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1. **What is HIV incidence?**

HIV incidence is the rate at which new HIV infections occur in a population (the number of new HIV cases divided by the number of HIV negative individuals at risk over a given time period, usually one year).

2. **What is HIV prevalence?**

HIV prevalence is the proportion of people who are living with HIV at a given time, within a given population. There are several approaches used to estimate HIV prevalence, including probability-based surveys, collection of sentinel surveillance data on people being treated at public health facilities (typically pregnant women at antenatal clinics) and/or specialized surveys of high-risk sub-populations.

3. **How does HIV incidence differ from prevalence?**

HIV incidence measures ongoing transmission (i.e. recent infections), rather than the total proportion of the population living with HIV at a given point in time. Prevalence offers important information about the overall HIV burden in a population. It is also easier to estimate than incidence and has therefore been more frequently used as an indicator. However, prevalence measurements are often subject to selection bias, and those based on population surveys can be confounded by migration, birth rates and the extended longevity offered by antiretroviral therapy.

4. **Why is HIV incidence useful as an indicator?**

HIV incidence provides critical insight into the current trajectory of the HIV epidemic and is thus invaluable in population surveillance, intervention targeting, prevention programme evaluation, clinical trial design and planning for future needs. The ability to accurately estimate HIV incidence at the national or subnational level offers one of the most promising mechanisms to assess and optimize the effectiveness of HIV prevention, treatment and care programmes.
5. How has incidence typically been measured and what are the drawbacks of these methods?

The public health community has experienced, and continues to face, fundamental difficulties in obtaining reliable estimates of HIV incidence.

Most often, HIV incidence is derived using indirect methods based on mathematical models that rely on the use of prevalence survey data. There are three typical approaches: one uses serial prevalence surveys, a second relies on assumptions about risk-taking behavior and HIV-1 transmission, and the third is based on prevalence in recently exposed populations. Most of these are subject to bias and a wide range of uncertainty, due partly to the fact that many countries do not have comprehensive population-based prevalence survey data or thorough information on migration and mortality of HIV-positive individuals.

It is possible to use a direct method of estimation, the theoretical ‘gold standard’ in HIV incidence measurement, which involves following a cohort of HIV-negative people over time and measuring the proportion that contract HIV. This method is rarely used: it is logistically challenging, expensive, subject to participation bias and takes many years to complete.

6. What is an HIV incidence assay?

An HIV incidence assay is a laboratory-based test that is used to distinguish ‘recent’ HIV infections from ‘non-recent.’ Incidence assays are usually only carried out on specimens already known to be HIV-positive, for example, following standard antibody detection tests. These assays are designed to identify the presence, or lack thereof, of specific biomarkers in the blood that can determine whether an HIV infection is newly acquired (i.e. typically less than one year) or established.

HIV incidence assays offer a way to estimate HIV incidence at a population level that is potentially game-changing. An effective HIV incidence assay would allow direct measurement rather than requiring mathematical modelling. This means estimations would be more accurate and could reflect current conditions, unlike present methods that rely on data that is often several years old. In theory, these assays could also be used to provide estimation of HIV incidence at the subpopulation level.

Several laboratory assays to estimate HIV incidence have been developed. However, in the modern context of antiretroviral treatment (ART) scale-up (since ART can affect incidence assay results), currently there is no single test that can be relied upon for accurate estimation of incidence as a stand-alone tool.

7. What is a recent infection test algorithm or RITA?

A recent infection test algorithm (RITA) is the generic term for a combination of two or more laboratory-based assays, at least one of which is an HIV incidence assay, that classifies HIV infections as recently acquired or not. RITAs are used as part of cross-sectional surveys, which provide information for a single point in time, rather than longitudinal data collection, which requires repeat measurements in individuals. Currently, this is the only reliable direct method of estimating incidence given that no single incidence assay meets established criteria for accuracy. However, the ideal is a standalone assay that does not require supplemental tests.

8. What is the mean duration of recent infection and why is it an essential parameter for estimating HIV incidence using a RITA?

The mean duration of recent infection or MDRI is the average time period, beginning from a defined point soon after the initial HIV transmission (for example, seroconversion or first detection of HIV RNA) until a few months later (usually 4-12 months), during which an infection is classified as recent by a given RITA. Ideally, this time period would be identical for every person. In reality, people vary in their immunological responses to HIV. In addition, characteristics of HIV such as the subtype influence the MDRI, which means that it varies depending on the predominant subtype of HIV circulating in the population. Since the MDRI affects the required sample size necessary to estimate HIV incidence in a given population, it is important when planning a survey to consider HIV subtype and other characteristics that may influence it.
9. What is a false recent ratio and why is it essential to calculate?

A false recent ratio (FRR, also sometimes referred to as the false recent rate or the proportion false recent) is a generic term for the proportion of individuals with longstanding infections (infected for longer than a defined period of time, usually two years) who are incorrectly classified by a RITA as recently infected. Misclassifications are often due to viral suppression (e.g. following successful treatment with antiretroviral drugs) or subtype variation. The MDRI and FRR are interrelated parameters that influence each other.

The FRR varies across populations and according to the assay(s) used. Therefore, for each survey, an FRR must be estimated for specific populations based on the proportion of ART-exposed, virally suppressed subjects and the predominant HIV subtypes found in that population. At present, calculating an FRR that is relevant to the test population and the assay(s) or RIITAs being used is one of the most significant challenges in using a RITA to estimate HIV incidence.

10. To what extent is the incorporation of viral load testing, and testing for presence of antiretroviral drugs helpful in reducing the uncertainty of incidence assay estimates?

RIITAs can incorporate the results of supplemental tests in order to reduce the FRR. The most commonly used supplemental test is viral load testing, which provides a way to exclude virally suppressed individuals who are misclassified as recently infected by an HIV incidence assay. In some instances, the addition of testing for the presence of antiretroviral drugs (ARV) has also been used, in combination with viral load.

A 2015 UNAIDS/WHO technical update on HIV incidence assays recommended the incorporation of viral load testing in RIITAs and suggested that there was increasing evidence in support of also testing for antiretroviral drugs. It is currently easier and cheaper to measure viral load, but surveys that already plan to include testing for the presence of ARV drugs (as South Africa has done) can apply that information in the RITA in addition to viral load testing. The guidance notes, however, that integration of supplemental information does not eliminate the need for estimating the false recent ratio of a RITA.

11. What HIV incidence assays are available and how reliable are they?

Numerous assays to detect recent HIV infection have been developed, yet only a few are commercially available. Evaluation of five of these assays showed that a limiting antigen (LAg) avidity enzyme immunoassay developed by the U.S. Centers for Disease Control and Prevention (CDC) had the lowest false recency ratio. However, no single assay fully met the recommended target product profile for an HIV incidence assay because of high false recency ratios in ART-exposed, virally suppressed subjects or in elite controllers (i.e. HIV-positive individuals who maintain undetectable viral loads without treatment, who comprise a very small proportion of people living with HIV). Therefore, the 2015 UNAIDS/WHO technical update on HIV incidence assays recommended the incorporation of viral load testing in RIITAs, as explained in #10 above.

A RITA that uses the LAg assay with viral load testing does meet the recommended target product profile for national surveys in some countries. These are countries with a high enough HIV prevalence and incidence that survey sample sizes have enough statistical power to account for the false recency ratio and provide an accurate incidence estimate. Currently, nearly 20 national HIV surveillance surveys using this type of RITA are being planned for high-prevalence countries from 2016 to 2020 for cross-sectional HIV incidence estimation. Surveys in low-prevalence countries, or countries where the predominant HIV subtype frequently gives false recent results, may require advancements beyond the currently available assays to enable accurate estimation of incidence.

The UNAIDS Reference Group on Estimates, Modelling and Projections has developed a tool that can be used by countries with assay-based HIV incidence data to inform estimates based on surveillance, survey and AIDS-related mortality data. It is likely that, until current RIITAs are strengthened or a new generation of HIV incidence assays is developed, triangulation of RITA-based HIV incidence estimates with data from mathematical modelling and cohort analysis will remain the best practice for developing accurate HIV incidence estimates.
12. Can currently available RITAs be used for subnational or subpopulation incidence estimates?

In theory, currently available RITAs can be used for incidence estimates in some subnational contexts or subpopulations. However, certain requirements must be fulfilled, such as the minimum sample size, and high HIV prevalence and incidence. As a result, using RITAs for subpopulation estimates, while possible in some contexts, is challenging because sample size requirements can be large— which gets expensive— unless incidence is very high. As well, HIV data are not always available for subpopulations of interest.

13. How can the use of available assays for estimating HIV incidence be optimized?

Promoting systematic implementation and impact analysis can help optimize the use of existing HIV incidence assays. This might include: supporting the development of detailed user guidance and harmonized training for implementers; developing and implementing an external quality assurance programme; conducting impact assessment to analyse the cost-benefits of using a RITA; providing support for organizations interested in implementation by helping them determine which, if any, of the currently available RITAs are appropriate for the populations they wish to assess; and/or supporting and enabling current HIV incidence assay manufacturers.

In addition, further study of current assays with the aim of determining assay performance in different geographic contexts (i.e. according to HIV subtype) will offer valuable insights into how and when to use different RITAs to estimate HIV incidence at the population level.

14. What new HIV incidence assays are in development?

Given that existing HIV incidence assays and RITAs are not appropriate for all HIV epidemic contexts, the research and development of new HIV incidence assays is a priority.

There are several discovery and development projects that are investigating new biomarkers, funded by the Bill and Melinda Gates Foundation, which are expected to reach the proof of concept stage by the end of 2015. The U.S. National Institutes of Health also funded several projects to find new biomarkers for HIV incidence assays and to reformat the LAg assay. Ideally one or more of these will be promising enough to pursue further, ultimately leading to the development and commercialization of a new assay that can simplify HIV incidence measurement in all epidemiological contexts.

15. What are the sustainability challenges for research on a new generation of HIV incidence assays?

There are several constraints and challenges associated with research and development of a new generation of HIV incidence assays. Despite the importance of HIV incidence as a national and global measure of importance (see #16 below), the market for HIV incidence assays is small, as it is mainly used for public health surveillance. National surveys that incorporate currently available assays tend to be expensive because they are large. For this reason, they are only conducted every 2-5 years. Without a large market, resource limitations faced by commercial producers (who are often small companies) present a major hurdle, and more significant financial support from the public sector will likely be needed going forward.

FIND has contracted an external party to provide an assessment of current and future market demand for these assays, which will be available by early 2016. This assessment will allow FIND and partners to better understand and demonstrate the value of investing in a new generation of HIV incidence assays.

16. Do major public health institutions (such as WHO and UNAIDS) and donors agree on the importance of HIV incidence as a key indicator?

A consensus is forming around the importance of HIV incidence estimates in global reporting. In May 2015, the WHO released guidelines detailing a set of 10 key indicators—one of which is HIV incidence—that have been prioritized as essential information in the HIV prevention, care, treatment and support continuum. These indicators, identified through a consultative process, are aligned to new programmatic recommendations and reflect the future of reporting requirements for measuring progress and for global accountability.
In addition, HIV incidence has been proposed as one of the indicators for the newly approved Sustainable Development Goals, which will guide global health and development priorities through 2030.

PEPFAR, UNAIDS, UNICEF, UNITAID, USAID, WHO and the Global Fund have confirmed their interest in incidence assays. In sum, major institutions agree on the need for both refinement and implementation of existing RITAs, as well as further research and development of new and improved assays to enable more

Measuring new infections is not only to evaluate impact... it should also be a critical part of knowing your epidemic.