UNAIDS/WHO Working Group
on Global HIV/AIDS and STI Surveillance

When and how to use assays for recent infection to estimate HIV incidence at a population level

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Abbreviations

AIDS	acquired immunodeficiency syndrome
ANC	antenatal clinic
ART	antiretroviral therapy
ARV	antiretroviral (drug)
BED-CEIA	BED capture enzyme immunoassay
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CoV	coefficient of variation
DNA	deoxyribonucleic acid
EA	enumeration area
EDTA	ethylenediaminetetraacetic acid
EIA	enzyme immunoassay
FRR	false recent rate
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
HSS	HIV sentinel surveillance
IDE	immunodominant epitope (assay)
IgG	immunoglobulin G
MARP	most-at-risk population
MSM	men who have sex with men
p24Ag	p24 antigen
RITA	recent infection testing algorithm
RNA	ribonucleic acid
STARHS	serological testing algorithm for recent HIV seroconversion
STI	sexually transmitted infection
UNAIDS	Joint United Nations Programme on HIV/AIDS
USA	United States of America
WHO	World Health Organization

Executive Summary

Why do we need to estimate HIV incidence? The estimation of HIV incidence, or the rate at which new HIV infection is acquired in a population, is required to evaluate the impact of HIV prevention measures, and to identify populations for recruitment into clinical trials of interventions to prevent infection or treat early infection. A number of different methodological approaches have been used to estimate HIV infection. All have their strengths and limitations.

Laboratory-based algorithms for classifying HIV infections as recently acquired: The assay-based approach involves the use of one or more serological laboratory tests that are able to classify HIV infections in a population according to whether or not they were acquired in the recent past (generally within four to 12 months). Classification using one or more assays of this kind, or a combination of assays and other relevant information about the recency of HIV infection, defines an HIV Recent Infection Testing Algorithm, or HIV RITA.

Characteristics of a RITA: For a specific population and HIV subtype, a RITA has a mean RITA duration, defined as the average length of time that people with newly acquired infection in the population are to be classified by the RITA as having recently acquired infection. A RITA also has a false recent rate (FRR), which is the proportion of non-recent HIV infections in the population incorrectly classified by the RITA as being recent.

Sources of the mean RITA duration and the FRR: In applying a RITA for the purpose of estimating HIV incidence, derivation of these two parameters must take into account the fact that they may vary across populations and HIV subtypes.

Application of an HIV RITA to the estimation of HIV incidence in a population: A RITA can be used to estimate HIV infection by first using the RITA to classify cases of HIV infection in the population as either recently acquired or not recently acquired, and then applying a mathematical formula to the resulting counts of recently acquired infection in the population. Both the mean RITA duration and the RITA FRR are required to estimate HIV incidence in a population based on the RITA results. Incidence ratios, comparing two populations or two time points, can be calculated without requiring the mean RITA duration, but would still require the FRR.

Sampling frames to obtain specimens for estimating HIV incidence using a RITA: Specimens for analysis using a RITA must be obtained using a well-defined sampling frame, either based on household survey methodology or systematic HIV testing in a specific population that does not depend on regular reporting of their clinical status.

Sample size requirements: Depending on the anticipated HIV incidence and the FRR of the RITA, the sample sizes required to apply a RITA for estimating the HIV incidence may be substantial.

When can laboratory-based methods be used to estimate HIV incidence? The use of serological laboratory-based methods is valid for estimating the HIV incidence provided (i) it involves a RITA for which the mean RITA duration and FRR have been well characterized for the population and HIV subtypes under consideration; (ii) a well-defined sampling frame has been used as the source of specimens; and (iii) the sample size is large enough to achieve a meaningful estimate of incidence.

1. Introduction

It is more than a decade since the first report appeared of a laboratory test aimed at distinguishing recently acquired HIV infections from infections of a longer duration.*(1)* Several expert groups have now developed tests of this kind, based on the underlying principle that the immunological response to HIV infection evolves for a number of months following infection, and that it is possible to identify a marker for the early period. This marker eventually disappears and can therefore serve as an indicator of recent infection.*(2,3)* For the purpose of these tests, "recent" generally means a period of up to a year after infection has been acquired.

Although the accuracy of the tests has been the subject of considerable debate, they have been applied widely in several different ways.*(4–6)* On an individual basis, the tests have been used to *stage HIV infection clinically* as being recently acquired, leading to decisions about treatment, contact tracing and clinical trial enrolment. From a public health perspective, the tests have been used to *estimate the proportion of those new HIV diagnoses that are recently acquired* in a population, and to epidemiologically describe these cases. The third application, and the focus of this guidance document, is the use of the tests to *estimate the rate of HIV incidence* in populations.

This guideline document first briefly reviews the public health importance of estimating the HIV incidence in populations, and the various methods that have been used for this purpose. It then provides guidance on the way in which laboratory tests for recent HIV infection may be used to estimate HIV incidence in populations, particularly with regard to sampling designs, sample size and the statistical analyses that should be employed.

This document has been prepared by the World Health Organization (WHO), through its Technical Working Group on HIV Incidence Assays that was established in 2008. The guidance is specifically aimed at those responsible for the funding, implementation or evaluation of HIV prevention programmes rather than the specialist in epidemiological or laboratory diagnostic methods; although a general understanding of these two subject areas is assumed of the readership.

Updates on this guidance and other relevant documents will be posted on the WHO Technical Working Group on HIV Incidence Assays web site at http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/

Throughout the document, HIV refers to HIV-1 only.

2. Estimation of HIV incidence

2.1 Uses of incidence estimates

HIV incidence is the rate at *which HIV infection is acquired in a population.* It is a quantitative index that measures the extent of ongoing HIV transmission in the population. Estimation of HIV incidence may be undertaken for three distinct purposes:

(1) Population surveillance
(2) Evaluation of the impact of preventive interventions
(3) Selection of a population for recruitment to a clinical trial on the efficacy of a new preventive intervention or early treatment.

Monitoring HIV infection through surveillance aims to identify patterns of incidence through comparisons over time or between population groups, and guide policy-makers in decisions about resource allocation. Surveillance may be undertaken for the general population, or for selected subpopulations that are perceived to be at higher risk for infection.

Incidence ratios can be used to evaluate the impact of an intervention by comparing the incidence between two time periods, or between two populations. On the other hand, the selection of populations for a prevention or early treatment trial depends on estimating the absolute incidence with some accuracy, as the power of the trial, and hence its feasibility, will depend on observing a specified number of new infections in the population.

2.2 The challenge of estimating HIV incidence

Despite the importance of incidence as a public health indicator, most prevention programmes and surveillance systems have focused on measuring the HIV prevalence in a population (the proportion currently living with HIV infection) rather than incidence. Prevalence is a useful public health index but does not directly reflect the rate of current HIV transmission, because it can be confounded by other factors such as survival, migration and birth rates. Moreover, with the large-scale expansion of antiretroviral therapy (ART) programmes in many countries, and the longer survival of HIV-infected people, measuring prevalence has become less relevant than earlier.

The explanation for the more limited role of incidence in public health surveillance lies in the fundamental difficulty of obtaining reliable estimates. Even in high incidence settings (above 0.5% per annum, or 1 new infection every year in every 200 people), the occurrence of a new or incident infection is a relatively rare event, so very large sample sizes are required.

A variety of approaches have been used for the estimation of HIV incidence, but all have limitations either in terms of their accuracy, their feasibility or their cost. The methodological options for estimation of incidence are described below.

Direct measurement of incidence

This approach involves longitudinal follow up of individuals who do not have HIV infection, with re-testing of those who are initially negative, to determine the proportion that has acquired infection. This method, considered to be the "gold standard" for incidence estimation, is difficult to apply on a large scale because of the resources required for longitudinal follow up. The estimates of incidence that are derived in this way are also of limited generalizability to the wider population, because of the selected nature of study participants, and the intensive engagement, often involving risk reduction counselling and other prevention

measures, which comes with enrolment in a cohort study or trial. Direct measurement of incidence can also be applied retrospectively to populations of individuals who have undergone re-testing, such as blood donors, but again the nature of these populations is likely to make them unrepresentative of the general population with regard to HIV risk.

Indirect estimation of incidence

- *Model-derived estimation of incidence from HIV prevalence in serial prevalence surveys* is based on the assumption that a change in population estimates of HIV prevalence observed in repeat cross-sectional surveys is the net effect of the incidence occurring between surveys, and deaths among people living with HIV, as well as any in-migration or out-migration of people with HIV. On this assumption, incidence can be estimated if information is available on the mortality and migration of people with HIV in the population. This approach has been used widely, particularly in countries that have ongoing routine serosurveys among pregnant women attending antenatal clinics (ANC surveillance) to project national HIV incidence estimates in the general population.*(7,8)* Recently, newer models have used HIV prevalence data from two sequential national population-based household surveys, where incidence was inferred for age cohorts, similarly using assumptions on mortality and migration.*(9,10)* There are several limitations to this approach. There is a lack of reliable information related to migration and mortality among people living with HIV infection. Relatively few countries conduct comprehensive population-based prevalence surveys and, when they do, the surveys generally take place at widely spaced time intervals. Incidence estimation can therefore only be undertaken relatively infrequently. Furthermore, using these methods, changes in incidence may not be detected until some time after they occur. The prevalence estimates used in ANC surveillance are available on an ongoing basis rather than being limited to widely spaced survey points, so incidence can potentially be estimated more frequently in the population, but the other limitations still apply.
- *Model-derived estimation of incidence using assumptions about risk behaviour and HIV-1 transmission* in populations with HIV prevalence estimates in the corresponding populations to produce estimates of the numbers of new infections associated with specific behaviours.*(11,12)* This approach depends on good information on risk behaviours and prevalence, as well as the transmission rates.
- I*ndirect estimation from HIV prevalence in young, recently exposed populations* involves focusing on populations in whom the time since first exposure to HIV infection is believed to be short, so that trends in prevalence approximate trends in incidence in the general population. For example, in populations where women on average first have sex at the age of 15 years, the trends in prevalence of HIV in those aged 15–24 years has been used as an approximation of trends in incidence in the broader male and female adult population, using regression methods to estimate the average change in prevalence per year. This approach depends on the availability of HIV testing for a large number of young people and assumptions about the recency of exposure.

Estimation using laboratory tests for recent HIV infection, the subject of this guidance document, involves estimating the number of people with recently acquired infection in a population using a laboratory test for recent HIV infection, and then using a mathematical formula to derive HIV incidence. The strength of this method is that it provides a direct measure of incidence, but does not require repeat measurements in individuals, and hence can be applied to specimens collected in cross-sectional surveys, rather than requiring longitudinal data collection on individuals. Its limitations are in the biases that can arise through the choice of sampling frame, and the potential for long-standing infections to be misclassified as recent (the so-called *"false recent rate"* [FRR]). These issues are addressed in detail in this guidance document. Another challenge to the use of this approach with currently available assays has been the variation in assay performance across HIV clades and population groups.

Though the guidance focuses on the estimation of HIV incidence using laboratory tests for recent infection, it is important to consider all available means of measuring incidence, and to interpret findings jointly, taking into account the strengths and limitations of each approach.*(13)* A finding regarding incidence that is derived from more than one method is likely to be more credible than one based on a single method. Inconsistencies between methods can also be illuminating, as the methodological differences may provide a satisfactory explanation.

3. Tests for recent HIV infection: terminology

Various terminologies have been used to describe laboratory assays for recent HIV infection and the methods used to estimate HIV incidence based on these assays. In April 2009, a subgroup of the WHO Technical Working Group on HIV Incidence Assays met to discuss statistical approaches to HIV incidence estimates and to establish a consensus on terminology. The report of this meeting and the terms that were discussed and refined further in July 2009 by the Technical Working Group are available at: http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html.

3.1 The new terminology

A summary of the consensus terms for describing assays for recent infection is presented in Table 3.1. The key new concept is the introduction of the term **RITA**, for "recent infection testing algorithm" to describe a laboratory assay, or a combination of one or more assays and clinical information, which is used to classify a case of HIV infection as being either recently acquired or not recently acquired.

The process of developing a RITA involves deriving the population mean of its "RITA duration", the time period which begins when infection is first acquired and lasts as long as the infection is classified by the RITA as recently acquired. Under a "perfect" RITA, this time period would be identical for all infections, and every infection in a population would be correctly classified by the algorithm as either recent or not recent.

In fact, people vary in their immunological response to HIV. Thus, RITAs are imperfect, with some recent infections being classified as non-recent and vice versa. Misclassification that occurs close to the mean RITA duration is not problematic for the purpose of incidence estimation, but may be a problem in cases of long-standing infection (i.e. those that are of a duration well beyond the mean RITA duration. A widely agreed rule of thumb has been to consider an infection that has been present for at least double the mean RITA duration to be long standing). Cases that are falsely classified as recent have the potential to seriously bias incidence estimates.

The solution to the misclassification problem is to obtain an estimate of the RITA's FRR, and incorporate it in the calculation of incidence. The accuracy of the incidence estimate will depend on having precise estimates of both the mean RITA duration and the RITA FRR. Also, the lower the FRR, the more precise is the estimate of incidence. **These issues are addressed in chapters 6, 8 and 9**.

Table 3.1. 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 infections occurring in a population, usually expressed as a rate of infection per person per unit time (e.g. "infections per 100 person-years")
Recent (or recently acquired) HIV infection	A state that begins at the moment when the biological process of HIV infection is first initiated. Its duration can be defined in purely chronological terms, e.g. six months after the moment infection was initiated; or in biological terms, on the basis of an observable biomarker that is present at the initiation of infection and then disappears (or vice versa). Under the biological definition, the duration of recency will vary among individuals.
Assay or test for recent HIV infection	A laboratory test that is used to classify a case of HIV infection as recent or not recent
Recent infection testing algorithm (RITA)	A laboratory test or combination of tests, or a combination of tests and supplementary laboratory and clinical information, used to classify an HIV infection as recent or not recent
Mean RITA duration	The mean duration of recent HIV infection, as defined by a RITA, in a population of people with HIV infection. *This parameter is essential for the estimation of HIV incidence using a RITA.* Ideally within the range of 4–12 months, the mean RITA duration can vary according to the specific RITA being used and, for each RITA, may vary by HIV subtype. A RITA should not be considered for use in estimating HIV incidence in a population if its mean duration has not already been determined for that population, and with respect to the predominant HIV subtypes in the population.
RITA false recent rate (FRR)	The fraction of long-term HIV infections in a population that is misclassified by the RITA as being recent. In this context, a long-term infection can be defined as an infection of duration longer than twice the mean RITA duration. *This parameter is essential for the estimation of HIV incidence using a RITA.*

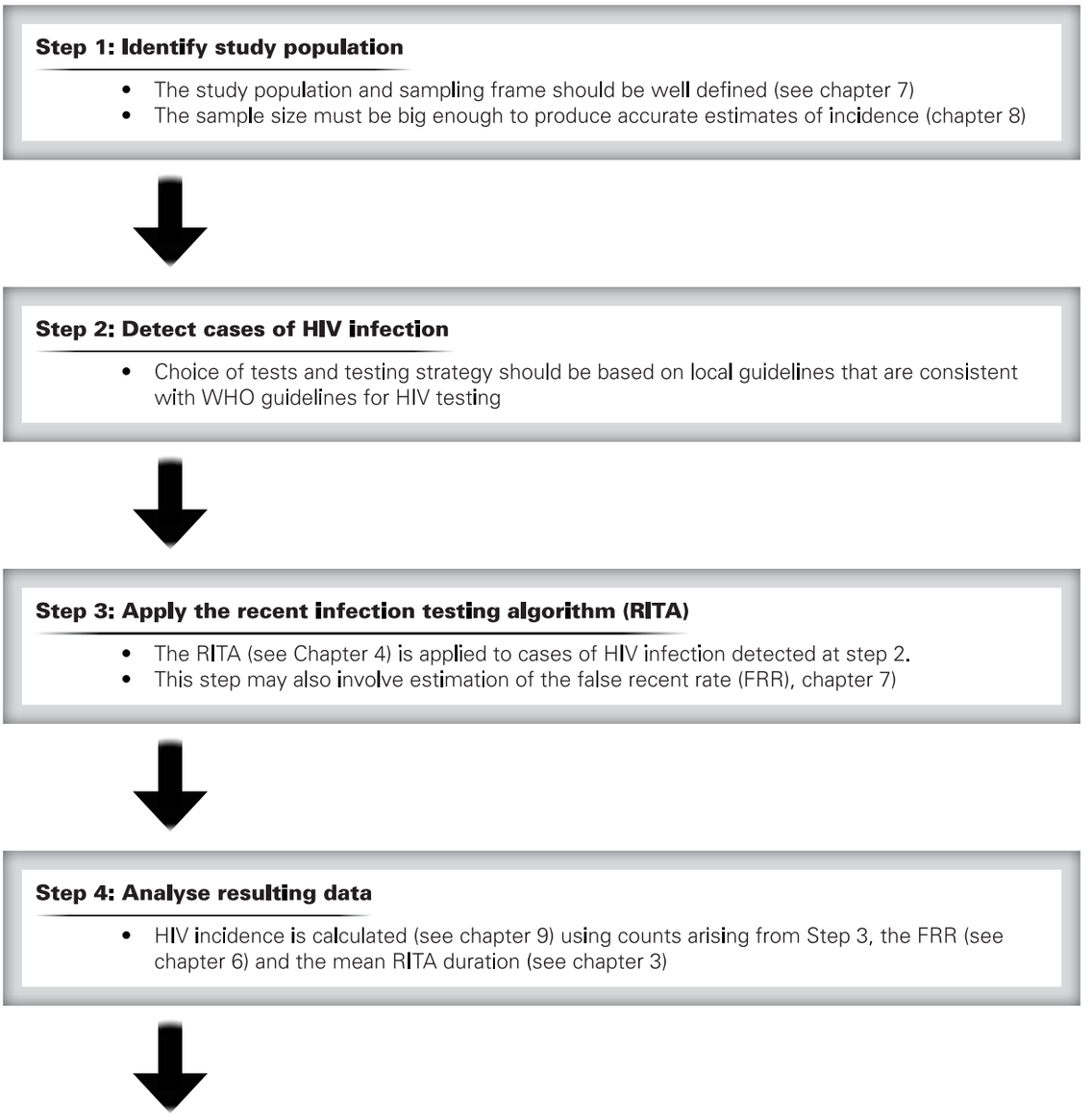
3.2 Previous terminology

In the past, the term STARHS (serological testing algorithm for recent HIV seroconversion) was adopted as the generic term for algorithms used to classify HIV infections as being recently acquired. The adoption of the term RITA (as the generic term for recent infection testing algorithms) recognizes that information other than serological test results may be used to classify an infection as recent or not recent. The term STARHS can still be used to refer to a serological test for recent infection that forms all or part of a RITA. Mean RITA duration was previously known as the "window period" or "incidence window period" for the recent infection assay, but had the potential to be confused with the more common use of the term "window period" to describe the period of time between the acquisition of HIV infection and its detection by standard diagnostic tests. RITA FRR was previously referred to as the "false-positive rate", a term which has the potential to be confused with false positivity in the diagnosis of HIV infection.

4. Application of a RITA to estimate HIV incidence

The estimation of HIV incidence using a RITA proceeds through a series of steps, as illustrated in Figure 4.1. The figure also indicates the chapters of this guidance document that are relevant to each step.

Figure 4.1. Steps involved in applying a RITA to estimate HIV incidence



5. Types of recent infection testing algorithms (RITA)

This chapter describes the different types of RITA that may be used for the purpose of estimating HIV incidence, and the requirements that must be met in each case.

It is beyond the scope of this document to make recommendations on specific tests for recent HIV infection and the way that they should be incorporated in a RITA. Generally, these decisions will be based on advice from the laboratory experts within the team responsible for estimation of incidence who, in turn, will be guided by the availability and accuracy of particular assays, as well as that of additional laboratory and clinical information, as indicated in section 5.2. Appendix 1 describes the handling of specimens for use in an assay for recent HIV infection, and Appendix 2 summarizes the types of assays for recent HIV infection which have been reported in the published literature, as well as their biological characteristics.

Regardless of which type of RITA is chosen for a survey, those responsible for the survey will need to be confident that the requirements outlined in this section have been met, and **should seek advice from laboratory experts** if there is any lack of clarity regarding the characteristics and performance of the tests for recent HIV infection and the way that they are being applied in the RITA.

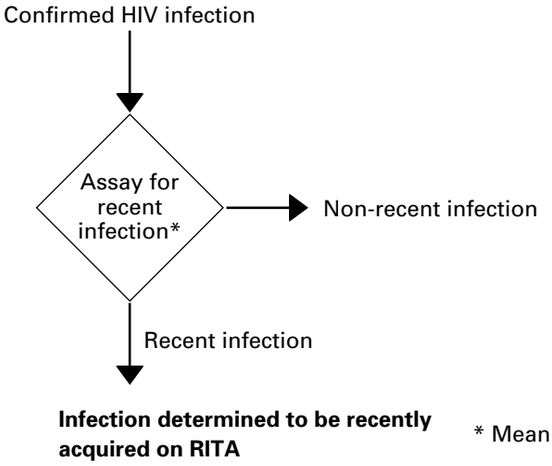
The following key points, already made in Chapter 2, should be noted for any application of a RITA:

- **An estimate of the mean RITA duration** must be available for the HIV subtypes present in the survey population.
- **An estimate of the RITA FRR** must be available for the survey population. This estimate is obtained either at the same time as the survey is conducted, or from an earlier survey in a comparable population (see Chapter 6).

5.1 RITA based on a single assay for recent infection

A single assay for recent HIV infection can be used as a RITA if the mean RITA duration of the assay is known for the HIV subtypes present in the survey population and its FRR is known for the population to which the RITA is to be applied. The RITA using a single assay for recent infection is shown in Figure 5.1.

Figure 5.1. Recent HIV infection testing algorithm (RITA) based on a single assay for recent infection



5.2 RITA based on a laboratory assay for recent infection combined with clinical, laboratory or historical information

This form of RITA involves an assay for recent infection combined with clinical, laboratory- or patient-provided information that can be used to classify HIV infections as recent or non-recent. The purpose of including the additional information is to reduce the FRR by assisting in identifying cases with long-standing infection.

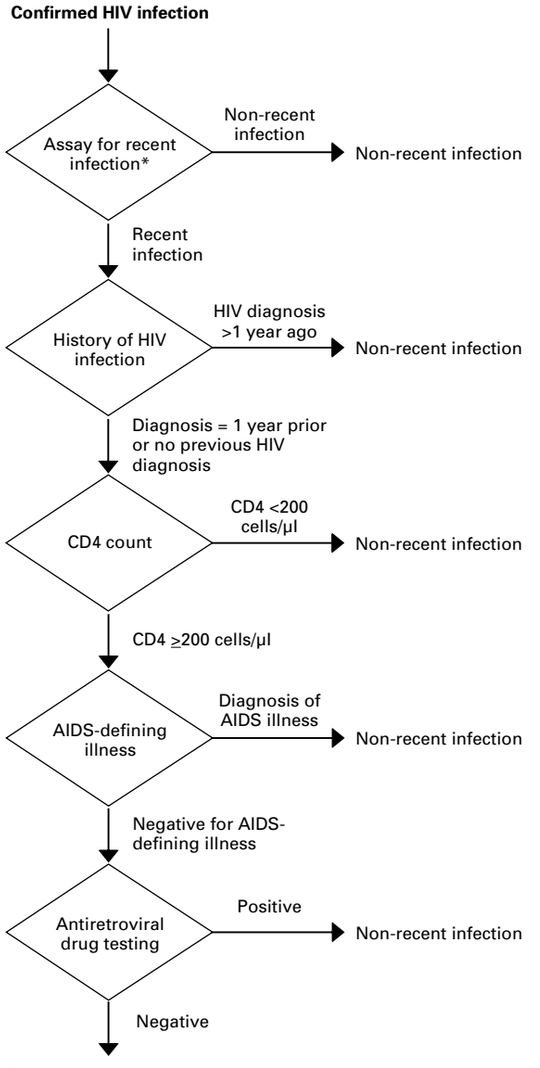
For example, a RITA could consist of the application of an assay for recent infection in combination with four criteria of supplementary clinical information. Cases that returned an assay-based result which indicated recent infection would be reclassified as non-recent if one or more of the following criteria were met:

1. CD4+ T cell count below 100 cells/µl
2. Presence of an AIDS-defining illness
3. Diagnosis of HIV more than one year ago, established by reliable clinical records
4. Receiving ART, established by either reliable clinical records or by testing biological specimens for the presence of antiretrovirals (ARVs).

The additional information cannot be assumed to eliminate misclassification of long-standing infection, so it is still necessary to estimate the FRR for the RITA as a whole.

The use of this form of RITA is shown in Figure 5.2.

Figure 5.2. An example of the application of a recent HIV infection testing algorithm (RITA) based on a laboratory assay for recent infection and additional clinical information. Clinical information used in this RITA example includes history of HIV infection, AIDS-defining illness, and testing of biological specimens for CD4 count and the presence of antiretroviral drugs.



* Mean RITA duration is known

5.3 RITA based on the use of a combination of two or more assays for recent infection

Another way to lower the FRR of a RITA is by using more than one assay for recent HIV infection in combination. The multiple assays for recent infection may additionally be combined with other information relating to the recency of infection, as in Figure 5.3.

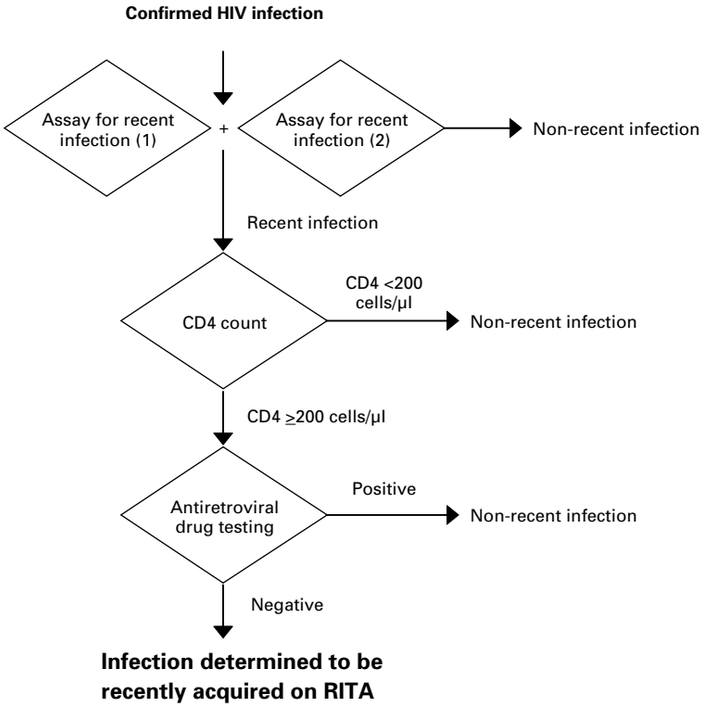
Combinations of assays may be in series (e.g. via a "screening and confirmation" approach) or in parallel, with the determination of recency on the basis of some specified set of results (e.g. "recent in at least one assay" or "recent in all assays"). It is beyond the scope of this document to make recommendations as to the strengths and weaknesses of the various options for combining assays

It is very important to note that RITAs composed of multiple assays will have a mean RITA duration that is not necessarily a simple function of the mean RITA durations of the individual assays. For example, it is unlikely that the mean RITA duration for the combination is the average of the durations or the shortest of the durations. Consequently, the RITA duration for the **specific algorithm, and combination of assays and clinical information** must be independently established if incidence is to be accurately estimated from the RITA.

Furthermore, since it is unlikely that the use of multiple assays and clinical information will eliminate all misclassifications, the FRR must be available **for the specific algorithm and combination of assays and clinical information** as applied to the survey population.

The application of a RITA based on the use of a combination of two or more assays for recent infection is demonstrated by the following example, with two assays for recent infection in combination with two criteria of supplementary clinical information – CD4 count and ART use.

Figure 5.3. An example of the application of a recent HIV infection testing algorithm (RITA) based on a combination of two or more assays for recent infection along with additional clinical information. The clinical information used in this example includes testing of biological specimens for CD4 count and the presence of antiretroviral drugs.



6. Calculation of the false recent rate (FRR)

There is substantial evidence that a proportion of people with long-standing HIV infection are misclassified as having recent infection by currently available assays for recent HIV infection. As a result, the FRR of RITAs that depend on these assays can never be assumed to be zero. The determinants of the misclassification of long-standing infection are not fully understood, but the phenomenon seems to occur more frequently in people with HIV infection who have one or more of the following characteristics:

- Late-stage infection, as defined by a diagnosis of AIDS or low CD4+ T cell count;
- Undergoing treatment with ART;
- "Elite controllers" who have low or undetectable viral loads.

The proportion of people in each of these categories varies across populations. Therefore, the rate of misclassification, and hence the FRR for any given RITA, is also likely to vary from one population to another and from one RITA to another. This variation has been demonstrated empirically for at least one frequently used assay for recent HIV infection (see Appendix 3).

The problem of misclassification was not initially recognized when tests for recent HIV infection first appeared, but there is now an appreciation of its importance. The formula for calculating HIV incidence based on the results of a RITA therefore incorporates the RITA FRR in the calculation of incidence. It is necessary to ensure that the FRR being applied is relevant to the RITA and to the population for which incidence is being estimated. For example, if an FRR is estimated for a subpopulation (e.g. pregnant women attending urban ANCs or a cohort of HIV-infected individuals with long-term HIV infection in a specific region in the country), this value should not be used as a proxy for an FRR for the country as a whole to estimate national incidence.

In general, the FRR of a RITA for a population should be reviewed at regular intervals (e.g. every five years) to take into account any change in the population characteristics, which may affect the FRR. In the absence of a recent measurement of FRR, or an FRR that is directly relevant to the setting, it will not be possible to reliably estimate incidence from the RITA.

The FRR for a RITA ($\mathcal{E}$) can be estimated using the formula $\varepsilon = R / P$, where P is the total number of cases of long-standing infection in the survey used for estimation of the FRR, and R is the number of these specimens that are classified as recent by the RITA. For the purposes of estimating the FRR, a long-standing infection can pragmatically be defined as an infection of duration longer than twice the mean RITA duration.*(14–16)* In addition to an estimate of the FRR, the coefficient of variation (CoV)[1] for the estimate of the FRR is required for calculating the confidence intervals (CIs) for the incidence estimate. A spreadsheet which performs both calculations can be downloaded at: http://www.sacema.com/page/assay-based-incidence-estimation

There are two main approaches that can be used to provide a locally relevant estimate of the RITA FRR in a population, as outlined below.

6.1 Estimation of the FRR within the same population as the one to which the RITA is being applied

This is the optimal approach, but is only feasible if:

- The FRR was determined in the same population as the population surveyed for incidence estimation.
- The FRR was determined for exactly the same RITA (i.e. the same combination of assays and clinical information used to determine the classification of recency) as will be used for incidence calculation.

1 The coefficient of variation (CoV) is a measure of precision of the estimate and is defined as the standard deviation divided by the mean. See glossary for more information on CoV.

- Appropriate sample sizes were used to estimate the FRR. As described earlier, the degree of uncertainty around the FRR will in turn influence the degree of uncertainty around the incidence estimate. See Chapter 8 for further details on the recommended sample size requirements for FRR studies.

Table 6.1 provides an example of two studies for which FRR estimates were derived within a larger cohort. It shows the country and population in which the FRR was obtained, study period, HIV-1 subtype of HIV specimens, the assay for recent infection and RITA used to derive the estimate, mean RITA duration of assay, FRR estimate and 95% CI of the FRR estimate.

6.2 Use of an externally derived FRR calculated in a population representative of the one in which you are applying the RITA to determine incidence

If an internally derived FRR is not available, an externally derived FRR of a RITA can be determined by applying the RITA to specimens from cases of long-standing HIV infection that are representative of the population in which the RITA is being applied to determine incidence.

This approach is only feasible if:

- The FRR was determined for exactly the same RITA (i.e. the same combination of assays and clinical information used to determine the mean recency duration for the RITA).
- The FRR was determined in a population representative of the one in which the incidence survey is being conducted, with respect to general demographics (e.g. age and sex distribution), HIV-1 subtypes, HIV epidemic history and, if provision of ART cannot be excluded, similar coverage of ART roll-out.
- Appropriate sample sizes were used to estimate the FRR. As described earlier, the degree of uncertainty around the estimate of the FRR will in turn influence the degree of uncertainty around the incidence estimate. See Chapter 8 for further details of recommended sample sizes for FRR studies.

Table 6.2 provides an example of a study in which FRR estimates were derived using a population that is representative of the one in which a future incidence survey will be conducted, using specimens collected from HIV sentinel surveillance in the country. It shows the country and population in which the FRR was obtained, study period, HIV-1 subtype of HIV specimens, the assay for recent infection and RITA used to derive the estimate, mean RITA duration of assay, FRR estimate and 95% CI of the FRR estimate.

6.3 Use of inclusion and exclusion criteria

We have noted that the FRR for a RITA should be estimated in a sample that is representative of people with non-recent infection from the population in which the incidence survey will ultimately be carried out.

Any inclusion or exclusion criteria used for the population in which the mean RITA duration was defined should also be applied in a consistent manner to the incidence survey sample. For example, if an FRR was estimated in a study involving only pregnant women, it would be inadvisable to use it to estimate incidence in a survey of a general population which includes men and women, since the immune response characteristics of pregnant women are likely to be different from those of people in a general population.

Another important example is the use of ART status as an exclusion criterion. **Earlier in this document, it was noted that clinical information such as ART status can be used in a RITA to reclassify people as having non-recent infection. It is, however, possible to use information of this type to exclude individuals from the sample (instead of reclassifying them).** If such exclusion criteria are applied consistently to both the estimation of the FRR and in the incidence estimation survey, the formulae described in Chapter 9 may be used to calculate an unbiased estimate of incidence.[2] However, as noted in the example of pregnant women above, it is not advisable to use an FRR that is estimated in a population in which people are excluded on the basis of a specific characteristic such as use of ART, but not excluded for the purpose of the incidence calculation.

2 Notably, when cases are excluded in this way, the calculation of CIs for the incidence estimate is affected. If information on the number of people excluded is discarded and the remaining counts are used in conjunction with the formulae in Chapter 9 to compute CIs, the intervals will be larger than necessary. It is, in principle, possible to derive expressions for CIs that incorporate the counts of people excluded, but this is beyond the scope of this document.

Table 6.1. Two studies for which internally derived false recent rate (FRR) estimates have been calculated. Details are given of the country of study, population for which the FRR was obtained, study period, major HIV-1 subtype in the population, definition of specimens used for calculating the FRR, assay for recent infection and recent infection testing algorithm (RITA) used to derive the estimate, mean RITA duration of assay, FRR estimate and 95% CI of the FRR estimate.

Reference	Country	Population	Study period	Common HIV-1 subtype in population	Specimens used for FRR calculation	Assay for recent infection	RITA applied	Sample size	Mean RITA duration (days)	FRR (%)	95% CI of FRR (%)
(Hargrove et al. 2008)*15*	Zimbabwe	Postpartum mothers	1997–2001	C	Specimens from people in the population who were HIV positive at baseline and HIV positive at the 12-month follow-up time point	BED	RITA based on a single assay for recent infection	2749	180	5.2	4.4–6.1
(Barnighausen et al. 2008)*14*	South Africa	Women (15–49 years old) and men (15–54 years old) who were living near Mtubatuba in the Umkhanykude district of KwaZulu-Natal	2003–2006	C	Specimens from people in the population who previously tested for HIV where the time between the first HIV-positive test and follow up was greater than the maximum BED progression time (306 days)	BED	RITA based on a single assay for recent infection	1065	153	1.7	1.0–2.7

Table 6.2. An example of how an externally derived false recent rate (FRR) estimate has been calculated in Viet Nam.[a] Details are given of the country of study, population for which the FRR was obtained, study period, major HIV-1 subtype in the population, definition of specimens used for calculating the FRR, assay for recent infection and recent infection testing algorithm (RITA) used to derive the estimate, mean RITA duration of assay, FRR estimate and 95% CI of the FRR estimate.

Reference	Country	Population	Study period	Common HIV-1 subtype in population	Specimens used for FRR calculation	Assay for recent infection	RITA applied	Sample size	Mean RITA duration (days)	Preliminary FRR (%)b	Additional preliminary findingsb
(Tuan et al. 2010)*17*	Viet Nam	National sample of pre-ART patients being followed for care at outpatient clinics	2009–2010	A/E	Specimens from HIV-infected persons being followed for care who have a documented HIV-positive test > than 12 months before enrolment	BED, rIDRM Avidity enzyme immunoassay (EIA)	RITA based on a single assay and combination of assays for recent infection	1284	155 (BED)	2.73% (95% CI 1.8, 3.6). No differences observed in FRR by age, sex, route of transmission, duration of infection, CD4 cell count	FRR varied by region (northern Viet Nam 5.1% vs southern Viet Nam 0.8%). Northern participants were more likely to be older, have been infected longer, and have higher rates of opportunistic infections (*Pneumocystis* pneumonia, *Candida* infections), tuberculosis, and diarrhoea compared with southern participants.

[a] A future incidence survey that applies this FRR will also exclude ART for comparability.

[b] Preliminary data reported only for the BED assay

7. Sampling frames for estimation of HIV incidence using a RITA

7.1 Sources of biological specimens for applying a RITA in estimating HIV incidence in a population

A RITA is applied to cases of HIV infection in populations for which incidence is to be determined. The source of specimens may be either a cross-sectional survey in which HIV testing is being undertaken on participants, or routine testing for HIV infection in a clinical setting.

Epidemiological considerations that play a role in the selection of populations for incidence estimation may include geographical area, age, gender and time frame of the survey. The sampling frame should be one that allows for generalization of the findings beyond the survey population, with the ideal being a random sample from the larger population of interest. The sample must also be big enough to allow the goals of the incidence estimation to be achieved, as described in Chapter 8.

This section describes three sources of biological specimens that can be tested for recent HIV infection and thereby used to derive a population estimate of HIV incidence based on a RITA: household surveys, sentinel surveillance in selected populations, and case-reporting surveillance systems. Each approach has strengths and weaknesses in relation to the goal of incidence estimation, as outlined below.

7.2 Household surveys among the general population

General population surveys, based on household sampling methodology, are used in a number of countries at a national or regional level to collect biological specimens for the purpose of providing national estimates of HIV prevalence and other health indicators. The same specimens can be used to carry out incidence estimation, using a RITA. This strategy is relevant to generalized epidemics, in which HIV is primarily transmitted heterosexually, and prevalence in adults is above 1%. Household surveys can provide estimates of HIV incidence, as well as an indication of participant characteristics, including social, behavioural and other biomedical factors that are associated with HIV incidence.

7.2.1 Planning a cross-sectional survey for estimating the incidence based on household sampling methodology in the general population

For detailed information on household survey methodology in relation to the measurement of HIV prevalence, refer to the Guidelines for measuring national HIV prevalence in population-based surveys.*18* As described in these guidelines, key components of survey design are as follows:

1. Survey location and timing:
   a. Geographical area to be sampled – all regions or a random sample of regions
   b. Survey population – gender of participants, age group, cultural group
   c. Time frames – duration of survey, time of the day the surveys are conducted, timing of repeat surveys.
2. Sampling methods:
   a. The population strata and clusters to be included in the sample
   b. Sampling units (individuals or households).
3. Sample size calculation, taking into account the response rate, expected prevalence and incidence in the population, mean RITA duration, the FRR and its coefficient of variation (see Chapter 8.
4. Data to be collected in the survey (e.g. demographic data, behavioural data and additional biomarkers, such as prevalence of sexually transmitted infections [STIs] and in some cases CD4 T-cell counts and viral load for HIV-infected persons). Data collected may include clinical information related to the RITA being used. Design the questionnaire to collect data.

5. Testing strategy:
   a. Liquid ("wet") specimens or dried blood spots (see Appendix 1)
   b. Assay to be used for detecting cases of HIV infection, with or without return of results
   c. RITA to be used for detecting cases of recent infection.

7.2.2 Limitations of household surveys among the general population

- Sampling from households may not adequately represent high-risk and mobile populations, such as those in prisons, hospitals or educational institutes, or the homeless, thereby potentially underestimating the HIV incidence in low-level or concentrated epidemics.
- The representativeness of the survey will depend on the response rate.
- Very large sample sizes may be required if countries with low HIV prevalence rates require provincial or regional HIV prevalence estimates.

7.2.3 Calculation of incidence in household-type surveys

Due to their scale, household surveys at a national level can generally provide good estimates of absolute incidence in generalized epidemics (adult prevalence above 1%). They are also able to provide comparisons between time points, calculated as an incidence ratio (see Chapter 10, 10.3, and Chapter 9, section 9.2) .

Household survey sampling methodology in the general population

Example: South Africa

South African National HIV Prevalence, Incidence, Behaviour and Communication Survey, 2008*

Background
The HIV epidemic in South Africa is generalized with an HIV prevalence of 16.2% in 2005. Heterosexual sex is the predominant mode of HIV transmission in South Africa followed by mother-to-child transmission. Young adults, in particular females, are at greatest risk for acquiring HIV. Injecting drug use is uncommon and is not a major source of transmission. Initial research into the burden of HIV among men who have sex with men (MSM) indicates a high prevalence.

A key objective of the 2008 National HIV Survey was to describe HIV prevalence, HIV incidence and risk behaviour in South Africa. The 2008 National HIV Survey is the third in a series of national population-based surveys conducted for surveillance of the HIV epidemic in South Africa. Previous national surveys were conducted in 2002 and 2005. The 2005 National HIV Survey also included incidence testing, allowing for trends in HIV incidence to be determined.

Sampling methodology

Study population: All people living in households or hostels in South Africa, including infants aged 2 years and below.

Study period: The survey was conducted between June 2008 and March 2009.

Sampling methods: Multi-stage cluster stratified sampling was used. The sample was stratified by province, settlement geography, and predominant race group in each area. Primary sampling units were 1000 enumeration areas which were selected from the 2001 population census database of 86 000 enumeration areas. Selection of enumeration areas was stratified by province and locality. Secondary sampling units were households. Within each household, only one person within each age group (child under 2 years, child aged 2–14 years, youth aged 15–24 years and adult aged 25+ years) was selected, if available.

Data collected: Six questionnaires, relating to the main objectives, were designed for this survey. All questionnaires, information sheets and informed consent forms were translated into the relevant local languages and pre-tested during the preliminary work.

Example taken from Shisana et al. 2010.(19)*

7.3 Sentinel and most-at-risk population (MARP) surveillance

Sentinel and MARP surveillance involves the collection of serological specimens from populations that are either of specific interest because they are at higher risk for infection or considered to be representative of a larger population. Sentinel and MARP surveillance may be facility-based or community-based.

Facility-based surveillance is generally undertaken through services that are used by the subpopulation of interest. For example, needle and syringe distribution programmes are a good point of contact with people who inject drugs, and sexual health clinics may provide access to MSM and sex workers. Pregnant women are considered a relatively good proxy for the general population in generalized epidemics and are accessible through antenatal services.*(20)* Military recruits may also be considered representative of the general population if they have been conscripted or selected randomly by draft or lottery. However, such recruitment often has substantial biases that need to be considered.

Community-based recruitment for sentinel surveillance is applicable for populations that are less likely to be accessible through a clinical or other facility-based setting. A variety of sampling methods may be used, including time–location sampling, snowball sampling and respondent-driven sampling.*(20,21)*

7.3.1 Planning a sentinel or MARP survey for incidence estimation

The Guidelines for conducting HIV sentinel serosurveys among pregnant women and other groups provides detailed information on the key steps to be followed in sentinel surveillance.*(20)* This group provides regular updates for established and new HIV surveillance methods. Key components of survey design are as follows:

1. Survey location, population and timing:
   a. Sentinel or community-based sites to be surveyed
   b. Population attending sites – e.g. gender, risk group, age group
   c. Sampling period.
2. Sampling methods (e.g. consecutive clients at a facility, convenience sample in a community setting, random sample)
3. HIV testing approach (unlinked or linked, with or without consent, with or without return of results to participants)
4. Sample size calculation, taking into account the response rate, expected prevalence and incidence in the population, mean RITA duration, the FRR and its CoV (see chapter 8)
5. Data to be collected in the survey (e.g. demographic, behavioural, additional biomarkers) and design of the questionnaire to collect data
6. Testing strategy:
   a. Liquid ("wet") specimens or dried blood spots
   b. Assay to be used for detecting cases of HIV infection
   c. RITA to be used for detecting cases of recent infection.

7.3.2 Limitations of sentinel surveys

- Within the sentinel population or MARP, those who are in contact with the survey may not be representative of the population group in general.
- There is high mobility among populations with high-risk behaviours and, given the stigmatized and illegal nature of some of these behaviours in many countries, they can be difficult to reach.
- If recruitment is in a clinical setting, there may be an overrepresentation of people seeking care due to symptoms (e.g. symptoms of STI), and hence a possible upward bias in the incidence estimate or a potential downward bias if persons are presenting to clinical settings with symptomatic, long-standing HIV disease. However, if an FRR is estimated for this population with an acceptable degree of precision, a valid estimate of incidence may be calculated.

Sentinel surveillance survey methodology*

Example: Cambodia

HIV incidence among ANC attendees in Cambodia, 2006

Background
HIV was first identified in Cambodia in the early 1990s. The first description of HIV transmission in the country was not available until 1995, when the first HIV sentinel surveillance (HSS) survey was completed. This first HSS survey in 1995 included eight sentinel groups, including sex workers, police and military personnel, and women attending ANCs. Prevalence of HIV among ANC attendees has been steadily decreasing since 2000.

The objective of this study was to determine the HIV incidence among women attending ANCs in Cambodia during 2006.

Sampling methodology

Sentinel sites: Specimens collected from the 2006 HSS survey from Cambodia were utilized for this study. The survey was carried out at sentinel sites in 22 provinces and cities in Cambodia.

Survey population: Women attending ANCs

Sampling period: Three-month period in 2006

Sampling methods: The sample size required was calculated using prevalence and incidence data from previous HSS surveys. The sample size was divided by 22 (representing each of the 22 provinces/cities) to calculate the number of ANC attendees required in each province/city. Pregnant women attending the designated ANC for their first pregnancy visit were recruited consecutively.

Example taken from Cambodia's HIV sentinel surveillance survey, 2006(22)*

7.4 HIV case reporting-based surveillance

HIV case reporting-based surveillance involves the reporting and analysis of newly diagnosed HIV infections in populations at a regional or national level. The same specimens used for HIV diagnosis may provide the basis for applying a RITA. Several resource-rich countries have used this approach to estimate HIV incidence, including the United States and France.*(23,24)* This approach is **not recommended** for countries that do not already have well-established case reporting-based HIV surveillance. Even for countries with such systems, there are major methodological issues that need to be taken into consideration before they are used for estimating HIV incidence via a RITA.

7.4.1 Limitations of HIV incidence estimation using case reporting-based surveillance

- Diagnoses of HIV infection that are obtained via case reporting-based surveillance depend strongly on the pattern of HIV testing and, as such, may not adequately represent populations less likely to access routine health services.
- People may seek testing due to risk behaviour or symptoms associated with primary HIV infection (seroconversion effect), potentially leading to overestimation of the population-level HIV incidence.
- In order to apply the incidence estimation formula, information is needed on the number in the population who test negative. This information is not usually provided by case reporting-based surveillance systems. It can be obtained from alternative sources, such as surveys of testing uptake, but may be difficult to obtain accurately, and thereby introduce further statistical uncertainty.

In summary, HIV incidence can be estimated by applying a RITA to HIV diagnoses detected through a case reporting-based surveillance system, provided the system is well established and the methodological approach has been customized to local conditions, the specific surveillance system and available resources. *(23,25)* Discussion of these methods is beyond the scope of this document.

7.5 Length of study period for estimating incidence using a cross-sectional study design

Estimation of incidence using a RITA assumes that the cross-sectional survey period is brief and conducted at one time point only. The period for enrolment of study participants for the purpose of estimating HIV incidence should be as short as possible. Generally the survey period should not exceed one year. The longer the study period, the less representative the sample is of instantaneous incidence.

For the purposes of identifying a trend in incidence, two successive surveys should be separated by sufficient time (more than 12 months) so that:

- there is reasonable expectation that a meaningful change may have occurred, if the objective is to detect a change in incidence
- the time between surveys is substantially larger than the time taken to perform each survey.

8. Sample size requirements for estimating HIV incidence using a RITA

Sample size requirements present one of the greatest challenges for estimating incidence using a RITA. In addition to the usual sampling variability, the statistical uncertainty around the mean duration of the RITA and FRR leads to further imprecision in the incidence estimates. Sample size calculations for the incidence survey should ensure that these parameters are estimated with an acceptable level of certainty.

In order to calculate the number of specimens required to estimate HIV incidence via a RITA with a pre-specified level of certainty, the following information is required:

- The anticipated (approximate) prevalence and incidence in the population of interest;
- The mean RITA duration and its CoV;
- The FRR of the RITA and its CoV.

The sensitivity of incidence estimation to the FRR estimate and the variability around the FRR have a significant impact on the sample size requirements for both the FRR survey and incidence estimation survey. Of note, high levels of the FRR can result in large uncertainty in the incidence estimate. RITAs that produce consistently low levels of the FRR (optimally <2%) in a variety of geographical settings and HIV-1 clades are more likely to produce valid estimates of incidence. In the example in Table 8.1, the prevalence is set at 10%, incidence ranges from 1% to 2%, the FRR ranges from 1% to 10%, the CoV of the FRR ranges from 10% to 30%, and the target CoV around the incidence estimate is 30%. A mean RITA duration of 200 days with a CoV of 5% around the mean RITA duration is assumed. For illustrative purposes, we have set a default CoV of 30%around the incidence estimate, but this may not be appropriate in every setting. As a general rule of thumb, to estimate incidence reliably, the CoV for the incidence estimate should not exceed 30%, since this corresponds to a 95% CI width of approximately 120% of the incidence estimate (i.e. the point estimate plus or minus 60% of the point estimate). For the same reason, the CoV of the FRR should not exceed 30%. In the case of a low FRR (point estimate of less than 1%), this rule of thumb may be relaxed, provided that the upper bound of the 95% CI for the FRR estimate is less than 1.5%.[1] Guidance on the optimal level of certainty one should apply for the FRR and incidence estimates will depend on critical discussions with programme managers and statisticians.

Table 8.1. Minimum sample sizes needed to estimate incidence at a CoV of 30%, by prevalence, incidence, level of FRR and CoV of FRR (assumes a mean RITA duration of 150 days with a CoV of 5%)

Prevalence (%)	Incidence (%)	FRR (%)	CoV of FRR 10%		CoV of FRR 20%		CoV of FRR 30%	
			Sample size for incidence survey	Sample size for FRR survey*	Sample size for incidence survey	Sample size for FRR survey*	Sample size for incidence survey	Sample size for FRR survey*
10.0	1.0	1.0	3 947	9 900	4 043	2 476	4 215	1 100
10.0	1.0	5.0	9 342	1 900	51 843	476	†	212
10.0	1.0	10.0	276 896	900	†	228	†	100
10.0	2.0	1.0	1 756	9 900	1 766	2 476	1 782	1 100
10.0	2.0	5.0	2 710	1 900	3 213	476	4 653	212
10.0	2.0	10.0	4 759	900	32 078	228	†	100

* Sample size is of long-standing HIV infections, defined as an infection longer than twice the mean RITA duration.

† In these instances it is not possible (at any sample size) to obtain an incidence estimate with a CoV equal to, or less than, 30%.

1 Confidence intervals for small FRRs will be asymmetrical. The formulae for computing the uncertainty of incidence assume Gaussian (symmetrical) uncertainty in the FRR. More sophisticated methods are, therefore, required to calculate accurate CIs for incidence.

These data highlight the important impact of the FRR on the incidence estimate. As the FRR of the RITA increases, the sample size required for estimating incidence at the specified level of certainty increases. Similarly, as the variability around the FRR increases, the sample size needed to estimate incidence reliably is impacted more, especially for high FRRs. Moreover, with increased variability around the FRR, there are scenarios where targets for incidence uncertainty can no longer be met (see † in Table 8.1). The high sample size requirements mean that it may not be feasible to estimate the FRR with an acceptable level of precision (CoV of FRR ≤30%) for some settings.

Example sample size charts for a range of incidence and prevalence levels are provided in Appendix 4 for general guidance on sample size requirements for incidence and FRR estimation surveys. These charts have fixed assumptions which will change according to the local context, and therefore should only be viewed as indicative of the minimum sample size targets one might expect in planning an incidence survey. Countries should utilize available spreadsheet tools that calculate sample sizes for realistic situations (available at: http://www.sacema.com/page/assay-based-incidence).

Example: A sentinel survey aims to estimate HIV incidence among ANC attendees in Cambodia. An example of how to calculate the sample size required for this study is outlined below, using data obtained from the 2006 HSS survey.*(22)*

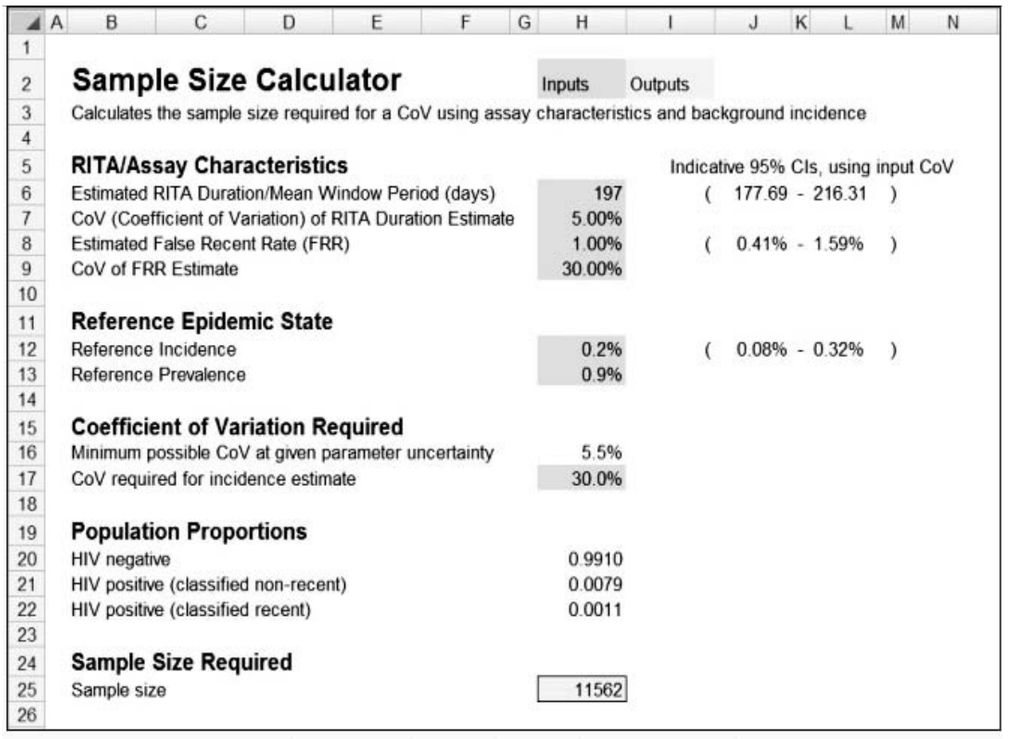
Step 1. Establish key data required for calculation

The key data required to calculate the sample size for this study are outlined below. **Note that the assumptions and data in this example are for illustrative purposes only.**

- Mean RITA duration = 197 days for BED assay (CoV of mean RITA duration = 5%)
- Estimated FRR = 1% (CoV of FRR=30%)
- Anticipated incidence = HIV incidence among Cambodian ANC attendees, 0.17%
- Anticipated prevalence = prevalence of HIV among Cambodian ANC attendees, 0.9%*
- CoV of incidence required: 30%

Step 2. Enter data into sample size calculator

The data obtained in step 1 can be used in the sample size worksheet of the spreadsheet (available at: http://www.sacema.com/page/assay-based-incidence-estimation) to calculate sample size.
For example:



Sample Size Calculator

Calculates the sample size required for a CoV using assay characteristics and background incidence

	Inputs	Outputs
RITA/Assay Characteristics		Indicative 95% CIs, using input CoV
Estimated RITA Duration/Mean Window Period (days)	197	(177.69 - 216.31)
CoV (Coefficient of Variation) of RITA Duration Estimate	5.00%	
Estimated False Recent Rate (FRR)	1.00%	(0.41% - 1.59%)
CoV of FRR Estimate	30.00%	
Reference Epidemic State		
Reference Incidence	0.2%	(0.08% - 0.32%)
Reference Prevalence	0.9%	
Coefficient of Variation Required		
Minimum possible CoV at given parameter uncertainty	5.5%	
CoV required for incidence estimate	30.0%	
Population Proportions		
HIV negative	0.9910	
HIV positive (classified non-recent)	0.0079	
HIV positive (classified recent)	0.0011	
Sample Size Required		
Sample size	11562	

* Crude HIV prevalence among ANC attendees, 2006 HSS survey 22

Step 3. Obtain sample size required

The spreadsheet calculates the sample size required for the study. For this example, 11 562 individuals are required to be enrolled in the survey and tested for the presence of HIV infection. It is important to note that for low-prevalence settings such as Cambodia, sample sizes of this magnitude may be difficult to obtain. It is important to carefully consider whether estimating the incidence is feasible and useful in such a setting. If separate estimates of incidence are required for subgroups of the population, for example, by age and gender categories, sample sizes within each stratum will need to be of the same magnitude.

The power to detect differences in incidence over time or between populations, as opposed to absolute incidence rates, depends similarly on the two RITA parameters (mean RITA duration and RITA FRR) and the epidemiological scenario (anticipated incidence and prevalence). Sample sizes required to achieve a specific level of power to detect a difference in incidence can be computed using power calculations. Spreadsheet tools to conduct power calculations may be downloaded from http://www.sacema.com/page/assay-based-incidence-estimation.

The use of survey designs other than simple random sampling has an impact on sampling variability and hence the sample size needed to achieve specific objectives. The spreadsheet tool does not take into account any such "design effect". However, by simply multiplying the calculated sample size by the design effect of the survey one can calculate the sample size needed for incidence surveys using sampling methods other than simple random sampling. Further discussion on design effects for two-stage cluster sampling design are discussed in the *Guidelines for measuring national HIV prevalence in population-based surveys.(18)*

9. Calculation of HIV incidence from the RITA results

When applying the incidence formula for estimation of HIV incidence using cross-sectional surveys, the following survey counts are used:

N is the number of people in the survey who are HIV negative, using standard HIV diagnostic tests.

P is the number of people in the survey who are HIV positive, using standard HIV diagnostic tests.

R is the number of people in the survey who are classified as having recently acquired HIV infection by the RITA.

Here we summarize the formulae used to calculate the basic estimates of interest.*(26,27)* For convenience, a spreadsheet is provided which performs these calculations and provides other tools for estimating sample sizes and computing other statistical quantities of interest (e.g. *P* value for incidence change). The spreadsheet may be downloaded from:
http://www.sacema.com/page/assay-based-incidence-estimation

9.1 Formulae for calculating incidence and appropriate adjustments

9.1.1 Incidence calculated as a rate

To calculate the incidence as an annual instantaneous rate (I_r), the following formula should be used:

$$I_r = \frac{R - \varepsilon P}{(1-\varepsilon)\omega N}$$

where the survey counts (N, P, R) are specified as follows:

N is the number of HIV-negative people in the survey

P is the number of HIV-positive people in the survey, and

R is the number of people classified as RITA positive,

and the calibration parameters are specified as follows:

ω is the mean RITA duration specified in units of years, and

ε is the FRR of the RITA.

9.1.2 Incidence calculated as an annual risk of infection

Incidence as an annual rate and the annual risk of infection (I_a) are related by the following conversion formula:

$$I_a = 1 - \exp(-I_r)$$

9.1.3 Formula for calculating 95% confidence interval *(CI)*

Confidence intervals are computed using a delta method approximation which may include the error, assumed to be normally distributed, associated with calibration parameters. The coefficient of variation (C_v) is computed as follows:

$$C_v = \sqrt{\frac{1}{P}\left(\frac{N+P}{N} + \frac{(P-R)R[1+\varepsilon/(1-\varepsilon)]^2}{[R-\varepsilon/(1-\varepsilon)(P-R)]^2}\right) + \frac{\sigma_\omega^2}{\omega^2} + \frac{\sigma_\varepsilon^2(P-R)^2}{(1-\varepsilon)^4[R-\varepsilon/(1-\varepsilon)(P-R)]^2}},$$

where

σ_ω is the standard deviation of the mean RITA duration (assumed normally distributed), and
σ_ε is the standard deviation of the FRR (assumed normally distributed).

The 95% CI for I_r is then computed as:

$$I_r \pm 1.96 \times I_r C_v .$$

The corresponding CI for the annual risk of infection can be computed by using the conversion formula on each of the CI limits computed for the rate.

9.1.4 How to handle missing samples

Under certain circumstances, it may not be possible to test all the HIV-positive samples using the test for recent infection. This situation may happen if samples are missing or unavailable for testing due to other reasons (such as contamination of the sample or insufficient volume).

In the case where the samples are "missing completely at random", it is appropriate to exclude those samples for which a test for recent infection was not conducted and to scale down the number of HIV-negative samples appropriately. Suppose M is the number of HIV-positive samples with a missing RITA test, then the following scaled counts should be used in place of the counts N, P and R when using the incidence and CI formulae above:

$$\overline{N} = \frac{N(P-M)}{P}$$

$$\overline{P} = P - M$$

$$\overline{R} = R .$$

The rescaled counts ensure that the incidence is correctly computed and that the statistical significance of the result is computed conservatively.

If the samples are not "missing completely at random", then more sophisticated techniques, which are beyond the scope of this document, must be used.

Calculation of incidence: example from South Africa

A regional household survey aims to estimate the HIV incidence among people resident in rural KwaZulu-Natal, South Africa. An example of how to calculate the incidence based on counts from a cross-sectional survey is outlined below.

Step 1. Establish key data required for calculation
The key data required to calculate the incidence required for this study are outlined below.*

- Mean RITA duration = The study used the BED assay and quoted the mean RITA duration as 153 days (CoV of mean RITA duration, 5%)
- Estimated FRR = The study estimated the FRR as 1.69% (CoV of FRR, approximately 20%)
- The number of HIV-negative individuals in the survey = 9236
- The number of HIV-positive individuals in the survey = 2519
- The number of HIV-positive individuals classified as recent by the RITA = 165

Step 2. Enter data into the incidence calculator
The data obtained in step 1 can be used in the incidence worksheet of the spreadsheet (available at http://www.sacema.com/page/assay-based-incidence-estimation) to calculate incidence. For example:

Prevalence/Incidence Calculator	Inputs	Outputs	
Calculates point estimate and confidence intervals for Incidence and Annual Risk of Infection			
RITA/Assay Characteristics		Indicative 95% CIs, using input CoV	
Estimated RITA Duration/Mean Window Period (days)	153	(138.01 - 167.99)	
CoV (Coefficient of Variation) of RITA Duration Estimate	5.00%		
Estimated False Recent Rate (FRR)	1.69%	(0.88% - 2.50%)	
CoV of FRR Estimate	24.48%		
Sample Counts			
HIV negative	9236		
HIV positive	2519		
RITA positive (i.e. classified recent)	165		
Total sample size	11755		
Estimated Prevalence			
Prevalence (95% Confidence Interval)	21.43%	(20.69% - 22.18%)	
CoV of prevalence	1.77%		
Estimated Incidence			
Instantaneous incidence (95% Confidence Interval)	3.22%	(2.33% - 4.10%)	
CoV of incidence	14.09%		
Annual Risk of Infection (95% Confidence Interval)	3.17%	(2.30% - 4.02%)	

Step 3. Obtain incidence estimate and CI
The spreadsheet calculates the annual risk of infection as 3.17% with a 95% CI of 2.30–4.02%

*Example survey counts and parameter values taken from Barnighausen et al.2008. *(14)*

9.2 Incidence ratio calculation

To compute an incidence ratio (sometimes called the hazard ratio) as observed for two surveys, the following formula should be used:

$$H = \frac{(R_1 - \varepsilon P_1)N_2}{(R_2 - \varepsilon P_2)N_1},$$

where the survey counts are specified as follows:

N_1 is the number of HIV-negative people in the first survey,

P_1 is the number of HIV-positive people in the first survey,

R_1 is the number of people classified as RITA positive in the first survey,

N_2 is the number of HIV-negative people in the second survey,

P_2 is the number of HIV-positive people in the second survey, and

R_2 is the number of people classified as RITA positive in the second survey.

Note that the above formula does not depend on an estimate of the mean RITA duration. Calculating the CIs for the hazard ratio is beyond the scope of this document. Please refer to the spreadsheet mentioned above for calculating the CI for the hazard ratio (available at: http://www.sacema.com/page/assay-based-incidence-estimation).

Calculation of incidence ratio: example from Cambodia

Successive cross-sectional surveys carried out in 2000 and 2002 aimed to estimate the HIV incidence among women attending ANC in Cambodia. An example of how to calculate an incidence ratio based on counts from two successive cross-sectional surveys is outlined below.*

*Note: the survey counts and RITA parameters used in this example are fictitious to demonstrate how an incidence ratio would be calculated.

Step 1. Establish key data required for the calculation
The key data required to calculate the incidence ratio required for this study is outlined below.

- Estimated FRR = 3% (CoV of FRR=10%)
- The number of HIV-negative individuals in survey 1 = 5776
- The number of HIV-positive individuals in survey 1 = 144
- The number of HIV-positive individuals classified as recent by the RITA (survey 1) = 33
- The number of HIV-negative individuals in survey 2 = 6412
- The number of HIV-positive individuals in survey 2 = 179
- The number of HIV-positive individuals classified as recent by the RITA (survey 2) = 49

Step 2. Enter data into the incidence ratio calculator
The data obtained in step 1 can be used in the hazard ratio worksheet of the spreadsheet (available at http://www.sacema.com/page/assay-based-incidence-estimation) to calculate the incidence ratio. For example:

1													
2	**Hazard Ratio Calculator**						Inputs	Outputs					
3	Calculates the Hazard Ratio of incidences as observed in two surveys												
4													
5	**RITA/Assay Characteristics**							Indicative 95% CIs, using input CoV					
6	Estimated False Recent Rate (FRR)						3.00%	(2.41% - 3.59%)					
7	CoV (Coefficient of Variation) of FRR Estimate						10.00%						
8													
9	**Sample Counts**						**Survey 1**	**Survey 2**					
10	HIV negative						5776	6412					
11	HIV positive						144	179					
12	RITA positive (i.e. classified recent)						33	49					
13	Total sample size						5920	6591					
14													
15	**Estimates**						**Survey 1**	**Survey 2**					
16	Coefficient of Variation (excluding parameter uncertainty)						19.50%	15.63%					
17													
18	Incidence Ratio (95% Confidence Interval)						1.37	(0.70 - 2.04)					
19	CoV of Incidence Ratio						25.00%						

Step 3. Obtain incidence estimate ratio and CI
The spreadsheet calculates the incidence ratio as 1.37 with a 95% CI of 0.70–2.04.

10. Application of HIV incidence estimates

10.1 Calculation of incidence

Following the methods described in this document, with due attention to sampling frames, sample size, and reliable estimates of the mean RITA duration and RITA FRR, the outcome should be a valid estimate of HIV incidence, including an uncertainty expressed as a CI.

As emphasized in Chapter 2, this estimate will nonetheless have limitations that must be kept in mind while applying it to the planning and evaluation of public health programmes, or the selection of populations for prevention trials.

Furthermore, the other methods of incidence estimation outlined in Chapter 2 may also produce estimates that can be used in conjunction with those derived from the methods based on assays for recent HIV infection. Joint interpretation of the findings from multiple methods of determining the incidence should be informed by an understanding of the strengths and limitations of each method, and the extent to which the requirements of each method were met.*(13)*

10.2 Comparison of HIV incidence between two different populations for the same time period

Comparison of HIV incidence can be made between different populations for the same time period. This comparison can be of value in evaluating a preventive intervention that has been applied to one of the populations, with the other serving as a form of control. Population surveillance also aims to identify differences between demographic subgroups, for example, as defined by age or gender.

When comparing two different populations in this way, incidence or hazard ratios are the appropriate measure. Identical methods should be used to estimate the incidence in each of the groups being compared.

The sample size of each group being compared should be large enough for the comparison to be made with adequate power. In interpreting any differences in incidence, it is also important to consider the composition of the groups being compared with regard to potential confounding variables. For example, geographically distinct populations may also have different age structures.

10.3 Comparison of HIV incidence in the same population for several time periods

The same population may be compared over several time periods. This comparison can also lead to the estimation of a time trend.

HIV incidence in a population can also be compared at two time points to evaluate the impact of a prevention intervention. For example, following a nationwide HIV prevention campaign, the HIV incidence before and after the campaign may be compared to determine its effectiveness.

For this purpose, relative incidence can be estimated using the calculation of the incidence ratio, as described in Chapter 9, section 9.2.

When comparing the HIV incidence of the same population between two time periods, the following conditions should be met in order to ensure that the comparison is meaningful:

- HIV incidence is measured in the same way. For example, using an identical RITA, a relative incidence rate can be calculated in this instance as either an increase or a decrease in incidence rather than an absolute incidence.
- It may not always be possible to use the same assay for both serosurveys due to changes in availability or improvement in assays. In these circumstances, RITA FRR and mean RITA duration should be known for each RITA.
- Because there are also trends over time in the composition of a population (e.g. an increasing proportion of elderly in the population), adjustments must be made for such changes before concluding that there are real differences in the incidence of HIV over time.

Appendix 1. Specimen quality and handling requirements for testing

Generally, assays for recent infection use plasma or serum specimens. However, dried blood spots have been validated for use in some assays for recent infection. Since assays for recent HIV infection measure HIV-specific antibodies (such as quantity and avidity), it is important to ensure that the integrity of specimens is maintained through the process of preparation, storage and transport of specimens. This process will ensure that the results obtained are accurate and reliable. Specifications for appropriate preparation, storage, transport and condition of liquid specimens and dried blood spots are described below. Additional guidelines on specimen collection are available.*(28)*

A.1. Liquid ("wet") specimens

A.1.1. Preparation

Serum or plasma should be separated from whole blood cells by centrifugation within eight hours of being drawn from the patient.

If the blood specimen cannot be processed immediately (e.g. no centrifuge is available or the specimens are collected in the evening), collect the blood in a purple-top tube with ethylenediaminetetracaetic acid (EDTA). Allow the blood to stand for 20 or 30 minutes and then carefully remove the plasma with a pipette, trying not to draw up any red blood cells in the pipette. To avoid haemolysis, process and test the specimen within 24 hours.

A.1.2. Storage

Specimens should be refrigerated on the same day as they are drawn from the patient. The specimen should be either frozen immediately in a non-frost-free freezer at –20oC or below, or stored at 4oC for no longer than one week before freezing.

Long-term storage of specimens should be done at –70°C in a non-frost-free freezer.

A.1.3. Transport

During shipping to a reference laboratory, specimens should be maintained at 4oC or below.

Ensure that the caps on the cryovials are tight during transport to avoid spillage and cross-contamination.
A.1.4. Condition of the specimen

Compromised specimens such as those stored under suboptimal conditions should not be tested to detect recent infection because of degradation of antibodies.

Limit the number of freeze-and-thaw cycles to five because multiple thawing may affect antibody levels and therefore test results.

The reliability of assays for recent HIV infection using specimens that have been frozen and thawed multiple times, or which are grossly lipaemic, haemolysed or cloudy is not known.

A.2. Dried blood spots

Dried blood spots or dried serum or plasma spots have been validated as appropriate specimens for use in some assays for recent HIV infection, such as the BED capture enzyme immunoassay (BED-CEIA).

A.2.1. Preparation

Dried blood spot specimens should be prepared from blood specimens obtained either by a finger-prick or venepuncture (using a coagulant) on a grade 903 card, a specially manufactured absorbent specimen collection (filter) paper. Specimens should not be caked or clotted.

Specimens must be air dried for at least three hours in a horizontal position. Depending on the climate, it might be necessary to allow the spots to dry overnight. Do not stack blood spots. Do not allow blood spots to touch other surfaces while drying. Do not heat blood spots.

Once the blood spots are completely dry, they should be stacked between sheets of glassine or wax paper so that the cards do not touch each other.

A.2.2. Storage

Ten to 15 cards can be packaged in gas-impermeable zip-lock bags containing desiccant packs and humidity indicator cards.

For short-term storage, the dried spots can be stored at 4oC in zip-lock bags with desiccant. For storage for over 90 days, the dried spots should be kept in the freezer at –20oC or below. Properly stored dried blood spots have been shown to be stable for at least two years.

A.2.3. Transport

The bags should be placed in an extra strong, tear-proof, air-permeable and water-resistant envelope for shipment.*(29)*

Appendix 2. Overview of tests for recent HIV infection

To date, eight types of assays have been developed as tests for recent HIV infection. Some assays have been developed specifically for the purpose of identifying recent infection, while others are modifications of commercially available assays used as HIV diagnostic tests (Table A.2.1). With the exception of a few assays, most of the assays listed below have not been appropriately evaluated to obtain rigorous values of the mean RITA duration and the FRR in diverse HIV-1 subtypes.*(30–32)*

Less-sensitive enzyme immunoassay (EIA)
Most standard antibody assays for HIV infection can be modified for use as a test for recent infection, using the principle that antibody titres increase for several months following the acquisition of infection. Janssen and colleagues first described this approach based on the test produced by Abbott laboratories (3A11), modified to create a less-sensitive HIV antibody test.*(1)* Confirmed HIV-1-positive specimens are retested with an EIA that has been made less sensitive by diluting the plasma sample to 1/20 000 and by reducing incubation times. People with recent HIV infection and an early immune response have low anti-HIV antibody titres and therefore test negative in the less-sensitive EIA.

Since the development of the less-sensitive EIA, other assays have been modified in this way to estimate incidence. The two immunoassays which have been commercially modified as less-sensitive EIAs are the Abbott 3A11 and Avioq HIV-1 microelisa (formally marketed as BioMerieux Vironostika HIV microelisa). The Abbott 3A11 is now out of production. HIV rapid antibody tests have also been modified for detection of recent HIV infection.

All of the assays based on this principle have used antigen from a single HIV subtype (B) and have therefore not been considered reliable for other subtypes. The mean RITA duration differs among divergent subtypes resulting in limitations of the application of these assays in international settings.

Proportional assays
Proportional assays measure the proportion of all the immunoglobulin G (IgG) in a person's serum that is directed specifically against HIV, based on the principle that this proportion is lower in early infection than in a long-standing one. The BED-CEIA is based on this principle, and was designed exclusively for the identification of recent HIV infection.*(33)* The BED-CEIA is an IgG antibody capture EIA, and uses a synthetic HIV peptide representative of different subtypes (B, E and D).

Avidity assays
Avidity refers to the strength of the bond between the antigen (viral protein) and HIV-specific antibody. Avidity assays are based on the premise that antibodies of low avidity are suggestive of recent infection. Following the measurement of total anti-HIV response, a denaturing agent is added to separate out antibodies with weak affinity. An avidity index can then be calculated.

IDE-V3 assay (immunodominant assay)
The IDE-V3 assay is based on two conserved immunogenetic sequences found in the envelope glycoprotein of HIV-1. One is the immunodominant epitope (hence IDE) of gp41, which comprises two oligopeptides of 30 amino acids; one from group M and the other from subtype D. The second is from the V3 loop of gp120, which contains five oligopeptides from subtypes A, B, C, D and E. This assay uses a mathematical formula that combines the quantitative responses to antigens from each region to distinguish recent from established infection.

p24 antigen

The p24 antigen (p24Ag) is usually detectable within a few days after onset of HIV viraemia and before detectable HIV antibodies are present. The level of p24Ag usually falls as the host immune system initiates a response. Detection of p24Ag in the absence of anti-HIV antibody may be used as a marker of recent infection. However, its presence is unreliable and brief (1–2 weeks) and therefore the test has limited utility in detecting recent infection.

HIV RNA

Detection of RNA in the absence of anti-HIV antibody can also be used to identify recent HIV infection. As HIV RNA can be detected earlier than p24Ag, a longer time period for classification of recency can be used. *(2)* Additionally, testing of pooled HIV RNA leads to an increase in the accuracy of RNA amplification assays and significantly lowers testing costs. However, use of this method to determine HIV incidence requires very large sample population sizes.

IgG3 anti-p24

IgG isotypes formed in response to an infection may vary during the course of the infection. Isotype IgG3 is usually present transiently during the first few months of HIV-1 infection and the antigen against which the IgG3 response is most reliable is p24. A simple EIA-based procedure has been developed where IgG3 to p24Ag is typically detectable for only the first one to four months of infection. The findings from initial studies of this assay have not yet been generalized to different populations with different subtypes of HIV infection.

Line immunoassay

A line immunoassay is similar to a western blot but uses a limited range of synthetic oligopeptides and recombinant antigens of both HIV-1 and HIV-2. Routinely, this type of assay is used as a confirmatory test to validate the presence of antibodies again HIV. The Inno-LIA™ HIV I/II Score, a line immunoassay, can be used to interpret results as either recent or non-recent infection.*(34)* This assay is costly but may be of value in settings where it is routinely used as the confirmatory diagnostic test.

Table A.2.1. Summary of the types of assays for recent HIV infection

Assay type	Principle	Component of the anti-HIV immune response being measured	Limitations
Less-sensitive enzyme immunoassay (EIA)	A diluted blood sample is used to identify low anti-HIV antibody titre. Low antibody titre correlates with recent infection.	Quantity	• Limited to use in populations with predominantly 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 due to different mean RITA durations of assay with non-B subtypes. • A proportion of people with long-standing infection, severe immunosuppression or those who are on ART are misclassified as having recent HIV infection.
Proportional assay e.g. BED-CEIA	Measures the ratio of HIV-specific IgG to total IgG. This ratio increases in recent infection.	Proportion	• A proportion of people with long-standing infection, severe immunosuppression or those who are on ART are misclassified as having recent HIV infection.
Avidity index	After measuring total anti-HIV response, a denaturing agent is added to separate weak- from strong-affinity antibodies, and calculated as an avidity index. This index increases during recent infection.	Avidity	• A proportion of persons with long-standing infection, severe immunosuppression or those who are on ART are misclassified as having recent HIV infection.

Immunodominant assay e.g. IDE-V3 assay	Measures total response to select gp41 and gp120 epitopes that induce the most consistent antibody responses	Anti-gp41/anti-gp120 V3 immunodominant responses	• Assay has a low sensitivity.
p24 antigen	Detects p24Ag in the absence of anti-HIV antibody	Presence of p24Ag, absence of anti-HIV antibody	• The period when a person is p24Ag positive and anti-HIV antibody negative is brief (1–2 weeks). • Large sample populations are required to obtain incidence estimates.
HIV RNA	Detects HIV RNA in the absence of anti-HIV antibody	Presence of HIV RNA, absence of anti-HIV antibody	• Large sample populations are required to obtain incidence estimates.
Anti-p24 IgG3	Measures a narrow and temporary response to p24 in a single subclass of IgG that is seen consistently in recent infection	Anti-p24 response	• The findings have not yet been validated among different populations with HIV infection with divergent subtypes.
Line immunoassay e.g. INNO-LIA™ HIV I/II Score	Measures reactivity with various synthetic oligopeptides and recombinant antigens	Reactivity with various antigens	• The assay is expensive unless it is routinely used as the confirmatory test.

Table A.2.2. Specific assays* for determining recent HIV infection, along with relevant references and contact details of the manufacturers

Assay	Type of assay	Reference	Company	Contact details
BED-CEIA	Proportional assay	Parekh et al. (2002)*(33)*	Sedia Biosciences Corporation	Contact: Sedia Biosciences Corporation 4900 NE 122nd Avenue Portland, OR 97230, USA Phone: +1 (503) 459-4159 For orders: E-mail: customerservice@sediabio.com
			Calypte Biomedical Corporation	Contact: Calypte Biomedical Corporation 16290 SW Upper Boones Ferry Road Portland, OR 97224, USA Phone: +1 (877) 225-9783 E-mail: customerservice@calypte.com Web site: www.calypte.com
Avioq HIV-1 microelisa system (modified commercial)	Less-sensitive enzyme immunoassay (LS-EIA)	Kothe et al. (2003)*(35)*	Avioq	Contact: Avioq 9700 Great Seneca HWY, Suite 115 Rockville, Maryland 20850, USA Phone: +1 (301) 947-0202 E-mail: admin@avioq.com Web site: www.avioq.com
OraQuick Advance Rapid HIV-1/2 assay	LS-EIA (modified rapid test)	Kshatriya et al. (2008), Soroka et al. (2005)*(36,37)*	OraSure	Contact: OraSure Technologies Web site: www.orasure.com
Anti-HIV 1+2 (modified commercial)	Avidity	Chawla et al. (2007)*(38)*	Ortho Clinical Diagnostics	Contact: Web site: www.orthoclinical.com
AxSYM HIV- ½ gO (modified commercial)	Avidity	Suligoi et al. (2002) Suligoi et al. (2003)*(39,40)*	Abbott	Contact: Abbott Diagnostics Web site: www.abbottdiagnostics.com
Avidity index assay and Limiting Antigen Avidity EIA using rIDR-M	Avidity	Wei et al. (2010) *(41)*	In-house assay	Please see reference for details.

Biorad HIV1/2+O Avidity EIA (modified commercial)	Avidity	Masciotra et al. (2010)***(42)***	In-house assay	Please see reference for details.
IDE-V3	Immunodominant assay	Barin et al. 2005***(43)***	In-house assay	Please see reference for details.
Anti-p24 IgG3	Anti-p24 IgG3	Wilson et al. 2004***(44)***	In-house assay	Please see reference for details.
INNO-LIA™ HIV I/II Score (modified commercial)	Line immunoassay	Schupbach et al. 2007***(34)***	Innogenetics	Contact : Innogenetics Phone: + 32-9 329 16 11 E-mail: customer_support@innogenetics.com Web site: www.innogenetics.be

*Not all commercially manufactured assays are available in all countries. Contact the regional offices of each company for more information on the availability of specific assays.

Appendix 3. Reported FRRs by RITA and population

Reported FRR by RITA and population	Year of survey	Major HIV subtypes	Sample size	Estimate of FRR	95% CI	CoV*	Reference
FRR for BED capture enzyme immunoassay							
Persons who inject drugs in China, Province A	2002–2005	A, B, Thai B, C, D, F, G, A/E, B/C	300	6.6	3.8, 9.4	21.65%	Xiao et al. (2007) ***(45)***
Pre-ART patients, San Salvador, El Salvador	2008	B	150	10.7	5.8, 15.6	23.36%	Ministry of Health, El Salvador (2009) ***(46)***
Pre-ART high-risk women cohort, Kigali, Rwanda	2006–2008	A, C, D	141	3.6	1.2, 8.1	48.89%	Braunstein et al. (2010)***(47)***
KwaZulu-Natal, South Africa	2003–2006	C	1065	1.7	1.0, 2.7	25.51%	Barninghausen et al. (2008)***(14)***
Pre-ART patients, Tygerberg, South Africa	2004–2006	C	430	11.2	8.3, 14.5	14.12%	Marinda et al. (2009)***(48)***
MSM in AIDSVAX B/B vaccine trial, US and Amsterdam	1999–2003	B	150	5.7	1.6, 9.8	36.70%	McDougal et al. (2006)***(16)***
Pre-ART participants in home-based AIDS cohort, Tororo, Uganda	2002–2005	A, D	226	12.4	8.4, 17.4	18.52%	Hladik et al. (2007)***(49)***
Pre-ART participants in the Rakai Community Cohort, Rakai, Uganda	2002–2003	A, D	473	16.1	12.8, 19.4	10.46%	Laeyendecker et al. (2009)***(50)***
Pre-ART patients, Ho Chi Minh City, Viet Nam	2009–2010	A/E	716	0.8	0.2, 1.5	41.45%	Tuan et al. (2010) ***(17)***
Pre-ART patients, northern provinces, Viet Nam	2009–2010	A/E	568	5.1	3.3, 6.9	17.51%	Tuan et al. (2010) ***(17)***
Pre-ART post-partum women participating in the ZVITAMBO cohort, Harare, Zimbabwe	1997–2000	C	2749	5.2	4.4, 6.1	8.34%	Hargrove et al. (2008)***(15)***
HIV-infected injecting drug users participating in the ALIVE cohort in Baltimore, Maryland , USA	1991–2007	B	488	10.2	7.5, 12.9	32.26%	Laeyendecker et al. (2010)***(51)***

FRR for the AxSYM HIV-1/2 gO Avidity index assay							
Pre-ART high-risk women cohort, Kigali, Rwanda	2006–2008	A, C, D	141	10.6	6.1, 17.0	69.24%	Braunstein et al. (2010)***(47)***
FRR for BED-CEIA and AxSYM HIV 1/2 gO Avidity index assay combined algorithm							
Pre-ART high-risk women cohort, Kigali, Rwanda	2006-2008	A, C, D	141	2.1	0.4, 6.1	69.24%	Braunstein et al. (2010)***(47)***
FRR for BED-CEIA and Biorad HIV1/2+O Avidity enzyme immunoassay combined algorithm							
Pre-ART participants in the Rakai community cohort, Rakai, Uganda	2002–2003	A, D	473	0.8	0, 1.6	51.02%	Laeyendecker et al. (2009)***(50)***
Pre-ART participants in the home-based AIDS care programme in Tororo district, Uganda	2002–2005	A, D	224	0.4	0, 1.2	76.53%	Hladik et al. (2007)***(49)***

* As a general rule, for an unbiased estimate of incidence, the CoV for FRRs should not exceed 30%. However, in the case of a low FRR (≤1.0%), this rule of thumb may be relaxed, provided that the upper bound of the 95% CI for the FRR estimate is ≤1.5%.

Appendix 4. Example sample size charts for estimating HIV incidence

Table A.4.1. Minimum sample sizes needed to estimate incidence with a RITA with a CoV of 30%, by level of prevalence, incidence and FRR[a]

Prevalence (%)	Incidence (%)	FRR (%)	10% FRR CoV		20% FRR CoV		30% FRR CoV	
			Sample size, incidence survey	Sample size, FRR survey[b]	Sample size, incidence	Sample size, FRR survey[b]	Sample size, incidence survey	Sample size, FRR survey[b]
25.00	2.50	1.00	1 992	9 900	2 065	2 476	2 198	1 100
		2.50	2 910	3 900	3 833	976	8 131	436
		5.00	5 754	1 900	*	476	*	212
		7.50	20 535	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
20.00	2.00	1.00	2 292	9 900	2 364	2 476	2 495	1 100
		2.50	2 916	4 900	3 347	1 228	4 443	548
		5.00	6 120	1 900	*	476	*	212
		7.50	16 859	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
15.00%	1.50	1.00	2 828	9 900	2 907	2 476	3 047	1 100
		2.50	3 967	3 900	4 853	976	7 729	436
		5.00	7 076	1 900	160 444	476	*	212
		7.50	16,600	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
10.00	1.00	1.00	3 947	9 900	4 043	2 476	4 215	1 100
		2.50	5 443	3 900	6 487	976	9 538	436
		5.00	9 342	1 900	51 843	476	*	212
		7.50	19 618	1 236	*	312	*	140
		10.00	276 896	900	*	228	*	100
5.00	0.50	1.00	7 374	9 900	7 535	2 476	7 819	1 100
		2.50	10 018	3 900	11 690	976	16 198	436
		5.00	16 643	1 900	57 781	476	*	212
		7.50	32 215	1 236	*	312	*	140
		10.00	150 695	900	*	228	*	100
2.50	0.25	1.00	14 277	9 900	14 572	2 476	15 091	1 100
		2.50	19 260	3 900	22 271	976	30 120	436
		5.00	31 537	1 900	94 463	476	*	212
		7.50	59 044	1 236	*	312	*	140
		10.00	219 727	900	*	228	*	100

[a]Assumes a mean RITA duration of 150 days with a CoV = 5% and baseline incidence that is 10% of prevalence

[b] Sample of long-standing HIV infections, defined as an infection period longer than twice the mean RITA duration

* In these instances, it is not possible (at any sample size) to obtain an incidence estimate with a CoV equal to, or less than, 30%.

Table A.4.2. Minimum sample sizes needed to estimate incidence with a RITA with a CoV of 30%, by level of prevalence, incidence and FRR[a]

Prevalence (%)	Incidence (%)	FRR (%)	10% FRR CoV		20% FRR CoV		30% FRR CoV	
			Sample size, incidence survey	Sample size, FRR survey[b]	Sample size, incidence	Sample size, FRR survey[b]	Sample size, incidence survey	Sample size, FRR survey[b]
25.00	5.00	1.00	873	9 900	880	2 476	893	1 100
		2.50	1 065	3 900	1 125	976	1 241	436
		5.00	1 458	1 900	1 912	476	3 976	212
		7.50	2 025	1 236	5 507	312	*	140
		10.00	2 988	900	*	228	*	100
20.00	4.00	1.00	1 010	9 900	1 018	2 476	1 030	1 100
		2.50	1 146	4 900	1 181	1 228	1 243	548
		5.00	1 638	1 900	2 060	476	3 609	212
		7.50	2 221	1 236	4 792	312	*	140
		10.00	3 154	900	*	228	*	100
15.00	3.00	1.00	1 253	9 900	1 261	2 476	1 274	1 100
		2.50	1 496	3 900	1 559	976	1 677	436
		5.00	1 979	1 900	2 409	476	3 774	212
		7.50	2 632	1 236	4 882	312	*	140
		10.00	3 624	900	*	228	*	100
10.00	2.00	1.00	1 756	9 900	1 766	2 476	1 782	1 100
		2.50	2 078	3 900	2 155	976	2 297	436
		5.00	2 710	1 900	3 213	476	4 653	212
		7.50	3 543	1 236	5 909	312	*	140
		10.00	4 759	900	32 078	228	*	100
5.00	1.00	1.00	3 294	9 900	3 310	2 476	3 337	1 100
		2.50	3 866	3 900	3 993	976	4 224	436
		5.00	4 978	1 900	5 779	476	7 894	212
		7.50	6 413	1 236	9 882	312	100 131	140
		10.00	8 440	900	31 795	228	*	100
2.50	0.50	1.00	6 387	9 900	6 417	2 476	6 467	1 100
		2.50	7 469	3 900	7 700	976	8 119	436
		5.00	9 561	1 900	10 997	476	14 670	212
		7.50	12 237	1 236	18 253	312	101 105	140
		10.00	15 959	900	50 673	228	*	100

[a]Assumes a mean RITA duration of 150 days with a CoV = 5% and baseline incidence that is 10% of prevalence

[b]Sample of long-standing HIV infections, defined as an infection period longer than twice the mean RITA duration

* In these instances, it is not possible (at any sample size) to obtain an incidence estimate with a CoV equal to, or less than, 30%.

Table A.4.3. Minimum sample sizes needed to estimate incidence with a RITA with a CoV of 20%, by level of prevalence, incidence and FRR[a]

Prevalence (%)	Incidence (%)	FRR (%)	10% FRR CoV		20% FRR CoV		30% FRR CoV	
			Sample size, incidence survey	Sample size, FRR survey[b]	Sample size, incidence	Sample size, FRR survey[b]	Sample size, incidence survey	Sample size, FRR survey[b]
25.00	2.50	1.00	4 723	9 900	5 150	2 476	6 064	1 100
		2.50	7 605	3 900	20 505	976	*	436
		5.00	34 210	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
20.00	2.00	1.00	5 422	9 900	5 844	2 476	6 717	1 100
		2.50	7 217	4 900	10 598	1 228	48 316	548
		5.00	28 737	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
15.00	1.50	1.00	6 680	9 900	7 134	2 476	8 045	1 100
		2.50	10 074	3 900	18 777	976	*	436
		5.00	28 706	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
10.00	1.00	1.00	9 307	9 900	9 863	2 476	10 954	1 100
		2.50	13 679	3 900	22 977	976	*	436
		5.00	34 293	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
5.00	0.50	1.00	17 372	9 900	18 291	2 476	20 062	1 100
		2.50	24 962	3 900	38 793	976	506 800	436
		5.00	56 811	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100
2.50	0.25	1.00	33 615	9 900	35 295	2 476	38 502	1 100
		2.50	47 813	3 900	71 970	976	455 726	436
		5.00	104 535	1 900	*	476	*	212
		7.50	*	1 236	*	312	*	140
		10.00	*	900	*	228	*	100

[a]Assumes a mean RITA duration of 150 days with a CoV = 5% and baseline incidence that is 10% of prevalence
[b]Sample of long-standing HIV infections, defined as an infection period longer than twice the mean RITA duration
* In these instances, it is not possible (at any sample size) to obtain an incidence estimate with a CoV equal to, or less than, 30%.

Table A.4.4. Minimum sample sizes needed to estimate incidence with a RITA with a CoV of 20%, by level of prevalence, incidence and FRR[a]

Prevalence (%)	Incidence (%)	FRR (%)	10% FRR CoV		20% FRR CoV		30% FRR CoV	
			Sample size, incidence survey	Sample size, FRR survey[b]	Sample size, incidence	Sample size, FRR survey[b]	Sample size, incidence survey	Sample size, FRR survey[b]
25.00	5.00	1.00	2 045	9 900	2 085	2 476	2 154	1 100
		2.50	2 545	3 900	2 915	976	3 848	436
		5.00	3 803	1 900	9 996	476	*	212
		7.50	6 571	1 236	*	312	*	140
		10.00	19 788	900	*	228	*	100
20.00	4.00	1.00	2 365	9 900	2 405	2 476	2 474	1 100
		2.50	2 710	4 900	2 910	1 228	3 319	548
		5.00	4 204	1 900	8 866	476	*	212
		7.50	6 806	1 236	*	312	*	140
		10.00	15 691	900	*	228	*	100
15.00	3.00	1.00	2 932	9 900	2 976	2 476	3 051	1 100
		2.50	3 555	3 900	3 934	976	4 782	436
		5.00	5 016	1 900	9 153	476	*	212
		7.50	7 723	1 236	*	312	*	140
		10.00	15 225	900	*	228	*	100
10.00	2.00	1.00	4 108	9 900	4 161	2 476	4 254	1 100
		2.50	4 927	3 900	5 383	976	6 366	436
		5.00	6 797	1 900	11 192	476	*	212
		7.50	10 057	1 236	*	312	*	140
		10.00	17 867	900	*	228	*	100
5.00	1.00	1.00	7 702	9 900	7 791	2 476	7 944	1 100
		2.50	9 150	3 900	9 894	976	11 444	436
		5.00	12 378	1 900	18 880	476	151 674	212
		7.50	17 731	1 236	597 319	312	*	140
		10.00	29 238	900	*	228	*	100
2.50	0.50	1.00	14 935	9 900	15 098	2 476	15 377	1 100
		2.50	17 664	3 900	19 012	976	21 785	436
		5.00	23 684	1 900	35 009	476	172 358	212
		7.50	33 453	1 236	338 274	312	*	140
		10.00	53 537	900	*	228	*	100

[a]Assumes a mean RITA duration of 150 days with a CoV = 5% and baseline incidence that is 10% of prevalence

[b]Sample of long-standing HIV infections, defined as an infection period longer than twice the mean RITA duration

* In these instances, it is not possible (at any sample size) to obtain an incidence estimate with a CoV equal to, or less than, 30%.

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Glossary of terms

Assay or test for recent infection: a laboratory method that is used in a recent infection testing algorithm (RITA) to classify HIV infection as recent, for the purposes of estimating incidence. Such assays generally produce a quantitative read out, for which a cut-off point is set to define whether the infection is classified as recent or not.

Calibration of an assay for recent infection involves the use of seroconversion panels to define the assay cut-off point that will give rise to the mean RITA duration of the assay. The standard error of the mean RITA duration can also be derived through the process of calibration.

Coefficient of variation: a measurement of the precision (or reproducibility) of a laboratory test or process. Precision is measured by a coefficient of variation which is expressed as a percentage. A higher (lower) coefficient of variation corresponds to a lower (higher) precision for the survey estimate.

Concentrated epidemic: an epidemic state in which HIV has a low prevalence in the general population, but has spread rapidly in defined subpopulations, confined primarily to people who engage in high-risk behaviour. In countries with concentrated epidemics, HIV prevalence is <1% in the general population and >5% in a least one defined population, such as MSM, people who inject drugs and sex workers.

Cross-sectional survey: a survey used to gather information on a population or sample of a population at a single point in time

Denominator: the lower portion of a fraction used to calculate a rate or ratio

Enumeration area (EA): the spatial area used by Statistics South Africa to collect census information on the South African population. An enumeration area consists of approximately 180 households in urban areas and 80–120 households in rural areas.

Enzyme immunoassay (EIA): an HIV test that identifies the presence of antibodies to HIV

Established infection: infection that lasts for longer than the mean RITA duration. It is greater than approximately six to 12 months post HIV infection, and may also include long-standing infection.

False recent rate (FRR): the fraction of non-recent infections that are incorrectly classified as "recent" as a result of applying a RITA

Generalized epidemic: the epidemic state in which HIV is established in the general population. In a generalized epidemic, the HIV prevalence in the general population is >1%.

Gp120: a glycoprotein exposed on the surface of the HIV envelope. Gp120 is essential for virus entry into cells as it binds to surface receptors on CD4 cells.

Incidence (HIV incidence): the rate at which new cases of HIV infection occur in a population per unit of time. In calculating the incidence, the numerator is the number of new HIV cases occurring in a population in a given time period and the denominator is the total population at risk during that time.

Incidence ratio: the ratio of the incidence in one population to the incidence in another population

Mean RITA duration: the average time that elapses between the acquisition of HIV infection and the classification of the infection as non-recent by the RITA

Numerator: the upper portion of a fraction used to calculate a rate or ratio

Prevalence: the percentage of people in a given population with a condition or disease at any time during a specific period

Recently acquired HIV infection: A state that begins at the moment when the biological process of HIV infection is first initiated. Its duration can be defined in purely chronological terms, e.g. six months after the moment infection was initiated; or in biological terms, on the basis of an observable biomarker that is present at the initiation of infection and then disappears (or vice versa). Under the biological definition, the duration of recency will vary among individuals.

Reporting delay: the time between diagnosis and the date on which the case is registered in the surveillance system

Recent infection testing algorithm (RITA): a laboratory test or combination of tests, or combination of tests and clinical information, intended to classify people as either having or not having recently acquired HIV infection, for the purposes of estimating HIV incidence. The algorithm is applied only to people who are already confirmed as having HIV infection by a recognized assay for anti-HIV antibody or HIV RNA or HIV DNA.

Sample: a selected subset of a population

Sample, representative: a sample of people whose characteristics correspond to those of the original or reference population

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