Technical Update

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WHO/UNAIDS Technical Update on HIV incidence assays for surveillance and epidemic monitoring

Contents

Summary points 1
Where are we with HIV incidence assays? 2
New developments 3
Future directions 4

Summary points

- **HIV incidence assays are only applicable to a population level and are not valid to estimate recent infection at individual level.**

- **There has been a lot of progress in understanding the issues related to the performance of HIV incidence assays. A newly available assay (Limiting-antigen Avidity EIA) performs better than the previously available BED-CEIA.**

- **Recent Infection Testing Algorithms (RITA) are recommended as they perform better than single assays.**

- **Results from assays or algorithms need to be corrected for "false recency".**

- **Assays and algorithms are being validated for use with dried blood spots.**

- **Large sample sizes are needed to detect incidence changes in populations with RITA.**

Where are we with HIV incidence assays?

Approximately 10 assays have been developed or adapted to estimate HIV incidence by classifying HIV-reactive specimens as being from recently or non-recently infected persons based on the maturation of the immune response. Most such assays are modified commercial HIV diagnostic assays and until recently only one dedicated incidence assay (BED-CEIA) was commercially available. The development of assays based on the maturation of the serological response has been challenged by several factors. Among those are:

1. Variable immune response among individuals: individual differences in the early immune response among people with HIV-1 infection, such as variation of anti-HIV antibody titre, or the rate of antibody production and maturation.

2. Variability by HIV-1 subtypes: different HIV-1 subtypes can exhibit different maturation kinetics on HIV incidence assays depending on the assay used resulting in variability of Mean Duration of Recent Infection (MDRI).

3. Variability by population: different populations can exhibit different antibody maturation kinetics as measured by particular HIV incidence assays and this can result in differences in MDRI

4. False recent status in persons with long-term infection can be due to:

- Elite controllers: individuals who naturally maintain low or undetectable HIV RNA levels have low antibody responses. Elite controllers have a survival advantage and will accumulate in a population over time and may reach 5% of people infected with HIV;
- Antiretroviral therapy (ART): administration of ART early after infection can prevent maturation of the antibody response, and long term suppression of HIV levels results in a decline in antibody levels;
- Advanced HIV disease (AIDS): advancing disease and the accompanying high viraemia and low CD4 count result in declining anti-HIV antibody titters;
- Unknown factors: the false recent rate (FRR) also can vary across HIV subtypes, populations and geographical areas due to reasons that are not well understood.

Due to the heterogeneity of the immune response to HIV infection among individuals, the application of incidence assays based on the serologic response has been challenged by poor performance in certain settings, and assay results may be interpreted improperly. To provide recommendations for overcoming these challenges the WHO Working Group on HIV Incidence Assays published guidelines in 2010[1] on when and how to use assays for recent infection to estimate HIV incidence at population level.

[1] UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance. When and how to use assays for recent infection to estimate HIV incidence at a population level. http://www.who.int/diagnostics_laboratory/hiv_incidence_may13_final.pdf

New developments

Several meetings organised around the 20th Conference on Retroviruses and Opportunistic Infections (CROI) in Atlanta in March 2013 reviewed new developments.

On 3 March, results from the evaluations of incidence assays conducted by the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) were presented to a panel of experts. The assays evaluated were the BED-CEIA assay, Limiting-antigen (LAg) Avidity EIA, Vitros-Less Sensitive and Bio-Rad Avidity Index EIA. Based on the preliminary analysis by CEPHIA, none of the assays evaluated have completely met the recommended target product profile for an incidence assay. However, the results presented demonstrated that the new LAg-Avidity EIA has a lower false-recent rate than the BED-CEIA assay when used on the same specimen sets.

On 7-8 March, the United States Centers for Disease Control and Prevention (CDC) Division of Global HIV/AIDS (DGHA) held a consultation to share more data specifically on the new CDC/DGHA-developed LAg-Avidity EIA, which is now commercially available. The results presented at the consultation demonstrated that the **LAg-Avidity EIA has a lower false-recent rate than the BED-CEIA assay. The incidence estimates based on LAg-Avidity assay results (corrected for FRR) appear closer to modelled estimates of national incidence, compared to similar estimates based on BED-CEIA assay results.**

Future Directions

- The MDRI of LAg-Avidity EIA initially reported by CDC/DGHA is 141 days (95% CI 119-160); however, CEPHIA and new CDC/DGHA data suggest that the MDRI may be shorter and it may be necessary to revise the MDRI estimate and/or to adjust the recommended assay cut-off. Experts from CDC and CEPHIA will review the calibration data for determination of MDRI with the intent to optimize the assay cut-off and the MDRI using new and existing data from longitudinal specimen panels from persons with documented incident infections, with appropriate attention to variability by subtype.

- Many countries supported by PEPFAR have been using the BED–CEIA for incidence surveillance. Considering the lower FRR of the LAg-Avidity EIA compared with the BED-CEIA assay, CDC/DGHA is recommending that countries outside the United States use LAg-Avidity EIA instead of the BED-CEIA. **Even though the false recent rate for the LAg-Avidity EIA is lower than for the BED assay, correcting for false recent results when calculating incidence remains necessary.**

- Currently CDC/DGHA is qualifying the new kit lots made by commercial partners to ensure the LAg-Avidity EIA kit quality. Additional measures to ensure kit quality may need to be implemented, including working with manufacturers and site inspections to ensure good manufacturing practices.

- CDC continues to recommend that state-of-the-art QC measures be put in place in laboratories that use the LAg-Avidity EIA or other incidence assays. **CDC/DGHA will provide training and technical assistance for laboratories wanting to use the LAg-Avidity EIA.**

- To improve the accuracy of recent HIV classification, **it is recommended that the LAg-Avidity EIA and other assays be used in an algorithm where assay-recent specimens are further tested for HIV RNA level and/or for the presence of ARTs to classify specimens with low viral load and individuals on ART as non-recently infected. These and additional algorithms will be validated by CEPHIA and CDC**.

- Although there is substantial interest in using incidence assays for testing dried blood spot (DBS) specimens commonly collected for surveillance purposes, the current LAg-Avidity EIA protocol is for serum/plasma specimens only. A DBS protocol is in development for LAg-Avidity EIA and **CDC/DGHA is working with the manufacturers of LAg-Avidity EIA to expedite the process and validate the use of DBS as soon as the protocol is available. DBS protocols for additional assays that may be used in recent infection testing algorithms (e.g. Bio-Rad Avidity Index EIA, HIV viral load, detection of antiretroviral drugs) have been developed, but still require evaluation for this intended use.**

- CEPHIA will continue data analysis on the initial evaluation of four assays, BED-CEIA, Lag-Avidity EIA, Vitros-Less Sensitive and Bio-Rad Avidity EIA and will provide more data on the evaluation of each assay. CEPHIA will also continue to evaluate other assays including assays based on new technologies that are currently in the developmental pipeline.

- **Large sample sizes are needed to derive precise incidence estimates from cross-sectional surveys in most settings. The UNAIDS Reference Group on Estimates, Modelling and Projections will develop methods to allow the joint analysis of assay-based estimates of incidence with other information about incidence levels and trends.**

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