

**METHODOLOGIC ISSUES: USING STARHS TO ASSESS INCIDENCE**

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The development of assays to detect recent HIV infection in cross-sectional specimens and thereby calculate HIV incidence represents a major advance in monitoring the HIV epidemic. Nevertheless, several methodologic issues must be taken in consideration in obtaining valid and unbiased estimates of incidence.

The source of the specimens used to measure incidence will in part determine how the results should be interpreted. In this regard, specimens may be collected from seroepidemiologic studies or in the context of HIV testing in various settings (e.g. diagnostic testing, STI clinics and blood donors).

The major challenges to the validity of the STARHS approach may be considered as follows: laboratory issues, data quality and completeness and selection bias. I will not deal with issues related to laboratory test performance. With respect to data quality and completeness, it is important that data on exposure category, in particular, be accurate and complete, since incidence in poorly defined or heterogeneous groups is not epidemiologically useful. Additional data is also helpful to analyze data by gender, age or region, for example. The STARHS result should be available for all or most specimens and, if incomplete, not subject to selection bias.

From an epidemiologic standpoint, the most critical element, especially when using specimens collected for other purposes, is selection bias. In particular, persons who test may not be representative of the underlying population, testing frequency may vary with HIV risk and persons may test selectively related to high-risk behaviour or symptoms associated with primary HIV infection ("seroconversion effect"). Analytic methods are available to control for, or otherwise take into account, the presence of selection bias related to seroconversion effect. Control of these biases is necessary so that the results of the STARHS assay can be correctly interpreted.

## INVESTIGATION OF IMMUNOASSAYS USED FOR DISTINGUISHING RECENT FROM ESTABLISHED HIV-1 INFECTION

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**Objectives:** To characterise the maturation of the humoral immune response to human immunodeficiency virus (HIV-1) infection, and to seek a specific antigen-antibody interaction as a marker of recent infection, we have examined in detail the antibody isotype-specific responses generated to HIV-1 antigens during seroconversion.

**Methods:** Western blots, ELISAs and Surface Plasmon Resonance (SPR) have been employed to thoroughly investigate the specificity and relative affinity of antibodies generated during seroconversion. This has permitted not only the identification of interactions specific to recent infection<sup>1</sup> but also allowed an analysis to be performed on the effect of the modifications applied to commercial assays for avidity<sup>2,3</sup> and detuned<sup>4,5</sup> testing.

**Results:** During maturation of the immune response to HIV-1 infection there is a rapid and sustained IgG response to all the major proteins transcribed by the *env*, *gag* and *pol* genes. The major antibody isotype contributing to this broad response is IgG<sub>1</sub>. Data obtained from panels of specimens collected longitudinally from individuals infected with HIV-1, has indicated that isotype-specific responses to different HIV-1 antigens appear at different time points following infection, mature at different rates and often only appear transiently.

**Conclusions:** We have found that the urea incubation used in the avidity assay appears to eliminate the detection of the early low affinity IgM and IgG<sub>3</sub> peaks of reactivity as well as significantly decreasing the IgG<sub>1</sub> reactivity to some antigens. Using a BIAcore to analyse the binding kinetics of antibodies in samples obtained from individuals where the approximate time of infection is known and correlating these data with antibody isotype and avidity results we have been able to explain some of the principles underling these assays. Results obtained in avidity assays can be explained to some extent by differences in the relative affinity of antibodies generated to individual antigens. Understanding the specific antibody-antigen interaction that occur early following infection is essential to the development of assays that can accurately provide an estimate of the incidence of HIV infection. This information is essential for epidemiological surveys as well as monitoring new infections during vaccine trials, managing treatment programmes and distinguishing between maternally derived antibodies and neonatal infection in the paediatric setting.

## **VALIDATING ASSAYS FOR ESTIMATING HIV-1 INCIDENCE**

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Conventionally, HIV incidence has been measured in longitudinal cohort studies; back-calculated from AIDS case reporting data coupled with estimates of the incubation time from initial infection to AIDS; and modeled using prevalence and survival data. The capacity to estimate HIV incidence from a single cross-sectional survey has advantages for surveillance, vaccine trials, and intervention studies.

A number of assays have now been developed for detecting recent HIV-1 infection and for estimating incidence using specimens from cross-sectional studies. These assays define the duration of a transient state related to early infection, either viremia before seroconversion (RNA or p24 antigen tests) or characteristics of the initial antibody response (antibody titer, proportion, specificity, isotype, or avidity).

The duration (“window period”) of this early state is determined, and the frequency of this transient state in the at-risk population divided by its window period gives an estimate of incidence (new infections per person per unit of time). Serologic techniques that use modified enzyme immunoassays (EIAs), include the sensitive-less sensitive EIA testing algorithm for recent HIV seroconversion (STARHS), the BED capture EIA, and the avidity index. Window periods were calibrated using specimen panels from recent seroconverters.

Preliminary validations comparing the accuracy of incidence estimates produced by these assays with an independently observed measure suggest that the assay methods tend to overestimate incidence. Key factors that affect the estimates include differences in window periods with different HIV-1 subtypes, rapidly changing incidence in the tested population, misclassification of longstanding or late infections, and effects of antiretroviral therapy. The ability to accurately identify recent infections in individuals requires optimization different from that needed to produce accurate population-based incidence estimates.

This presentation will, summarize preliminary results from validations with different HIV subtypes and examine alternatives for improving assay-based incidence estimates. These include using confirmatory algorithms of sequential testing with assays that rely on different principles, modifying assay cutoffs, and calculating adjustments for misclassification based on imputed sensitivity and specificity.

**RECENT APPLICATION OF SEROLOGICAL AND NAT STRATEGIES FOR CROSS-SECTIONAL INCIDENCE STUDIES OF HIV AND HCV INFECTIONS IN HIGH RISK AND BLOOD DONOR POPULATIONS**

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The STARHS testing algorithm using “detuned” or less-sensitive (LS)-EIAs has proven very useful for estimating HIV incidence based on testing of confirmed HIV seropositive specimens identified from cross-sectional and longitudinal screened population.

This presentation will first summarize recent applications of STARHS (using the OTV-LS-EIA) to US and international (Brazil and South Africa) blood donor populations. This approach has allowed 1) determination of HIV incidence over time and by donation type (first time, repeat, lapsed) and demographic subgroups of donors; 2) estimation of residual HIV transmission risk from screened transfusions; 3) development of policies for safe donor recruitment and implementation of nucleic acid amplification testing (NAT); and 4) identification of recently infected donors for focused studies of risk factors and viral characteristics (genotype, resistance profiles) of incident infection. An alternative approach of using window period ratios to project residual risk and NAT yield will be presented which has several advantages over the classic incidence-window period calculations used in STARHS.

The presentation will then review data on the effect of HAART on LS-EIA seroconversion. When HAART is administered in the early post-infection period, subsequent seroconversion is aborted and seroreactivity may revert by LS-EIA and also by sensitive EIAs and Western blots. Subsequent discontinuation of HAART results in brisk seroconversion by LS-EIA at a rate exceeding that of primary seroconversion, indicating an anamnestic response. Although less pronounced, similar seroreversion by LS-EIA occurs following HAART therapy of patients with long-standing infections, suggesting a potential role for LS-EIA approaches to monitor antiviral therapy.

Finally, the presentation will summarize our group’s work to develop cross-sectional incidence testing strategies applicable to HCV infection. Although an assay (Ortho’s Vitros ECi system) was identified that is able to semiquantify evolving HCV antibody levels for 50-100 days post-seroconversion, the application of this assay to incidence estimation is confounded by 1) highly variable patterns of acute viremia and seroconversion, and 2) the fact that ~20% of HCV infected persons resolve viremia, which leads to seroreversion by this assay such that the antibody profile of HCV resolvers is very similar to that of recent seroconvertors. Although a strategy of using a combination of RNA and ECi antibody levels could prove useful for HCV incidence estimation, we have focused on an alternative approach that uses HCV NAT assays to detect incident cases in the ~60 day high-titer viremic phase preceding HCV EIA seroconversion. Application of this approach to blood donor and high risk (e.g., injection drug user) populations has demonstrated excellent correlations of incidence rates derived from rates of detection of NAT window phase samples and observed incidence derived from prospective follow-up studies. This approach is now being used in blood donor and high risk populations to estimate HCV incidence and to identify HCV incident cases for pathogenesis and treatment studies.

**EXPLORING HORIZONS BEYOND HIV**

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In recent years the development of approaches to identifying a recent viral infection have focused on HIV. A range of approaches have been developed and applied, and their strengths and weaknesses explored. However, the methods described for HIV have mostly been adopted, adapted and refined from those developed for the investigation of other viruses. One of the earliest serological approaches to identifying a recent infection was to demonstrate an increase in virus-specific antibody titre of 4-fold or more. Both the 'detuned' and 'BED' are fundamentally refinements of this approach. More recently, the need to distinguish between primary infection and re-infection with rubella was the catalyst for an effective test to assess the avidity of anti-rubella antibodies. This approach has been applied to a range of viral infections, including HIV. The potential to apply these and other approaches to other sexually-transmitted and blood borne viral infections will be explored.