HIV INCIDENCE ESTIMATES FOR SOUTH AFRICA: UPDATE ON LABORATORY METHODS AND POST-TEST ALGORITHMS

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Introduction

One of the key indicators of the South African National Strategic Plan on HIV, STIs and TB (2012–2016) is a reduction by 50% of new HIV infections.[1] The South African strategic plan is in line with the Millennium Development Goal 6. Thus, the May 2013 WHO/UNAIDS Technical Update on HIV Incidence Assays for Surveillance and Epidemic Monitoring has provided a new lease of life for laboratory-based measures of incidence.[2] The Technical Update followed the presentation of results from the evaluations of HIV incidence assays conducted by the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) at the Conference for Retroviruses and Opportunistic Infections (CROI) held in March 2013. The assays evaluated by CEPHIA were the BED capture enzyme immunoassay (BED-CEIA), limiting-antigen avidity EIA (LAg AI), Vitros-Less Sensitive and Bio-Rad Avidity Index EIA. Based on the preliminary analysis by CEPHIA, none of the assays evaluated completely met the recommended target product profile for an incidence assay. Nevertheless, the assays can be used in an algorithm (see discussion below on the Recent Infection Testing Algorithm [RITA]) that would provide credible estimates of HIV incidence. This is in contrast to the UNAIDS statement of 2005 which cautioned against the use of the BED-CEIA for purposes of estimating HIV incidence at a population level.[3] Subsequent to the 2005 statement, there have been several developments at a laboratory level (i.e. assay development with at least 10 assays described) and at the post-test laboratory level (i.e. mathematical and statistical tools for the establishment of, for example, the mean duration of recent infection) that culminated in the 2013 Technical Update. The critical importance of HIV incidence estimates is explored in detail below. The National Institute for Communicable Diseases (NICD) currently applies incidence assays to various surveys, including the national annual HIV antenatal prevalence surveys and the general household surveys conducted by the Human Sciences Research Council (HSRC), described below. In addition, incidence testing has been applied to interventions studies such as the roll-out of male circumcision.

National HIV prevalence surveillance systems

National HIV surveillance comprises three major programmes in South Africa. Firstly, one of the long-standing and major contributors to our understanding of the HIV epidemic in South Africa is the annual antenatal survey. Antenatal-based HIV surveillance was initiated in 1990 and is a national, anonymous, unlinked surveillance system that focuses on first-time antenatal attendees. The surveys are conducted in 52 districts and currently aim to recruit 36,000 pregnant women. The participants are recruited from 1445 public sector antenatal clinics annually in the month of October. There have been several refinements to the surveys, with the following objectives: to determine (1) the national point prevalence, (2) the prevalence in each province and district, (3) age-related prevalence, and (4) trends. The justification of the use of such surveys is that approximately 89% of pregnant mothers in South Africa utilise the public service, and of these 90% are black women. The most recent report showed a prevalence of 29.5% for 2011 which was similar to the prevalence of 30.2% for 2010.[4] In addition to the antenatal surveys, there was the national household survey for HIV prevalence in 2002 and the HIV prevalence and incidence surveys in 2005, 2008 and 2012, all led by the Human Sciences Research Council (HSRC). The reported national HIV prevalence for these surveys was 11.4% (2002), 10.8% (2005), 10.6% (2008) and 12.3 % (2012).[5] In general, one can conclude from both types of surveys that South Africa has a hyperendemic, generalized and mature HIV epidemic with approximately 6.4 million people infected. Within this epidemic, one can identify subsets that are at higher risk based on higher prevalence compared with the general population. The most-at-risk populations (MARPS) include African females aged 20–34 years,

African males aged 25–49 years, men who have sex with men (MSM), high-risk drinkers, people who use drugs for recreational purposes, and people with disabilities.

The epidemic has been addressed in several ways; one programme was the introduction of antiretroviral treatment in 2004 and, in 2007, the National Strategic Plan (NSP) established universal access targets (defined as the annual enrolment of 80% of those newly eligible onto antiretroviral therapy) over five years. An important estimate in the HSRC study that may explain the increase in prevalence is that approximately 2.1 million individuals are currently on antiretroviral therapy. The effect of expanded antiretroviral therapy coverage on life expectancy with a concomitant increase in prevalence has been noted in South Africa.[6] In the case of prevention of mother-to-child transmission, antiretroviral therapy was introduced in 2002 and the programme has undergone various iterations to keep pace with policy changes. The third national surveillance system is the South African Prevention of Mother-To-Child Transmission Effectiveness (SAPMTCTE) study, which examines the effectiveness of the prevention of mother-to-child transmission of HIV. Two surveys have been conducted to date, and a third is in the process of completion. The initial surveys show a declining trend, with transmission rates of 3.5% for 2010 and 2.7% for 2011.[7] A limitation is that the data apply to 4–8 weeks post birth period and do not take into account HIV transmission in the post-weaning phase. Nevertheless, a target for elimination of mother-to-child transmission has been set for 2015.

The leading edge of the epidemic: HIV incidence
The HIV prevalence surveillance studies have contributed to understanding the nature and magnitude of the epidemic, the identification of geotypes that are affected (e.g. rural versus urban), as well as the age groups and genders affected. The three general household surveys in particular have certainly provided critical information that has complemented the ongoing annual antenatal surveys. It is nevertheless recognised that prevalence studies have limitations. HIV prevalence, based on the data from the antenatal surveys and general population surveys, has reached a plateau in South Africa. There is thus a need to

understand the underlying dynamics of the epidemic, especially in the context of various interventions, such as the expanded antiretroviral programme. A critical approach for understanding the dynamics of the epidemic is to measure incidence; i.e. the number of infections/person-years of observation or as an annual percentage of the population that acquire infections.[8] The information that can be derived from incidence measurements includes an understanding of transmission patterns, enabling a rational basis for the introduction of interventions (e.g. prevention and treatment programmes), the subsequent evaluation of these interventions, and the projection of the burden of HIV infections.

Estimated and observed HIV incidence in South Africa
The UNAIDS Report on the Global AIDS Epidemic 2013 reports a 52% reduction in new HIV infections among children, and a combined 33% reduction among adults and children since 2001. A summary of the estimates for South Africa is presented in table 1. The estimates are based on the Global AIDS Response Progress Reporting (GARPR), an online tool to which countries submit their most recent data on global indicators, and are also based on modelled HIV estimates created in standard modelling software by national epidemiological teams. The reported estimates of new infections for all ages in 2012 are 350,000, compared with 640,000 in 2001.

Other approaches in modelling incidence include, for example, a modelled cohort approach. The cohort approach was applied to the HSRC national household surveys conducted in South Africa in 2002, 2005 and 2008. In the case of the 2008 study, the extent of antiretroviral use was taken into account in the calculations.[9] In the period 2002–2005, the HIV incidence rate in men and women aged 15–49 years was estimated to be 2.0 infections per 100 person-years. The highest incidence rate was in women in the age group 15–24, with an estimate of 5.5 per 100 person-years. The incidence for the 2005–2008 period for the 15–49 age group was 1.3/100 person-years, but this decline was not significant. Nevertheless, when the estimate was calculated in women in the 15–24 age group, there was a 60% decline in incidence (2.2/100

person-years) which was statistically significant. By contrast, data obtained from longitudinal studies have shown a high and sustained HIV incidence in rural KwaZulu-Natal, whether at a general population level with an incidence of 3.4 per 100 person-years over a 5-year period, or in a cohort study of women where the incidence of 6.5 per 100 person-years and 6.4 per 100 person-years in rural and urban settings, respectively,

was observed.[10,11] The household survey incidence estimates have generated lively debate as to the validity of the different approaches; e.g., statistical modelling versus laboratory-based prevalence and its use in HIV incidence estimates.[12,13] An additional and critical limitation is that the incidence estimates are retrospective, which highlights the need for more real-time estimates.

Table 1: UNAIDS Modelled HIV estimates for South Africa: Comparison of 2001 and 2012 estimates*

Category	2001			2012		
	Estimate	Lower Estimate	Upper Estimate	Estimate	Lower Estimate	Upper Estimate
Prevalence estimates in adults (15-49)	15.3	14.7	15.8	17.9	17.3	18.4
People living with HIV (all ages)	4 300 000	4 100 000	4 700 000	6 100 000	5 800 000	6 400 000
Adults (15+ living with HIV)	4 100 000	3 900 000	4 400 000	5 700 000	5 500 000	6 000 000
New infections (all ages)	640 000	600 000	700 000	370 000	340 000	420 000
New infections (15+)	560 000	530 000	620 000	350 000	320 000	390 000
Percentage of 15-24 living with HIV females/males for 2012	13.9	12.9	16.8	3.9	2.5	5.7
AIDS deaths	200 000	190 000	230 000	240 000	220 000	270 000

*http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf

Laboratory-based incidence measurements
Prospective cohorts that include HIV negative partici-pants that are followed up at regular intervals can be used to directly measure incidence based on serocon-version or detection of virus components e.g. p24 or RNA. There are several disadvantages to such an approach, for example: cohorts may not necessarily reflect trends in the general population; there is difficulty in sustaining large cohort studies; it is costly to sustain cohorts; the introduction of bias caused by behaviour change can result in a lower incidence relative to the population under study. An important consideration is that the window period for these viral markers is relatively brief, 2–4 weeks prior to antibody detection, and would thus require substantial numbers to be tested.[14] In the case of p24, the protein is relatively variable in its detection making it an unreliable marker for detecting primary HIV infections (PHI). Nevertheless, the identification of primary HIV infection (PHI) is

important because it is during this phase of HIV infection that individuals are highly infectious and contribute significantly to transmission dynamics.

In order to improve the efficiency of detection of PHI, pooling strategies have been devised to reduce costs. Estimates of PHI in South Africa have varied in different settings; for example, estimates of 2.9% were observed in a high-risk setting.[15] The ideal would be a laboratory-based incidence method(s) that can distinguish between recent HIV infection and established or long-term infection with a sufficiently long window period in order to measure incidence.[16] The requirements of an incidence assay are different from the standard diagnostic assay because the incidence estimates are applied at a population level and thus a high degree of sensitivity and specificity, as is the case of a diagnostic assay, is not required. Standard serological testing for HIV infection cannot distinguish between recent

(incident) HIV infections and long-term infections. Following exposure to HIV, individuals enter a transitory period in which there are specific immune responses such as antibody maturation that can distinguish between recent HIV infection and long-term infection. The duration of these responses is known as the mean duration of recent HIV infection (MDRI) and is highly heterogeneous in a population. The MDRI is an important parameter in incidence calculations (see below). The MDRI varies and depends on the method(s) used for detection. Factors that affect the MDRI include the population under study and the virus subtype. Nevertheless, an MDRI of 4–12 months duration is regarded as ideal in terms of providing information in real time.

Several serological assays have been described and used for the estimation of HIV incidence at both an individual and population level.[17] The underlying principles of the assays used for incidence estimates include for example, quantity/titre of antibody, antibody avidity, IgG subtype and epitope-specific antibodies. Although several assays have been described several are either in-house or commercially available assays used off-label. The BED capture EIA (CEIA) and LAg AI assays have been specifically developed for HIV incidence testing and are commercially available. The principal underlying the BED CEIA assay is to determine the proportion of IgG present.[18] By using a specific normalised cut-off value and a defined window period, incidence can be calculated. To overcome the issue of subtype the assay includes three peptides of the transmembrane gp41 glycoprotein of HIV subtype B, CRF_01AE and subtype D (hence the name for assay, "BED"). Nevertheless, there are differences in window period for the different subtypes but these are not as marked as for the detuned assay, which was amongst the first of the quantitative assays to be used in incidence measurements.[19] The LAg AI assay includes antibody avidity in the presence of limiting antigen on coated wells. The antigen is the same as for the BED assay but is linearized to improve antibody binding.[20] Compared to a conventional two-well avidity assay the LAg AI assay is performed in a single well in the presence of a chaotropic agent. The LAg AI assay has undergone initial validation using a defined protocol by

the CDC and recently by CEPHIA (see above). These two assays cannot be used in isolation to calculate incidence because there are variations in the MDRI and misclassification can occur when using these assays. These complications have led to post-test methods/algorithms (termed RITA) to calculate incidence.

Recent infection testing algorithm (RITA)
An important observation in the use of the BED CEIA was the overestimation of HIV incidence, the result of misclassification.[21] Thus, a second key post-test parameter that is included in the HIV incidence calculation is the misclassification rate or false recent rates (FRR). Four conditions contribute to the false recent rate. Firstly, there is heterogeneous immune response in a population. Thus, when considering the evolution of maturation of antibodies, the majority of individuals will fall within a defined MDRI but there is a small proportion of individuals (2%-5%, depending on various states of the epidemic), such as elite controllers, where immune responses do not evolve. Elite controllers will be falsely classified by a laboratory test as recently infected despite a duration of infection greater than the MDRI. The second condition is the use of antiretroviral drugs that suppress viral replication and antigen production, leading to a reduction of anybody responses. Thirdly, advanced HIV disease (AIDS) with a loss of CD4 cells and antibody responses and, fourthly, unknown factors such as HIV subtype, populations and geographic region, appear to influence responses although the underlying mechanisms are not known.

The current iteration for calculating HIV incidence is known as RITA (Recent Infection Testing Algorithm) and takes into account not only the MDRI but also the FRR (figure 1).[22] An example of RITA would be the use of two different assays, the use of multiple epitope-based assays and would include additional information such as CD4 count, HIV viral load and data on antiretroviral drugs.[23] Some of the incidence formulae are similar but it is recommended that specific FRR need to be determined. However, this may be an onerous requirement to fulfil and would entail testing individuals from the same population who have been infected for periods greater than the MDRI e.g. >12 months. The calculation of accurate incidence would require large

sample sizes compared to that of prevalence given that incident HIV infections are 10-fold less than prevalent infections.[24] For an HIV incidence assay to be suitable for use it is recommended that the FRR should be less than 2%. The algorithm the NICD intends to use will comprise a single incidence assay and the use of an HIV viral load cut-off to exclude elite controllers/viral suppression (figure 2).

$$I_r = \frac{R - \varepsilon P}{(1 - \varepsilon)\omega N}$$

Survey counts:

N = no. of HIV-negative persons in survey
P = no. of HIV-positive persons in survey
R = no. of specimens classified as recent

Calibration parameters:

ω = mean duration of recent infection (MDRI, in years)
ε = False recent ratio (FRR)

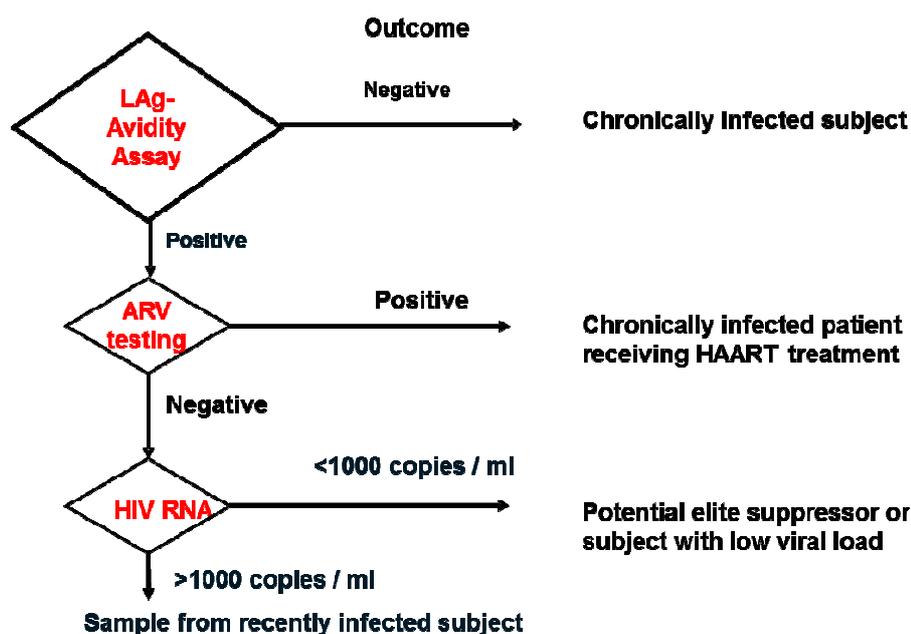Figure 1: Formula for estimating assay-based HIV incidence rates.

Figure 2: Recent Infection Testing Algorithm (RITA) for calculating HIV incidence.

Conclusion

The HIV epidemic in South Africa has now matured into a diffuse and hyperendemic phase. In addition, there have been multiple interventions and more recently an expanded antiretroviral programme. In this context the usefulness of prevalence surveys to understand the epidemic becomes more difficult with increasing prevalence as reported in the recent HSRC General Population Survey.   Obtaining incidence estimates becomes more relevant in understanding the dynamics of the epidemic, patterns of transmission, identifying risks and assessing interventions. This is especially so in the era of rapid evolution of policy, for example the recent WHO recommendation to initiate treatment at CD4 counts of 500 cells/mm^3 or less.[25]

Establishing estimates of incidence has not been straightforward. Initial mathematical models for incidence will become more complicated as various factors such as the effect of antiretroviral therapy are taken into

account. An alternative and appealing approach to link to HIV incidence estimates is the concept of community viral load i.e. population-level aggregate measures of viral load. There is a plausible relationship between suppression of viral load, transmission and HIV incidence but the straightforward relationship belies the complexity underlying this concept.[26] Shortcomings in deriving community viral load estimates include the fact they may not represent actual viral loads, possibly because of dropouts in the treatment cascade and variability of the viral loads in individuals over time. Alternative approaches to incidence estimates include possible biomarkers (e.g. cytokines) or viral diversity and sequence ambiguity. [27,28] In the case of viral diversity, the development of suitable indices that would sufficiently discriminate between early and late infections have been described but are likely to be complicated in that not all settings are there. For example, single founder viruses and super-infections may complicate the interpretation of data. The ideal for HIV incidence estimation remains a cost-effective laboratory-based approach that produces near real time estimates that can inform policy decisions concerning identification of hot spots, transmission patterns and evaluation of interventions. Recent advances such as the LAg AI assay in combination with post-test factors do provide optimism because challenges that have beset incidence measurements to date are being addressed.

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