# Using recency assays for HIV surveillance

## 2022 technical guidance

# Contents

# Acknowledgments

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# Abbreviations

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|---|---|
| Ag/Ab | antigen/antibody |
| AIDS | acquired immunodeficiency syndrome |
| CDC | United States Centers for Disease Control and Prevention |
| CEIA | capture enzyme immunoassay |
| CEPHIA | Consortium for the Evaluation and Performance of HIV Incidence Assays |
| CI | confidence interval |
| FIND | Foundation for Innovative Diagnostics |
| FRR | false recent ratio (or false recent rate) |
| HIV | human immunodeficiency virus |
| IgG | immunoglobulin G |
| LAg | limiting antigen |
| MCAR | missing completely at random |
| MDRI | mean duration of recent infection |
| PEP | post-exposure prophylaxis |
| PEPFAR | United States President's Emergency Plan for AIDS Relief |
| PHIA | population-based HIV impact assessment |
| PrEP | pre-exposure prophylaxis |
| RITA | recent infection testing algorithm |
| TRACE | Tracking with Recency Assays to Control the Epidemic |
| UNAIDS | Joint United Nations Programme on HIV/AIDS |
| UPHIA | Uganda population-based HIV impact assessment |
| WHO | World Health Organization |

# Executive summary

Recency assays use one or more biomarkers to identify whether HIV infection in a person is recent (usually within a year or less) or longstanding. Recency assays have been used to estimate incidence in representative cross-sectional surveys and in epidemiological studies to better understand the patterns and distributions of new and longstanding HIV infections.

Surveillance strategies to accurately estimate HIV incidence are critical to global efforts to end the AIDS epidemic. Detecting recent HIV infections can be a useful addition to surveillance when analysed appropriately. Uses of recency information include the following:

‣ To better understand the current transmission patterns of HIV in a specific country, geographical area or subpopulation (i.e. to recognize areas where people have more recently acquired HIV).

‣ To monitor the proportion of the population living with HIV who are diagnosed early versus late in infection.

There are a number of advantages to using recency assays as part of representative population-based surveys or programmatic settings with high HIV status ascertainment and consistent testing, such as antenatal clinics:

‣ They can be used to estimate incidence (including trends over time) when used as part of representative cross-sectional surveys. This is a major advantage because cross-sectional surveys are much less expensive and easier to implement than the more classic strategy of observing incidence through a longitudinal cohort.

‣ They can be used to directly measure recency in a large number of people. Without recency assays, this is typically done by relying on models or other projections of incidence, which are often extrapolated from a much smaller group of people, or require assumptions based on other studies. Using recency assays also provides an alternative to surveys when HIV incidence reaches a low level, when surveys would be less cost-effective for this purpose.

‣ They can be used to identify possible geographical or demographic subpopulation with higher proportions of recent infections than other areas. Although this must be done carefully to ensure the findings are being interpreted correctly, this type of strategy to identify populations with high levels of recent HIV infection can allow for programmatic intervention and effective prioritization of limited resources. For example, in a country with a generalized epidemic with one dominant HIV subtype, a similar recent infection testing algorithm (RITA) in use countrywide, and high testing rates in antenatal care settings, comparing the proportion of people testing recent in antenatal clinics in different cities or provinces could be informative.

Recency assays come with a number of challenges and potential biases. When used within representative surveys for purposes of incidence estimation, the following considerations apply:

‣ Very large sample sizes are required. The sample size required increases as incidence decreases, which causes more problems as HIV incidence in a country reaches a low level.

‣ Incidence estimates can be biased if calculations use parameters that are not applicable to the local context. Incidence estimation using recency assays requires both a mean duration of recent infection (MDRI) and a false recent ratio (FRR) for the RITA being used. If an inappropriate MDRI and FRR are chosen (e.g. those used in another setting with a much different epidemic), this could bias incidence estimates upwards or downwards.

These challenges are well-documented for incidence estimation, and best practices are described in the literature for use of recency assays for this purpose.

Recency assays used within HIV testing services or case surveillance come with some challenges and warnings:

‣ There are potential biases when interpreting recency results from HIV testing services, which may result in misunderstandings or mistaken appropriation of resources. One of the fundamental challenges with recency testing as part of HIV testing programmes is that people who access services are not randomly selected from the greater population. For example, a higher proportion of recent infections in a particular age, gender or sub-population might indicate higher HIV incidence for that group, or it might indicate differences in HIV testing behaviour, mobility, use of pre-exposure prophylaxis (PrEP) or antiretroviral therapy, or other factors. Similarly, associating the recency results with particular key populations might be biased by self-reporting of behaviours that are heavily stigmatized or criminalized. Recency findings from programme data should be interpreted with caution and analysed separately for different populations, and all analyses should be done only within the context of that specific population.

‣ The recency testing assay or RITA being used may be complex, expensive or difficult to run with high quality in a programmatic setting. It is important that recency assays used in programmatic settings are conducted according to good laboratory practices, with test technicians receiving initial and ongoing training, and with quality assurance programmes in place. Assays must not be used on their own to determine recency but rather as part of a RITA that incorporates additional clinical data (at a minimum, viral load) to help reclassify people with a false recent result on their assay.

‣ There may be a risk associated with collecting recency data if confidentiality or criminalization is an issue. Extra care must be given to how recency results will be stored and who will have access to them. It is important to recognize that there is currently no evidence to support use of recency results for prioritizing initiation of antiretroviral therapy, partner services, index testing or additional services.

This guidance provides several best practices to guide the use of recency assays for surveillance purposes. Some of the major updates to previous guidance include the following best practices:

‣ RITAs must include additional clinical data to identify and screen out false recent assay results. At a minimum, viral load results must be incorporated into the RITA.

- Assay parameters for incidence estimation (MDRI and FRR) must be based on the local context. Published numbers from other contexts should not be used without careful consideration.

- Recency results from nonrepresentative (programmatic) settings should be interpreted with caution. Recency indicators calculated from programmatic settings should be compared only when it is known that antiretroviral therapy coverage, HIV testing coverage, PrEP use (including long-acting injectable PrEP) and subtype are comparable through time or space, and the RITA being used is consistent in both settings.

- When calculating a "proportion recent" from programmatic settings to be used as a proxy for HIV incidence, the denominator should be all people at risk for recent infection (those testing recent + those testing HIV-negative), rather than only people who tested positive for HIV at the site.

Recency testing is a complex undertaking and has demonstrated utility in population-based surveys to measure HIV incidence. Use of recency testing in programmatic settings, however, should be considered only when existing HIV testing coverage of the population being studied is high, a cost-effective RITA can be implemented to reduce the number of false recent results, and analysis plans make appropriate statistical adjustments and infer population-specific trends in recent infection.

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Ultimately, countries must ask a series of questions before implementing a recency testing initiative:

- How will results be used, and is there an easier or more cost-effective way to meet those aims?

- If recency testing will be used, what is the most cost-effective RITA that will effectively answer the questions at hand?

- Will the programme or study design allow accurate interpretation of the recency results to answer these questions, and how will the findings be presented to protect and improve health for and with members of civil society?

- What is the practical ability to respond to the results of recency testing/surveillance and over what time frame?

- Will the results be sufficiently robust to inform program decisions even when triangulated with additional data sources?

- Does the country and programme have the programme, lab and data analysis capacity to implement a recency initiative?

# Introduction

Surveillance strategies to accurately estimate HIV incidence or detect patterns of recent transmission are critical to global efforts to end the HIV epidemic. These calculations are useful, however, only if they are timely and accurate, with potential biases clearly defined and accounted for. Calculations that are considerably higher or lower than reality (i.e. biased estimates) may result in incorrect interpretations of the data and potential misalignment of resources.

Since the release of the last technical guidance from the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) on the use of HIV recency assays in 2011 *(1)*, the field of HIV recency testing has changed substantially. Many of the assays that were available in 2011 are no longer available or have been replaced by other, newer technologies. Several challenges have been identified related to the performance of recency assays when used for population-based incidence estimation, including the impact of undisclosed use of antiretroviral medicines or pre-exposure prophylaxis (PrEP), and the significant impact of epidemiological context on assay performance.

Many recency assays are simple to use, but interpretation and use of the results is often not as easy as for diagnostic tests. For example, the non-random nature by which people access HIV testing programmes requires special attention to reduce the effect of systematic biases on the accuracy of estimates derived from the use of recency assays within HIV testing services sites. With this in mind, this updated document provides guidance to assist country programmes in using recency assays for surveillance purposes according to best practices based on currently available evidence.

## Focus of this guidance

This technical guidance outlines best practices regarding the appropriate use of HIV recency assays for surveillance purposes within:

‣ Population-based surveys for estimating HIV incidence.

‣ Programme data from HIV testing services *(2)* for population- or programme-level monitoring.

This guidance also identifies uses of recency testing for which there is a lack of high-quality supporting evidence in the published literature. The methods and summary of results of a systematic review of evidence around recency testing have been published separately *(3)*. This guidance is intended to complement rather than supplant the UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance guidance on monitoring HIV impact using population-based surveys *(4)*. It does not change existing WHO consolidated guidelines on HIV testing services, which focus on clinical HIV testing services programmes.

This guidance does not cover the use of recency testing for clinical management of people living with HIV, or risk-network service differentiation of people who receive a recency test, or their partners. Clinical management includes the return of results to clients, counselling messages about recent infection, prioritizing initiation of antiretroviral therapy and additional services based on recency results, and prioritizing or altering partner services and index testing based on recency results.

The intended audience of this guidance is both planners of HIV surveillance (Parts 1–3) and implementers of recency surveillance (Part 4). A summary of the best practices is available in Annex 1.

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WHO does not recommend the use of recency testing for the clinical management of people living with HIV or their partners, as there is currently insufficient evidence of their clinical utility. Information on clinical HIV testing guidelines can be found in two consolidated HIV guidelines *(5, 6)*.

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## Structure of this guidance

This guidance has been organized into four parts.

**Part 1** focuses on designing a surveillance strategy, including determining the setting, population of interest, and most appropriate recent infection testing algorithm (RITA) to use.

**Part 2** focuses on strategies for maximizing accuracy of the data collected using a RITA, including minimizing selection bias of people being tested with recency assays, properly collecting and transporting specimens for testing, and preparing for HIV incidence estimation by determining the most appropriate mean duration of recent infection (MDRI) and false recent ratio (FRR) to be used for the local context.

**Part 3** focuses on interpreting and reporting findings from these analyses, whether HIV incidence has been estimated or other indicators of recency have been calculated from HIV testing services programme data.

**Part 4,** which is intended for epidemiologists and statisticians who are using recency assay or RITA results to estimate HIV incidence, or who are assisting with the design of studies or surveillance programmes that incorporate recency assays for surveillance use cases.

## History and challenges of using recency assays

Recency assays use one or more biomarkers to identify whether an HIV infection in a person is recent or longstanding. These assays typically use a measure of the evolution of the immune response following initial infection. The accuracy of estimates of HIV incidence depends on the accuracy of two parameters of the algorithm being used: MDRI (average time after infection that people are classified as recently infected) and FRR (proportion of people with longstanding infection misclassified as recently infected). The precision of the estimate (the certainty that exists about its value) is sensitive to these same parameters *(7)*.

To explore the challenges in accurate HIV incidence estimation using recency assays, the Incidence Assay Critical Path Working Group was convened by WHO and UNAIDS.

In 2011 the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) was formed to encourage development of new, improved recency assays and independently evaluate the performance of existing recency assays to determine whether any assays met a predetermined target product profile *(8, 9)*.

As of July 2021, there are nine commercially available recency assays (Table 1), including two that are immunochromatographic (lateral flow) formats of the limiting antigen (LAg) avidity test, designed for point-of-care use. Other assays that are locally validated or were previously commercially available have also been evaluated by CEPHIA *(10, 11)*. Note there is no WHO prequalified recency assay, as these tests are for surveillance use only and the WHO prequalification process is limited to diagnostic tests.

**Table 1.**
Commercially available recency assays, 2022

| Product | Manufacturer | Assay type |
|---------|-------------|------------|
| Asanté™ HIV-1 Rapid Recency® Assay | Sedia Biosciences | Rapid, point of care |
| Maxim Swift™ HIV Recent Infection Assay | Maxim Biomedical | Rapid, point of care |
| Sedia® HIV-1 Limiting Antigen (LAg)-Avidity EIA | Sedia Biosciences | Laboratory-based |
| Maxim HIV-1 Limiting Antigen Avidity (LAg-Avidity) EIA | Maxim Biomedical | Laboratory-based |
| Genetic SystemsTM HIV-1/HIV-2 PLUS O EIA [a] | Bio-Rad Laboratories | Laboratory-based |
| ARCHITECT HIV Ag/Ab Combo [a] | Abbott Laboratories | Laboratory-based |
| VITROS® Anti-HIV 1+2 [a] | Ortho Clinical Diagnostics | Laboratory-based |
| Geenius™ HIV 1/2 Supplemental Assay [a] | Bio-Rad Laboratories | Laboratory-based |
| INNO-LIA® HIV I/II Score [a] | Fujirebio | Laboratory-based |

[a] *For recency determination, these assays must be used in a way that deviates from the manufacturer's instructions for use.*

No recency assay has fully met the target product profile for HIV incidence estimation developed by the Foundation for Innovative Diagnostics (FIND) and WHO in 2016 *(9, 12)*. Several factors have been identified that adversely affect recency assay performance (typically by raising the FRR), including elite control of HIV (i.e. natural viral suppression), HIV-1 subtype, specimen type, advanced HIV disease, and use of antiretroviral therapy or PrEP *(8, 13–17)*. The effect of antiretroviral therapy on recency assay FRRs appears to be more pronounced when antiretroviral therapy is started very soon after infection *(18, 19)*. This impact is being increasingly felt due to the impressive advancements worldwide in antiretroviral therapy coverage, policy changes supporting same-day or early initiation of antiretroviral therapy upon diagnosis and increasing use of PrEP and post-exposure prophylaxis (PEP). These challenges may be exacerbated further when long-acting injectable antiretroviral medicines and PrEP and other future treatment innovations become widely available. Other potential factors that impact assay MDRI and FRR have been raised but are not confirmed, including sex, pregnancy and comorbidities *(20–22)*.

Annex 2 outlines the performance, strengths and weaknesses of some of the commercially available laboratory-based recency assays, as evaluated by CEPHIA in 2016 *(8)*, according to the FIND target product profile.

Multiple strategies have been suggested to address the challenges to recency assay performance, including making statistical adjustments to the aggregated recency results, and using multiple types of recency assays or other contextual information in a recent infection testing algorithm or RITA (23, 24).

## Consensus terminology for describing HIV recency assays

For consistency, Table 2 summarizes the consensus terms for describing HIV recency assays and their use for HIV surveillance purposes.

**Table 2.**
Consensus terminology and definitions for use of recency assays

| Term | Definition |
|---|---|
| Recent (or recently acquired) HIV infection | State that begins at the moment HIV infection begins. Its duration can be defined in chronological terms (e.g. 6 months after the moment infection occurred) 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 between people. The definition of recent infection can also include elements intended to reduce false recent classifications, such as no previous HIV diagnosis or absence of antiretroviral medicines detected in the blood, which can impact the duration of recency. The term "incident infection" is sometimes used synonymously with recent infection, but its use is less standardized and is susceptible to misinterpretation. |
| Acute HIV infection | HIV infection where viral RNA or viral antigen (p24 protein) is detectable, but anti-HIV antibodies have not yet developed. This state lasts from shortly after exposure to several days to weeks after infection, depending on the methods of viral RNA, viral antigen and antibody detection. When acute HIV infection is identified, it can be considered a recent HIV infection without relying on a recency assay. |
| Longstanding HIV infection | State that begins when a person transitions out of the recent state and is considered to have HIV infection of longer duration. As with recent HIV infection, the time at which a person transitions to the longstanding state can be defined in purely chronological terms or in biological terms. The terms "long-term" and "longstanding" infection are used interchangeably. |
| Recency assay | Assay used to classify a case of HIV infection as recent or longstanding. The previously used term "incidence assay" has fallen out of favour because these assays may be used for purposes other than incidence calculation. |
| RITA | A combination of one or more assays and clinical information (usually viral load) used to classify an HIV infection as being recently acquired or longstanding. |
| MDRI | Average duration of the recent state among people infected for less than a specified cutoff time (T) for a specific RITA in a population of people living with HIV. This parameter is essential for estimating HIV incidence with a RITA. Ideally within the range of 4–12 months, the MDRI for a RITA can vary according to the specific RITA being used and, for each RITA, may vary by HIV subtype. MDRIs are not constant but are context-dependent. The MDRI was previously known as the "window period" or "incidence window period" for a recency assay, but this term 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 serology assays; therefore, these terms should be avoided. |

| Term | Definition |
|------|------------|
| FRR | Proportion of people with longstanding HIV infection (infected for longer than a specified cutoff time T) in a population who are misclassified by the RITA as being recent. This parameter is essential for the estimation of HIV incidence using a RITA and, as with MDRI, is context-dependent. A RITA's FRR has previously been referred to as the "false positive rate"; this term has the potential to be confused with false positivity in the diagnosis of HIV infection and should be avoided. |

## Confidentiality and ethics

All uses of recency testing informed by this guidance must assure confidentiality and security of data. All testing for HIV should be conducted on a voluntary basis and never in a coercive or mandatory setting. When recency testing is offered, it should be offered only to people diagnosed with HIV. People should be aware that they can refuse recency testing and that refusal will have no effect on their care or access to services. From a surveillance perspective, anonymous routine testing of all specimens from people newly diagnosed as HIV-positive can be considered, since recency test results have not been shown to have individual benefit and are not recommended for use at the individual level.

Unless clear protocols are implemented to guarantee the confidentiality of health records and personal information, recency testing may expose people to intimate partner and gender-based violence, and even prosecution in criminalized contexts *(25)*.

Any demographic or clinical characteristics included with datasets must be aggregated enough so as not to identify individuals. Any publications, reports, presentations or communications on the use of recency testing for surveillance purposes should be produced in collaboration or consultation with the communities most impacted by the results, such as communities of people living with HIV, and must avoid the use stigmatizing language.

While keeping the above in mind, anonymized and de-identified data should be made available to the public.

As for any other test, recency testing must adhere to ethical and human rights principles. WHO and UNAIDS continue to highlight that all HIV testing services must adhere to the WHO "5 Cs" *(26)*:

‣ Counselling and consent: people offered recency testing must give informed consent. This means they must voluntarily agree to testing after the process and benefits of testing, the meaning and limitations of the results, and the right to refuse testing without consequences have been explained fully. Protocols should be in place to assist adolescents and young people who are able to consent to recency testing without prior parental authorization.

‣ Confidentiality: testing services must be confidential, meaning the client's test results and identity must not be disclosed to anyone beyond health-care providers without the client's consent. Recency test results may be linked to individual health records in the context of HIV surveillance. The use of identifiable information should be limited to the short term and to the scope and context of surveillance activities. Recency test results must not be linked to larger medical records or other personal information and must not be used to inform any criminal complaints or court proceedings on HIV nondisclosure, exposure or transmission. Communications around recency testing must avoid the use of any stigmatizing language against, or bias towards, historically marginalized or criminalized populations, including gay

men and other men who have sex with men, people who use drugs, transgender women and sex workers *(27)*.

‣ Correct results: WHO and UNAIDS do not recommend the return of recency assay results to individuals, or prioritization of initiation of antiretroviral therapy, partner services, index testing or additional services based on recency results. Recency testing has considerably limited accuracy in dating the time of infection, and there is insufficient evidence to support use in these ways.

‣ Connections: people who are offered recency testing must be linked with treatment, care and support services.

Legal and policy reforms may be necessary to ensure recency testing adheres to ethical and human rights principles. This may include reform of laws criminalizing HIV nondisclosure, exposure or transmission, and people from key populations. Other measures may be necessary, such as sensitizing the police on gender-based violence and the harmful impact of criminalization; training judges and prosecutors on the limitations of recency test results in meeting evidentiary standards; and training health-care workers on confidentiality and ethical standards *(28)*.

# 1. Critical aspects to consider in developing a surveillance strategy using recency testing

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## 1.1 Choosing what data will be measured

Historically, recency assays have been used mainly within the context of population-based surveys with representative sampling, and then used to estimate HIV incidence for a country, region or key population.

More recently, many countries and organizations have become interested in using recency assays within routine HIV testing services for case surveillance and measuring other indicators beyond estimated incidence, such as epidemiologically linked clusters, geographical hotspots or subpopulations with relatively high, ongoing or emerging transmission to inform prioritization of HIV prevention and testing interventions. However, using recency assays in this way requires a number of best practices for appropriate use, and cautions in interpretation that are described further in parts 2 and 3.

WHO does not recommend the use of recency assays for clinical use or management of people living with HIV or their partners. In this context, this includes return of results to clients; counselling messages about recent infection; and prioritizing initiation of antiretroviral therapy, partner services, index testing or additional services based on recency results.

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### 1.1.1 Using recency assays to estimate HIV incidence within population-based surveys

**National household surveys and other population-based surveys**
In 2015, UNAIDS and WHO released guidance on monitoring the impact of the HIV epidemic using population-based surveys *(4)*. These guidelines included directions for estimating HIV incidence based on the 2011 WHO guidelines *(1)*. Two technical updates from UNAIDS and WHO in 2015 provided additional details on the application of RITAs in population-based surveys and other programming monitoring activities *(29, 30)*. Since 2013, results have been published from multiple population-based surveys that used this approach; the majority were population-based HIV impact assessment (PHIA) surveys supported by the United States President's Emergency Plan for AIDS Relief (PEPFAR) *(31)*.

PHIA surveys involve cross-sectional, nationally representative household surveys of adults and adolescents aged 15 years and over that measure HIV prevalence and incidence, risk factors, treatment coverage and other characteristics. Several surveys have also included children aged 0–14 years. All PHIA surveys have been conducted in countries with a high HIV burden in sub-Saharan Africa, with the exception of Haiti *(32)*. PHIA participants receive home-based HIV testing and counselling based on the country's national HIV testing algorithm. All HIV seropositive samples undergo laboratory-based RITA. In the first three PHIA surveys in Zimbabwe, Malawi, and Zambia, RITA initially included HIV-1 LAg avidity plus viral load. Qualitative data on detection of antiretroviral medicines were subsequently added to better distinguish recent and longstanding infections.

In PHIA surveys, incidence estimates were obtained from RITA results using a standard cross-sectional incidence estimator *(7)* (see Part 4 for more information) and used a mean duration of recent infection of 130 days (95% confidence interval (CI) 118–142 days), time cut-off of 1 year, and residual proportion false recent of 0.0%, which is assumed and not based on local data. No adjustment was made for subtype-related variation in MDRI, except in Uganda (Case study 1), where MDRI was adjusted to 153 days to reflect the weighted average of subtypes A and D *(33)*. Survey weights are used for all estimates to account for the complex sampling design.

There is strong evidence that a best practice for using recency in population-based surveys is to estimate a locally adapted MDRI and FRR before calculating HIV incidence except for countries with largely non-B and non-C subtype epidemics *(20)*. PHIA surveys used a standard MDRI and FRR (regardless of country or local context) despite this evidence. An example of a national population-based survey that used locally adapted MDRI and FRR for HIV incidence estimation is the Human Sciences Resource Council survey in South Africa in 2017 *(34)*.

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## Case study 1
## Uganda population-based HIV impact assessment (UPHIA), 2016–2017

The UPHIA in 2016–2017 was a nationally representative, cross-sectional population-based survey of households throughout Uganda (33). Two of its primary objectives were to estimate HIV incidence among adults aged 15–64 years and progress towards the 90–90–90 UNAIDS targets.

The survey used a two-stage, stratified cluster sample design. It was administered to 12 386 households, where 96% of eligible adults were interviewed, and 99% of those interviewed (29 024 adults) provided blood for diagnostic and recency HIV testing. The sample size was calculated to provide a representative national estimate of HIV incidence among adults with a relative standard error less than or equal to 30%.

To assess recency, people aged 18 months to 64 years who tested positive for HIV were tested with the Sedia LAg-Avidity EIA (Sedia Biosciences Corporation, Beaverton, OR, United States of America). Test results were incorporated into a RITA with viral load. People with viral load below 1000 copies/mL were classified as longstanding, regardless of their LAg-Avidity results.

As antiretroviral therapy coverage increased in Uganda, it became apparent that misclassifications on the recency assay could occur as a result, even if the viral load was over 1000 copies/mL. Based in part on data from multiple PHIA surveys in various sub-Saharan African countries, the UPHIA RITA was updated to include detection of antiretroviral medicines as a second criterion under which a person would be classified as longstanding.

Since MDRI varies by subtype, a Uganda-specific MDRI was calculated for UPHIA as a weighted average of the MDRIs for subtypes A and D:

$$MDRIUganda = WA \times MDRIA + WD \times MDRID$$

where W and MDRI are the proportions and MDRIs for each HIV subtype.

For subtype A, an MDRI of 130 days (95% CI 118–142 days) was used, consistent with previous PHIA surveys. For subtype D, an MDRI of 244 days (95% CI 166–326 days) was used, based on the mean of estimates from several sources, including CEPHIA (22), Johns Hopkins University (15) and unpublished data from the United States Centers for Disease Control and Prevention (CDC) (personal communication, 2022).

The resulting weighted average MDRI used for UPHIA incidence estimation was 153 days (95% CI 127–178 days). As with other PHIAs, an FRR of 0.0% was assumed, since viral load and detection of antiretroviral medicines had been incorporated into the RITA to reduce FRR to negligible levels.

The UPHIA estimates were weighted for probability of sample selection and adjusted for nonresponse and noncoverage. Results for HIV diagnosis (but not recency results) were returned to survey participants with home-based testing and counselling.

Annual incidence of HIV among adults was estimated to be 0.40% (0.46% among women, 0.35% among men), corresponding to approximately 73 000 new cases of HIV during the year among adults in Uganda. The estimated incidence of HIV among adults was 0.44% (95% CI 0.20–0.68%) in urban areas and 0.39% (95% CI 0.21–0.58%) in rural areas. The study was not powered to compare incidence estimates across demographic subgroups beyond sex.

**Key or sentinel population surveys**

Current practices for estimating HIV incidence rely on evidence from use of RITAs in large population-based surveys such as PHIAs. A large limitation of population-based surveys is that even in relatively high-burden countries, the sample sizes required for a national HIV incidence estimate can be prohibitively large. The sample size necessary for incidence estimates disaggregated by age, sex or subnational area may also be too large to be practical or cost-effective. These sample size considerations pose a challenge for estimation of HIV incidence among key or sentinel populations within a national survey.

The methods described above can be used in representative surveys focused on more narrowly defined populations, such as monitoring HIV incidence in key populations (e.g. gay men and other men who have sex with men, sex workers, transgender people, people who inject drugs, people in prisons and other closed settings),

sentinel populations (e.g. people in antenatal clinics, military recruits, people in major hospitals), or specific subpopulations in broader epidemics (e.g. adolescent girls and young women), including people in blood donation programmes (35, 36) and people accessing routine HIV services *(37–39)*.

Although it is possible to estimate HIV incidence through these more targeted surveillance strategies, there are three major challenges in the application of RITAs in these cases:

‣ It can be difficult or impossible to recruit truly representative samples using probability sampling in these settings. Generally, there is selection bias, which means the sample is representative not of the entire key population but of a subset of the population. For site-based surveys, the sample will be representative only of the type of people who present for specific services at the included sites. For surveys using respondent-driven sampling or other network-based survey methods, they may be biased by the choice of seed, network characteristics (including bottlenecks), or the potential for all members of that key population to not be networked together. It may also be true that highly stigmatized populations such as gay men and other men who have sex with men and people who inject drugs may choose to mask their membership of those groups when presenting for care or deciding whether to enrol in a survey. These factors may bias or limit the generalizability of the findings overall and should be noted explicitly as a limitation if it is suspected that these factors could not be avoided.

‣ It can be difficult or impossible to test people in quantities that are large enough for incidence estimates to be meaningful. Before implementing a surveillance strategy using recency assays in these settings, power and sample size calculations should be done to ensure it will be possible to generate estimates of HIV incidence that are precise enough to be useful.

‣ To reach a large enough sample for analysis, extended data-collection periods in routine care settings may be needed, rather than what would be needed with a true cross-sectional survey. If data are collected over an extended period of time, the incidence estimate produced is a weighted average of incidence over the period. A detailed explanation of this issue is available (35).

There are applications of recency assays to estimate HIV incidence in key or sentinel populations that go beyond simple population surveillance or programme planning. In one example of this, researchers in the United States used a RITA to assess where HIV incidence increased in first-time blood donors after changes to a Food and Drug Administration recommendation related to blood donation by gay men and other men who have sex with men in 2015 *(35)*. For 15 months before and 2 years after implementation of the new policy, which allowed gay men and other men who have sex with men to donate blood if they had not had sex with another man for at least 12 months, HIV-positive donations underwent a RITA.

Incidence was estimated during both periods, and factors associated with incident infection were assessed using Poisson regression. The study found that HIV incidence in first-time donors did not change significantly, at 2.62 cases per 100 000 person-years (95% CI 1.53–3.93 cases/100 000 person-years) before implementation of the 12-month deferral, and 2.85 cases per 100 000 person-years (95% CI 1.96–3.93 cases/100 000 person-years) after implementation of the 12-month deferral. Although UNAIDS and WHO guidance did not recommend using recency assays in low-incidence populations (anticipated incidence below 0.3 cases/100 person-years) at the time of this study (30), the study demonstrated utility of this approach in a very low-incidence population.

### 1.1.2 Using recency assays within HIV testing programmes

In many settings, surveillance staff have been monitoring patterns and trends in recent infection using programme data and not calculating HIV incidence. Other indicators have been used, such as the proportion recently infected among people at risk for HIV infection (37, 40–46). Ireland used recency indicators in its national routine surveillance system from 2016–2018 (40, 41). These metrics can be a helpful indicator, but they have been shown to be inaccurate proxies for HIV incidence (47).

See Part 3 for more information on the challenges in interpreting these proportion-based indicators.

There is increasing interest in the use of recency assays within HIV testing programmes, but research evaluating the best practices for this work is limited. More evidence on using HIV recency assays for this purpose will likely emerge from the PEPFAR Tracking with Recency Assays to Control the Epidemic (TRACE) initiative between 2021 and 2025 (48). Since 2019, the TRACE initiative has been introduced into 27 countries (as of April 2022) with PEPFAR-funded programmes (49).[1] Through TRACE, the recency assay is conducted as a supplementary assay for people who consent to recency testing within routine HIV testing services. PEPFAR guidance recommends that viral load testing is incorporated into RITAs to reduce misclassification (50). The considerations and best practices for conducting recency assays within routine HIV testing services apply to all programmatic settings and are further described in parts 2 and 3 of this guidance.

Sequencing of HIV RNA from new diagnoses (molecular surveillance) is a method for more conclusively identifying epidemiologically linked clusters of HIV infection within a population. This approach is expensive and may not be feasible in resource-limited settings. If recency testing is used within surveillance programmes to detect hotspots of infection (which may be epidemiologically linked clusters of HIV transmission), programmes may consider storing viral load sample remnants from the RITA for a certain duration (e.g. 6–12 months) in case a molecular surveillance analysis is considered important and affordable for outbreak investigation (51).

## 1.2 Selecting a RITA

### 1.2.1 RITA terminology

Various terms have been used to describe laboratory assays that test for recent HIV infection and methods used to estimate HIV incidence based on these assays. Previously, the term "serological testing algorithm for recent HIV seroconversion" (STARHS) was adopted as the generic term for algorithms used to classify recently acquired HIV infections. This has been almost entirely replaced by "RITA", which recognizes that information other than serological test results may be used to classify an infection as recent or longstanding. The term "RITA" describes a combination of one or more assays and clinical information used to classify a case of HIV infection as being recently acquired or longstanding.

### 1.2.2 Types of RITA

WHO and UNAIDS do not suggest specific commercial assays for recent HIV infection or suggest how they should be incorporated in a RITA. Generally, these decisions will be based on advice from laboratory experts, who should be guided by the availability

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1 Brazil, Burundi, Cambodia, Cameroon, Democratic Republic of the Congo, El Salvador, Eswatini, Ethiopia, Guatemala, Honduras, Kenya, Lao People's Democratic Republic, Lesotho, Malawi, Namibia, Nicaragua, Nigeria, Panama, Philippines, Rwanda, South Africa, Thailand, Uganda, United Republic of Tanzania, Viet Nam, Zambia, Zimbabwe.

and accuracy of particular assays, their cost and requirements for use, and availability of additional laboratory and clinical information such as viral load, testing for antiretroviral metabolites or clinical records.

Annex 3 describes the handling of specimens for use in a recency assay. Annex 4 summarizes the types of recency assay reported in the published literature and their biological characteristics.

People who are virally suppressed as a result of antiretroviral therapy may be misclassified as recently infected *(8, 17, 50, 52)*. This is particularly true for people treated for longer duration or early in infection *(18, 19)* and for people with natural viral suppression (elite control) *(52)*. A common approach to reducing false recency associated with treatment or natural viral suppression is to include a viral load threshold in the RITA, which is associated with substantial reductions in the FRR *(53)*. Although not feasible in many settings, testing for exposure to antiretroviral medicines using liquid-based chromatography is often used additionally in contexts where there is substantial antiretroviral therapy coverage and significant numbers of people may not be fully virally suppressed, which would reduce the effectiveness of a viral load threshold in reducing the FRR *(33, 54–63)*.

To further reduce the FRR of a RITA, additional clinical information (at a minimum, viral load) or historical infection information should be incorporated to assist in identifying cases at or below the recency cut-off of the assay that should still be counted as longstanding cases. For example, a RITA could incorporate client- or provider-reported information in addition to viral load and recency assay results (Figure 1).

Note that Figure 1 highlights only one RITA, although comparably strong variations might be selected reflecting local conditions. For example, in many settings, a recency assay will automatically be run on every client who tests positive for HIV, and medical history (whether there is a previous diagnosis or presence of an AIDS-defining illness) will be consulted only if a client has an assay result at or below the recency cut-off and a viral load at or above 1000 copies/mL to determine whether they should be reclassified as having longstanding infection. Different algorithms will have different cost and workflow implications.

**Figure 1.**

Example of RITA based on client- or provider-reported information about previous diagnosis or AIDS-defining illnesses, one recency assay and viral load
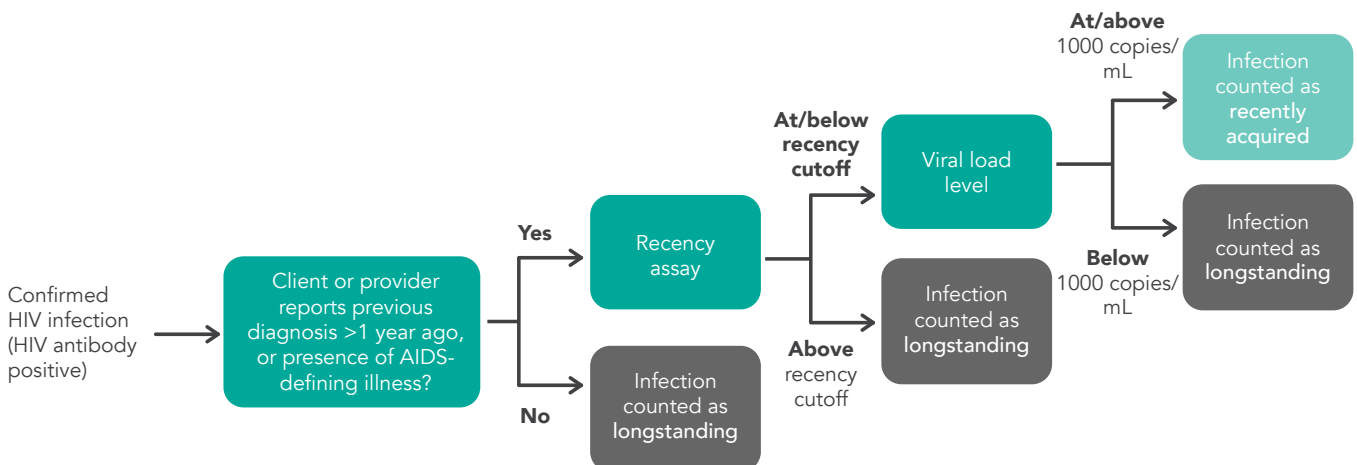
Depending on the information selected for inclusion in the RITA, cases that return an assay-based result of recent could be reclassified as longstanding if one or more of the following criteria is met:

‣ Viral load below 1000 copies/mL.

‣ CD4+ T-cell count below 200 cells/μL.

‣ Client- or provider-reported presence of an AIDS-defining illness.

‣ Record of HIV diagnosis more than a year ago, established by client or provider report, or cross-checking with case surveillance records.

‣ Record of receiving antiretroviral therapy (for purposes other than PrEP or PEP), established by client or provider report, or by testing biological specimens for the presence of antiretroviral medicines.

Even with the inclusion of viral load, testing for exposure to antiretroviral medicines, or self-reported antiretroviral therapy status in a RITA *(63)*, some degree of misclassification is inevitable, so it is still necessary to estimate the FRR for the RITA as a whole. See Part 4 for more information.

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**Best practice 1**
**Historical or clinical information (at a minimum, viral load results from the time of HIV diagnosis) must always be incorporated into any RITA.**

Reclassifying cases that return a "recent" assay-based result as longstanding based on clinical or historical information can reduce the rate of false recent results from the RITA. Single assays should never be used on their own to estimate HIV incidence or other indicators of recency.

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If it is not possible to incorporate historical or clinical or laboratory information beyond viral load into the RITA, another way to lower the FRR of the RITA is by using more than one recency assay, although the RITA must still include viral load results. Combinations of assays may be in series (the second assay is run only if the first assay indicates recent infection) or in parallel (both assays are run at the same time), with the determination of recency based on the country-defined RITA (e.g. recent in at least one assay or recent in all assays). Recency assays that are combined in a RITA should generally be based on different kinds of biomarkers (e.g. viral marker and antibody marker, or antibody titre and antibody avidity) to increase accuracy of recent infection detection. Combining two or more assays based on the same principles of detection is not likely to improve accuracy of the RITA.

Ideally, specific RITAs will be chosen by a combination of laboratory, programme, epidemiology and data analysis experts to incorporate the maximum amount of informative data available and to minimize the effect of bias from imperfect data collection or assay results.

**Best practice 2**
**National HIV surveillance managers should decide on a RITA based on a clear cost–benefit analysis.**

In addition to concerns about accuracy and ease of use, implementers should consider the cost efficiency of different RITAs. Since recency testing results should only be analysed based on full RITA results (at minimum including viral load results), the benefits regarding quality and accuracy and the cost implications of laboratory testing versus point-of-care testing should be considered carefully.

The costs of assays fluctuate over time, making it difficult to summarize the cost analysis in this guidance. Countries should consider the following before deciding on laboratory versus point-of-care testing:

‣ Cost per test.

‣ Cost of transporting tests to sites.

‣ Cost of transporting results to laboratories.

‣ Laboratory costs.

‣ Accuracy and performance of tests.

‣ Capacity of staff in clinics and laboratories to read tests.

‣ Benefit of clinic staff having the results.

‣ Ethical implications of clinic staff having individual recency test results before they have been incorporated into a RITA, where reclassification may occur.

When using a RITA for incidence calculation, any combination of assays used should have calibration data available that will allow estimation of MDRI and FRR for the full RITA. RITAs using multiple recency assays may have an MDRI that is different from a simple calculation using the MDRI of the individual assays (i.e. do not simply use the average of the durations or the shortest of the durations as the RITA MDRI).

See Part 4 for details on estimating the MDRI for a RITA.

## 1.3 Community consultations around HIV recency testing

Through collaboration and community intelligence, involvement of the community in recency testing efforts adds value and enhances the expertise of technical staff, researchers, service providers and others involved in public health programmes or research. Community involvement serves as a critical piece of this work and must be coupled with science to drive policy changes. Country programmes must have a community engagement plan in place with communities of people living with or affected by HIV.

A community engagement plan should include initial consultations to introduce recency testing and routine follow-up meetings. Concerns and considerations should be addressed before and during programme implementation to elicit community buy-in for recency testing and use of results. Initial consultations with community members should introduce the purpose of recency testing among people newly diagnosed with HIV, advantages of recency testing, and potential effects of using the results for surveillance.

Routine community consultations should be used to discuss the communication of recency results in reports, and any concerns about stigmatization of communities or subpopulations linked to recency clusters or a high proportion of recent HIV infection. The structure and scope of community consultations will be context specific. Community consultations must ensure the community is involved in decisions on how data are collected, stored, analysed, interpreted and reported on or used.

Community consultations should apply the following best practices:

‣ Country programmes should ensure various community groups are engaged in discussions around the legal and social contexts of HIV recency testing.

‣ Country programmes should allow the community sufficient time to review and provide input to relevant documents about HIV recency programmes (e.g. information sheets, data collection forms, standard operating procedures, data analysis plans, data use plans) before and during programme implementation.

‣ Country programmes should provide routine updates to the community regarding the progress of recency testing and use of data.

**Best practice 3**
**Conduct consultations with stakeholders, including at a minimum with communities of people living with or affected by HIV about the public health and human rights-based approach to recency testing, including the role, results and use of recency surveillance.**

Before initiating recency testing, it is important to get input from the population that will be impacted most by the results. Stigmatizing and blaming populations must be avoided, in consultation with organizations of people living with HIV. Consultations should be held with ministries of justice to clarify the legal status of criminalization of HIV transmission and to develop regulations and evidentiary standards around the allowance of recency test results in criminal or civil cases related to HIV transmission or exposure. Ministries of justice should issue guidance that highlights that recency testing at an individual level is too inaccurate to be used in criminal or civil proceedings.

# 2. Considerations for collection of accurate recency data

## 2.1 Minimizing selection bias for recency testing in programme settings

Although not applicable for population-based surveys with representative sampling, if recency assays are to be used within HIV testing services, it is important to minimize selection bias. Selection bias is an epidemiological term that means the sample of people included is not representative of the entire population of interest, but rather shows information about a selection or subset of people who have presented for HIV testing services at the sites included in the programme and ultimately consented to recency testing. Selection bias is a problem because it is rarely consistent across sites—meaning that although a recency indicator may give a signal about what is happening in one site, it is difficult to appropriately compare this with results in other sites. Similarly, if testing programmes change over time, it will not be possible to determine time trends.

Selection bias related to the characteristics of people who come for HIV testing cannot be avoided. It must be assessed, acknowledged and clearly described when reporting findings to aid in the proper interpretation of results. For example, are the people coming in for testing mostly pregnant young women; or men who frequent a specific HIV testing site; or people who had sex with high risk for HIV transmission in the past few months; or a certain demographic of people who are more comfortable using the testing facilities?

It is advisable to look at an age/sex pyramid for the selected sample and compare it with the larger group for which you are trying to estimate incidence, to recognize and describe any potential selection bias related to these factors. It is important to assess whether testing behaviour or treatment initiation timing has changed in this group over time, which would affect interpretation of recency findings across time within this group.

**Best practice 4**
**Examine differences between the selected group of people who received recency testing and the larger group for which you are trying to infer rates of recent HIV acquisition, to assess and be able to report potential selection bias.**

For example, look at an age/sex pyramid for the selected sample and compare it with the larger target population. Be sure to describe HIV incidence or other indicators only for the population truly represented by the group tested, and do not overgeneralize to parts of the population that were absent from the recency testing strategy.

Selection bias cannot be eliminated in situations other than through surveys with well-designed probability sampling, but it is best minimized wherever possible. One way selection bias can be reduced is through design approaches that are conducting HIV testing for reasons unrelated to HIV risk factors or disease such as pregnant women undergoing HIV testing as part of antenatal care or military recruits. Another measure is to maximize the likelihood that all or most people who are newly diagnosed as HIV-positive are tested with the recency assay. Systematic differences between people who agree to recency testing and those who are eligible (have newly tested HIV antibody-positive) but decline recency testing can skew findings, making them misleading.

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**Best practice 5**
**Minimize selection bias by striving to include all people newly diagnosed as HIV-positive in recency testing.**

From a surveillance perspective, anonymous routine testing of all specimens from people newly diagnosed as HIV-positive can be considered, since recency test results have not been shown to have individual benefit and are not recommended for use at the individual level. This would reduce the selection bias associated with individual consent for recency testing, but it would not eliminate the bias related to which types of people receive HIV testing in a particular setting.

Where anonymous routine testing is not possible and either an opt-in or opt-out approach for recency testing is required, testing providers should have simple, clear talking points to explain recency testing, and differentiate it from testing for diagnosis.

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Finally, consider the ways that specimen collection and clinic flow related to recency testing can be streamlined, so that time demands or concerns about confidentiality (e.g. "my friend or family will know the test is positive because it is taking so long to get this recency test") are minimized and will not impede consent for recency testing (64). In all cases, recency testing programmes should be planned in consultation with the community and informed by those who will be directly impacted by programmatic decisions, especially people living with or affected by HIV.

## 2.2 Considerations for specimen collection, transport and processing

In addition to important considerations related to study design, planning and implementation of recency assays for HIV surveillance, thought must be given to the logistics of using these assays in the setting of intended use. All centres offering HIV testing for diagnosis are used to the practical considerations of laboratory testing, specimen transport and training of personnel. As with all testing methods, however, recency assays have their own set of requirements that need advanced planning, training and attention.

It is important to take steps to ensure specimens for recency testing are collected and transported properly, with laboratory equipment maintained and laboratory staff trained regularly, to avoid inaccurate or invalid results:

- Plan ahead to transport specimens under cold chain (when applicable).

- Ensure all required equipment is available, fully serviced, maintained and calibrated.

- Ensure laboratory staff are trained in assay performance and monitored regularly to confirm compliance with the testing protocol.

- Monitor equipment temperatures regularly.

- Develop and implement robust internal quality control procedures including inter-reliability of reading recency assays. Accurate interpretation of results can be especially difficult with rapid assays.

- Take part in an external quality assessment scheme specific to the assay being used (if available).

- Record and share any invalid runs or unusual results with the assay manufacturer so they can be investigated and troubleshooted and retesting initiated if needed *(65)*.

A study by the Measurement and Surveillance of HIV Epidemics (MeSH) Consortium outlined in detail a series of lessons learnt during real-world implementation of a RITA in three routine service-delivery settings in Kenya and Zimbabwe. Despite a successful integration of the RITA into services, the enzyme immunoassay being used was initially more difficult than expected to operationalize, with issues related to storage, specimen transport, equipment cleaning, and correctly following assay protocols, despite comprehensive training of personnel. In the course of the implementation study, these challenges were overcome. The authors offered a list of issues with associated suggested actions and additional considerations (Table 3) *(66)*.

**Table 3.**
Potential issues with field implementation of a RITA, with suggested actions and considerations

| | Issue | Suggested actions | Additional considerations |
|---|---|---|---|
| Recency assay | Assay availability may be limited | Discuss number of tests required with assay manufacturer well in advance of launch of study | This requires an understanding of the population to be tested and the number of people expected to test positive during a time period |
| | Import of assay into country may not be permitted | Ensure import permits are in place and product is registered at an early stage | Delays to imports may affect performance of assay if it is not stored in appropriate condition when awaiting customs clearance |
| Transport | Assay transport within country | Ensure cold chain is maintained for assay when transported to local laboratories (if applicable) | Minimizing number of laboratories performing the assay may make this easier |
| | Specimen transport within country (if applicable) | Some specimens should be transported under cold chain

For dried blood spot specimens, the specimen must be fully dried before transport and maintained with a desiccant | Depending on other assays required, different specimens may need to be transported under different conditions |
| Training and performance | Before testing is undertaken, staff should be trained in performance of assay | All users should receive training from experienced user before undertaking real-world testing

CDC offers training panels to help users achieve competency

CDC can help review aggregate data for multiple quality indicators to assure data quality | All staff (especially nonclinical, non-laboratory personnel) should be monitored regularly to confirm compliance with testing protocol

Laboratories must ensure standard operating procedures are in place for each assay, detailing all steps and conditions undertaken in the laboratory |
| | Some assays need only basic laboratory equipment but can be very sensitive to issues such as inadequate washing | Ensure all required equipment is available, fully serviced, maintained and calibrated

Equipment should be itemized before testing begins and reviewed by an experienced person to ensure it is suitable for use | Service contracts may not be in place for some pieces of equipment; monitoring the performance of equipment is critical to ensure performance as expected

Even common items such as pipettes should be serviced and calibrated before use |
| | Reagents and test kits should be stored as per instructions for use | Reagents and test strips must be stored at the temperature required for the specific test

Temperature monitoring should be in place for all freezers and refrigerators | Unless the whole test kit is being used at one time, unused reagents must be returned to appropriate storage conditions as soon as possible |
| Quality control | Temperature control | Regular monitoring of equipment temperatures should be recorded and incubators confirmed to have reached temperature before starting assay (if applicable) | |

| | Issue | Suggested actions | Additional considerations |
|---|---|---|---|
| Quality control | Internal quality control | Although many recency assays come with control material, when batch testing users should follow good laboratory practice and include some specimens of known reactivity in every test to ensure reproducibility over time | These results should be plotted and analysed to look for any trends in performance |
| | External quality assessment | Each testing laboratory should partake in an external quality assessment programme for the assay (if available)<br><br>This enables interlaboratory comparison and provides evidence to foster confidence in the results issued by each laboratory | |
| Quality control | Assay failures | Laboratories should record and share any invalid runs with the manufacturer (even if using an adapted protocol)<br><br>This will help to identify whether any systematic errors occur with the assay and enhance ability to troubleshoot the assay | Ensure details of all equipment used (including basics such as pipettes) is recorded and items are identifiable by serial numbers<br><br>Lot and batch numbers of assays should be recorded if not done by company-supplied software |
| | Assay-linked analysis software | Users should ensure they are using the correct analysis software associated with the assays (if applicable)<br><br>Each assay has different validation criteria; use of incorrect manufacturer's software may lead to errors in validation and interpretation | Users should be aware that software for plasma and serum specimens and dried blood spot specimens often differ, so caution should be taken to use the correct software |
| | Unusual results | All unusual results should be investigated, and retesting undertaken if warranted<br><br>Unusual results may include specimens that offer a low optical density (which require retesting to confirm the specimen contains antibodies to HIV-1), specimens where the screening normalized optical density is significantly different from that of the confirmatory assay, or specimens where the control line does not appear | Ensure only HIV-1-positive specimens are tested<br><br>Many assays do not differentiate HIV-1 from HIV-2; care should be taken where HIV-2 is prevalent |

*Source: based on De Wit MM, Rice B, Risher K, et al. Experiences and lessons learned from the real-world implementation of an HIV recent infection testing algorithm in three routine service-delivery settings in Kenya and Zimbabwe. BMC Health Serv Res. 2021;21(1):596.*

# 3. Interpreting and reporting recency findings

## 3.1 Preparing to estimate HIV incidence

### 3.1.1 Application of a RITA to estimate HIV incidence

In any incidence estimation, a cut-off time $T$ must be chosen for a RITA. The MDRI and FRR of a RITA are defined by the selected $T$. People vary in their immunological response to HIV, and the MDRI captures the average duration rather than the duration for each person. The FRR captures the proportion of people infected for longer than $T$ who are incorrectly classified as recently infected.

Ongoing HIV transmission and improvements in the HIV care cascade could both produce increased proportions of people being classified as recent among the total number of people susceptible to HIV. It is difficult to distinguish evidence of ongoing transmission (high HIV incidence) in specific demographic or geographical groups from the impact of increased testing coverage and an increased proportion of "already known infection" in the region. Changes in testing coverage may result in over- or underestimation of recency or assumed transmission.

**Avoiding biased incidence estimates due to incorrect FRR**
Similar to the rate at which assays for diagnosis have some ratio of results that will be falsely reactive (false positive), recency assays and RITAs have an FRR among people infected for more than a prespecified cut-off time ($T$, often 2 years). The FRR of any RITA is impacted by local HIV subtype distribution, sex distribution, and level of exposure to antiretroviral medicines (including use of PrEP).

These contextual variations must be accounted for when estimating the FRR of a RITA in a particular geographical area or among a specific population of people before using RITA results for HIV surveillance, otherwise an incorrect FRR estimate may bias incidence estimates.

Use of viral load testing in a RITA further reduces the FRR and should be part of all RITAs (see Best practice 1).

**Avoiding bias due to incorrect MDRI**
The MDRI is the average duration of the recent state among people infected for less than cut-off time T for a specific RITA in a specific population of people. Several MDRI estimates have been published for commercially available recency assays used in isolation or in combination with a viral load threshold. MDRI has been demonstrated to vary, however, according to HIV subtype and sex, and potentially pregnancy and postpartum status, which can affect the progression of certain biomarkers. Although

subtype confirmation is not logistically feasible in most settings, assumptions can usually be reasonably made about the subtype mix in an area.

MDRI should be estimated using data with a similar subtype and sex distribution as the population in which the RITA will be applied, or statistically adjusted for differences between calibration data and the population of interest. See Part 4 for more details.

The MDRI can vary based on changes in the testing and treatment response when the RITA includes such markers to reduce false recency. For example, if the algorithm includes prior HIV diagnosis as a determination of recency, the distribution of time from infection to diagnosis in the population will impact the average time people remain in the "recent" state for that RITA in that population. This problem worsens as the distribution becomes narrower (as people are diagnosed earlier in infection). If antiretroviral medicine testing or viral load is included in a RITA, the distribution of time from infection to treatment initiation can impact the MDRI in a population, especially if a significant proportion of people initiate treatment within a period similar to the MDRI of the recency assay being used.

Although MDRI is conceived as a biological property of any assay, the effective MDRI is influenced by testing behaviour and the treatment cascade and may be reduced relative to the biological MDRI of the assay, affecting the accuracy of incidence estimates. This concern appears to be most pertinent in situations where the average time from infection to diagnosis (or to treatment initiation) is short. Typically, when the average time from infection to diagnosis or treatment is less than the time cut-off $T$ for that RITA, an adjustment to the MDRI is necessary (see Best practice 10).

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**Best practice 6**
**Estimate MDRI and FRR for the RITA that are specific to the local context before using the results for an incidence calculation.**

Best practice for calculating HIV incidence using a RITA is not to directly use an MDRI or FRR for an assay that has been published for another setting. Detailed guidance about establishing a local MDRI or FRR is provided elsewhere in this guidance. A straightforward mechanism for doing this has been established and is available at https://worldhealthorg.shinyapps.io/recency_test_properties/.

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In many cases—particularly when people who have tested recently or have any recent history of use of antiretroviral medicines have been excluded from testing—the FRR may be very close to zero, in which case setting it to zero for an incidence analysis may not introduce much bias. When a fixed value of 0.0% is assumed for FRR and no uncertainty bounds are placed around that value, however, the precision of the incidence estimate can be substantially overestimated. In these cases, the effect of such an assumption should be quantified with a formal sensitivity analysis, and the findings of that sensitivity analysis should be reported along with incidence results and interpretation. A common practice is to specify a plausible range for MDRI and FRR and estimate incidence using the values in that range (low, middle and high) to evaluate the sensitivity of the incidence estimate to potential biases in the MDRI or FRR parameters. If the incidence estimate is highly sensitive to MDRI or FRR values within the plausible ranges, caution should be exercised in interpretation. Even if a point estimate of 0.0% is used, uncertainty in that estimate can be specified and incorporated in the uncertainty of the incidence estimate by making use of the

parametric bootstrapping functionality (See https://worldhealthorg.shinyapps.io/recency_test_properties/).

Although in most population-based surveys the effective MDRI probably does not deviate from the biological MDRI sufficiently to warrant any adjustment, in settings where rapid diagnosis and treatment initiation is the norm, such as Eswatini and Malawi *(67)*, and in surveys of people from key populations that are targeted with very intensive testing programmes, such as female sex workers in South Africa *(68)*, adjustment of the MDRI may be required (see Part 4).

A general analysis of the impact of the distribution of time from infection to diagnosis in a population suggests that when the median time from infection to diagnosis is less than the time cut-off T in terms of which MDRI is defined, an adjustment to the MDRI is necessary to reduce bias. See Part 4 for a simple method to estimate the effective MDRI.

### 3.1.2 Common problems in using a RITA to estimate HIV incidence

The most common problems in estimating HIV incidence in cross-sectional surveys using recency assays and RITAs are outlined in Figure 2. See Parts 3 and 4 for more details on implementing the strategies in the right-hand column.

**Figure 2.**
Common problems when using a RITA to estimate HIV incidence

| Major problems | Strategies to solve problems |
|---|---|
| **1** Specificity of HIV screening test or algorithm is poor, resulting in people who are misdiagnosed ("false positives") being counted as recently infected.<br><br>*Result: biased incidence estimates* | **1** Select a testing algorithm of assays with different strengths and weaknesses. A strategy with three reactive tests for HIV diagnosis is now recommended by WHO for all settings, regardless of population prevalence. |
| **2** Viral load is not incorporated into RITA, resulting in high FRR for RITA.<br><br>*Result: biased incidence estimates* | **2** Before introducing recency testing into a surveillance programme, ensure there is a sustainable plan to include viral load testing as part of any RITA. |
| **3** MDRI and FFR not appropriately adapted to local context or population.<br><br>*Result: biased incidence estimates* | **3** Estimate or select MDRI and FRR estimates carefully, using local data where possible as calibration data. |
| **4** Sensitivity (window period) of HIV screening test or algorithm is not accounted for when estimating MDRI.<br><br>*Result: biased incidence estimates* | **4** Adjust MDRI based on relative window periods of the HIV-positive case definitions, both in the survey or study and in calibration data. |
| **5** FRR is incorrectly assumed to reduce to zero when viral load or antiretroviral medicine exposure testing are included.<br><br>*Result: biased incidence estimates* | **5** Estimate FRR from calibration data using the same RITA, and then adapt estimates to the local context. If local data are not available for this purpose, conduct sensitivity analyses to test assumptions made when adapting the FRR. |
| **6** Poorly performing RITAS are used, such as those with short MDRI or large FRR.<br><br>*Result: incident estimates are highly uncertain; incidence differences cannot be detected* | **6** Select RITAs that perform well so there is enough power to detect differences in incidence, given the survey or programme design. |
| **7** Sample size is too small to detect differences in incidence between survey rounds or populations.<br><br>*Result: uninformative incidence surveillance* | **7** Select a sample size based on careful power analysis, accounting for RITA performance. Note that sample sizes must often be exceptionally large to detect small changes in incidence, which is increasingly common as HIV incidence rates become low. See Part 4 for more information on sample size requirements and procedures. |

## 3.2 Estimating and interpreting HIV incidence

### 3.2.1 Analytical choices during HIV incidence estimation

HIV incidence can be estimated accurately from recency assays only when data are collected through population-based surveys or other representative data compilation, and when appropriate MDRI and FRR estimates are included in the calculations. In other settings, such as HIV testing services, and without accounting for potential false recent results, HIV incidence calculations should generally not be conducted.

Once recency data from a population-based survey have been collected with sufficient sample size and a locally appropriate MDRI and FRR have been selected, incidence can be calculated. This should be done using an estimator (a formula or rule for calculating the estimate of HIV incidence) that is well-documented and has been shown to minimize bias in the findings by accounting for local nuances that can affect incidence results.

The currently accepted incidence estimator for cross-sectional studies using RITA data was introduced by Kassanjee and colleagues in 2012 *(7)*. This estimator incorporates the MDRI, FRR and T as specific parameters. The choice of T for a RITA is theoretically arbitrary but must be selected so the vast majority of people will have progressed to the longstanding state by T after becoming infected.

A new incidence estimator has been developed, which was available for review in a preprint at the time of writing this guidance *(69)*. This estimator incorporates a local area's testing history directly and internally modifies the FRR and MDRI accordingly, and therefore does not require locally adapted FRR and MDRI parameters to be used. This may be a promising advancement for the field once it has been peer-reviewed and studied further.

More details about the use of the Kassanjee estimator and tools to aid in incidence estimation, including methods for local adaptation of the FRR and MDRI parameters, are available elsewhere in this guidance.

To estimate whether there is a statistically significant change in incidence over time, a test statistic must be selected. The established approach to examine change in HIV incidence is to calculate the difference between the two incidence measurements (incidence difference) and determine whether it is statistically different from zero. Another potential approach is to calculate the ratio between the two measurements (incidence ratio) and determine whether the ratio is statistically different from one. A simulation study applied to the context of comparing RITA-based incidence to cohort-based incidence in HIV prevention trials showed that smaller sample sizes can be tolerated when the test is based on the ratio *(70)*. It is therefore more conservative to compute power and required sample sizes using the established incidence difference test. Both approaches are implemented in the web tool at https://worldhealthorg.shinyapps.io/recency_incidence_difference/.

### 3.2.2 Best practices for interpreting and reporting HIV incidence estimates

The goal of estimating HIV incidence is to use the information for resource prioritization, programme planning or other epidemiological analyses. To ensure estimates can be understood correctly and used appropriately to compare populations, it is critical that estimates are reported according to a series of best practices.
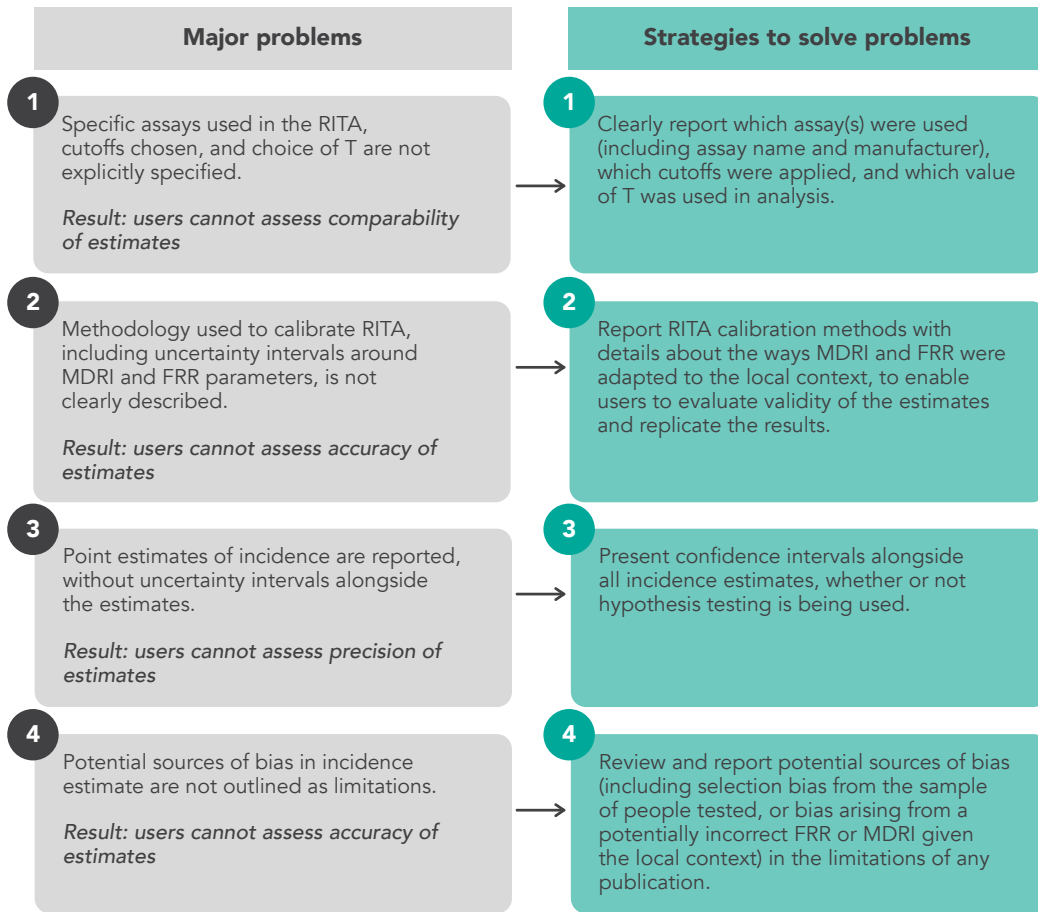
All recency assays have different properties, and the assumptions made about those properties will influence incidence estimates. This is true even for assays with the same name that are produced by different manufacturers. For this reason, it is imperative

that when incidence results are being reported, findings are presented alongside details of the assays (and manufacturers) used in the RITA, the cut-offs applied, and the values of T, MDRI and FRR used in the analysis. Details of how the MDRI and FRR were estimated are necessary so that users can assess the accuracy of estimates.

Figure 3 summarizes common problems when reporting HIV incidence findings, and strategies to solve those problems.

**Figure 3.**

Common problems when reporting findings from HIV incidence analyses using recency assays

| Major problems | Strategies to solve problems |
|---|---|
| **1** Specific assays used in the RITA, cutoffs chosen, and choice of T are not explicitly specified.<br><br>*Result: users cannot assess comparability of estimates* | **1** Clearly report which assay(s) were used (including assay name and manufacturer), which cutoffs were applied, and which value of T was used in analysis. |
| **2** Methodology used to calibrate RITA, including uncertainty intervals around MDRI and FRR parameters, is not clearly described.<br><br>*Result: users cannot assess accuracy of estimates* | **2** Report RITA calibration methods with details about the ways MDRI and FRR were adapted to the local context, to enable users to evaluate validity of the estimates and replicate the results. |
| **3** Point estimates of incidence are reported, without uncertainty intervals alongside the estimates.<br><br>*Result: users cannot assess precision of estimates* | **3** Present confidence intervals alongside all incidence estimates, whether or not hypothesis testing is being used. |
| **4** Potential sources of bias in incidence estimate are not outlined as limitations.<br><br>*Result: users cannot assess accuracy of estimates* | **4** Review and report potential sources of bias (including selection bias from the sample of people tested, or bias arising from a potentially incorrect FRR or MDRI given the local context) in the limitations of any publication. |

## 3.3 Estimating and interpreting surveillance metrics other than HIV incidence

### 3.3.1 Selecting an appropriate recency indicator

All RITAs will misclassify some people with longstanding infection as recently infected, and further research is required to evaluate how best to account for this misclassification in interpreting routine recency testing data, when FRR and MDRI parameters are not incorporated into calculations.

Simple proportion-based indicators such as [number of people testing recent] / [number of people testing recent + number of people testing HIV-negative] assume there are no false recent results by the RITA, and they may be misleading if calculated over time as a measure of epidemic trends related to ongoing transmission or programme effectiveness, in the case where the FRR of the assay used is changing.

This is because rates of testing and entry points for testing within routine programme implementation may also be changing over time, which affects the FRR of any assay being used.

In antenatal clinics and other settings with a stable age and sex distribution in the population and consistently high HIV testing rates, these indicators may work well, as FRR is likely to be constant. Deliberate policy changes (e.g. starting a programme of active partner notification services), increases in self-testing outside the clinic environment, and large-scale programme disruptions due to test kit shortages or non-HIV outbreaks (e.g. Ebola, COVID-19) have a profound impact on the selection of people accessing HIV testing services, however, with an unknown and unmeasurable influence on the probability of testing recent versus longstanding infected people at various timepoints being compared.

## Best practice 7
**When calculating a proportion-based indicator of recency from HIV testing services or case surveillance, calculate the "proportion recent" as the number of recent infections divided by the total number of people at risk for HIV (people testing recent + people testing HIV-negative), rather than the total number of people newly diagnosed.**

It has been demonstrated that use of the total number of people newly diagnosed with HIV as a denominator may be very inaccurate and may even have a trend in the opposite direction to HIV incidence *(47)*.

The indicator can be adjusted for recency test coverage by multiplying the number of RITA recents in the numerator and the denominator with 1/recency coverage. This is especially important if the recency coverage changes over time, in which case a change in the indicator may reflect that change in recency testing coverage rather than a change in HIV incidence.

## 3.3.2 Interpreting and visualizing differences in HIV recency across geographical regions

A potential use of recency testing is to understand the geographical distribution or clustering of new HIV infections. Interpreting this distribution to identify areas for programmatic focus will be meaningful only if routine HIV testing and recency testing reflect underlying population incidence equally in all regions. In most cases, voluntary use of HIV testing services will vary depending on a person's perceived risk of HIV acquisition. This is likely to vary by geographical area, education level, access to testing and many other variables, resulting in an unequal chance of being tested for HIV prevalence and, subsequently, testing for HIV recency.

Similarly, provider-initiated HIV testing will vary across regions if one provider is more targeted with testing clients for HIV (i.e. finding more people who have been infected recently) than providers in other regions.

When comparing two geographical areas, there is the added challenge that where people test and where they live or work may not be the same. This can be mitigated by collecting data on each person's residence in addition to the testing site location. In such scenarios, comparing the proportion of recent infections among people at risk

of acquisition across different regions will not be meaningful and may be interpreted incorrectly. For example, as country programmes strive to improve testing coverage and diagnose more people earlier in HIV infection, an increase in the proportion of people testing recent could be a sign of great success rather than a sign of a worsening problem.

Regardless of the denominator used, recency proportions should be used as only one data point among many triangulated measures (e.g. findings from population-based surveys, population-size estimation analyses, biobehavioural surveillance surveys, molecular surveillance analyses). Direct comparisons of recency proportions as a proxy for differing HIV incidence between geographical areas or subpopulations (or a single population at two or more timepoints) should be made only when it is reasonable to assume that antiretroviral therapy coverage, HIV testing coverage and PrEP use (including long-acting injectable PrEP) are comparable between the two groups being compared.

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Programmatic data from women attending antenatal clinics can be used to assess changes in HIV incidence in settings where all attendees not already known to be living with HIV are generally offered and accept testing for HIV. This will provide a measure of incidence among women attending antenatal clinics. If the proportion of pregnant women attending antenatal clinics in the region is high, the measure can inform the geographical distribution of recent HIV infections among pregnant women in areas with generalized epidemics.

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**Best practice 8**
**Analyse recency findings from HIV testing services programme data separately (disaggregated) for different populations in a geographical area, programme or study and report findings within the context of each specific population.**

For example, recency results might be reported "among women attending antenatal clinics in the region, where 46% of clients are aged under 25 years".

Failure to do this may result in inappropriate allocation of prevention and public health response resources to a group or area that does not actually have higher rates of HIV transmission because it is assumed two different populations are being compared evenly.

If not adjusted by collecting residence data, attention should be paid in the discussion or limitations to the potential for people testing to be different from people who live in the geographical region being assessed.

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As a best practice for reporting tabular results across different geographical areas, recency results could be presented in a format similar to Table 4.

**Table 4.**
Dummy data of proportion of recent infections among people at risk for HIV acquisition [a] in Country X

| Population | 2017 | 2022 |
|---|---|---|
| | % recent (number recent/number at risk) | % recent (number recent/number at risk) |
| Women attending antenatal clinics | | |
| Women aged 15–24 years in Province A | 0.48% (42/8750) | 0.42% (37/8810) |
| Women aged 25–34 years in Province A | 0.25% (25/9804) | 0.22% (24/10 714) |
| Women aged 15–24 years in Province B | 0.16% (67/42 949) | 0.14% (63/43 750) |
| Women aged 25–34 years in Province B | 0.09% (51/56 667) | 0.06% (32/57 143) |
| Men attending voluntary counselling and testing sites | | |
| Men aged 15–24 years in Province A | 0.17% 19/11 310) | 0.19% (21/10 938) |
| Men aged 25–34 years in Province A | 0.33% (31/9226) | 0.30% (30/10 033) |
| Men aged 35–44 years in Province A | 0.44% (22/4977) | 0.45% (20/4444) |
| Men aged 45+ years in Province A | 0.39% (5/1263) | 0.34% (5/1449) |
| Men aged 15–24 years in Province B | 0.07% (18/27 273) | 0.04% (12/30 008) |
| Men aged 25–34 years in Province B | 0.13% (16/12 308) | 0.08% (12/14 998) |
| Men aged 35–44 years in Province B | 0.06% (11/18 333) | 0.04% (19/20 455) |
| Men aged 45+ years in Province B | 0.03% (2/7143) | 0.03% (2/7141) |

[a] *Number at risk for HIV acquisition defined as (HIV-negative test results + HIV recent test results).*

This type of table is strongly advised over those that are commonly seen, where specific populations are conflated, making it erroneous to compare across groups. As an example, Table 5 presents the same findings as those in Table 4, but the information is much less easily interpreted. Readers are unable to see differences in gender by province, or age differences for people testing in antenatal clinics in a single province. The use of such tables should be avoided.

**Table 5.**

Example of in-apporpriate presentation of recency results from HIV testing services

**Avoid**

| Population | 2017 | 2022 |
|---|---|---|
| | % recent (number recent/number at risk) | % recent (number recent/number at risk) |
| Gender | | |
| Men | 0.13% (124/91 832) | 0.11% (111/99 466) |
| Women | 0.16% (185/118 169) | 0.13% (156/120 417) |
| Age | | |
| 15–24 years | 0.16% (146/90 281) | 0.14% (133/93 505) |
| 25–34 years | 0.14% (123/88 004) | 0.11% (98/92 889) |
| 35–44 years | 0.14% (33/23 311) | 0.12% (29/24 899) |
| 45+ years | 0.08% (7/8405) | 0.08% (7/8590) |
| Province | | |
| A | 0.32% (144/45 330) | 0.29% (137/46 388) |
| B | 0.10% (165/164 672) | 0.07% (130/173 494) |
| Setting | | |
| Antenatal clinics | 0.16% (185/118 169) | 0.13% (156/120 417) |
| Voluntary counselling and testing sites | 0.13% (124/91 832) | 0.11% (111/99 466) |

[a] *Number at risk for HIV acquisition defined as (HIV-negative test results + HIV recent test results).*

# 4. Statistical best practices for estimating HIV incidence using representative samples

Even in the case of the results reported as in Table 4, it is important to describe any contextual differences that might bias results. Imagine for example that voluntary counselling and testing clinics in Province B in Table 4 launched a campaign in 2019 to encourage younger people to test for HIV every 3 months, but the same was not true for voluntary counselling and testing clinics in Province A. In this case, the differences in testing frequency would make it difficult to interpret differences in recency in the two provinces, even among men in the same age group attending similar voluntary counselling and testing clinics.

## 4.1 Determining sample size

Although cross-sectional HIV incidence estimation is frequently embedded in representative surveys with objectives to measure indicators beyond HIV incidence, it is important to ensure sample sizes are sufficient to support informative incidence estimates or detection of incidence difference.

To calculate the size of the sample required to achieve a specified level of precision, which can be defined as a certain relative standard error on incidence or confidence interval (CI) width, it is necessary to specify the following:

‣ Target relative standard error, variance or CI width around the incidence estimate.

‣ Hypothesized or expected incidence.

‣ Hypothesized or expected prevalence of HIV.

‣ Proportion of people living with HIV identified in the survey who will receive recency testing.

‣ MDRI and relative standard error on MDRI for the RITA to be used.

‣ FRR and relative standard error on FRR for the RITA to be used.

‣ Time cutoff T for recency classification (MDRI and FRR estimates must be consistent with chosen value of T).

‣ Design effects on prevalence and prevalence of recency (i.e. variance inflation associated with complex sampling frames).

‣ Significance level $\alpha$.

‣ Computing the required sample size for a certain power to detect incidence differences requires that the following are specified:

‣ Desired power (e.g. 80%).

‣ Hypothesized incidence in the two settings or surveys.

- Hypothesized HIV prevalence in the two settings or surveys.

- Proportion of people living with HIV identified in the survey who will receive recency testing in each survey.

- MDRI and FRR estimates relevant to the RITAs in each survey; and whether these test property estimates are shared or estimated independently, or only one of the test property estimates is shared while the other is independently estimated.

- Time cutoff $T$ for recency classification.

- Design effects on prevalence and prevalence of recency (i.e. variance inflation associated with complex sampling frames) in each survey.

- Significance level $\alpha$.

Tools are available to help countries compile these data and use the information to estimate sample size requirements for their HIV incidence estimation, including the inctools R package *(71)* and the web tool [https://worldhealthorg.shinyapps.io/recency_sample_size/](https://worldhealthorg.shinyapps.io/recency_sample_size/).

## 4.2 Estimating locally appropriate estimates of MDRI and FRR for a RITA

RITAs should only be applied to people who have been diagnosed as living with HIV. RITAs should be selected to collect the maximum possible information, while balancing resource availability, feasibility, cost and other logistical concerns. The MDRI and FRR of the RITA should be estimated for the local context, before proceeding to analysis.

### 4.2.1 Estimating MDRI for a RITA

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**Best practice 9**
**Use the inctools R package *(71)* or other similar methods to directly calculate the MDRI of a RITA from local longitudinal data of seroconverters when possible.**

Several approaches have been proposed for estimating the MDRI using longitudinal data from seroconverters, which can be broadly classified as interpolation, survival analysis and parametric regression *(72)*. All methods require that the time of infection (which could be defined as either the time of infectious exposure or time of first detectability of infection on a reference HIV test) is estimated for all people contributing longitudinal data for recency assay calibration.

A common approach is to use the midpoint between the dates of a last HIV-negative test and a first HIV-positive test for a given person as the estimated time of seroconversion, assuming seroconversion could have occurred at any time during the interval with uniform probability *(73)*. More sophisticated methods are available that account for the variable window periods of different assays for diagnosis to estimate the time of first detectability on a reference assay *(74, 75)*.

The MDRI can be expressed as follows:

$$\Omega_T = \int_0^T P_R(t)\ dt$$

where PR(t) is the probability of being alive and classified as recently infected by the test or algorithm in question at time t after infection *(7)*. This function can be inferred directly by fitting a model to dichotomous outcomes (recent versus longstanding results) or indirectly by modelling continuous biomarker measurements and then estimating the probability of obtaining a result below the recency discrimination threshold *(72)*. Linear binomial regression has the advantage of being easily applied to complex multi-assay RITAs and seamlessly accounting for multiple transitions from recent to longstanding (and the reverse). The widely used open-source inctools package for the R statistical programming language implements the binomial regression approach *(71)*.

**Best practice 10**
**If local calibration data are not available, adapt published MDRI estimates to the specific context of a survey or study, with regard to the case definition of HIV-positive that triggers use of recency testing, sensitivity of the RITA being used, subtype and sex distribution, the choice of T, and the distribution of times from infection to diagnosis or infection to treatment initiation in the local area.**

In most cases, researchers will not estimate MDRI de novo using longitudinal data but will rely on published estimates for the RITA being implemented. Numerous MDRI estimates have been published for commercially available recency assays used in isolation or in combination with a viral load threshold *(10, 16, 21, 53, 73, 76–82)* and combinations of recency assays and immune markers with or without viral load *(45, 83–86)*.

MDRI has been demonstrated to vary according to HIV subtype *(20, 53, 73, 87)* and sex *(22)* or potentially pregnancy or postpartum status *(21)*. The web tool https://worldhealthorg.shinyapps.io/recency_test_properties/ can assist with easy adaptation of published MDRI to the local context.

**Adjusting for the case definition of HIV-positive that triggers use of recency testing**
A RITA may also include defining HIV RNA-positive/antibody-negative people as recently infected, which would result in a longer MDRI *(35)*. This is usually a matter of adding or subtracting a certain number of days. For example, if a published MDRI estimate is based on an HIV case definition of fourth-generation antigen/antibody (Ag/Ab) positivity but is used in a study where people who are reactive on an RNA test but non-reactive on an Ag/Ab test are classified as recently infected, the MDRI needs to be adjusted by adding the duration of the RNA detectable, pre-Ag/Ab seroconversion window period.

**Adjusting for the sensitivity of the HIV screening assay or algorithm, which may be different from the reference assay used in test calibration**
The consistent estimation of MDRI requires that the time of infection (or seroconversion on a specific reference test) is estimated for people contributing specimens to

calibration panels. Although a naive approach is possible (e.g. using the midpoint between the date of the last HIV-negative antibody test and first HIV-positive antibody test as an estimated date of seroconversion *(73, 77)*, assuming a uniform distribution of the probability of infection during this interval), the proliferation of HIV screening assays and assay classes (e.g. RNA, p24 antigen, antibody, Ag/Ab combination assays) has created complexity in estimating time of infection *(52, 74, 75, 88)*.

If the last negative HIV test and first positive HIV test took place on the same day (i.e. there are discrepant results on different assays), or when negative and positive tests have different window periods, the boundaries of the interval should account for these variable window periods. For example, if on a certain date a person tested negative by an RNA assay, and on a later date tested positive by an antibody assay (i.e. an assay with a relatively short and a relatively long window period, respectively), the implied interval during which infection could have occurred is asymmetrically adjusted from the test dates. Window periods for a large number of diagnostic tests have been published *(88–90)*, and a public tool is available for estimating infection time intervals *(53)*.

Furthermore, a reference time is analytically chosen (e.g. estimated date of infectious exposure, of first RNA detectability, or of seroconversion on a fourth-generation Ag/Ab combination assay). When applying a RITA in the field, the HIV screening algorithm in use impacts the appropriate MDRI (i.e. when a less sensitive screening algorithm is used, the MDRI is shorter than when a highly sensitive RNA screening test is used *(69)* (A. Welte, personal communication, 2021; R. Kassanjee and A. Welte, personal communication, 2022).

An example of estimating infection time is given in Table 6. This shows diagnostic test results for a person who had discrepant results on tests with different window periods on a single date.

**Table 6.**

Example discrepant results for Subject A due to differing window periods of two HIV diagnostic tests

| Date | Test | Result | Window period [a] |
|------|------|--------|-------------------|
| 10 January 2017 | Aptima HIV-1 Quant Dx RNA | Positive | 1.4 days |
| 10 January 2017 | Uni-GoldTM HIV | Negative | 25.1 days |

[a] *Window period calculated relative to the window period of a hypothetical RNA assay with a detection threshold of 1 copy/mL.*

In Table 6, Subject A has a negative and a positive test result on the same day. If the window periods of the tests are known, plausible bounds can be placed on the time of infection. In this example, the relative window period of each of the assays is known relative to a highly sensitive RNA assay with a detection threshold of 1 RNA copy/mL (1.4 days for the Aptima HIV-1 Quant Dx RNA assay and 25.1 days for the Uni-Gold HIV diagnostic point-of-care test). The negative test with the smallest window period and the positive test with the largest window period on any given date are informative for bounding the infection time.

Subject A's detectable Aptima result indicates they could not have become detectably infected later than 1 day before the test date, and their negative Uni-Gold HIV result indicates they could not have become detectably infected earlier than 25 days before the test date. Subject A is therefore said to have an earliest plausible date of detectable infection of 16 December 2016 and a latest plausible date of detectable

infection of 9 January 2017. A point estimate for the date of first detectability is the midpoint of this interval and known as the estimated date of detectable infection (28 December 2016).

The Infection Dating Tool (https://tools.incidence-estimation.org/idt) provides a more sophisticated implementation of this method that derives point estimates and 95% credible intervals for the date of detectable infection.

When MDRI estimation is performed using a different case definition of HIV-positive than the screening tests used in cross-sectional incidence estimation, the same relative window periods can be used to adjust the MDRI estimate. For example, an MDRI estimated from data in which estimated dates of detectable infection are estimated as above is expressed relative to a hypothetical RNA assay with a detection threshold of 1 copy/mL. If the survey screens for HIV using the Uni-Gold HIV rapid diagnostic test, 25 days should be deducted from the MDRI estimate before application in the survey context, since infections would be detected on average 25 days later post-infection than in the calibration dataset.

### Adjusting for the distribution of HIV subtypes in the population of interest
As subtype has been shown to affect MDRI (20), a published subtype-specific MDRI should be used if possible. If the only available published MDRIs reference different subtype distributions than is true for the population of interest, a weighted average of subtype-specific MDRI estimates can be used.

### Adjusting for the distribution of sex in the population of interest
The sex distribution in a population has been shown to affect the MDRI (20). Especially if the study population is solely or largely composed of one sex, a sex-specific or weighted average of sex-specific MDRI estimates should be used.

### Accounting for the choice of T
Since MDRI and FRR are defined in terms of the time cutoff for the recency calculation, the MDRI must be estimated using the same value of $T$ as will be used in the study design and incidence estimation. If not, a different choice of $T$ should be selected for which there are data.

### Adjusting for the distribution of time from infection to diagnosis or treatment in the local area
If the chosen RITA classifies diagnosed or treated people as longstanding, and the number of days from HIV infection to HIV diagnosis (or HIV treatment) is such that a substantial proportion of people who would otherwise have been classified as recent are classified as longstanding, the MDRI should be adjusted. This typically happens when the time from infection to diagnosis or treatment is shorter than $T$ for more than half of people living with HIV in the population of interest, although this is only a rule of thumb. In most situations, the effective MDRI of a RITA will not deviate greatly from the biological properties of the primary test for recent infection. If a population is subject to high testing or treatment coverage resulting in the median time from infection to diagnosis or treatment initiation being less than $T$, however, the effective MDRI may deviate meaningfully from the biological MDRI, and adjustment should be made to calculate a local MDRI.

Note that distribution of times from infection to diagnosis is relevant if the RITA includes classification of people living with HIV with previous diagnoses (ascertained from self-report or medical records) as longstanding, and the distribution of times from infection to treatment initiation is relevant if the RITA includes detection of antiretroviral medicines, viral load, or records of or self-reported treatment status.

If the MDRI needs to be adjusted for bias arising from one of these distributions, this can be achieved by specifying a function for the probability of diagnosis or treatment (or viral suppression) as a function of time. This is likely to be relatively easy if information on this distribution is available from the same survey used to estimate incidence. Considering that the function $P_R(t)$ given above describes the probability of testing recent on the primary biomarker as a function of time since infection in the absence of rapid diagnosis and treatment, we can define the function $P'_R(t)$ as the probability that the person has not exited the recent state by being diagnosed, treated or virally suppressed (as appropriate) and tests recent according to the laboratory tests(s):

$$P'_R(t) = \left(1 - P_d(t)\right) \cdot P_R(t)$$

where $P_d(t)$ is the probability of diagnosis, treatment or viral suppression as a function of time since infection, allowing the calculation of an adjusted MDRI $\Omega'$ as follows:

$$\Omega' = \int_0^T P'_R(t)\, dt$$

The function for the probability of not leaving the recent state by being diagnosed, treated or virally suppressed ($1 - P_d(t)$) can be a Weibull survival function with shape and scale parameters chosen to roughly fit the available data. A shifted (three-parameter) Weibull could also be used. If high-quality data are not available, expert opinion or model-based estimates may be used. For ease of use, a Weibull survival function with use-inputted shape and scale parameters is implemented in the web tool at https://worldhealthorg.shinyapps.io/recency_test_properties/.

The degree of adjustment to the MDRI (i.e. the degree to which the effective MDRI deviates from the biological MDRI) is shown in Figure 4 for the Sedia LAg-Avidity EIA for a range of median times to diagnosis when the assay is used in a RITA that classifies previously diagnosed people living with HIV as longstanding.

**Figure 4.**
Effective (adjusted) MDRI relative to biological MDRI for different distributions of time from infection to diagnosis using a Weibull survival function
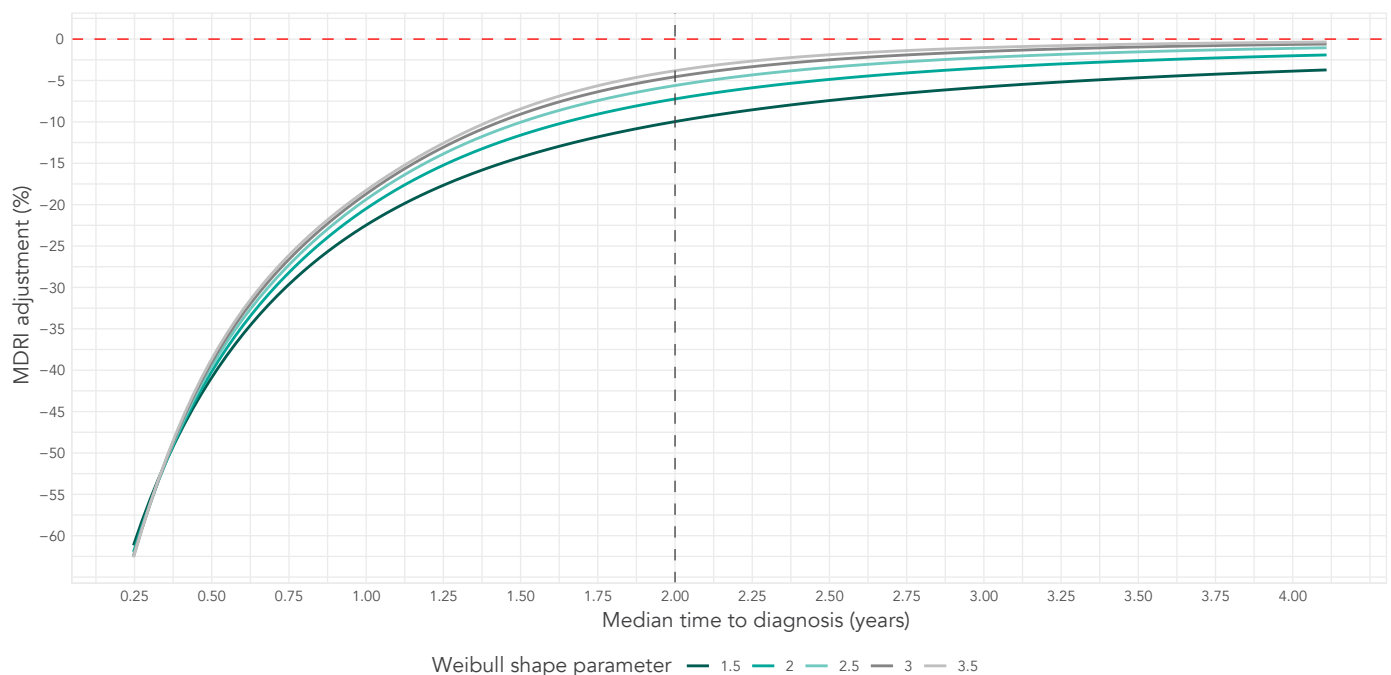
### 4.2.2 Estimating FRR for a RITA

There is substantial evidence that a proportion of people with longstanding HIV infection are misclassified as recently infected by currently available recency assays *(91–93)*. It is critical to establish the FRR of a recency assay to avoid overestimating incidence, although a risk of overadjustment also exists, which would result in an underestimate of population-based incidence *(94)*. Notably, RITA calibration data are most often generated using panels drawn from biospecimen repositories constructed using remnants from earlier HIV cohort studies. Because many of these cohort studies were executed at a time when antiretroviral therapy was much less widely available, and before guidelines recommended treatment initiation for all people testing positive for HIV, a naive FRR estimate based on these panels has the potential for significant bias.

The precision of incidence estimates is very sensitive to FRR. In general, FRRs greater than a few per cent result in highly imprecise incidence estimates, which may make incidence trend analysis impossible through a reduction in power to detect incidence change. It is therefore critical for cross-sectional incidence estimation that a RITA be chosen with a sufficiently large MDRI and sufficiently small FRR to support informative incidence estimates.

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**Best practice 11**
**Estimate the FRR for the local context in which the RITA will be applied.**

FRR is inherently context-specific, and the naive application of FRRs from the literature could result in biased incidence estimates. The FRR is driven by the probability of obtaining a recent result as a function of time transitioning from close to one at early times post-infection to close to zero (in a well-performing test). This function does not necessarily reach zero by T, however, and in most instances has a long tail and a substantial probability of a recent result at times shortly after T. For this reason, the distribution of times since infection in the population infected for longer than T can substantially affect the FRR *(20, 53, 80)*. Furthermore, the proportion of people in the population with longstanding infection who are on antiretroviral therapy, who exhibit natural viral suppression (elite controllers) or who have late-stage infection greatly affects the FRR in that population.

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Estimating incidence in subpopulations such as key populations (e.g. gay men and other men who have sex with men) or other vulnerable populations (e.g. adolescent girls and young women), or using routine HIV testing services programme data rather than population-based surveys with random sampling presents additional challenges. Performance of a RITA in these populations may differ from performance in the general population, due to biological and epidemiological differences.

In general, the FRR of a RITA for a population should be reviewed at regular intervals, taking into account any change in the population characteristics that may affect the FRR. The length of these intervals will depend on the local setting. For example, it might be most appropriate to revisit FRR estimates during each round of a large population-based survey. If there are no natural cycles for FRR review, an interval length may be prespecified depending on how quickly trends are changing in the region (e.g. in some areas with a more established epidemic, it may not be necessary to review FRR more than every 5 years or even longer; in areas with more dynamic epidemics, however, it may be useful to review the FRR annually or every 2–3 years).

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.

Both naive and context-specific FRR estimation methods have been implemented in publicly available software tools, most prominently the inctools R package *(71)*. It is critical that the uncertainty in FRR estimates is quantified, usually expressed as a relative standard error, in order for CIs on incidence estimates to be calculated correctly. Delta method approximation and bootstrapping approaches are available within inctools.

**Estimation of FRR within the same population as that to which the RITA is being applied**

The optimal estimation approach involves directly estimating the FRR within the same population as that to which the RITA is being applied. Examples exist in the literature of using data from cohorts drawn from the same population in which incidence will be estimated (Table 7). This is feasible only 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.

‣ Key characteristics of the population (such as proportion treated) did not change substantially between the time of estimating FRR and the time of incidence estimation.

‣ The FRR study is based on a sufficiently large sample of people with longstanding infection to support accurate and precise estimation of the FRR.

Table 7 provides examples of studies where FRRs used in incidence estimation were derived from cohorts recruited from the same population.

**Table 7.**
Four studies with internally derived FRR estimates used in incidence estimation

| Reference | Country | Population | Study period | Common HIV-1 sub-types | Assay used in RITA | RITA | MDRI (days) | FRR (%) | 95% CI on FRR |
|---|---|---|---|---|---|---|---|---|---|
| Hargrove et al. 2008 *(95)* | Zimbabwe | Postpartum women | 1997–2001 | C | BED | Single assay | 180 | 5.2 | 4.4–6.1 |
| Kim et al. 2011 *(96)* | Kenya, Uganda | General population | 2003–2007 | C (Kenya) A, D (Uganda) | BED | Single assay | 155 | 14.9 | 12.2–17.5 |
| Laeyendecker et al. 2019 *(87)* | Uganda | General population | 2008–2009 | A, D | LAg | LAg + viral load | 187 | 1.1 | Not reported |
| Laeyendecker et al. 2019 *(87)* | Uganda | General population | 2012–2013 | A, D | LAg | LAg + viral load | 187 | 4.8 | Not reported |

As described earlier, the degree of uncertainty around the FRR will influence the degree of uncertainty around the incidence estimate. The relative standard error on the FRR should not exceed 25%, although sample size and power calculations should be performed using standard methods to assess whether a particular RITA has sufficient performance to support incidence estimation.

Use of an externally derived FRR calculated in a population representative of that in which the RITA is applied to determine incidence

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

This approach is feasible only if:

‣ The FRR was determined for exactly the same RITA—this is a challenge for RITAs incorporating testing for exposure to antiretroviral medicines, since calibration data that include this are generally not available.

‣ The FRR was determined in a population representative of that in which the incidence survey is being conducted, with respect to general demographics (e.g. age and sex distribution), HIV-1 subtype, HIV epidemic history and, if use of antiretroviral therapy cannot be excluded, similar coverage of antiretroviral therapy.

‣ Appropriate sample sizes were used to estimate the FRR.

Since datasets of sufficient size and appropriately representative of the population in which incidence is being estimated are not generally available, it may be preferable to use all available calibration data and apply mathematical techniques to estimate context-specific FRR, as described below.

**Adapting externally derived FRR to a local population using mathematical techniques**
A general procedure of estimating context-adapted FRR using mathematical techniques has been well-described in the literature *(53, 80, 82)*. The procedure relies on deriving a weighting function that captures the distribution of times since infection in the population, consistent with HIV prevalence and recent incidence, in order to derive the FRR in the untreated population. A $P_R(t)$ curve is then fitted to calibration data, and this function is weighted by the inferred weighting function for the distribution of times since infection in the untreated population. This procedure accounts for the fact that there may be a substantial residual probability of obtaining a recent result at times greater than T post-infection, but the numbers of people (or proportion of the untreated population living with HIV) who have these durations of infection may vary widely between contexts, depending on the history of the epidemic and treatment coverage.

The FRR in the untreated group is obtained by integrating the weighted function from *T* to infinity. The FRR in the treated population can be estimated separately (and may be very close to zero, depending on the algorithm), with the overall FRR as a weighted average of the untreated and treated FRRs *(20)*.

This can be expressed as follows:

$$FRR_{untreated} = \int_{T}^{\infty} \rho(t)P_R(t)\,dt$$

where

$$\rho(t) = \frac{f(t)}{\int_{T}^{\infty} f(t)dt}$$

and *f(t)* is an appropriate survival function (e.g. a Weibull function) expressing survival in the untreated state. The parameters of this function are chosen to produce the known treatment coverage in the population, consistent with recent incidence and prevalence. The final FRR estimate is:

$$FRR = (1 - c) \times FRR_{untreated} + c \times FRR_{treated}$$

where c is the proportion of the population living with HIV receiving antiretroviral therapy *(53, 80)*.

Although a statistician may be able to implement this method manually, for ease the web tool https://worldhealthorg.shinyapps.io/recency_test_properties/ implements this method automatically, using parameters inputted by the user.

### 4.2.3 Incorporating inclusion and exclusion criteria into FRR and MDRI estimates

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

Another important example is the use of antiretroviral therapy status as an exclusion criterion. Clinical information such as exposure to antiretroviral medicines can be used in a RITA to reclassify people as having longstanding infection. It is, however, possible to use information of this type to exclude people from the sample rather than 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 Section 4.2.2 may be used to calculate an unbiased estimate of incidence. As noted above in the example of pregnant women, however, when calculating incidence using recency assays, it is advisable to exclude data from anyone who meets criteria for which they would have been excluded from the population from which the chosen FRR was derived.

## 4.3 Analysing and reporting incidence data resulting from RITA application

### 4.3.1 Calculating HIV incidence using RITA results

Incidence calculated as a rate

The Kassanjee estimator *(7)*, which is the currently accepted and widely used cross-sectional biomarker-based incidence estimator, can be expressed in terms of sample counts, as follows:

$$\hat{I}_T = \frac{n_R - \beta_T n_+}{n_S(\Omega_T - \beta_T T)}$$

where:

$I_T$ is the estimated incidence.

$n_R$ is the number of recently infected people in the survey.

$n+$ is the number of people living with HIV in the survey.

$n_s$ is the number of HIV-negative people in the survey.

$\beta_T$ is the FRR of the RITA.

$\Omega_T$ is the MDRI of the RITA.

$T$ is the time cutoff for recency classification.

Technically, the incidence estimate is a weighted average of the incidence over the period $t_0 - T$ to $t_0$ if the survey is conducted at time $t_0$, weighted primarily by the temporal dynamics of the biomarker (essentially a flipped version of the $P_R(t)$ curve extending into the past from the time of the survey). For further details, see the source literature *(7)*.

A major disadvantage of the estimator expressed in terms of survey counts is that it is difficult to account for complex sampling frames that deviate from simple random sampling and the use of survey weights, since applying survey weights produces population-level count estimates, which cannot be plugged into the estimator as individual survey counts. This could result in incorrect estimates of the variance of the incidence estimate (and thus the resulting confidence intervals). Survey sampling methodology is beyond the scope of this guidance.

In the past, spreadsheet-based tools that relied on survey counts to implement the incidence estimator and related tools were used widely, such as the Assay-based Incidence Estimation Tools. Due to the disadvantages of using survey counts for complex surveys, a version of the estimator was developed that relies on survey proportions that can be estimated from standard complex survey analysis tools available in statistical analysis environments such as SAS, Stata and R. When combined with appropriate variance estimates for the survey proportions and the test property estimates, delta method-based variance and CI estimation for the incidence estimate are available for surveys with complex sampling frames. The estimator expressed in terms of proportions is as follows:

$$\hat{I}_T = \frac{P_+ \cdot (P_{R|+} - \beta_T)}{(1 - P_+) \cdot (\Omega_T - \beta_T T)}$$

where $P_+$ is the prevalence of HIV infection and $P_{R|+}$ is the prevalence of recency among people living with HIV.

This estimator is implemented in the inctools R package *(71)*, and the web tool at https://worldhealthorg.shinyapps.io/recency_incidence_calculator/ but it could easily be implemented manually in other statistical analysis environments.

**Incidence calculated as an annual risk of infection**

Incidence as an annual rate and the annual risk of infection (the probability that a person will become infected over the course of a year) are related by the following conversion formula:

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

where $I_a$ is the annual risk of infection and $I_T$ is the annual incidence rate outputted from the Kassanjee estimator. The inctools R package reports the annual risk of infection when computing incidence rate estimates *(71)*.

**Formula for calculating 95% CI**

The CI is computed using a delta method variance approximation with this estimator. The error in both sample counts or sample proportions and recency test properties is assumed to be distributed normally. The standard deviations of RITA properties and sample proportions are required to calculate the variance of the incidence estimate. The variance of the incidence estimator (using sample counts) is as follows:

$$Var\left[\hat{I}_T\right] \approx \left(\frac{P_R - \beta_T P_+}{P_S(\Omega_T - \beta_T T)}\right)^2 \times \left[\frac{1}{n(P_R + P_{NR})}\left(\frac{1}{P_S} + \frac{P_R P_{NR}}{(P_R - \beta_T(P_R + P_{NR}))^2}\right) + \sigma_{\hat{\Omega}_T}^2\left(\frac{1}{\Omega_T - \beta_T T}\right)^2 + \sigma_{\hat{\beta}_T}^2\left(\frac{P_{NR}\Omega_T - P_R(T - \Omega_T)}{(P_R - \beta_T(P_R + P_{NR}))(\Omega_T - \beta_T T)}\right)^2\right]$$

where $\sigma_{\hat{\beta}_T}^2$ is the variance of the MDRI estimate and $\sigma_{\hat{\Omega}_T}^2$ is the variance of the FRR estimate *(7)*.

Assuming normally distributed error, the 95% CI around the incidence estimate can be obtained as follows:

$$\sigma_{\hat{I}} = \sqrt[2]{Var[\hat{I}_T]}$$

$$\hat{I}_T \pm 1.96 \times \sigma_{\hat{I}}$$

The formula for delta method-based variance of the incidence estimator using sample proportions is similar and can be obtained in the documentation of the inctools R package *(71)*. An alternative to delta method variance approximation is parametric or empirical bootstrapping (see below).

**Handling missing specimens**

Under certain circumstances, it may not be possible to test all the HIV-positive specimens within a study or survey using a recency assay. This situation may occur if specimens are missing or unavailable for testing due to, for example, contamination of the specimen or insufficient volume.

If specimens are missing completely at random (MCAR), it is appropriate to exclude those specimens for which a test for recent infection was not conducted when using the specimen counts-based estimator, and to scale down the number of HIV-negative samples appropriately. When specimens are MCAR, there is assumed to be no relationship between the missingness of the data and any other values (observed

or missing). In the case of missing specimens, this would mean that specimens are missing randomly, and missingness is not associated with any particular characteristic of study participant. When using proportions and standard survey methodology, estimating the prevalence of recency among people living with HIV using a subset analysis (the subset of people living with HIV who have a recency result) where missingness is MCAR should not bias results, although it will reduce precision of the estimates as it reduces sample size.

In the case where specimens are missing at random (missingness is contingent on a known and measured variable), multiple imputation or inverse probability weighting may be necessary.

When data are assumed to be not missing at random (missingness is expected to be non-random but the pattern is unknown or depends on unmeasured variables), Bayesian methods of analysis should be used.

### 4.3.2 Calculating incidence difference and incidence rate ratio, and detecting incidence change

The conventional approach to detecting change in HIV incidence between two surveys conducted at different times in the same population, or to distinguish incidence in two populations, is the incidence difference, defined simply as:

$$\widehat{\Delta}_I = \hat{I}_A - \hat{I}_B$$

The test statistic, denoted *Z* and distributed *N*(0,1), is as follows and allows computation of *P* values and CIs:

$$Z = \frac{\widehat{\Delta}_I}{\sqrt{\widehat{Var[\widehat{\Delta}_I]}}}$$

In computing the variance of $\hat{\Delta}_I$, it is important to note whether MDRI and FRR estimates are shared between the two surveys or independently estimated. The inctools R package documentation provides formulas for the variance of $\hat{\Delta}_I$ in three cases: shared MDRI and FRR estimates, independently estimated MDRI and FRR, and a shared MDRI estimate but independently estimated FRRs *(71)*.

Statistical hypothesis testing for comparing incidence difference between two surveys includes the following:

‣ Specifying the null hypothesis that the incidence is the same in the two surveys.

‣ Remaining agnostic about the value of this shared incidence.

‣ Estimating the prevalence independently from the data in each survey.

‣ Performing a two-tailed test using the test statistic Z described above, given there is no basis for predetermining the direction of the incidence difference, even if there is a reasonable suspicion of the likely direction.

It is also possible to compute an incidence rate ratio (or hazard ratio) as:

$$\hat{R} = \frac{\hat{I}_A}{\hat{I}_B}$$

Specifying the null hypothesis that the incidence is the same in the two contexts ($R_0 = 1$, where $R_0$ is defined as the incidence rate ratio under the null) allows for the use of a new test statistic that has recently been proposed *(70)*. This test statistic may

be preferable to the test statistic for incidence difference, although this work had not been peer-reviewed at the time this guidance was written. The test statistic, denoted and distributed $N(0,1)$, allows for calculation of P values and hypothesis tests on the incidence ratio:

$$\tilde{Z} = \frac{\log(\hat{R}) - \log(R_0)}{\sqrt{Var\widehat{[\log(\hat{R})]}}}$$

As with incidence estimates in a single survey, when calculating incidence differences and incidence ratios from multiple surveys (used to detect incidence change or variation between contexts or populations), bootstrapping is a valid alternative to delta method variance approximation and may in some cases represent uncertainty better than other methods. Note that both parametric bootstrapping (drawing values of estimates from distributions, e.g. normal distributions with specified means and standard deviations) and resampling observations with replacement from the underlying data could be used. The bootstrap scheme should account for any complex sampling frame; for example, in a survey design where observations are clustered in primary sampling units, the bootstrapping scheme should replicate this sampling frame.

It is also important to account for uncertainty in the MDRI and FRR, neither of which can be known with certainty. In a bootstrapping procedure, sampling from distributions of MDRI and FRR estimates is appropriate. In delta method-based approaches, uncertainty can be addressed by incorporating the standard errors on MDRI and FRR into the calculation.

### 4.3.3 Best practices for reporting HIV incidence calculations

Following the methods described in this guidance should result in valid HIV incidence estimates, provided due attention is paid to sampling frames, sample size, and reliable estimates of the MDRI and FRR of the recency assay or RITA, including contextual adaptation as necessary.

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**Best practice 12**
**Report all estimates, including incidence estimates and incidence difference or incidence ratio estimates, with uncertainty expressed as CIs, relative standard errors or variance.**

MDRI and FRR estimates used in the calculation should also be reported with uncertainty.

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CIs (or credible intervals) are the most easily interpreted. P values may be reported for hypothesis tests (e.g. of incidence being different from zero or of incidence differing between two contexts or timepoints). If P values are reported, choice of test statistic and assumptions, including any assumption of normally distributed error, should be reported so that P values can be interpreted. When more than one comparison is being made, a statistical correction for multiple comparisons should be considered.

It is clear from the complex analytical choices and uncertain parameters involved in cross-sectional HIV incidence estimation that any estimate will have limitations, even if all best practices are followed. Sensitivity analyses are suggested when possible,

especially with regard to the chosen MDRI and FRR, given the difficulty of appropriately estimating these parameters for a local context. If, for example, a conclusion that incidence declined between two timepoints is highly sensitive to the FRR estimate and is not robust to plausible ranges of that parameter, the conclusion should be treated with scepticism. CIs are not sufficient to account for potential sources of systematic error, including biased RITA property estimates.

When alternative or complementary methods of estimating incidence in the same population are available, these should be pursued to the extent possible so that a triangulation approach may be followed in the planning, implementation and evaluation of public health programmes, HIV prevention interventions, and selection of populations for prevention trials. Triangulation of evidence on HIV incidence should involve careful assessment of the methodology used in producing estimates, inherent strengths and limitations of different approaches, and the quality of reporting.

# Annex 1
# Summary of best practices

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## Designing a surveillance strategy using recency testing

**Best practice 1**
Historical or clinical information (at a minimum, viral load results from the time of HIV diagnosis) must always be incorporated into any RITA. Reclassifying cases that return a "recent" assay-based result as longstanding based on clinical or historical information can reduce the rate of false recent results from the RITA. Single assays should never be used on their own to estimate HIV incidence or other indicators of recency.

**Best practice 2**
National HIV surveillance managers should decide on a RITA based on a clear cost–benefit analysis. In addition to concerns about accuracy and ease of use, implementers should consider the cost efficiency of different RITAs. Since recency testing should only be analysed alongside viral load results, the benefits regarding quality and accuracy and the cost implications of laboratory testing versus point-of-care testing should be considered carefully.

**Best practice 3**
Conduct consultations with communities of people living with or affected by HIV about the public health and human rights-based approach to recency testing, including the role, results and use of recency surveillance. Before initiating recency testing, it is important to get input from the population that will be impacted most by the results. Stigma and blaming of populations must be avoided, which is best done in consultation with organizations of people living with HIV.

## Considerations for collection of accurate recency data

**Best practice 4**
Examine differences between the selected group of people who received recency testing and the larger group for which you are trying to infer rates of recent HIV acquisition, to assess and be able to report potential selection bias. For example, look at an age/sex pyramid for the selected sample and compare it with the larger target population. Be sure to describe HIV incidence or other indicators only for the population truly represented by the group tested, and do not overgeneralize to parts of the population that were absent from the recency testing strategy.

**Best practice 5**
Minimize selection bias by striving to include all people newly diagnosed as HIV-positive in recency testing. From a surveillance perspective, anonymous routine testing of all specimens from people newly diagnosed as HIV-positive can be considered, since recency test results have not been shown to have individual benefit and are not recommended for use at the individual level. This would reduce the selection bias associated with individual consent for recency testing, but it would not eliminate the bias related to which types of people receive HIV testing in a particular setting.

Where anonymous routine testing is not possible and either an opt-in or opt-out approach for recency testing is required, testing providers should have simple, clear talking points to explain recency testing, differentiate it from testing for diagnosis, and explain whether results will be available to the client. WHO recommends that recency results be used for surveillance purposes only and not disclosed to clients.

## Interpreting and reporting recency findings

### Best practice 6
Estimate MDRI and FRR for the RITA that are specific to the local context before using the results for an incidence calculation. Best practice for calculating HIV incidence using a RITA is not to directly use an MDRI or FRR for an assay that has been published for another setting. Detailed guidance about establishing a local MDRI or FRR is provided elsewhere in this guidance. A straightforward mechanism for doing this has been established and is available https://worldhealthorg.shinyapps.io/recency_test_properties/.

### Best practice 7
When calculating a proportion-based indicator of recency from HIV testing services or case surveillance, calculate the "proportion recent" as the number of recent infections divided by the total number of people at risk for HIV (people testing recent + people testing HIV-negative), rather than the total number of people newly diagnosed. It has been demonstrated that use of the total number of people newly diagnosed with HIV as a denominator may be very inaccurate and may even have a trend in the opposite direction to HIV incidence *(47)*.

The indicator can be adjusted for recency test coverage by multiplying the number of RITA recents in the numerator and the denominator with 1/recency coverage. This is especially important if the recency coverage changes over time, in which case a change in the indicator may reflect that change rather than a change in HIV incidence.

### Best practice 8
Analyse recency findings from HIV testing services programme data separately (disaggregated) for different populations in a geographical area, programme or study and report findings within the context of each specific population. For example, recency results might be reported "among people attending antenatal clinics in the region, where 46% of clients are aged under 25 years".

Failure to do this may result in inappropriate allocation of prevention and public health response resources to a group or area that does not actually have higher rates of HIV transmission because it is assumed two different populations are being compared evenly.

If not adjusted by collecting residence data, attention should be paid in the discussion or limitations to the potential for people testing to be different from people who live in the geographical region being assessed.

## Statistical best practices for estimating HIV incidence using representative samples

### Best practice 9
Use the inctools R package *(71)* or other similar methods to directly calculate the MDRI of a RITA from local longitudinal data of seroconverters when possible. Several approaches have been proposed for estimating the MDRI using longitudinal data from seroconverters, which can be broadly classified as interpolation, survival analysis and

parametric regression *(72)*. All methods require that the time of infection (which could be defined as either the time of infectious exposure or time of first detectability of infection on a reference HIV test) is estimated for all people contributing longitudinal data for recency assay calibration.

A common approach is to use the midpoint between the dates of a last negative test and a first positive test for a given person as the estimated time of seroconversion, assuming seroconversion could have occurred at any time during the interval with uniform probability *(73)*. More sophisticated methods are available that account for the variable window periods of different assays for diagnosis to estimate the time of first detectability on a reference assay *(74, 75)*.

The MDRI can be expressed as follows:

$$\Omega_T = \int_0^T P_R(t)\ dt$$

where $P_R(t)$ is the probability of being alive and classified as recently infected by the test or algorithm in question at time t after infection *(7)*. This function can be inferred directly by fitting a model to dichotomous outcomes (recent versus longstanding results) or indirectly by modelling continuous biomarker measurements and then estimating the probability of obtaining a result below the recency discrimination threshold *(72)*. Linear binomial regression has the advantage of being easily applied to complex multi-assay RITAs and seamlessly accounting for multiple transitions from recent to longstanding (and the reverse), although there are other methods that also allow multiple transitions. The widely used open-source inctools package for the R statistical programming language implements the binomial regression approach *(71)*.

**Best practice 10**
If local calibration data are not available, adapt published MDRI estimates to the specific context of a survey or study, with regard to the case definition of HIV-positive that triggers use of recency testing, sensitivity of the RITA being used, subtype and sex distribution, the choice of *T*, and the distribution of times from infection to diagnosis or infection to treatment initiation in the local area.

In most cases, researchers will not estimate MDRI de novo using longitudinal data but will rely on published estimates for the RITA being implemented. Numerous MDRI estimates have been published for commercially available recency assays used in isolation or in combination with a viral load threshold *(10, 16, 21, 53, 73, 76–82)* and combinations of recency assays and immune markers with or without viral load *(45, 83–86)*.

MDRI has been demonstrated to vary according to HIV subtype *(20, 53, 73, 87)* and sex *(22)* or potentially pregnancy or postpartum status *(21)*. The web tool at https://worldhealthorg.shinyapps.io/recency_test_properties/ can assist with easy adaptation of published MDRI to the local context.

**Best practice 11**
Estimate the FRR for the local context in which the RITA will be applied. FRR is inherently context-specific, and the naive application of FRRs from the literature could result in biased incidence estimates. The FRR is driven by the probability of obtaining a recent result as a function of time transitioning from close to one at early times post-infection to close to zero (in a well-performing test). This function does not necessarily reach zero by *T*, however, and in most instances has a long tail and a substantial probability of a recent result at times shortly after *T*. For this reason, the distribution of times since infection in the population infected for longer than *T* can substantially affect the FRR *(20, 53, 80)*. Furthermore, the proportion of people in the population

with longstanding infection who are on antiretroviral therapy, who exhibit natural viral suppression (elite controllers) or who have late-stage infection greatly affects the FRR in that population.

**Best practice 12**
Report all estimates, including incidence estimates and incidence difference or incidence ratio estimates, with uncertainty expressed as CIs, relative standard errors or variance. MDRI and FRR estimates used in the calculation should also be reported with uncertainty.

Table A2.1 was adapted from a publication by CEPHIA published in 2016, after CEPHIA evaluated 10 assays with respect to their performance according to the FIND target product profile for HIV recency assays, endorsed by WHO in 2016.

# Annex 2

# Non-performance-based characteristics of HIV recency assays as evaluated by CEPHIA

**Table A2.1.**
Summary of Assay performance according to FIND target product profiles

| Specification | Sedia HIV-1 Limiting LAg-Avidity EIA | Sedia BED HIV-1 Incidence EIA | Bio-Rad Genetics Systems HIV-1/HIV-2 Plus O EIA (avidity protocol) | Ortho VITROS Anti-HIV 1 + 2 (avidity protocol) | Bio-Rad Geenius HIV-1/2 Confirmatory (modified protocol) | Abbott ARCHITECT HIV Ag/Ab Combo (avidity protocol) | Asanté HIV-1 Rapid Recency Assay |
|---|---|---|---|---|---|---|---|
| Specimen type | Serum or plasma / Dried blood spot | Serum or plasma / Dried blood spot | Serum or plasma / Dried blood spot | Serum or plasma | Serum or plasma | Serum or plasma | Whole blood/serum or plasma |
| Specimen volume | 40 µL total | 40 µL total | 20 µL total | 40 µL total | 10 µL total | 40 µL total | 5 µL total |
| Infrastructure requirements | Commercial assay / General laboratory equipment | Commercial assay / General laboratory equipment | Modified commercial assay / Automated platform | Modified commercial assay / Automated platform | Company-supplied reader and access to software to obtain band data | Modified commercial assay / Automated platform | Can be performed locally without laboratory equipment / Proprietary reader may be required a |
| Storage and shipping conditions | Some reagents are stored frozen | Some reagents are stored frozen | 4–25 °C | 2–25 °C | 4–25 °C | 4–25 °C | 2–30 °C |
| Incubation temperature | 4–25 °C | 4–37 °C | 4–37 °C | Incubation within automated platform | 4–25 °C | Incubation within automated platform | 15–37 °C |
| Shelf-life b | >18 months | 9 months | 12 months | 12 months | >18 months | >18 months | 18 months |

| Specification | Sedia HIV-1 Limiting LAg-Avidity EIA | Sedia BED HIV-1 Incidence EIA | Bio-Rad Genetics Systems HIV-1/ HIV-2 Plus O EIA (avidity protocol) | Ortho VITROS Anti-HIV 1 + 2 (avidity protocol) | Bio-Rad Geenius HIV-1/2 Confirmatory (modified protocol) | Abbott ARCHITECT HIV Ag/Ab Combo (avidity protocol) | Asanté HIV-1 Rapid Recency Assay |
|---|---|---|---|---|---|---|---|
| Training | Technician proficient with 1 week's training | Technician proficient with 1 week's training and approved | Technician proficient with 1 week's training | Technician proficient with 1 week's training following company-approved course | Minimal training to conduct assay | Technician proficient with 1 week's training following company-approved course | Technician proficient with 1 week's training |
| Regulatory pathway | Assay produced in GMP facilities and approved for research use only. Each new kit lot approved for release by CDC | Assay produced in GMP facilities and approved for research use only. Each new kit lot approved for release by CDC | None for modification. In most countries, standard assay is approved for HIV diagnosis but not for detection of recent infection | None for modification. In most countries, standard assay is approved for HIV diagnosis but not for detection of recent infection | None for modification. In most countries, standard assay is approved for HIV diagnosis but not for detection of recent infection | None for modification. In most countries, standard assay is approved for HIV diagnosis but not for detection of recent infection | Assay produced in GMP facilities and approved for research use only. Each new kit lot approved for release by CDC |

*a* The use of a reader will mean the assay only reaches the desired characteristics of the target product profile.

*b* Once a commercial assay is modified by a user, the determination of the shelf-life must be determined by the user, taking into account the impact of their modifications on the assay.

*GMP: good manufacturing practice.*

*Key:*

| Ideal performance | Acceptable performance | Outside target product profile acceptability criteria |
|---|---|---|

# Annex 3
# Specimen quality and handling requirements for testing

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Most assays for recent infection use plasma or serum specimens, but dried blood spots and capillary (fingerprick) or venepuncture whole blood have been validated for use in some assays for recent infection. Since assays for recent HIV infection measure properties of HIV-specific antibodies (such as quantity and avidity), it is crucial to ensure the integrity of specimens is maintained throughout the process of preparation, storage, transport and testing. This process ensures the results obtained are accurate and reliable. Where specimens are tested using modified commercial assays, the specimen handling conditions must be at least as stringent as (and potentially more stringent than) those required for the assay used to screen for or aid in diagnosis. This is because the exacting conditions required for accurate performance of HIV recency assays may be more likely to be adversely affected by the quality of the specimen than the diagnostic assay in its unmodified form. 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 *(97)*.

## Liquid (wet) specimens

**Preparation**
Serum or plasma should be separated from whole blood cells by centrifugation within 8 hours of being drawn. If the blood specimen cannot be processed immediately (e.g. no centrifuge is available or specimens are collected in the evening), collect the blood in a purple-top tube with ethylenediaminetetraacetic acid (EDTA). Allow the blood to stand for 20–30 minutes and then carefully remove the plasma with a pipette, trying not to draw up any red blood cells. To avoid haemolysis, process and test the specimen within 24 hours. Assays where whole blood specimens are used are normally collected by fingerprick via a capillary tube and used immediately.

**Storage**
Specimens should be refrigerated on the day they are drawn. The specimen should be either frozen immediately in a non-frost-free freezer at −20 °C or below or stored at 4 °C for no more than a week before freezing. Long-term storage of specimens should be done at −70 °C in a non-frost-free freezer.

**Transport**
During shipping to a reference laboratory, specimens should be shipped frozen and maintained below 0 °C during transport if they have been stored frozen. Samples can be transferred at 4 °C or below if they are to be transferred and tested within 7 days. Ensure the caps on the cryovials are tight during transport to avoid spillage and cross-contamination.

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

Limit the number of freeze/thaw cycles to five because multiple thawing may affect antibody levels and therefore test results. There is only limited information on the reliability of assays for recent HIV infection using specimens that have been frozen and thawed multiple times or stored in suboptimal conditions, and there is no information for all recency assays *(98)*.

In general, specimens that are grossly lipaemic, haemolysed or cloudy should not be used with recency assays since one or more of the assays in a RITA may be adversely impacted and results may not be reliable.

### Dried blood spots
Dried blood spots (and in some cases dried serum or plasma spots) have been validated as appropriate specimens for use in some assays for recent HIV infection, such as the LAg-Avidity and the BED-capture enzyme immunoassay (CEIA). As manufacturers' specimen requirements may vary, it is crucial to ensure the appropriate specimens are used for each assay.

### Preparation
Dried blood spot specimens should be prepared from blood specimens obtained by a fingerprick or venepuncture (typically using an anticoagulant) on a grade 903 card, a specially manufactured absorbent specimen collection (filter) paper. Filter papers that are not recommended or validated for a specific assay should not be used for collection of dried blood spots. Specimens should not be caked or clotted.

Specimens must be air-dried for at least 3 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 the cards do not touch each other.

### Storage
Between 10 and 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 4 °C in zip-lock bags with desiccant. For storage for more than 90 days, the dried spots should be kept in a freezer at −20 °C or below. Properly stored dried blood spots have been shown to be stable for at least 2 years. Dried blood spots stored at room temperature should not be used for incidence assays *(99)*.

### Transport
The bags should be placed in an extra strong, tear-proof, air-permeable and water-resistant envelope for shipment *(100)*.

# Annex 4
# Overview of assays for recent infection

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Multiple approaches to distinguish recent from long-term infection have been described. Some of these are based on the presence or absence of markers of infection, while others measure aspects of the immune response to HIV infection (Table A4.1). 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 A4.2).

With the exception of a few assays, most of the assays listed below have not been evaluated appropriately to obtain rigorous values of the mean RITA duration and the FRR in diverse HIV-1 subtypes (76, 101, 102).

An HIV-positive status per national diagnostic algorithm is required to support interpretation of HIV recency assays. As recency assays are not approved for HIV diagnosis, they should not be incorporated into testing algorithms to diagnose HIV infection. The ability to incorporate HIV diagnosis and recency determination into a multiplex assay has been described (103). As a confirmed HIV diagnosis is a prerequisite for all HIV recency assays, the incorporation of a diagnostic test result into the recency assay reduces the potential for anti-HIV negative specimens to be falsely labelled as recent HIV infections. This technology also has the added potential to incorporate many different recency approaches into a single assay. The lack of regulatory approval for the aid for diagnosis component of the assay, however, means this assay may currently be limited to use in research studies.

In recent years, further approaches and technologies to identify HIV recency using alternative biomarkers, such as using molecular methods and microarrays, have been described, but these are early in their development and have not been evaluated widely and are not discussed here.

## Less sensitive enzyme immunoassay

Most standard antibody assays for HIV infection can be modified for use as an assay for recent infection, using the principle that antibody titres increase for several months following the acquisition of infection. This approach was first described based on the assay produced by Abbott laboratories (3A11), modified to create a less sensitive HIV antibody assay (104). Confirmed HIV-1-positive specimens are retested with an enzyme immunoassay that has been made less sensitive by diluting the plasma specimen 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 enzyme immunoassay. Since the development of the less sensitive enzyme immunoassay, other assays have been modified in this way to estimate incidence. The two commercial immunoassays that have been modified as less sensitive enzyme immunoassays are the Abbott 3A11 and the Avioq HIV-1 Microelisa (formally marketed as BioMerieux Vironostika HIV Microelisa). The Abbott 3A11 is now out of production.

HIV rapid diagnostic tests have also been modified for detection of recent HIV infection. All of the assays based on this principle have used antigens from a single HIV subtype (B) and therefore have 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. In recent years, HIV antibody-only assays have been phased out and replaced with Ag/Ab assays. The increase in sensitivity of newer generations of HIV antibody assays and the adoption of Ag/Ab assays has made it increasingly difficult to develop less sensitive assays.

## Rapid recency assays

The use of the immunochromatographic (lateral flow) format has allowed the development of rapid recency assays. These assays use similar antigens to those found in more common enzyme immunoassays using microtitre plates, but the antigens are immobilized on a thin membrane (often nitrocellulose). The specimen may be mixed with a small amount of buffer and added to one end of the membrane, or the specimen may be added directly to the end of the membrane followed by a buffer solution.

The specimen, with the buffer, is moved along the membrane by a wicking action, and specific antibodies in the specimen bind to antigens on the solid phase. The long-term/recent line of the assay is coated with antigen at a limited concentration. Due to the limited concentration of the antigen, and the speed at which the sample passes across the long-term/recent line, only highly avid or high-titre HIV-specific antibody will bind to the antigen. Highly avid and high-titre antibody are correlated with longstanding infection. A verification line containing other HIV antigens will encourage the binding of HIV antibodies regardless of the recency status. Detector molecules can then be added to the membrane, washing away any unbound antibody and attaching to any antigen/antibody complexes. The bound antigen/antibody complexes give a visible line on the membrane that can be detected by eye or an electronic reader.

These assays typically give a result in less than 20 minutes and can be performed near to the person without the use of laboratory equipment.

## 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. Avidity assays can be configured in different formats.

Dual-well avidity assays (e.g. Bio-Rad Avidity) determine an avidity index from the reactivity of the total anti-HIV response in one well (the control well) and the reactivity in an identical well that has been treated with a chaotropic agent, which disrupts ionic, hydrophobic or hydrogen bonds between the antigen and antibody (the test well). In recently acquired infection, the chaotrope will disrupt the Ag/Ab binding and lead to lower reactivity, giving a low avidity index. Using a predetermined cutoff for the avidity index, samples can be separated into recent and longstanding HIV infections.

In single-well avidity assays (e.g. LAg-Avidity), a new concept of limiting the amount of antigen is used, which prevents binding of low-avidity antibodies while permitting binding of high-avidity antibodies *(73, 77, 105)*. The LAg-Avidity enzyme immunoassay and rapid tests for recent infection are based on this principle and use a multi-subtype recombinant protein developed specifically for these assays. The laboratory-based LAg assay also uses a disassociation buffer that will disrupt Ag/Ab complexes from recent HIV infections with low avidity and will reduce the level of reactivity in the well.

Using calibrators and a predetermined level of reactivity, the assay can be used to differentiate between recent and longstanding infection.

## High dynamic range immunoassays

The introduction of platforms that detect using chemiluminescent and fluorescent signals, rather than colorimetric signals, to indicate reactivity means the level of signal can now be determined over a much greater dynamic range. The level of the signal is proportional to the amount of bound antibody, so the differentiation between a low titre of HIV specific antibody in a specimen and a high titre of antibody can be determined easily. From comparison with other HIV recency assays and evaluating specimens from people with known dates of HIV infection, it has been possible to determine levels of reactivity from the immunoassay (e.g. Abbott ARCHITECT) that will also determine whether a specimen is likely to have come from a person with longstanding or more recently acquired HIV infection.

## 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 than a longstanding infection. The BED-CEIA is based on this principle and was designed exclusively for the identification of recent HIV infection (106). The BED-CEIA is an IgG antibody CEIA and uses a synthetic HIV peptide representative of different HIV subtypes.

## 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 of gp41, which comprises 2 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 5 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 longstanding infection. This assay has been used as part of the French national HIV case surveillance system since 2003.

## p24 antigen

The p24 antigen (p24Ag) is usually detectable within a few days of 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 antibodies may be used as a marker of recent infection. Its presence is unreliable and brief (1–2 weeks), however, with a high rate of false positives unless reactivity is confirmed using neutralization. Therefore, the test has limited use in detecting recent infection. In addition, p24Ag can be detected in late-stage AIDS following failure of the immune system.

## HIV RNA

Detection of RNA in the absence of anti-HIV antibody can be used to identify recent HIV infection. As HIV RNA can be detected a few days earlier than p24Ag, the MDRI for this marker is about 2–3 weeks (107). Additionally, testing of pooled HIV RNA has been shown to be feasible for identifying acute infection (108) and significantly lowers

testing costs. Use of this method to determine HIV incidence is technically complex and expensive and 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 enzyme immunoassay-based procedure has been developed where IgG3 to p24Ag is typically detectable for only the first 1–4 months of infection. The findings from initial studies of this assay were not reproducible, resulting in no commercialization of this concept.

## 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. This type of assay is routinely used as a confirmatory test to validate the presence of antibodies against HIV. The Inno-LIA HIV I/II Score, a line immunoassay, can be used to interpret results as recent or longstanding infection *(109)*. This assay is costly but may be of value in settings where it is routinely used as the confirmatory diagnostic test.

**Table A4.1.**
Summary of recency assay types and characteristics [a]

| Assay type | Principle | Component of anti-HIV immune response being measured | Limitations |
|---|---|---|---|
| Less-sensitive enzyme immunoassay | Diluted blood sample is used to identify low anti-HIV antibody titre<br><br>Low antibody titre correlates with recent infection | Antibody titre | Limited to use in populations with predominantly subtype B HIV-1 infection<br><br>Assays require separate calibration with predominant subtypes found in sub-Saharan Africa (subtypes A, C, D, E), India (subtype C) and South-East Asia (subtype E) due to different mean RITA durations of assay with non-B subtypes<br><br>A proportion of people with longstanding infection, with severe immunosuppression or on antiretroviral therapy are misclassified as having recent HIV infection |
| Proportional assay (e.g. BED-CEIA) | Measures ratio of HIV-specific IgG to total IgG<br><br>Ratio increases in recent infection | Proportion of HIV-specific antibody | A proportion of people with longstanding infection, with severe immunosuppression or on antiretroviral therapy are misclassified as having recent HIV infection<br><br>Factors that alter total antibody production may impact results |
| 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 | Avidity | A proportion of people with longstanding infection, with severe immunosuppression or on antiretroviral therapy are misclassified as having recent HIV infection |

| Assay type | Principle | Component of anti-HIV immune response being measured | Limitations |
|---|---|---|---|
| | This index increases during recent infection | | Variation in kit batches may impact assay<br><br>There are no built-in controls or calibrators for recency classification in the kit, but users can incorporate self-validated controls |
| Single-well avidity | Measures antibody avidity by limiting antigen that facilitates binding of only high-avidity antibodies<br><br>Recency is inferred from reactivity below a fixed normalized cutoff | Avidity | Assay is calibrated to determine cutoff<br><br>A proportion of people with longstanding infection, with severe immunosuppression or on antiretroviral therapy are misclassified as having recent HIV infection |
| High dynamic range immunoassays | Specimens from people recently infected have a low antibody titre<br><br>Wide dynamic range of these assays allows better differentiation between recent and longstanding infection | Antibody titre | Assay batch changes may impact performance<br><br>A proportion of people with longstanding infection, with severe immunosuppression or on antiretroviral therapy are misclassified as having recent HIV infection<br><br>High cost of automated platform |
| Immunodominant assay (e.g. IDE-V3 assay) | Measures total response to selected gp41 and gp120 epitopes that induce most consistent antibody responses | Anti-gp41/anti-gp120 V3 immunodominant responses | Assay has low sensitivity<br><br>Not commercially available<br><br>Limited data on use cases |
| p24 antigen | Detects p24Ag in absence of anti-HIV antibody | Presence of p24Ag, absence of anti-HIV antibody | Period when a person is p24Ag-positive and anti-HIV antibody-negative is brief (1–2 weeks)<br><br>Large sample populations required to obtain incidence estimates due to short MDRI<br><br>P24Ag can appear in late-stage AIDS after failure of immune system |
| HIV RNA | Detects HIV RNA in absence of anti-HIV antibody | Presence of HIV RNA, absence of HIV antibody | Large sample populations required to obtain incidence estimates<br><br>HIV RNA present for only short time before antibody seroconversion |
| Anti-p24 IgG3 | Measures narrow, temporary response to p24 in single subclass of IgG that is seen consistently in recent infection | Subclass specific anti-p24 response | Findings have not been reproducible<br><br>Short period in which recent infection can be inferred<br><br>Not commercially available |
| Line immunoassay (e.g. INNO-LIA HIV-I/II Score) | Measures reactivity with various synthetic oligopeptides and recombinant antigens | Reactivity with various antigens | Assay is expensive unless it is used routinely as confirmatory test<br><br>Interpretation of reactivity is subjective |

[a] Availability of assays in each of these categories can be found in Tables A4.2 and A4.3.

**Table A4.2.**

Commercially available recency assays and methodologies [a]

| Product | Manufacturer | Assay type | Reference |
|---|---|---|---|
| AsantéTM HIV-1 Rapid Recency® Assay | Sedia Biosciences Corporation, Beaverton, OR, United States | Rapid testing for recent infection—avidity | http://www.sediabio.com/products/asante-rapid-hiv-1-recency-assay |
| Maxim SwiftTM HIV Recent Infection Assay | Maxim Biomedical, Rockville, MD, United States | Rapid testing for recent infection—avidity | https://www.maximbio.com/swift-hiv-recent-infection-assay-kit |
| Sedia® HIV-1 Limiting Antigen (LAg)-Avidity EIA for Plasma/Serum | Sedia Biosciences Corporation | Laboratory-based, manual enzyme immunoassay—avidity | http://www.sediabio.com/products/lag-avidity-eia |
| Maxim HIV-1 LAg-Avidity EIA Kit for Plasma/Serum | Maxim Biomedical | Laboratory-based, manual enzyme immunoassay—avidity | https://www.maximbio.com/Products/92001/Maxim-HIV-1-Limiting-Antigen-Avidity-(LAg-Avidity)-EIA-Kit%2C-192-Tests |
| Maxim HIV-1 LAg-Avidity EIA Kit for DBS | Maxim Biomedical | Laboratory-based, manual enzyme immunoassay—avidity | https://www.maximbio.com/Products/92003/Maxim-HIV-1-Limiting-Antigen-Avidity-(LAg-Avidity)-DBS-EIA-Kit%2C-192-Tests |
| ARCHITECT HIV Ag/ Ab Combo (avidity protocol or unmodified protocol) | Abbott Laboratories, Abbott Park, IL, United States | Laboratory-based, proprietary automated platform CEIA—avidity and high dynamic range | Suligoi B, Regine V, Raimondo M, et al. HIV avidity index performance using a modified fourth-generation immunoassay to detect recent HIV infections. Clin Chem Lab Med. 2017;55(12):2010–2019 <br><br> Grebe E, Welte A, Hall J, et al. Infection staging and incidence surveillance applications of high dynamic range diagnostic immuno-assay platforms. Acquir Immune Defic Syndr. 2017;76(5):547–555 |

| Product | Manufacturer | Assay type | Reference |
|---|---|---|---|
| Genetic Systems HIV-1/HIV-2 PLUS O EIA (Avidity protocol), multiple methods | Bio-Rad Laboratories, Redmond, WA, United States | Laboratory-based, manual enzyme immunoassay—avidity | Shepherd SJ, McAllister G, Kean J, et al. Development of an avidity assay for detection of recent HIV infections. J Virol Methods. 2015;217:42–49<br><br>Masciotra S, Dobbs T, Candal D, et al. Antibody avidity-based assay for identifying recent HIV-1 infections based on Genetic Systems™ 1/2 Plus O EIA. Presented at Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 2010 |
| VITROS® Anti-HIV 1+2 (avidity protocol) | Ortho Clinical Diagnostics, Raritan, NJ, United States | Laboratory-based, proprietary automated platform CEIA—avidity | Chawla A, Murphy G, Donnelly C, et al. Human immunodeficiency virus (HIV) antibody avidity testing to identify recent infection in newly diagnosed HIV type 1 (HIV-1)-seropositive persons infected with diverse HIV-1 subtypes. J Clin Microbiol. 2007;45:415–420 |
| GeeniusTM HIV-1/2 Supplemental Assay (modified protocol) | Bio-Rad Laboratories | Laboratory-based, lateral flow enzyme immunoassay—relationship between HIV antigens | Keating SM, Kassanjee R, Lebedeva M, et al. Performance of the Bio-Rad Geenius HIV1/2 supplemental assay in detecting "recent" HIV infection and calculating population incidence. J Acquir Immune Defic Syndr. 2016;73(5):581–588 |
| INNO-LIA® HIV I/II Score | Fujirebio US, Malvern, PA, United States | Laboratory-based, manual enzyme immunoassay—relationship between HIV antigens | Schupbach J, Gebhardt MD, Tomasik Z, et al. Assessment of recent HIV-1 infection by a line immunoassay for HIV-1/2 confirmation. PLoS Med. 2007;4(12):e343 |

[a] When modified procedures or interpretative criteria are used, the assays are being used off-label and the results obtained are not diagnostic. Modified assay may not be supported by the manufacturer. Bespoke recency assays do not have claims for HIV diagnosis.

**Table A4.3.**

Previously described methodologies (noncommercial or no longer available) [a]

| Product | Manufacturer | Assay type | Reference |
|---|---|---|---|
| IDE-V3 | Not applicable | Laboratory-based, manual enzyme immunoassay—relationship between HIV antigens | Barin F, Meyer L, Lancar R, et al. Development and validation of an immunoassay for identification of recent human immunodeficiency virus type1 infections and its use on dried serum spots. J Clin Microbiol. 2005;43(9):4441–4447 |
| 3A11-LS assay (less sensitive modification) | Abbott Laboratories | Laboratory-based, proprietary automated platform, manual enzyme immunoassay—antibody titre | Janssen RS, Satten GA, Stramer SL, et al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA. 1998;280(1):42–48 |
| Vironostika-LS (less sensitive modification of the Vironostika HIV-1 EIA) | bioMérieux, Inc., Durham, NC, United States | Laboratory-based, manual enzyme immunoassay—antibody titre | Kothe D, Byers RH, Caudill SP, et al. Performance characteristics of a new less sensitive HIV-1 enzyme immunoassay for use in estimating HIV seroincidence. J Acquir Immune Defic Syndr. 2003;33(5):625–634 |
| AxSYM HIV ½ gO (modified protocol) | Abbott Laboratories | Laboratory-based, proprietary automated platform enzyme immunoassay—avidity | Suligoi B, Galli C, Massi M, et al. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. J Clin Microbiol. 2002;40:4015–4020 |
| IgG3 (informal name used for an unnamed IgG3-specific anti-p24 EIA) | Not applicable | Laboratory-based enzyme immunoassay—presence of particular IgG subclass | Wilson KM, Johnson EI, Croom HA, et al. Incidence immunoassay for distinguishing recent from established HIV-1 infection in therapy-naive populations. AIDS. 2004;18(17):2253–2259 |

[a] *This is not an exhaustive list but includes assays that have been widely used previously.*

# Annex 5
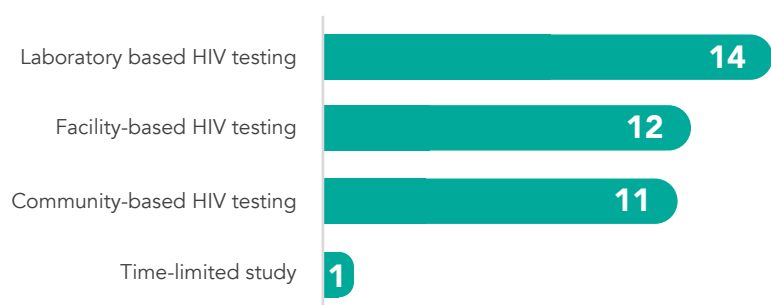# Country-level data submitted in response to a WHO survey about recency assays (November 2020–January 2021)

**Figure A5.1.** The charts below summarize various aspects of recency assays across all WHO member states, health jurisdictions or Technical Assistance partner institutions participating in a survey about recency assays[2] (more detail about methods is available in section 1.5).

**Recency Testing.** Among the 47 total respondents, 60% (n=28) currently use recency testing in their respective country.

**60%**
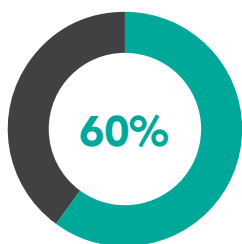
of respondents (28 of 47 total respondents) are using recency testing in their country

**HIV testing services.** Respondents using recency testing offer testing services in laboratory-based, facility-based, and/or community-based settings. (Total respondents = 25, results are not mutually exclusive).

| | |
|---|---|
| Laboratory based HIV testing | 14 |
| Facility-based HIV testing | 12 |
| Community-based HIV testing | 11 |
| Time-limited study | 1 |

**Program Scale.** Most recency testing services operate at a scale of more than 1,000 clients. (Total respondents = 23).

| <100 clients | 100 to 1,000 clients | > 1,000 clients |
|---|---|---|
| 9% | 26% | 65% |
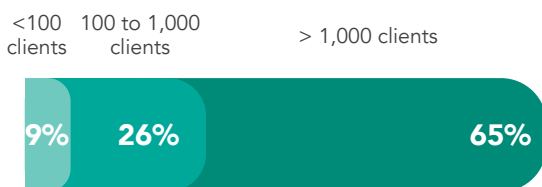
**Population tested.** Recency tests are commonly offered to all HIV Testing Services (HTS) clients testing positive or to select HTS client subgroups. (Total respondents = 24)

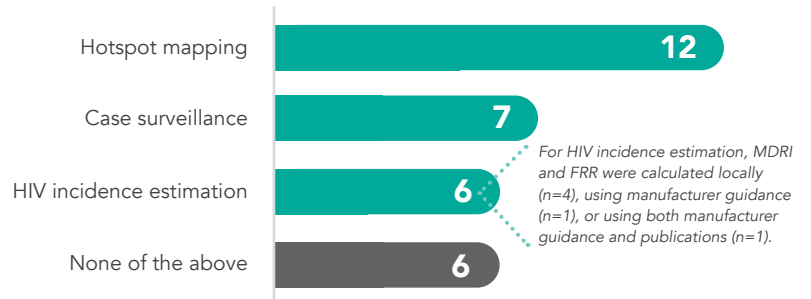| | |
|---|---|
| All clients testing HIV-positive through HTS | 10 |
| Select groups of HTS clients | 10 |
| All HIV-positive participants in population or targeted survey(s) | 3 |
| Select groups in population or targeted survey(s) | 1 |
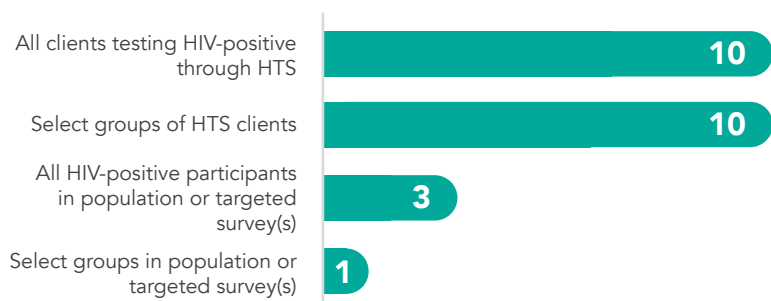
2    Respondents included: Argentina, Australia (Monash University), Bangladesh, Belgium (Gent Uni), Bhutan, Callen Lorde, Cambodia (CDC), Cameroon, Democratic Republic of Congo (TRACE), England, England (PHE), EQAPOL, Eswatini (FHI360), Eswatini (PSI), Eswatini (TRACE), Ethiopia (PHI and WHO), Ethiopia (PSI-USAID), India, Indonesia, Ireland (NVRL and HPSC), JHPIEGO, Kenya, Malawi, Zimbabwe (MSF), Kenya_NASCOP, Lesotho (TRACE), Malawi (iTECH), Maldives, MSF, Myanmar, Nepal, Nepal (FHI360), Netherlands, Nigeria_SHARP TO2 (FHI360), Nigeria_SIDHAS (FHI360), Paul Drain—SA (UWashington),PEPFAR (representing all 50+ countries), South Africa, South Africa (HSRC), South Korea, Sri Lanka,Thailand, Thailand (CDC),Timor Leste, Ukraine (UKR), United States, Vietnam (FHI 360), Vitalant Research Institute, Zimbabwe (PSI).

**Surveillance.** Eleven respondents used recency results to inform targeted HIV interventions (n=9) and/or additional surveillance (n=5).
(Total respondents = 19, results are not mutually exclusive).
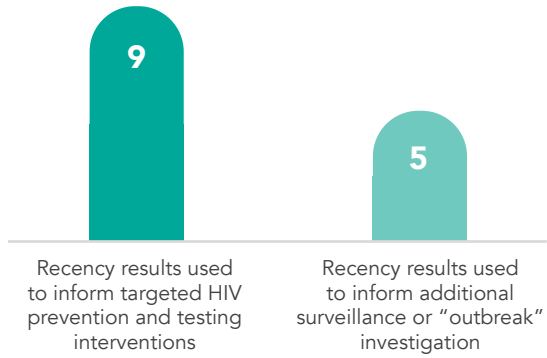
**Uses of recency results.** For those using recency testing (n=28), 64% apply it to hotspot mapping, case surveillance, and/or HIV incidence estimation (results are not mutually exclusive).



| | |
|---|---|
| **9** | **5** |
| Recency results used to inform targeted HIV prevention and testing interventions | Recency results used to inform additional surveillance or "outbreak" investigation |



Hotspot mapping — 12
Case surveillance — 7
HIV incidence estimation — 6
None of the above — 6

*For HIV incidence estimation, MDRI and FRR were calculated locally (n=4), using manufacturer guidance (n=1), or using both manufacturer guidance and publications (n=1).*

# Glossary

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| | |
|---|---|
| **Acute HIV infection** | HIV infection where viral RNA is detectable but no viral antigen (p24 protein) is detectable or no humoral immune response (anti-HIV antibodies) has developed. This state lasts from shortly after exposure to several days to several weeks after infection, depending on the methods of viral RNA, viral antigen and antibody detection, and can be included in the definition of recent HIV infection without relying on a recency assay in these cases. |
| **Calibration** | calibration of an assay for recent infection involves the use of seroconversion panels to define the assay cutoff point that will give rise to the assay MDRI. The standard error of the MDRI can also be derived through the process of calibration. |
| **Concentrated epidemic** | epidemic state in which HIV has a low prevalence in the general population but has spread rapidly in defined subpopulations. In countries with concentrated epidemics, HIV prevalence is less than 1% in the general population and more than 5% in at least one defined population such as gay men and other men who have sex with men, people who inject drugs, or sex workers. |
| **Cross-sectional survey** | survey used to gather information on a population or sample of a population at a single point in time. |
| **Denominator** | lower portion of a fraction used to calculate a rate or ratio. |
| **Enzyme immunoassay** | HIV test that identifies the presence of antibodies to HIV. |
| **Established infection** | longstanding infection. |
| **False recent ratio (or rate) (FRR)** | proportion of people with longstanding HIV infection (infected for longer than a specified cutoff time denoted T) in a population that are misclassified by the RITA as having recent infection. This parameter is essential for the estimation of HIV incidence using a RITA and, as with MDRI, is context-dependent. |
| **Gp120** | 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. |
| **Grey literature** | literature that is not formally published in peer-reviewed journal articles or books, such as government reports, conference presentations or website content. |

| | |
|---|---|
| **Incidence** | frequency with which HIV infection is acquired in a population. Incidence is defined as either a proportion (probability of infection occurring before a given time) or a rate (number of new cases of infection during a specified amount of person-time, e.g. per 100 000 people per year). HIV incidence is a quantitative way to measure the extent of ongoing HIV transmission in a population. |
| **Incidence ratio** | ratio of incidence in one population to incidence in another population. |
| **Key population** | group that often has substantially higher HIV prevalence or incidence than the general population, usually the focus of targeted HIV prevention or care interventions, including testing. UNAIDS considers gay men and other men who have sex with men, sex workers, transgender people, people who inject drugs, and people in prison and other incarcerated people as the five main key population groups that are particularly vulnerable to HIV and frequently lack adequate access to services. |
| **Longstanding (or long-term) infection** | state that begins when a person transitions out of the recent state and is considered to have HIV infection of longer duration. As with recent HIV infection, the time at which a person transitions to the longstanding state can be defined in purely chronological terms or in biological terms. |
| **Mean duration of recent infection (MDRI)** | average duration of the recent state in people infected for less than a specified cutoff time (known as T), for a specific RITA in a population of people with HIV infection. This parameter is essential for the estimation of HIV incidence using a RITA. MDRIs are not fixed but are context-dependent, and they may vary by subtype or other population characteristics. |
| **Molecular surveillance** | method of cluster identification that involves genetic sequencing of HIV RNA from people receiving new diagnoses of HIV infection. |
| **Numerator** | upper portion of a fraction used to calculate a rate or ratio. |
| **Point-of-care test** | test that can be used outside of a traditional laboratory, at the bedside or within a community setting, and run while the person waits for the results. |
| **Population-based survey** | survey designed to produce nationally or regionally representative estimates of HIV incidence or other epidemic characteristics, especially for comparison with other countries or regions. Population-based surveys typically have large sample sizes and are often conducted in households sampled from a catchment area, with trained interviewers administering interviews using standardized data collection instruments. |
| **Prevalence** | percentage of people in a population with an infection at any time during a specific period. |

| **Rapid test for recent infection** | recency assay designed for rapid results (e.g. 20 minutes) and that can be used as a point-of-care test or run in a centralized laboratory. |
|---|---|
| **Recency assay** | HIV immunoassay designed to detect recent infection— that is, whether a person was infected recently (before a predetermined time T, e.g. 2 years) or has a longstanding infection. |
| **Recent infection testing algorithm (RITA)** | 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 longstanding. |
| **Recently acquired (or recent) HIV infection** | state that begins at the moment the biological process of HIV infection is initiated. Its duration can be defined in purely chronological terms, such as 6 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 varies between people. |
| **Sample** | selected subset of a population. |
| **Sample, representative** | a sample of people whose characteristics correspond to those of the original or reference population. |
| **Selection bias** | epidemiological term that means the people with data in the study or programme are representative not of the general population but of a subset that has been preferentially selected for inclusion. If not identified and discussed as part of findings, selection bias can mean that results (e.g. estimates of recent infection as a proxy for ongoing HIV transmission in the group) are misinterpreted to mean something about a larger group of people when this is inaccurate. |
| **Sensitivity** | a diagnostic test's ability to correctly identify a person with true infection. A test with 99% sensitivity will produce a false negative result 1% of the time by producing a negative result when in fact infection exists. Highly sensitive tests are desirable for ensuring the smallest possible number of infections are missed by a screening programme. This term is not applicable for use with recency assays. |
| **Sensitivity analysis** | statistical technique designed to determine how different parameters or assumptions in a statistical model (i.e. sources of uncertainty in the model) affect results. Commonly, a series of different but plausible assumptions are tried for unknown parameters, with results reported for a range of plausible values, but other methods (e.g. Bayesian bias analysis) can be used. |

| **Sentinel population** | population of people at a single facility or a small number of facilities, chosen for surveillance or study because they are believed to be representative of the surrounding population or to function as an early warning sign of increasing area trends of HIV incidence. |
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| **Specificity** | diagnostic test's ability to correctly identify a person who is not truly infected. A test with 99% specificity will produce a false positive result 1% of the time, by producing a positive result when in fact the person does not have any infection. Highly specific tests are desirable for confirming HIV infection or ensuring people tested with a RITA are living with HIV. This term is not applicable for use with recency assays. |
| **Surveillance** | strategy to systematically collect and analyse data about a specific public health issue, and to regularly interpret the data and disseminate findings to guide policy-makers, funders and programme planners in decisions about intervention prioritization and resource allocation. |

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