

Beyond detuning: 10 years of progress and new challenges in the development and application of assays for HIV incidence estimation

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Introduction

In the ongoing battle against HIV/AIDS, it is critical that we are able to measure and monitor HIV incidence, that is the number of new infections during a period of time, usually expressed as number of infections/person-years of observation or as an annual percentage of the population that acquire infection. Knowledge of HIV incidence is necessary to understand transmission patterns; to provide a rational basis for targeting prevention efforts; to evaluate interventions to reduce transmission; and to predict or project the burden of HIV infection in different demographic and risk populations. Reliable information on HIV incidence is especially important to support prevention programs in the low-income and middle-income countries that continue to bear a disproportionate share of the global burden of the HIV epidemic. Improved estimates of HIV incidence are essential to evaluate

ongoing HIV prevention and treatment programs in these resource-constrained settings and to guide the most effective use of the billions of dollars that will be spent on the epidemic in the coming years [1].

Direct measurement of incidence through prospective follow-up of cohorts of HIV-negative persons is expensive, generally unrepresentative of the larger population, and difficult to sustain, even in resource-rich settings. Furthermore, enrollment of persons into cohort studies can introduce selection bias or induce behavior change that can result in a lower observed HIV incidence than is representative of the population from which the cohort is drawn.

As a consequence of the shortcomings of longitudinal studies, HIV incidence estimates have frequently been provided by mathematical models based on epidemiological information about HIV prevalence and AIDS

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diagnoses or death rates. When input parameters are well established, these model-based approaches can provide reasonably accurate indirect incidence estimates [2]. Most recently, an epidemiological model for incidence estimation has been described that compares prevalence data in two sequential cross-sectional prevalence surveys [3]. This method has been validated using simulated data and through comparison of its estimates with measurements of incidence in several community-based cohort studies. Measuring the impact of antiretroviral treatment on age-specific HIV prevalence levels has further improved the epidemiological HIV incidence estimation from survey data [4]. However, the application of this method to estimate HIV incidence is limited to the general population at the national level, because there is a fundamental assumption about individuals in different age groups being comparable in the surveys. This situation would not be the case for prevalence data obtained from subpopulations in which individuals may enter and exit as they age (such as injecting drug-users or sex workers). Thus, classic and newer modelling approaches to HIV incidence estimation are logistically challenging, are difficult to standardize over time, and/or require complicated statistical methods.

Given the complexities and limitations of epidemiological and modeling approaches to measure HIV incidence, there has long been a strong argument for laboratory methods that can distinguish recent from established or long-term HIV infection, in order to estimate HIV incidence. In this Review, we present a summary of past and present efforts to develop and apply HIV incidence assays, and discuss what it will take to develop improved HIV incidence assays and algorithms, and to support accurate application of these methods in various research and surveillance settings.

Insights that led to development of cross-sectional HIV incidence strategies

In the early 1990s, our understanding of the dynamics of early HIV infection began to improve. The concepts of 'incidence-window period' (I-WP) modeling were first framed out by Petersen *et al.* [5] at the Centers for Disease Control and Prevention (CDC) and collaborators in the United States blood banking community. To estimate the window period preceding development of HIV antibodies, these investigators used data from recipients of blood transfusions who became infected with HIV, i.e., so-called 'look-back' investigations (i.e., tracing and follow-up testing of transfusion recipients who received blood from donors who subsequently seroconverted). The infectious window period was estimated to last 56 days prior to HIV seroconversion based on first-generation antibody assays and 45 days prior to

seroconversion for second-generation HIV antibody assays. When this information was combined with longitudinal cohort data on HIV incidence in repeat blood donors, the residual risk of transmitting HIV from preseroconversion 'window-phase donations' could be calculated ($\text{residual risk} = I \times \text{WP}$) [6].

Subsequent detailed studies of viral nucleic acid, p24 antigen and antibody dynamics in acute HIV infection led to refined understanding of the evolution of laboratory markers during the first several months of HIV infection [7,8]. For example, Fiebig *et al.* [8] tested panels of specimens from seroconverters against an array of readily available laboratory assays for HIV viremia and antibodies. They were able to generate precise point estimates with confidence intervals (CIs) for serial stages of acute infection, as defined by the results of these multiple HIV tests. Fiebig observed the ability of different HIV assays to 'close the preseroconversion window period,' and projected how the use of different tests might affect the yield of testing in populations with different rates of HIV incidence ($\text{Yield} = I \times \Delta \text{WP}$). This approach was widely embraced by test manufacturers, regulators and policy makers, who shared the common goal of closing the preseroconversion window period to enhance blood safety.

The window period concept was first inverted to estimate HIV incidence by Brookmeyer and Quinn [9]. They developed an approach for testing seronegative samples for p24 antigen and used the rate of detection of these incident infections (i.e., those in the preantibody seroconversion, antigenemic phase) and the known duration of the preseroconversion window period, to calculate incidence. Although innovative, this approach was problematic owing to the logistics and expense inherent in testing large numbers of seronegative samples for p24 antigen, which turned out to be detectable for only 5–10 days prior to antibody appearance. Moreover, these authors noted that the rate of detection of p24 antigen-positive/antibody-negative samples appeared to overestimate incidence, relative to rates of seroconversion documented on follow-up. They attributed this to test seeking during the acute symptomatic phase of infection (resulting in higher cross-sectional incidence estimates) and/or to modified risk following initial testing and counseling (resulting in lower observed incidence rates). In recent years, a number of HIV testing programs have implemented similar tests for acute HIV infection, using highly sensitive HIV RNA tests to detect viremic/preseroconversion HIV testing clients [10,11], and reviewed in Zetola and Pilcher [12]. This experience has consistently shown that early, preseroconversion HIV infections are indeed greatly overrepresented in high-risk testing settings.

Concurrent with Brookmeyer and Quinn's work, Janssen *et al.* [13] developed and validated a 'detuned'

or less-sensitive HIV antibody assay, which could discriminate recent from long-standing infections, as a tool to estimate incidence in large cross-sectional populations. In a landmark 1998 article published in *JAMA*, Janssen *et al.* [13] presented this new approach to measuring HIV incidence. The technique, subsequently termed the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS), was based on the principle that HIV antibody titers evolve in a predictable fashion over the months following initial seroconversion. Laboratory assays could, thus, be developed that would differentiate recent from long-standing HIV infections in a testing population, based on antibody evolution. The first such assay employed a second generation commercial HIV antibody assay (Abbott HIV 3A11) performed using less-sensitive or 'detuned' conditions (high dilution, reduced incubation periods, high cutoff). Specimens from recently infected persons test reactive on the standard antibody assay protocol, but nonreactive on the less-sensitive assay protocol. Based on the analysis of a large number of seroconversion panels and specimens, a series of 'detuned window periods' were calculated for different cutoffs of the modified less-sensitive assay. For example, at a standardized optical-density cutoff of 1.0, the window period from seroconversion on the standard assay to seroconversion on the less-sensitive assay was 129 days (95% CI: 109–149 days). The rate of detecting persons in the 'detuned window period' (less sensitive-enzyme immunoassay (LS-EIA) WP) could then be used to calculate incidence in the tested population using the simple formula: $I = \text{rate of recent seroconverters} / \text{LS-EIA WP} / 365$, analogous to the approach suggested by Brookmeyer and Quinn. A similar approach was later developed for a second licensed HIV-1 antibody assay (the BioMérieux Vironostika EIA) [14,15]. Both assays were widely used to calculate incidence projections for various populations in the late 1990s and early 2000s (reviewed by McDougal *et al.* [16] and Le Vu *et al.* [17]).

With broad use of the STARHS approach, this testing was found to have a number of potentially serious limitations. Most critically, detuned assays tended to misclassify as 'recent' persons with late-stage AIDS (with depleted HIV immunity and hence waning antibodies), as well as persons with viral suppression owing to antiretroviral therapy (ART) in whom antibodies also wane [18]. The performance of STARHS using the earliest detuned assays was also found to vary according to viral subtypes in the tested population. These findings led to the need to exercise caution in selection of populations for application of STARHS – exclusion of AIDS patients and ART-treated patients, and evaluation of subtype-specific window periods for each recent infection assay used to calculate incidence. These limitations led to efforts to develop alternative laboratory methods that might be less susceptible to these confounding factors.

Current laboratory methods for detection of recent HIV infection and their limitations

Other immune-response maturation approaches to identifying recent HIV-1 infection have been developed over the last 6 years (Fig. 1). These include assays based on characterizing the proportion of total bound immunoglobulin G (IgG) antibodies that capture a synthetic branched gp41 peptide constructed to express immunodominant epitopes of HIV-1 subtypes B, E, and D (the 'BED' capture enzyme immunoassay) [19]; quantification of the avidity of anti-HIV antibodies using modified second or third generation anti-HIV assays [20–22]; measurement of the antibody response to a gp41 immunodominant epitope (IDE) and various gp120–V3 loop peptides (the 'IDE-V3' assay) [23–25]; measurement of isotype IgG3 anti-HIV, which is present early in the immune response [26]; and quantification of anti-HIV antibodies on a line immunoassay (Inno-LIA HIV adaptation) [27]. All of these assays have shown promise and many have been employed in screening programs to detect recent seroconverters for clinical, public health, and research purposes, and to measure and track HIV incidence (recently reviewed in [17,28,29]). Several rapid HIV antibody assays have also been adapted for detection of recent seroconversion and incidence estimation [30,31]. The most recent proposal is a new avidity assay developed by CDC in 2009 [32]. Of the assays that are currently available, the BED is the most commonly used. A summary of available assays and their limitations are presented in Table 1.

Each of these approaches continues to be challenged by the variability of the immune response among recent HIV-1-infected persons, by differential performance in populations infected with different HIV-1 subtypes, and by the impact of ART, late-stage AIDS immunosuppression, and 'elite controller' status on individual subject misclassification. It remains unclear exactly how, or to

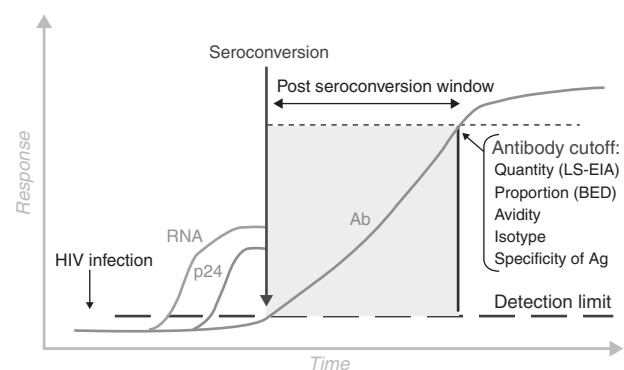


Fig. 1. Principle of assays that discriminate recent from long-standing HIV infections, based on maturation of HIV-specific antibody responses, for use in cross-sectional incidence estimation. LS-EIA, less-sensitive-enzyme immunoassay.

Table 1. Summary of tests for recent infection and their limitations.

Assay type	Principle	Component of the anti-HIV immune response being measured
Less-sensitive or detuned EIAs	A diluted blood sample is used to identify low anti-HIV antibody titre. Low antibody titre correlates with recent infection.	Quantity
Proportional assay e.g., BED-CEIA	Measures the ratio of HIV-specific IgG to total IgG. This ratio increases in recent infection.	- Limited to use in populations with predominant subtype B HIV-1 infection. - Assays require separate calibration with the predominant subtypes found in sub-Saharan Africa (subtypes A, C, D, and E), India (subtype C) and south-east Asia (subtype E). This is owing to different mean RITA durations of assay with non-B subtypes.
Avidity index	After measuring total anti-HIV response, a denaturing agent is added to separate weak from strong affinity antibodies and calculates an avidity index. This index increases during recent infection.	Proportion
Immunodominant assay e.g., IDE-V3 assay	Measures total response to select gp41 and gp120 epitopes that induce the most consistent antibody responses.	Avidity
p24 antigen	Detection of p24 antigen in the absence of anti-HIV antibody.	- Antigp41/antigp120 V3 immunodominant responses. - Presence of p24 antigen, absence of anti-HIV antibody. - Assay has a low sensitivity.
HIV RNA	Detection of HIV RNA in the absence of anti-HIV antibody.	- The period where a person is p24 antigen positive and anti-HIV antibody negative is brief (1–2 weeks). - Large sample populations are required to obtain incidence estimates.
Antip24 IgG ₃	Measures a narrow and temporary response to p24 in a single subclass of IgG that is seen consistently in recent infection.	Large sample populations are required to obtain incidence estimates.
Line immunoassay e.g., INNO-LIA HIV I/II Score	Measures reactivity with various synthetic oligopeptides and recombinant antigens.	- The findings have not yet been validated among different populations infected with divergent subtypes. - Assay is expensive unless it is routinely used as the confirmatory test.

EIAs, enzyme immunoassay; GP, glycoprotein; IDE-V3, immunodominant epitope V3; IgG, immunoglobulin G; RITA, Recent Infection Testing Algorithm.

what extent each of these factors influences the precision and specificity of available serologic assays in determining recent HIV seroconversion. However, such factors can lead to an overall lack of specificity in identifying persons with recent infection. Moreover, there is mounting evidence that many STARHs assays (and particularly the BED assay) are susceptible to high rates of misclassification of individuals with long-standing infections as recently infected [i.e., high 'false recent rates' (FRR)], impairing their performance in many settings.

To address this issue, several groups have recently proposed using test algorithms employing two or more incidence assays in sequence, as part of a Recent Infection Testing Algorithm (RITA) to reduce the FRR and increase the accuracy of incidence estimations [1]. An example of the application of a RITA based on two different incidence assays in combination with additional clinical information on CD4 cell count and antiretroviral treatment is shown in Fig. 2. This approach is reasonable and merits further evaluation, but may increase the complexity and cost of applying tests for recent infection [33]. The inclusion of CD4 cell counting in the testing algorithm requires whole blood samples, which implies that this information would not be available with dried blood spot specimens typically collected in national population-based surveys. In addition to these problems with assay performance, problems persist with standardization and quality control, and with the cost and continued availability of the commercial assays that have been adapted for incidence testing [28].

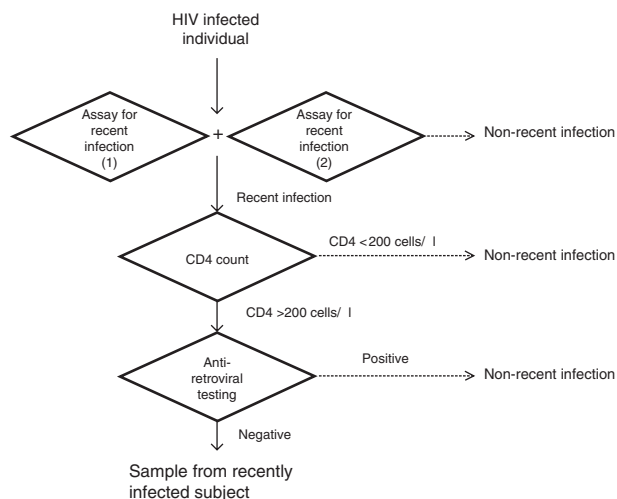


Fig. 2. Representative testing algorithm incorporating two incidence assays and available clinical data (CD4 count and antiretroviral treatment history) for determination of recent HIV infection status of specimens evaluated for HIV incidence estimation. Assay 1 and assay 2 represent two assays for recent infection, preferably based on different principles for discriminated recent from non-recent infections.

The WHO working group on HIV incidence assays

A number of leading public health organizations and scientists involved in incidence assay development and application have recently launched a concerted effort to overcome technical barriers, and to develop robust approaches based on immune maturation testing for incidence estimation. To advance this effort, in 2008, WHO formally convened a Technical Working Group on HIV Incidence Assays (http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html). This group is made up of epidemiologists, laboratory specialists, and public health officials. Several gaps were identified in assay development, validation, and commercialization. For example, there has been a lack of standard terminology and no clear consensus on statistical methods to use for HIV incidence estimation. It was also evident that the assays currently available and employed have not yet been rigorously validated using appropriate, comprehensive sample sets that would allow a standardized comparison of their performance, and a consideration of the benefits of combining assays into RITAs. The Bill & Melinda Gates Foundation (Seattle, Washington, USA), recognizing the pressing need for assays for estimation of HIV incidence to support prevention and intervention trials, has provided funding and support for the Working Group to tackle these limitations and challenges.

Following an initial review of the literature on this topic [34], the Working Group has addressed five domains: standardized terminology, market forces for assay development, potential new biomarkers for HIV incidence detection, a database and virtual repository of specimens to be used for HIV incidence validation, and procedures to validate incidence assays. A subgroup was also convened to address current challenges in statistical methods for cross-sectional HIV incidence estimation based on testing for recent infection.

Standardized terminology

Following initial development of assays for recent HIV infection in the late 1990s, various terms have been used to describe assays and methods to estimate HIV incidence. In order to harmonize terms, WHO convened several meetings to discuss the most appropriate terms to describe assays for recent infection. The results of this meeting are summarized in Table 2 (http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html).

Market assessment

In 2009, Family Health International (FHI) conducted a global landscape and market assessment, to estimate demand for HIV incidence assays (http://www.who.int/diagnostics_laboratory/links/assays_to_estimate_hiv_incidence.pdf). Qualitative interviews with key informants,

Table 2. Consensus terminology and definitions for the application of assays for recent infection to estimate HIV incidence.

Term	Definition
HIV incidence	The number of new HIV cases occurring in a population per person-time at risk, usually expressed as a rate. The natural units for expressing incidence are ‘probability of an infection occurring, per person, per unit time’.
Recent Infection Testing Algorithm	A laboratory test or combination of tests, or combination of tests and clinical information, intended to classify individuals as recently or not recently infected, for the purposes of estimating HIV incidence. The algorithm would usually only be performed on specimens from individuals who have been classified as HIV infected, for example by conventional serology. An ‘ideal’ RITA is one which has the property that all individuals transition from the RITA-recent state to the RITA-nonrecent state, and remain RITA-nonrecent until death.
Recent (or recently acquired) HIV infection	A transient period soon after HIV exposure. The rate at which the susceptible population enters this transient state is the incidence of HIV infection. Its duration varies between individuals and depends on the method used for detection. In the absence of an ‘ideal’ testing algorithm, a transition from recent to nonrecent infection may be unobservable in some individuals, even though the number of individuals who are recently infected can be systematically estimated.
Assay or test for recent HIV infection	A laboratory method that is used in a RITA to classify HIV infection as recent, for the purposes of estimating incidence.
RITA false recent rate)	The fraction of nonrecent infections in a given population that are classified as ‘RITA-recent.’
Mean RITA duration	The mean duration of the ‘state of recency’, which critically depends on the specific RITA being used. When some individuals remain indefinitely in the RITA-recent state, and/or some revert from the RITA-nonrecent to the RITA-recent state, this can still sensibly be defined as the mean duration within the subpopulation for which it is finite or less than a specified cutoff.

RITA, Recent Infection Testing Algorithm.

conducted to assess the potential market for HIV incidence assays, revealed that conceptions of acute and recent HIV infection are often conflated, and terms are used inconsistently. The BED assay was the most widely known and the most commonly used incidence assay among interviewees. Although the BED assay was widely known, acceptance and use of this assay were intensely disputed. Although some praised its low cost and convenience, many investigators familiar with its use strongly criticized the BED assay’s tendency to overestimate HIV incidence. Interviewees uniformly voiced a desire for assays with improved performance and were willing to accept incremental increases in cost. About half of interviewees expected that assay performance improvements would lead to increased demand for their use. Donor endorsements and expanded surveillance activities were cited as potential influences on demand.

In contrast to HIV diagnostics that are intended for clinical management, demand for HIV incidence assays is driven almost exclusively by public health and research needs. FHI projected the 5-year demand for three scenarios:

- (1) Scenario 1: The current situation, in which HIV incidence assays are used only on HIV-seropositive specimens and demand is influenced by suboptimal performance.
- (2) Scenario 2: An improved HIV incidence assay is widely available at a low cost and performs consistently well across HIV-1 subtypes. This new assay is used only on HIV-seropositive specimens.
- (3) Scenario 3: A novel HIV incidence assay is developed that can be used both to determine HIV-seropositivity and to identify how recently a given sample was

infected. This assay would be used for public health and research purposes, rather than individual patient diagnosis.

Estimates of potential market size under each of the three scenarios described above are provided in Table 3. The results indicate that an assay that could be used to identify a sample as HIV-seropositive and from a recent infection could result in an increased demand of 6–12 million specimens tested over 5 years. This increased demand would be owing, in part, to the use of such assays to screen specimens of unknown HIV-serostatus, as opposed to use only on HIV-seropositive specimens as for current HIV incidence assays. However, the limitations of the assays that are currently available have been widely noted and reviewed. Normative bodies have not fully endorsed their use, and consequently, current demand is far below potential future demand.

Possible new biomarkers

The overwhelming majority of work on testing for recent HIV infection has focused on the use of modified serologic assays for detection of HIV proteins, nucleic acids, and antibodies. Additional approaches, based on alternate biomarkers, have been proposed for measuring incidence that may merit future evaluation:

- (1) Cytokine profiles: these may evolve in predictable patterns, in parallel to early serologic responses in acute HIV infection [35]. Data collected to date suggests that changes in cytokine profiles are limited to the very early postinfection period, potentially limiting their usefulness for incidence estimation.
- (2) Intraindividual virus genetic diversity: viral diversity typically expands in the months following infection and

Table 3. Estimated number of specimens to be tested for HIV incidence over 5 years, by scenario and demand component.

Demand component	Scenario 1		Scenario 2		Scenario 3	
	Lower	Upper	Lower	Upper	Lower	Upper
Population-based surveys	8000	16 000	20 000	50 000	500 000	1 000 000
Sentinel surveillance	75 000	100 000	250 000	500 000	4 000 000	8 000 000
Case-based surveillance	200 000	350 000	300 000	500 000	300 000	500 000
Special studies	25 000	50 000	150 000	300 000	1 400 000	2 800 000
Total	308 000	516 000	720 000	1 350 000	6 200 000	12 300 000

Scenarios defined in the text.

expands dramatically around the time of the first appearance of neutralizing antibody responses. Whether simple assays for assessment of HIV quasispecies diversity can be developed, and whether difference in diversity measured by such assays are more or less accurate than current serologic assays, remain to be determined.

- (3) Changes in population level genetic diversity over time: it has recently been proposed that it might be possible to measure population-level HIV incidence by measuring how population level viral sequence diversity changes over time. Advanced 'phylodynamic' methods have recently been developed that might be adapted for incidence estimation (e.g., Hughes *et al.* [36]). Although sequencing is very expensive, the fact that many surveillance programs already collect sequence data suggests that resistance databases could be used to passively collect data for incidence estimation.

To date, none of the above approaches have been subjected to rigorous evaluation with regard to their application in incidence estimation. For the moment, the WHO Working Group remains primarily focused on the further development and validation of approaches based on serologic assays for recent infection. The group will explore a supplemental role for one or more of these alternative biomarkers, as well as the use of HIV RNA (to identify individuals with very low-level viremia in elite controllers and HAART-treated patients) and CD4 cell count data (to detect advanced AIDS cases), for identification and exclusion of cases of long-standing infection that are misclassified as recent infection (i.e., reduce the FRR of RITAs).

Assessment of specimens available for use in measuring and validating test/recent infection testing algorithm performance

An essential element of the development and evaluation process for the calibration and validation of assays for recent infection is the availability of relevant biological sample sets. An ongoing research program has been launched to identify and catalogue studies that could potentially contribute specimens to panels that could be used to develop, calibrate, and evaluate assays for recent HIV infection. Results of literature search and survey results from this effort are available in a report to be made

available at http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html. We were able to identify scores of studies capable of collecting and storing specimens that would be highly relevant to evaluation of tests for recent infection (e.g., seroconverter cohorts and studies enrolling known nonrecently infected patients, including elite controllers and patients with advanced disease). However, the overwhelming majority of these existing research studies maintain archived plasma specimens in only limited quantities and for limited numbers of individuals.

In recognition of these results, the WHO Working Group has organized an effort to collect large numbers of well pedigreed and large-volume blood specimens, sufficient to allow head-to-head evaluation of currently available assays. The specimen repository that is currently being planned will optimally include some archived specimens, but will also include specimens collected prospectively for this purpose by collaborating cohorts and blood donation programs with ready access to specific types of patients.

Development of appropriate statistical methods for incidence estimation

In April 2009, a statistical methodology subgroup of the WHO Working Group met to discuss the latest progress in the theoretical underpinnings of RITA-based cross-sectional incidence inference. If the RITA characteristics – now described as the mean RITA duration or 'RITA interval,' and the FRR – are known with precision, in principle this allows incidence to be estimated with minimal bias. Two subtly different versions of this method have been described [37,38], and it has been shown that this approach addresses limitations of previous approaches [39]. Crucially, it is now possible to calculate the uncertainty of incidence estimates by systematically taking into account the statistical effects of sampling in the cross-sectional survey, as well as the uncertainty with which the performance characteristics of the RITA are known. Spreadsheets implementing the methods agreed by the WHO Working Group can aid in the calculation of CIs, *P*-values, sample sizes and power, and are available on the website of the South African Centre for Epidemiological Modeling and Analysis, <http://www.sacema.com/page/assay-based-incidence-estimation>.

A framework for systematically characterizing a RITA in terms of a mean RITA interval and a FRR also facilitates an improved analysis of the fundamental trade-offs faced by developers of better tests. In particular, it is desirable to have the mean duration of the test-defined recent state to be as long as possible (to enable small survey sample sizes) while having a low FRR (thus reducing the statistical blurring of any systematic adjustment for this effect). These two crucial factors are fundamentally in conflict, and this is the main source of difficulty in identifying suitable biomarkers and thresholds.

The deterioration of statistical power with an increasing FRR is particularly dramatic, from which it follows that imprecise estimates of the FRR can lead to considerable bias in incidence estimation. No useful analysis of incidence can be done without a reasonably precise estimate of a relatively small FRR, of the order of one or two percentage, although statistical tests for trends can at least be performed without precisely estimating the FRR in the tested population.

Challenges ahead

Although the need for laboratory approaches to measure HIV incidence in populations is apparent, the use of tests for recent infection is greatly hindered by problems affecting test performance, and by the lack of sufficient data to guide the selection, use and interpretation of optimal laboratory-based methods. In order to resolve this critical impasse, the WHO Working Group on HIV incidence assays strongly recommends that a concerted effort be organized to rigorously evaluate the performance of current laboratory assays for recent infection; to develop specific recommendations for assay/algorithm use and interpretation for surveillance and research applications; and to develop working partnerships between private industry, public institutions, private foundations and academia to move the field forward.

Necessity frequently breeds invention. In this case, the need for recent infection testing is most clearly felt by the public health organizations, which need better information on HIV incidence. Importantly, investigators and sponsors of intervention and vaccine trials need to identify high incidence subpopulations in which to conduct studies and then accurately measure incidence as important efficacy endpoints. Because of the lack of market incentive for development and evaluation of recent infection assays, it is likely that public institutions and private foundations will need to assume a greater role to advance development and ensure availability and appropriate application of RITAs for incidence estimation. But this effort, now over a decade in evolution, is vital for the control of the HIV pandemic.

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