

**TECHNICAL UPDATE
ON HIV INCIDENCE ASSAYS
FOR SURVEILLANCE
AND MONITORING PURPOSES**

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Introduction

This document is intended for epidemiologists, statisticians and laboratory technicians responsible for the use of HIV incidence assays for surveillance and epidemic monitoring purposes. People that are not technical experts in this field may prefer to first read the UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance published guidelines on When and How to Use Assays for Recent Infection to Estimate HIV Incidence at a Population Level [1] for a basic introduction to this topic.

The purpose of this technical update is to summarize recent findings and offer new recommendations in the field of HIV incidence assays. This update is based on recently published literature and presentations at the WHO HIV Incidence Assay Working Group Meeting in October 2014 in Barcelona, Spain. This meeting was sponsored by the World Health Organization (WHO), the US Government's Centers for Disease Control and Prevention (CDC), the Consortium for the Evaluation and Performance of HIV Incidence (CEPHIA) and the Joint United Nations Programme on HIV/AIDS (UNAIDS). A detailed meeting report is available at

http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/.

This document is divided into four sections. The first provides a brief summary of where we are at in the development and use of HIV incidence assays for surveillance and epidemic monitoring. The second section summarizes recent findings from the literature and the October 2014 WHO HIV Incidence Assay Working Group Meeting in Barcelona, Spain. The third section provides a summary of key recommendations from the meeting report reflecting consensus among participants on key topics. The fourth section describes future directions and research needs arising from the meeting.

This technical update supersedes the previous WHO/UNAIDS technical update on HIV incidence assays for surveillance and epidemic monitoring published on 30 May 2013.

Development and use of HIV incidence assays

Development of HIV incidence assays has been challenging because of a host of factors that can lead to misclassification of individuals who actually have long-standing infections as being recently infected. These factors include variability in immune responses at an individual and population level, variability by HIV-1 subtype, access to antiretroviral therapy, decreases in the genetic diversity of the HIV virus in the era of antiretroviral therapy, advanced HIV disease and other factors that are not well understood. Previously, the inability to account for these factors when measuring incidence led to incorrect conclusions about the level and patterns of new infections in many countries [2].

Although about 20 different assays have been developed or adapted to detect recent HIV infection, only six assays have been used to estimate cross-sectional incidence in studies and cross-sectional surveys. Until recently, only one dedicated incidence assay – the BED-capture enzyme immunoassay (Calypte, USA; Sedia BioSciences, USA) – was commercially available. However, in 2012, a second assay – the limiting antigen (LAg) avidity enzyme immunoassay (Sedia BioSciences, USA) – was commercially released. The remaining assays are modified commercially-available HIV assays.

More recently, measures of HIV incidence have improved owing to the development of newer assays, coupled with strategies for reducing misclassification using recent infection algorithms that take into account HIV viral load and exposure to antiretroviral therapy. Recent infection testing algorithms that include use of two incidence assays, in combination with CD4 and viral load, for a total of four assays, have been applied in randomized trial and cohort-based studies [3-5].

Starting in 2015, the United States Government is investing in population-based surveys that use a recent infection testing algorithm of LAg and viral load to measure the health impact of HIV programs in at least 12 sub-Saharan African countries. Elsewhere, the use of appropriate recent infection testing algorithms in other population-based settings and surveys continues to be debated.

Summary of recent findings

The list below highlights recent findings from the October 2014 WHO HIV Incidence Assay Working Group Meeting in Barcelona, Spain, and the literature that were used to inform the new UNAIDS/WHO recommendations.

- **Evaluation of single assays:** Results from an independent evaluation of the performance and operational characteristics of five HIV incidence assays – LAg, BED-CEIA, Less-sensitive/Detuned Vitros, Vitros Avidity and BioRad Avidity – showed that LAg had the lowest false recency rate[6]. However, none of the assays fully met the recommended target product profile for an HIV incidence assay because of misclassification of elite controllers and those receiving ART. Higher false recency rates were found for sub-type A1 and D specimens, although larger sample sizes will be required to confirm the extent of subtype misclassification.
- **Modifications to LAg test kit performance characteristics:** When using LAg in cross-sectional studies, a cut-off normalized optical density of 1.5 and a mean duration of recent infection of 130 days (95% confidence interval 118–142) is now recommended (article in press, PlosOne, and as reflected in test kit instructions for use). A LAg kit is also available for use with dried blood spot specimens.
- **Application of RITA in country settings:** An assay's false recency rate can be significantly reduced by using a combination of results from a single assay and viral load testing to determine recency of infection, because low viral load may signal exposure to antiretroviral therapy or elite controllers. Testing for antiretroviral therapy exposure in addition to viral load may further reduce the false recency rate, although additional research is needed to determine whether this additional testing is recommended.
- **Validation of recent testing infection algorithms in the field:** In Kenya[7] and South Africa[8], recent testing infection algorithms using LAg, viral load and antiretroviral therapy testing have produced estimates of HIV incidence in national population surveys that are consistent with mathematical model and synthetic cohort analyses of HIV incidence. In Swaziland[9], the estimate of LAg derived incidence with viral load only from a national population survey was similar to that observed in a follow-up longitudinal cohort study in the same population.
- **Changes in the formula to calculate incidence:** The current recommended formula for calculating incidence assuming a post-infection cut-off time of one year[10] can lead to an unnecessarily high false recency rate for many assays. A cut-off time of two years was found to reduce the false recency rate while not substantially decreasing the mean duration of recent infection in these assays.

- **Powering population-surveys to detect changes in incidence:** Sample sizes required to detect changes in HIV incidence over time are large, suggesting that application of recent testing infection algorithms to estimate incidence in settings where HIV prevalence and incidence is low may be limited.
- **Reconsideration of the mean duration of recent infection estimate:** Although not a new issue, the current estimate of the mean duration of recent infection does not take into account the time from infection to seroconversion and therefore detection by HIV serology assays; this period could be as long as one month, whatever the type of assay. Mean duration of recent infection estimates should be based on time from infection, and additional research is required to estimate this period for different assays.
- **Triangulation of HIV incidence estimates:** The UNAIDS Reference Group on Estimates, Modelling and Projections has developed methods in the Spectrum software tool (software and documentation available at <http://www.avenirhealth.org/software-spectrum.php>) to allow countries with assay-based estimates of incidence to inform national and sub-national modelled estimates of incidence based on other HIV-related data sources, including surveillance, survey and AIDS-related mortality data.

Key recommendations

To promote accuracy and comparability of HIV incidence estimates for surveillance and epidemic monitoring globally, in 2010 the UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance published guidelines on When and How to Use Assays for Recent Infection to Estimate HIV Incidence at a Population Level [1]. These guidelines, in tandem with the recommendations outlined below, reflect the most up-to-date guidance on using HIV incidence assays for surveillance and epidemic monitoring purposes. They include that:

- Countries should use a recent testing infection algorithm that incorporates viral load testing to estimate HIV incidence in population-based surveys.
- Although there is increasing evidence to recommend testing for the presence of antiretroviral therapy in recent testing infection algorithms – especially in the context of increasing coverage globally – further research is needed.
- A recent infection testing algorithm that uses multiple HIV incidence assays with testing for viral load and antiretroviral therapy requires additional independent and field validation in population-based survey settings before they can be recommended for this purpose.
- Population-based surveys to measure the impact of programs on HIV incidence over time need to be powered appropriately, accounting for the survey design and uncertainty around the false recency rate.
- A post-infection cut-off time of two instead of one year should be assumed when calculating HIV incidence in a cross-sectional survey.
- Even though the proportion testing falsely recent using a recent infection testing algorithm is low, correcting for this misclassification when calculating incidence remains necessary.
- Caution should be taken when interpreting results in geographic areas where HIV sub-types A1 and D predominate, as HIV incidence assays have higher reported levels of misclassification in these populations.
- State-of-the-art quality assurance measures should be put in place in laboratories that use incidence assays.
- Despite improvements in the performance of recent testing infection algorithms, triangulation of data from this approach with estimates obtained from other methods (e.g. modelling and cohort analyses) is critical.

Future directions and research needs

This technical update, including the new recommendations provided here, illustrate the substantial progress that has been made since 2013 towards the improved use of HIV incidence assays for surveillance and epidemic monitoring.

Critical to continuing this progress is addressing future directions and research needs, as agreed upon by key stakeholders attending the October 2014 WHO HIV Incidence Assay Working Group Meeting in Barcelona, Spain. These include that:

- Experts from CDC and CEPHIA will jointly review data from their respective studies to develop consensus on mean duration of recent infection, including variability by subtypes, if any. Additional studies with increased sample sizes are planned by CDC, to understand performance of LAg in subtypes' A1 and D, and other circulating recombinant forms.
- CEPHIA will continue to analyse data on the initial evaluation of the five assays, and will extend their work to include five additional assays. Comparative performance evaluations may also include different specimen types (e.g. dried blood spots, oral fluid, urine and faeces). They will also provide new data on the evaluation of selected recent testing infection algorithms using viral load and the presence of antiretrovirals.
- The sample size calculation tool currently recommended by the Working Group and available at <http://www.incidence-estimation.org/page/tools-for-incidence-from-biomarkers-for-recent-infection> will be revised by the tool's developer, The South African Centre for Epidemiological Modelling and Analysis, to allow for estimation of required samples sizes that take into account cluster-based survey designs.
- CDC will continue to work to qualify the new kit lots made by commercial partners to ensure LAg quality management systems during manufacturing processes.
- In collaboration with CEPHIA and CDC, the External Quality Assurance Program Oversight Laboratory based at Duke University will begin a proficiency testing programme for the LAg assay. Other assays may be added to the scheme as the need for external quality assessment is demonstrated.

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