Consider a recency assay that is more likely to classify more recently infected individuals as “recent” compared to individuals who have been infected longer. Denote $R$ as a random variable indicating the event where a randomly selected individual in the population is HIV-positive and reactive for recency at time of cross-sectional survey (irrespective of their true timing of seroconversion or treatment status), $T$ (with realization $\omega$) as the time since HIV seroconversion in years. $T$ is negative for HIV-negative individuals and $-\infty$ those that will not become infected in the future and so $p(T = t)$ is the incident case rate $t$ years before the cross-sectional survey. This work assumes an infinitely large population so that $p(T = t)$ is a probability density. $H$ is defined as the event that the randomly selected individual is HIV-positive at the time of the cross-sectional survey, and $q(t)$ as the probability that an individual in the reference population who seroconverted $t$ years ago tests recent on the assay. Incidence at the time of the cross-sectional survey is then defined as:

$$\lambda = \frac{p(T = 0)}{1 - P(H)}.$$

One detail to note is that incidence here is defined in terms of HIV seroconversion (i.e., the point in an infection at which the HIV test starts being positive) not the time of infection.

If the performance of the recency test does not change over time, and the probability an individual in the study population infected $t$ years ago tests recent is the same as in the reference population (i.e., $q(t) = P(R | T = t)$), the rate of seroconversions (the number of seroconversions per time unit per person) averaged over $q$ is

$$\int_0^\infty p(T = t) q(t) dt = \int_0^\infty \frac{p(T = t) P(R | T = t) dt}{\int_0^\infty q(x) dx} = \frac{P(R)}{\Omega},$$

where $\Omega = \int_0^\infty q(x) dx$ is the mean duration of the recency test. Thus, the averaged rate of infections is simply the ratio of the proportion of the total population classified as recent over the average duration an individual spends classified as recent.

Adjusting this averaged seroconversion rate for the size of the susceptible (HIV-negative) population in order to translate the rate of seroconversions to incidence (the number of seroconversions per time unit per susceptible individual) results in

$$\tilde{\lambda} = \frac{P(R)}{(1 - P(H))\Omega}.$$

If the susceptible population size is constant, then $\tilde{\lambda}$ is the averaged incidence. Otherwise, it can be interpreted as the averaged seroconversion rate adjusted to incidence using the current susceptible population size.

If $q$ is known, Equation (1) can be used in conjunction with a cross-sectional survey to estimate $\tilde{\lambda}$ by replacing the
probabilities with observed sample proportions. Estimation of $q$ among untreated individuals is achieved via a calibration study measuring recency test results in individuals with known time of seroconversion. Many researchers have used such studies to estimate $q$ for various assay types and across geographies (see Kassanjee et al.\textsuperscript{15} and references therein).

While it is often assumed that the reference population from the calibration study is representative of the untreated study population, there may be some divergence. Population-specific factors like age, sex, comorbidities, and HIV subtype may affect an assay’s performance. One approach to overcome this limitation is to weight the calibration data to match that of the study population. As an example, Voetsch et al.\textsuperscript{16} adjusted $q$ for a PHIA in Uganda to match the study population’s HIV subtype distribution.

A limitation of these analyses in the context of HIV is that reference data tends to become very sparse as $t$ gets larger. It is rare, even earlier in the HIV pandemic, for an individual with known seroconversion date to be followed for many years without receiving treatment. Thus, there is considerable uncertainty about the shape of the tail of $q$, and the thickness of the tail can have a large impact on the resulting $\Omega$. Furthermore, if the rate at which long-term infections test recent is constant after some point, $\Omega$ is infinite.

The issue of a potentially infinite $\Omega$ can be solved by truncating $q$ at some cutoff points $\tau$. Hereafter, it is assumed that the seroconversion rate ($p(T = t)$) is constant over the last $\tau$ time units. Under this assumption, incidence can be expressed as

$$\lambda = \frac{P(T < \tau | H) P(H)}{(1 - P(H)) \tau},$$

by applying equation (1) to a hypothetical case where $R$ is the event $T < \tau$. Rearranging gives an equation for the proportion of HIV-positive individuals who are long-term cases

$$P(T \geq \tau | H) = 1 - \frac{\lambda \tau (1 - P(H))}{P(H)}.$$  \hspace{1cm} (2)

The probability that an HIV-positive individual is classified as “recent” by a recency assay ($P(R|H)$) can be decomposed into those who have been infected for shorter than $\tau$ and those infected longer than $\tau$

$$P(R|H) = P(RT < \tau | H) + P(RT \geq \tau | H).$$

Incidence can be expressed as

$$\lambda = \frac{p(T = 0)}{1 - P(H)}$$

$$= \int_0^\tau \frac{p(T = t) q(t)}{1 - P(H)} dt$$

$$= \frac{P(R \ & T < \tau)}{(1 - P(H)) \Omega}.$$

$$= \frac{(P(R|H) - P(R \ & T \geq \tau|H)P(H)}{(1 - P(H)) \Omega}, \hspace{1cm} (3)$$

where $\Omega = \int_0^\tau q(t) dt$ is the mean recency conditional on $T < \tau$, the reference “MDRI,” and $\beta_r = P(R|T \geq \tau, H)$ is the FRR among long-term infections ($T > \tau$).\textsuperscript{7} The third equality is obtained under the assumption that $q(t) = P(R|T = t)$, and the last equality is obtained by substituting in Equation (2) and solving for $\lambda$.

$\beta_r$ and $\Omega$, are not estimable from a single cross-sectional survey, and therefore, appropriate values for FRR and MDRI must be drawn from reference studies. Typically, these studies assume that $P(R|T = t)$ is constant for all $t > \tau$, which allows the recency rate among a set of known long-term cases to be used to estimate, $\beta_r$.\textsuperscript{10,12}

Adjusting for Recent Infection Testing Algorithms

Given that values must be sourced from reference studies, FRR and MDRI would ideally be stable across populations and time. However, FRR particularly depends on the proportion of the population that is on ARV therapy, who have a high probability of being classified as “recent” even when infected for long durations. Elite controllers also have increased FRRs. In the context of the LAg-Avidity assay, Kassanjee et al.\textsuperscript{10} found a 58% FRR in treated individuals, with a 47% FRR for those with low viral load. However, they did not find evidence of elevated FRR in those with low CD4 counts.

In an attempt to correct the classification of these false recents, modern surveys have adopted recent infection testing algorithms (RITAs\textsuperscript{14–16}). The most common of these algorithms involve an additional screening component designed to remove treated individuals and elite controllers from those classified as recent infections. Voetsch et al.\textsuperscript{16} adopted the nomenclature of RITA2 for a RITA that classifies individuals with ARV biomarkers or viral loads <1000 copies per milliliter as nonrecent. The recency assay may be performed on all screened-in individuals but is more commonly performed on all HIV-positive participants.

This article utilizes a slightly more restrictive RITA compared to RITA2, which we will call RITA3. In RITA3, individuals are screened out if any of the following conditions hold:

1. The subject is HIV-negative.
2. The subject has been previously diagnosed, as determined by either self-report or ARV biomarkers.
3. The subject is virally suppressed (viral load <1000).
4. The subject has progressed to AIDS.

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The reasons for this more restrictive screening algorithm are threefold. First, in many populations, the majority of newly diagnosed individuals immediately initiate ARV treatment and so the difference between screening based on ARVs compared with previous diagnosis may be minimal. Second, FRRs for cases who were on ARV therapy, but are not currently taking the drugs is unknown and could be potentially higher than never treated cases. Third, it is mathematically convenient to have a process where cases only transition from screened in to screened out, and not the other way around.

If external information on the time between diagnosis and treatment is available, the methods developed here can be extended to the RITA2 algorithm. See eAppendix 1; http://links.lww.com/EDE/C19 for details.

Figure 1 diagrams the decomposed effects of the screening step and assay recency on how individuals are classified by the algorithm and applies to any of the RITA algorithms. To the left of $\tau$ is the time frame deemed recent, and to the right nonrecent. The height of each colored area represents the proportion of cases whose time since seroconversion is $T$ that are screened in/out and/or are assay recent/nonrecent.

Initially, all HIV cases are assay recent and screened in (i.e., undiagnosed) and are thus considered “recent” cases by the RITA algorithm (dark salmon). As time progresses, individuals leave for other categories either because they start testing nonrecent on the assay (blue), become diagnosed (light salmon) or both (white).

The residual FRR is the height of the curve in area (d). If no screening was done, more errors would have occurred, as area (b) would also be included in the residual false recents.

The left side of the plot shows the correctly classified recents in area (c). The area under this curve is the context-specific MDRI. The area (a) represents true recents that are classified as nonrecent due to being screened out. Absent screening, the areas of (a) and (c) would be combined and so the effect of the screening process is to reduce context-specific MDRI.

Let $S$ be the event where an individual passes the screening (i.e., they have never been diagnosed, have viral load $\geq 1000$ copies/ml and are, therefore, screened in). Again assuming constant $p(T = t)$ over the last $\tau$ time units, the probability that an individual infected in the last $\tau$ time units is screened in is

$$P(S|T < \tau, H) = \int_0^\tau P(S|T = t) p(T = t|T < \tau, H) dt$$

$$= \frac{1}{\tau} \int_0^\tau P(S|T = t) dt$$

$$= \frac{\Omega_s}{\tau}, \quad (4)$$

where $\Omega_s = \int_0^\tau P(S|T = t) dt$ is the mean duration of screening in up to $\tau$. $P(S|T = t)$ is an important quantity in this development and in RITA3. It represents the probability that an individual remains undiagnosed $t$ years after seroconversion, and can thus be interpreted as a survival function.

The probability that a screened-in individual is long-term is

$$P(T \geq \tau|S) = 1 - P(T < \tau|H, S)$$

$$= 1 - P(S|T < \tau, H) \frac{P(T < \tau|H)}{P(S|H)}$$

$$= 1 - \frac{\Omega_s \lambda (1 - P(H))}{P(S \& H)}, \quad (5)$$
with the last equality obtained by substituting in equations 2 and 4. The above two relationships are useful in the derivations of residual FRR and incidence below.

A standard approach to RITA\textsuperscript{16} is to simply replace \( R \) by \( R \land S \) in equation 3, so that the formula becomes

\[
\lambda = \frac{\left( P(R \land S; H) - P(R \land S; T \geq \tau, H) \right) P(H)}{(1 - P(H))(\Omega_{r,s} - P(R \land S; T \geq \tau, H) \tau)},
\]

where

\[
\Omega_{r,s} = \int_0^\tau q(t)P(S|T = t)dt
\]

is the mean period an individual both tests recent and is screened in up to \( \tau \) time units from infection (i.e., context-specific MDRI), assuming that the reference study assay performance is representative of the screened in population (i.e., \( q(t) = P(R; S, T = t) \)). \( P(R \land S; T > \tau, H) \) is the residual FRR.

Deciding what values to use for the residual FRR and context-specific MDRI has been a challenge. A naive approach would be to use the reference values in their place. However, historically the rule of thumb that has been applied is to use the reference values in their place. However, Giguère et al.\textsuperscript{19} provide median populations there may be an external estimate of this that can be used. For example, Giguère et al.\textsuperscript{19} provide median time-to-diagnosis estimates for 40 countries in sub-Saharan Africa. Under the assumption that time-to-diagnosis follows an exponential distribution, the rate parameter of the exponential distribution is the natural log of 2 divided by median time-to-diagnosis, which can form the basis for a constructed survival curve.

Equations 7 and 8 are key relationships that provide insight both into the spread of infection within the population and the dynamics of a RITA algorithm. Equation 8 provides us with a formulation of incidence that depends only on the reference FRR and not on the more fluid residual FRR. Equation 7, which itself depends on incidence, defines the relationship between reference and residual FRR as mediated by the screening process.

**Estimating \( P(S|T = t, H) \)**

RITA3 screens out individuals based on their diagnosis status. Estimating \( \Omega_{r,s} \) requires the time from seroconversion to diagnosis survival function \( P(S|T = t, H) \). In some populations there may be an external estimate of this that can be used. Fellow et al.\textsuperscript{20} proposed that the distribution of time-since-last-test among HIV-negative individuals, under restrictive assumptions, could be used as a proxy for the time between seroconversion and the next positive regular test. They combined this time-to-diagnosis-via-regular-testing, with an assumption that individuals not diagnosed via regular testing would be diagnosed at progression to AIDS. Thus, the diagnosis process is modeled as a competing risk between regular testing and diagnosis due to the presentation of severe symptoms.

EAppendix 4; http://links.lww.com/EDE/C19, develops a formal framework for the time-to-diagnosis-via-regular-testing distribution. For the population that engages in regular testing, and assuming a non-time varying HIV infection probability distribution, under a renewal theory framework it is shown that if HIV infection risk is independent of the time-between-tests distribution, then the time-since-last-test distribution among HIV individuals is the same as the time-to-diagnosis-via-regular-testing distribution.
If HIV risk and time between tests are not independent, then the time since last test among undiagnosed HIV cases can be leveraged. Assuming that time-to-diagnosis-via-regular-testing distribution is exponential, the expected time to diagnosis is half the expected time since the last test among the undiagnosed population at the time of the cross-sectional survey.

Thus, if

\[ p(A = x | T = 0) = \lambda_a e^{-\lambda_a x}, \]

is the probability that time from seroconversion to diagnosis in the testing population \( A \) is \( x \) among cases incident at the time of the cross-sectional survey, then the rate \( \lambda_a \) may be estimated as

\[ \hat{\lambda}_a = \frac{2}{m_1}, \]

where \( m_1 \) is the estimated average time since last test among undiagnosed individuals. Alternately, if there are outliers or potentially miss-reported data at the tails of the time-since-last-test data, the median \( (\Omega_2) \) may be used by solving the equation

\[ 5 = e^{-\lambda_a \tau} (\lambda_a Q_2 + 1) \quad (9) \]

for \( \lambda_a \).

To get the regular testing survival function for the whole population (not just those engaged in regular testing) is adjusted for the size of the non-testing population. The proportion of non-testers among incident cases is estimated as the proportion of individuals reporting never having had an HIV test among the HIV-negative population \( (\omega) \). The degree to which the HIV-negative population is representative of the incident case population in this regard may vary depending on the population under study. The estimated survival function is then

\[ \hat{d}(x) = 1 - \hat{P}(A \leq x | T = 0)(1 - \hat{\omega}). \]

Time to diagnosis due to AIDS symptoms is modeled as a Weibull distribution with median 10.052 and mean 10.319 years (scale = 1/0.086, shape = 2.516).\(^{21}\) The survival function is denoted as

\[ a(t) = P_{\text{Weibull}}(X > t | \text{shape} = \frac{\text{median}}{\text{scale}}, \text{scale} = 2.516). \]

If AIDS progression and the likelihood of being diagnosed due to regular screening are independent, the probability that an individual has not been screened out at time \( t \) is then

\[ \hat{P}(S|T = t, H) = a(t)\hat{d}(t). \]

Incorporating diagnosis at AIDS is done to be consistent with\(^{20}\) and its impact is typically small given the comparatively short \( \tau \) periods used in recency assay studies.

Whether time to diagnosis \( (\hat{P}(S|T = t, H)) \) is estimated via an external source or estimated from testing history, the mean duration of screening can then be calculated as

\[ \hat{\Omega}_s = \int_0^T \hat{P}(S|T = t, H)dt, \]

and the mean duration of recency is

\[ \hat{\Omega}_{rx} = \int_0^T q(t)\hat{P}(S|T = t, H)dt. \]

Inferring the distribution from testing history requires a number of strong assumptions. One assumption is that individuals transitioning to AIDS become diagnosed. While this may be questionable in practice, the influence this has on \( \hat{\Omega}_s \) and \( \hat{\Omega}_{rx} \) is typically minimal. This is because the probability of transitioning to AIDS in the first \( \tau \) years is typically small, and the \( \hat{\Omega}_s \) quantities only depend on the distribution up to time \( \tau \).

Constructing \( \hat{d}(t) \) from testing history has the benefit of not relying on other external parameters, but comes with strong assumptions. These are spelled out mathematically in eAppendix 4; http://links.lww.com/EDC19. Some of the major assumptions are as follows. First, the testing history estimators are derived within a renewal process framework at equilibrium where time to diagnosis is exponentially distributed and that the renewal process has been at equilibrium for at least \( \tau \) years. Second, it assumes that the probability of HIV infection is flat. If the testing histories of the HIV-negative population are used, it assumes that HIV risk and testing frequency are independent. If the testing histories of the undiagnosed population are used, those with high HIV risk may test at higher rates. To estimate \( \omega \) we also assume that the proportion of individuals that never engage in regular HIV testing among incident HIV infections is the same as in the HIV-negative population. Finally, HIV testing history must be accurately reported.

These are strong assumptions to be sure, and they may not be reasonable in many populations. That said Fellows et al.\(^{20}\) found that this framework of testing history can be useful when applied to large population representative surveys. For the purposes of this article, we present both testing history and external parameter based estimates of \( \hat{\Omega}_s \) and \( \hat{\Omega}_{rx} \), but do not assert superiority of one or the other. We do assert that an estimate of \( \hat{P}(S|T = t, H) \) is needed to adjust for a RITA screening process.

We obtain a RITA aware incidence estimator for RITA3 is by substituting mean duration estimates into Equation 5 along with the survey-derived proportion estimates \( \hat{P}(R|S) \), \( \hat{P}(S) \) and \( \hat{P}(H) \)

\[ \hat{\lambda}_{RITA3} = \frac{\hat{P}(R|S) - \beta \hat{P}(S)}{(1 - \hat{P}(H))(\hat{\Omega}_{rx} - \beta \hat{\Omega}_s)}. \quad (10) \]

We can similarly estimate the residual FRR by plugging in estimates and proportions into equation 4.
\[ P(\mathbf{R} \cap S|T \geq \tau, H) = \frac{\hat{P}(S|H)\hat{P}(H) - \lambda_{\text{RITA}2}\Omega_s(1 - \hat{P}(H))}{\hat{P}(H) - \lambda_{\text{RITA}2}(1 - \hat{P}(H))}. \] (11)

The key advantage of these estimators is that the only external dependencies that they have is for the reference FRR and \( q(t) \). The only additional requirement is either that the survey collects participants’ testing history, or that an external estimate of the time-from-seroconversion-to-diagnosis distribution is available.

These equations can be generalized in a rather straightforward manner to the case of the less restrictive RITA2 that only screens out individuals based on ARV biomarkers and viral load. However, additional external information is required on the distribution of times between diagnosis and treatment. See eAppendix 1; http://links.lww.com/EDE/C19 for details.

**APPLICATION: THE POPULATION-BASED HIV IMPACT ASSESSMENT**

The Population-based HIV Impact Assessment (PHIA) surveys are similarly designed population representative surveys to estimate HIV prevalence, incidence, and viral load suppression in high HIV burden countries in sub-Saharan Africa. All PHIA survey protocols, consent forms, screening forms, refusal forms, referral forms, recruitment materials, and questionnaires were reviewed and approved by in-country ethics and regulatory bodies and the institutional review boards of Columbia University Medical Center, Westat, and the CDC. In the primary survey analyses, incidence estimation was performed using the LAg-Avidity assay combined with viral load (>1000 copies/ml) and absence of ARV biomarkers (i.e., RITA2). Details of the survey methods are presented in Patel et al.\(^2\) and Sachathep et al.\(^2\)

In this section, the PHIA surveys in Cameroon, Côte d’Ivoire, Eswatini, Ethiopia, Lesotho, Malawi, Namibia, Rwanda, Tanzania, Zambia, and Zimbabwe, restricted to respondents aged 15 to 49 years, are reanalyzed. The primary survey analyses of Voetsch et al.\(^1\) assumed that the residual false recent rate was zero and that the context-specific mean duration was 130 days, which we will call the “Historical” strategy. The Uganda PHIA study was not included in the analysis due to the different reference MDRI used (153 days).

The screening process is stricter in the current analysis than in the RITA2 analysis of\(^6\) as any individuals with previous diagnosis were screened out, whereas Voetsch et al.\(^1\) only screened out those recently on ARV. Survey standard errors are calculated using jackknife and study supplied replicate weights.

**\( q(t) \) and \( \beta \) Parameter Values**

\( q(t) \) was constructed by reanalysis of the data from, Duong et al.\(^8\) who estimated reference MDRI in untreated individuals to be between 130 and 137 days depending on the analysis method used.

A smoothed generalized additive model with a binomial family and cubic regression splines was used to predict the probability of a recent classification on the assay given time since seroconversion. The smoothing parameter was estimated using Mallows’ Cp. Figure 2 shows the observed conditional proportions and the fitted generalized additive model curve.

The estimated reference MDRI from the generalized additive model curve given \( \tau = 2 \) years is 134.2 days. This is consistent with the seven analyses presented in the originating paper.\(^8\) The utilization of this curve implies the assumption that the reference population is representative of the screened-in study population.

\( \beta \) is estimated using the results of\(^8\). They found two false recents from among 362 long-term (>2 years), untreated individuals with detectable viral loads with CD4 counts > 200. Based on this, a reference FRR of \( \beta = 2/362 = 0.55\% \) was used.

**Time to Diagnosis**

Three different time-to-diagnosis distribution estimates are presented, which are then used to construct RITA aware incidence estimates using equation 10) First, we leverage the median time-to-diagnosis estimates reported by Giguère et al.\(^19\) as external estimates combined national HIV testing program data longitudinally across 40 countries with 183 population-based surveys for their model, which did not use reported time since the last test as an input.

The model-based median years-to-diagnosis estimates for the countries during the first year of their PHIA study were: Cameroon 1.3, Cote d’Ivoire 2.9, Eswatini 0.49, Ethiopia 2.3, Lesotho 0.55, Malawi 1.7, Namibia 1.1, Rwanda 1.0, Tanzania 1.5, Zambia 1.5, and Zimbabwe 1.5. These medians were then used to construct \( P(S|T = t, H) \) assuming an exponential distribution, which we call the “External” estimate.

We computed time since last test as the difference between the survey date and the reported date of last negative test. The day of the month for the last HIV test was not recorded in the surveys, and so we imputed the midpoint of the month if the last test occurred earlier then the calendar month and year of the interview. If it occurred during the calendar month and year of the interview, the midpoint between the start of the month and the interview date was used.

Two testing history based estimates were also constructed. The “Negative” estimate uses the empirical distribution of time since last test in the HIV-negative population as the estimate for time from seroconversion to diagnosis. The “Undiagnosed” estimate uses median time since the last test in the undiagnosed HIV-positive population and equation 9. The median is used to avoid potential outliers and recall bias in the right tail of the distribution as well as to mute any potential effects from the imputations done at the left side of the distribution.
Table 1 shows the mean time to diagnosis truncated at a \( t \) of 2 years (\( \Omega_s \)). \( \Omega_s \) is an important parameter in the estimation as well as a summary of the rate of diagnosis over the relevant window as values of \( P(S|T=t,H) \) do not effect estimation for any \( t > \tau \). Both of the testing history methods show good agreement with the external estimate, with a concordance correlation between “Undiagnosed” and “External” of 80% and a concordance correlation between “Negative” and “External” of 80%. The concordance between “Negative” and “Undiagnosed” was similarly high at 81%.

### Incidence Analysis

Context-specific MDRI and residual FRR estimates are displayed in Table 2. RITA3 screening has the effect of reducing both MDRI and FRR relative to their reference values. Within country, the percent reduction in MDRI and FRR are similar across estimation methods (“External,” “Negative,” and “Undiagnosed”). Between countries on the other hand, the reduction ranges quite widely. In Cote d’Ivoire, we see an MDRI reduction of 5%–7% compared with Eswatini, where the reduction is 29%–30%. FRR ranges from a 52% reduction to a 90% reduction.

The standard errors in context-specific MDRI tend to be larger using the “Undiagnosed” testing history method, likely due to this being a much smaller subpopulation than the HIV negatives. The standard errors for the residual FRR between the two testing history methods are similar. The absolute size of the standard errors for the methods are small, indicating that sampling variability in testing history will likely not inflate the variability of the incidence estimate by a large amount.

Table 3 shows incidence estimates. The “RITA Aware” estimates use equation 10, which implicitly uses the adjusted FRR and MDRI values developed in this work. In the absence of a rigorous framework for how a RITA affects FRR and MDRI, many studies have historically used a rule of thumb that FRR equals 0 and and MDRI equals 130.16 This is displayed in Table 2 as the “Historical” estimator. The “Naive” estimate ignores the RITA3 screening and assumes that the residual FRR and context-specific MDRI are equal to their reference values.

Using the reference values for the residual FRR and context-specific MDRI values (i.e., Naive) results in very low incidence estimates. The point estimate for Ethiopia is negative because the observed proportion recent is lower than the assumed reference FRR value. There is very poor agreement between the RITA aware Undiagnosed, Negative and External estimates compared to the Naive estimates, with a concordance correlation coefficient of just 51%, 53%, and 53%, respectively. Given that residual FRR is greatly reduced by the RITA3 screening, using the reference FRR cannot be advised.

The Historical and RITA aware estimates are remarkably similar. The Historical estimate’s residual FRR was below that of the RITA aware estimates, while the MDRI estimate was larger. The first creates a downward bias, while the second creates an upward one. The combination of these two effects resulted in close, but not perfect, agreement.
The concordance correlation coefficient between the Historical estimates and the Undiagnosed, Negative and External estimators was 93%, 95%, and 95%, respectively. The two countries with the largest deviation between the RITA aware and historical estimators was Eswatini and Lesotho, both countries that experienced very high testing volume during the study period. Incidence in Eswatini was estimated at 1.41%–1.43% by the RITA aware estimators, which was markedly higher than the 1.09% from the historical estimator. Similarly, the RITA aware estimators ranged from 1.33%–1.47% in Lesotho compared to just 1.08% for the historical estimator. A scatter plot of the comparison of the RITA aware estimates to the Historical and Naive estimates is included in eAppendix 2; http://links.lww.com/EDE/C19.

It is useful to assess how sensitive the incidence results are to the specification of the time-to-diagnosis survival function as this relies on either self-reported testing history or an external estimate. We explored the effect of potential misspecification by adding a multiplicative scaling factor to MDRI (Ω_r) and FRR (β) values are calculated from reference studies in untreated populations. Context-specific MDRI (Ω_r,s) and FRR (P(R & S|T ≥ τ, H)) values are estimated for the study population after the application of RITA3 screening. Time to diagnosis estimation is estimated using either the testing history of the undiagnosed HIV-positive population, the testing history of the HIV-negative population, or from an external study. Reduction is the percent reduction of the context-specific (or residual) value compared to the reference value. (se) indicates jackknife standard error.

| Country | Method | Reduction | Context-specific (se) | Ref. | Reduction | Residual (se) | Ref. |
|--------|--------|-----------|----------------------|------|-----------|--------------|------|
| Cameroon | External | −14 | 115.6 (0) | 134.20 | −59 | 0.23 (0.012) | 0.55 |
| | Negative | −12 | 118.5 (0.4) | 134.20 | −61 | 0.22 (0.013) | 0.55 |
| | Undiagnosed | −15 | 114.1 (2.3) | 134.20 | −60 | 0.22 (0.012) | 0.55 |
| Cote | External | −7 | 125.3 (0) | 134.20 | −52 | 0.27 (0.02) | 0.55 |
| d’Ivoire | Negative | −5 | 127.2 (0.3) | 134.20 | −52 | 0.26 (0.02) | 0.55 |
| | Undiagnosed | −7 | 124.3 (1.7) | 134.20 | −52 | 0.27 (0.02) | 0.55 |
| Eswatini | External | −30 | 94.1 (0) | 134.20 | −89 | 0.06 (0.004) | 0.55 |
| | Negative | −29 | 95.7 (0.6) | 134.20 | −90 | 0.05 (0.005) | 0.55 |
| | Undiagnosed | −29 | 94.8 (7.2) | 134.20 | −90 | 0.06 (0.004) | 0.55 |
| Ethiopia | External | −8 | 123.2 (0) | 134.20 | −81 | 0.1 (0.013) | 0.55 |
| | Negative | −9 | 122 (0.3) | 134.20 | −81 | 0.1 (0.013) | 0.55 |
| | Undiagnosed | −9 | 121.7 (7.2) | 134.20 | −81 | 0.1 (0.013) | 0.55 |
| Lesotho | External | −27 | 97.5 (0) | 134.20 | −83 | 0.09 (0.004) | 0.55 |
| | Negative | −30 | 94.6 (0.6) | 134.20 | −84 | 0.09 (0.005) | 0.55 |
| | Undiagnosed | −34 | 88.4 (0.3) | 134.20 | −83 | 0.09 (0.004) | 0.55 |
| Malawi | External | −11 | 119.6 (0) | 134.20 | −83 | 0.1 (0.007) | 0.55 |
| | Negative | −17 | 112.1 (0.5) | 134.20 | −82 | 0.1 (0.007) | 0.55 |
| | Undiagnosed | −20 | 107.9 (0.9) | 134.20 | −82 | 0.1 (0.006) | 0.55 |
| Namibia | External | −15 | 113.5 (0) | 134.20 | −88 | 0.07 (0.008) | 0.55 |
| | Negative | −16 | 113.2 (0.4) | 134.20 | −88 | 0.06 (0.008) | 0.55 |
| | Undiagnosed | −21 | 106 (8.4) | 134.20 | −88 | 0.07 (0.008) | 0.55 |
| Rwanda | External | −17 | 111.3 (0) | 134.20 | −87 | 0.07 (0.009) | 0.55 |
| | Negative | −10 | 120.9 (0.3) | 134.20 | −88 | 0.06 (0.009) | 0.55 |
| | Undiagnosed | −13 | 116.9 (2.1) | 134.20 | −88 | 0.07 (0.009) | 0.55 |
| Tanzania | External | −12 | 118.1 (0) | 134.20 | −67 | 0.18 (0.01) | 0.55 |
| | Negative | −12 | 118.1 (0.3) | 134.20 | −67 | 0.18 (0.01) | 0.55 |
| | Undiagnosed | −15 | 113.7 (1.3) | 134.20 | −67 | 0.18 (0.01) | 0.55 |
| Uganda | External | −20 | 107.7 (0) | 134.20 | −79 | 0.11 (0.008) | 0.55 |
| | Negative | −17 | 111.7 (0.4) | 134.20 | −81 | 0.11 (0.009) | 0.55 |
| | Undiagnosed | −22 | 104.7 (0.4) | 134.20 | −80 | 0.11 (0.009) | 0.55 |
| Zambia | External | −12 | 118.1 (0) | 134.20 | −77 | 0.13 (0.008) | 0.55 |
| | Negative | −17 | 111.2 (0.4) | 134.20 | −77 | 0.13 (0.008) | 0.55 |
| | Undiagnosed | −25 | 100.8 (4.5) | 134.20 | −76 | 0.13 (0.008) | 0.55 |
| Zimbabwe | External | −12 | 117.6 (0) | 134.20 | −79 | 0.12 (0.006) | 0.55 |
| | Negative | −16 | 112.7 (0.4) | 134.20 | −79 | 0.12 (0.006) | 0.55 |
| | Undiagnosed | −20 | 107.3 (2.8) | 134.20 | −78 | 0.12 (0.006) | 0.55 |
the estimated probability that an individual gets screened out 
\( (1 - \hat{P}(S|T = t, H)) \) and observing how that affects the resulting incidence estimates. A multiplicative factor of +20% thus increases the probability an individual is diagnosed at each time since seroconversion by 20% in the incidence estimation compared to the curve estimated by the “Undiagnosed” method. Table 4 shows how much effect each percent change in the probability of being diagnosed has on the resulting incidence estimate. For small errors within ±10%, there is little effect on the incidence estimates, with the largest error being a 5% difference. For moderate errors of ±20%, most estimates remain within 5%, but a few are larger, with the largest being 11%. Extreme errors of ±50% or ±100% can result in large changes to the incidence estimate. Interestingly though, even with an error of −100% most biases remain muted, with eight of the 12 estimates remaining with 20%.

**DISCUSSION**

Estimating incidence from cross-sectional surveys has become an important component of our understanding of the HIV pandemic in sub-Saharan Africa. The validity of these estimates has been questioned due to uncertainty around appropriate input parameters for FRR and MDRI. The previous solution, implemented in the PHIA surveys, was to assume there are no false recents (FRR = 0) after application of RITA and that MDRI is not affected by the screening. However, it is implausible that the RITA screening perfectly removes all false recents and so residual FRR must be some

### TABLE 3. Incidence Estimates (%) With Different Residual FRR and Context-specific MDRI Values

| Country    | Undiagnosed | Negative | External | Historical | Naive     |
|------------|-------------|----------|----------|------------|-----------|
| Cameroon   | 0.26 (0.08) | 0.25 (0.08) | 0.25 (0.08) | 0.24 (0.07) | 0.19 (0.07) |
| Cote d’Ivoire | 0.02 (0.02) | 0.02 (0.02) | 0.02 (0.02) | 0.03 (0.02) | 0 (0.02)   |
| Eswatini   | 1.42 (0.35) | 1.41 (0.33) | 1.43 (0.33) | 1.09 (0.24) | 0.51 (0.23) |
| Ethiopia   | 0.02 (0.02) | 0.02 (0.02) | 0.02 (0.02) | 0.02 (0.02) | -0.02 (0.02) |
| Lesotho    | 1.47 (0.33) | 1.38 (0.31) | 1.33 (0.3)  | 1.08 (0.22) | 0.58 (0.22) |
| Malawi     | 0.35 (0.1)  | 0.34 (0.1)  | 0.32 (0.09) | 0.32 (0.08) | 0.15 (0.08) |
| Namibia    | 0.41 (0.13) | 0.39 (0.12) | 0.38 (0.12) | 0.36 (0.1) | 0.16 (0.1)   |
| Rwanda     | 0.08 (0.04) | 0.08 (0.03) | 0.09 (0.04) | 0.08 (0.03) | 0.04 (0.03) |
| Tanzania   | 0.24 (0.06) | 0.23 (0.05) | 0.23 (0.05) | 0.23 (0.05) | 0.16 (0.05) |
| Uganda     | 0.49 (0.1)  | 0.46 (0.09) | 0.48 (0.09) | 0.41 (0.08) | 0.31 (0.08) |
| Zambia     | 0.67 (0.13) | 0.61 (0.12) | 0.58 (0.11) | 0.57 (0.1) | 0.37 (0.1)   |
| Zimbabwe   | 0.42 (0.11) | 0.4 (0.1)  | 0.38 (0.1)  | 0.4 (0.09) | 0.16 (0.09) |

The “RITA Aware” estimates are calculated using equation 10 and takes into account the estimated reductions in FRR and MDRI from reference to adjusted. Adjustment is performed using either the testing history of the undiagnosed HIV-positive population, the testing history of the HIV-negative population, or using an external study. The “Historical” estimates assume the context-specific MDRI is equal to 130 days and the FRR is 0, a strategy that has been commonly applied in previous publications. The “Naive” estimates assume that the residual FRR and context-specific MDRI are equal to their reference values. Jackknife standard errors are presented in the parenthesis.

### TABLE 4. Percent Change in the RITA Aware (Undiagnosed) incidence Estimate Resulting From Different Percent Changes in the Estimated Probability of Being Screened Out Given Time Since Seroconversion (1 − \( \hat{P}(S|T = t, H) \))

| Country    | % Change | % Change | % Change | % Change | % Change | % Change | % Change | % Change |
|------------|----------|----------|----------|----------|----------|----------|----------|----------|
| Cameroon   | −14      | −8       | −3       | −10      | 2        | 3        | 9        | 20       |
| Cote d’Ivoire | −7       | −4       | −1       | −10      | −1       | −2       | 9        | 5        |
| Eswatini   | −28      | −17      | −7       | −10      | −2       | −3       | 11       | 69       |
| Ethiopia   | −9       | −5       | −2       | −10      | −3       | −4       | 5        | 31       |
| Lesotho    | −33      | −20      | −9       | −10      | −4       | −5       | 6        | 11       |
| Malawi     | −19      | −10      | −4       | −10      | −5       | −6       | 7        | 29       |
| Namibia    | −20      | −11      | −5       | −10      | −6       | −7       | 1        | 16       |
| Rwanda     | −12      | −7       | −3       | −10      | −8       | −8       | 2        | 20       |
| Tanzania   | −15      | −8       | −3       | −10      | −9       | −9       | 3        | 34       |
| Uganda     | −21      | −12      | −5       | −10      | −10      | −10      | 4        | 43       |
| Zambia     | −24      | −14      | −6       | −10      | −12      | −12      | 5        | 30       |
| Zimbabwe   | −19      | −11      | −5       | −10      | −13      | −13      | 6        | 40       |
number greater than zero. Additionally, the RITA screening process leads to a context-specific MDRI that is lower than the reference MDRI, and this value varies depending on testing and treatment coverage in the population. Exactly how much larger than zero the residual FRR should be has been an open question, and previous work has not addressed reducing the MDRI to account for the screening process.

This article developed explicit relationships between reference and residual FRR and context-specific MDRI values, which provided insight into the key drivers that determine how far the adjusted values stray from their reference counterparts due to the screening process involved in RITAs. Using these relations, a convenient formula for incidence was discovered that only depends on external parameters derived in an undiagnosed, treatment-naive, nonelite controller population. The formula is designed to be applied in the context of a cross-sectional survey. Application of these methods to recency assays deployed as part of a routine surveillance system is not recommended at this time but is an area of future research.

The new incidence formula requires an estimate of the time between HIV seroconversion and diagnosis in the study population. This may be obtained from an external source estimate if one is available. Giguère et al. published methodology for estimating longitudinal time to diagnosis estimates in 40 African countries.

Alternatively, building on the work of Fellows et al., it was demonstrated how self-reported testing history, which is routinely collected in cross-sectional incidence surveys, can be leveraged to estimate time to diagnosis. Two versions of the testing history based estimation were presented. One used the testing histories of the HIV-negative population, and one used testing histories among the undiagnosed HIV-positive population. Both methods showed good agreement with the external estimates.

Using testing history to construct the time to diagnosis curve necessarily requires strong assumptions. Practitioners should use time to diagnosis estimates that are accurate in the context that they are studying. However, we do not recommend using the “Historical” estimator as that implicitly assumes a time to diagnosis distribution where all individuals are diagnosed more than $\tau$ years after seroconversion.

Given a correct time to diagnosis survival function, the new formula requires few major new assumptions. We assume that the infection rate is stable over the last $\tau$ years, and that the reference $\beta$ and $q(t)$ are applicable to the relevant subset of the study population. In the case of RITA, this subpopulation is the screened-in individuals.

While our method contrasts with the previous practice of setting residual FRR to zero and setting context-specific MDRI equal to a reference MDRI (the Historical estimator), the two estimates showed strong agreement across many of the PHIA surveys examined, with concordance correlations of $\geq 93\%$. There was some amount of good fortune to this—in the case of these existing PHIA studies, the effect of setting the FRR parameter too low (FRR = 0) was counteracted by the effect of setting MDRI too high. There was considerable divergence between the RITA aware estimators and the Historical estimator in two of the countries (Lesotho and Eswatini), where the Historical estimator estimated a much lower incidence rate.

The agreement between the methods in some datasets is no guarantee of agreement in future studies or of agreement in any sub-populations. Setting FRR to zero when it is known to be greater than zero, as the Historical method does, could potentially lead to statistical criticism of the results. As such, it is recommended to use the methods described here, which explicitly handle the competing risk process underlying the RITA algorithm.

The incidence estimation equation (equation 10) is very simple in form; however, there is some computational complexity involved in calculating the context-specific MDRI using the testing history distribution. To facilitate application of the method an R package (“recent”) has been developed, which implements incidence point estimates and uncertainty intervals for the target population, as well as any sub-populations of interest. It is available at https://github.com/fellstat/recent.

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