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**SEROLOGIC TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION IN ESTIMATING INCIDENCE OF HIV-1 AMONG ADULTS VISITING A VCT CENTRE AT A KENYAN TERTIARY HEALTH INSTITUTION**

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**ABSTRACT**

**Objective:** To determine HIV high risk groups among adults visiting Kenyatta National Hospital Voluntary Counselling and Testing Centre by use of Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS).

**Design:** A cross-sectional study of adults.

**Setting:** Kenyatta National Hospital Voluntary and Counselling Centre.

**Results:** Of the 6,415 adults screened for antibodies to HIV at Kenyatta National Hospital VCT Centre between July 2002 and February 2003, 728 tested positive in the two HIV screening tests used at the center, indicating a prevalence of 11%. Of these seropositive cases, 355 consented to participate in the study. Using STARHS, 34 (9.6%) of the plasma samples were classified as being from individuals with recent infection (within 170 days), giving an annual estimated HIV-1 incidence in this population of 1.3 infections per 100 person-years with a 95% CI of 0.872–1.728%. Young adults had a higher rate of new infection than older adults. Young females were infected much earlier in life, with a peak age of new infections of 26 years, versus. 31 years for young males.

**Conclusion:** This study confirms our hypothesis that STARHS or Detuned assay can be used to determine HIV incidence in this population. The HIV high risk groups as identified by this study are young women between ages 16 to 26 years old and men between ages 45 to 55 years of age.

**INTRODUCTION**

Information on the incidence of human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), is limited in many parts of the world, and especially sub-Saharan Africa, the region considered to have the highest rate of new infections. Accurate incidence data are critical to public health surveillance, as they allow for more accurate assessment of HIV high-risk groups, and thus better targeting of HIV prevention programmes. Incidence data also permit more accurate estimation of sample sizes for large-scale AIDS vaccines trials.

Patterns of HIV-1 transmission in populations can be better followed and measured with incidence data. Indeed, several studies (1,2) indicate that stable HIV-1 prevalence can sometimes mask substantial incidence in population sub-groups with a continuous turnover of members, such as injecting drug users (IDU) and childbearing women (CBW), among others.

However, prevalence data are often easier to collect than incidence data (1). Heyward *et al.* (3) have shown that HIV-1 prevalence statistics for sub-Saharan Africa are increasingly available through longitudinal and cross-section studies, whereas empirical estimates of HIV-1 incidence remain rare. This may

be attributed in part to the lack of rapid methods for obtaining incidence statistics. Historically, three methods, namely longitudinal cohort studies (4), clinical diagnosis for acute retroviral syndrome (5), and HIV-1 p24 antigen or RNA-PCR (6), have been used to measure incident HIV-1 infection.

The need to find a diagnostic tool that could be used to determine incidence of HIV infection led the US Centers for Disease Control and Prevention (CDC) to develop an HIV enzyme immunoassay (EIA) testing strategy, the so-called Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS), also known as the 'detuned test' (7). STARHS permits the determination of the number of very recently infected persons (incidence) from among those with serologic evidence of HIV-1 infection. The procedure uses a sensitive followed by a less sensitive EIA. It takes advantage of the progressive development of HIV-1 antibody response during the initial phase of infection, whereby recently infected individuals are seropositive using the sensitive EIA, but non-reactive using a less sensitive EIA. Those with long-standing HIV-1 infection test positive with both tests (7). STARHS can be used to identify persons who seroconverted on average within the past 170 days (8).

The main benefit of STARHS is that for the first time, incident HIV-1 infection can be estimated through a single cross-section survey, saving on both cost and time. We used the STARHS to estimate HIV-1 incidence in subjects visiting the Kenyatta National Hospital Voluntary Counselling and Testing (VCT) Centre, and thereby determined HIV-1 high-risk groups in this population.

## MATERIALS AND METHODS

*Study population:* Study participants were recruited at the Kenyatta National Hospital VCT after obtaining ethical clearance from The Kenyatta National Hospital Ethics and Research Board. The subjects were enrolled in the study after obtaining a signed informed consent. Blood samples were taken from each subject and screened for antibodies to HIV using two rapid tests, namely Abbott Determine HIV-1/2 (Abbott Diagnostic Division, The Netherlands) and Trinity Biotech Uni-Gold (Trinity Biotech plc) and were enrolled in the study if their test results indicated that they were reactive using both tests. Those with any acquired immunodeficiency syndrome (AIDS)-defining conditions or are suffering from tuberculosis (TB) or on TB treatment were excluded from the study.

*HIV-1/2 screening tests:* Venous blood from 355 subjects was drawn and two aliquots of 0.5ml of plasma were frozen. One aliquot was used for HIV-1/2 confirmation test and the detune screen assay

(sensitive), and the other was used for the detune test (less sensitive).

*Assay controls:* Seroconversion samples. To test the reliability of the detune assay in a population where HIV-1 clade B virus is not known to be common, we included samples obtained from a cohort of commercial sex workers. Fourteen seroconversion samples and 26 samples from those with longstanding infection. For the seroconversion samples, seroconversion was estimated to have occurred midway between the last seronegative test and the first seropositive test dates on ELISA, as determined from study records. Long-standing infection samples were taken from individuals who had been infected for at least one year.

*CDC controls:* The Centers for Disease Control and Prevention (CDC) provided a calibrator and a low positive and high positive as controls.

*Sensitive assay:* Vironostika Uni-Form II (Organon Teknika). This is a fourth-generation EIA and was used in this study as a confirmatory test and the sensitive assay. The EIA was performed according to the manufacturer's instructions as follows: plasma samples were diluted by the addition of 100µl of specimen diluent into the micro wells, followed by addition of 50µl of specimens or controls into their respective wells. The samples were incubated at 37°C for one hour. Unbound antibodies were washed followed by addition of 100µl TMB substrate into each well. Adding 100µl 1M sulphuric acid to all wells stopped the reactions and the results were read at a single wavelength of 450nm. Plasma reactive with this assay were confirmed as HIV-1/2 seropositive and were then processed for the detune assay.

*Less-sensitive or detuned assay:* Low-sensitivity screening assay (Vironostika, OTV-LS EIA) was used to determine whether the subjects had recent or longstanding HIV infections. All samples confirmed by ELISA to have antibodies to HIV were subjected to a single run at a DILSIM dilution of 1:20,000. This helped exclude samples with long-standing infection from those with recent infection. Samples were tested in the OTV EIA protocol as described by CDC (8), with its subsequent modifications to include a calibrator and a low positive control. Briefly, 200µl of the 1:20,000 dilutions of controls and sample sera were transferred to the corresponding wells of the Vironostika HIV-1 antigen coated plate. The samples and controls were then incubated at 37°C for 30 minutes. This was followed with a wash step to remove any unbound antibodies: 300µl of the wash solution was added to each well four times, either manually or by automated washer. To each well, 150µl of prepared conjugate was added then immediately the plate was incubated at

the same conditions as before. Unbound conjugate was washed either manually or using an automated washer as described above. To each well, 150µl of substrate was then added, followed by incubation of the plates for 10 to 13 minutes in the dark at room temperature. Lastly, 150µl of stop solution was added to each well to stop the reaction and immediately the plate was read first by blanking the micro-ELISA reader on air (without strip holder and strips) and then reading the absorbance of the solution in each well at 450nm. By using a cut-off of 2.00 for standardized optical density (SOD), specimens with SODs > 2.00 were considered to be from individuals with long-standing HIV infection and were excluded from further detuning. Specimens with SOD < 2.00 were subjected to the detuned assay procedure.

*'Detuned' assay procedure:* In this assay sample sera and controls were tested in triplicate at 1:20,000 dilutions. Serum samples from patients and CDC controls (calibrator, LPC and HPC) were diluted to 1:20, 1:400 and 1:20,000 before being tested. 200µl of 1:20,000 dilution and control samples were transferred into the corresponding Vironostika HIV-1 antigen coated plate. The rest of the assay was performed as for the 'low sensitive screening assay' described above. Specimen results were expressed as SOD calculated from the formula (mean specimen OD

– mean negative control OD)/mean calibrator OD. In this assay (LS EIA) an SOD value of <1.00 was used to define those patients as LS EIA non-reactive, consistent with infection within the last 170 days.

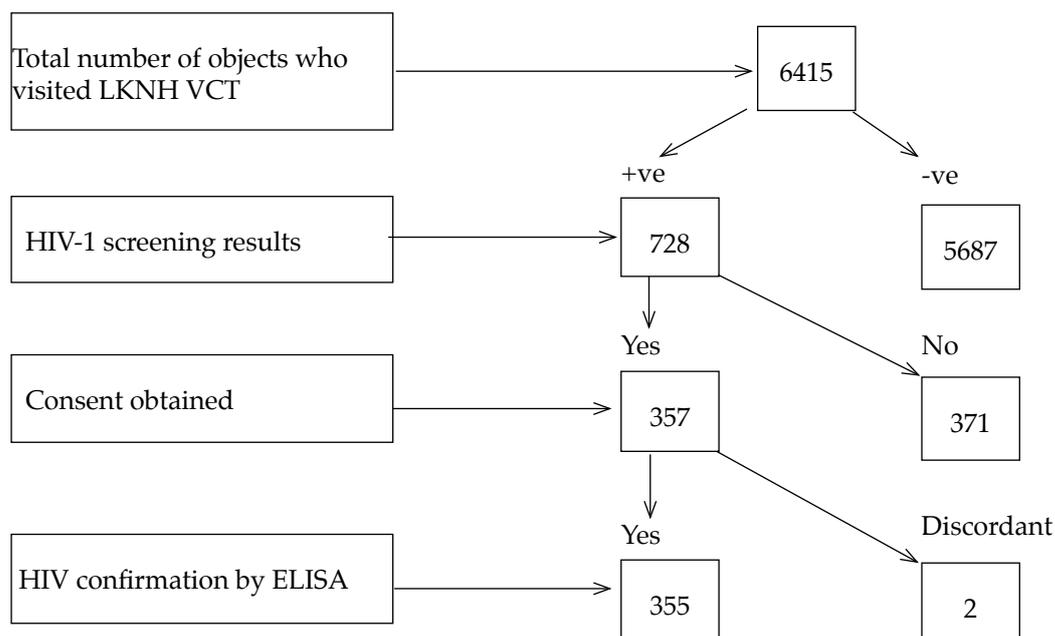
*Data analysis:* Statistical analysis was done using SPSS for windows package (SPSS Inc. Chicago). Incident HIV-1 infection was calculated using the statistical module in Microsoft Excel (Microsoft Corp. Seattle, WA). Annual estimated HIV incidence was calculated as described by Janssen *et al* (7). The mean time to seroconversion used in this study was 170 days (8).

## RESULTS

*Study population:* Between July 2002 and February 2003, a total of 6,415 men and women were screened for antibodies to HIV-1 at Kenyatta National Hospital VCT. Out of the 728 seropositive subjects, 355 individuals aged between 16 and 65 years met the criteria to participate in our study (Figure 1) 144 (41%) males with a median age of 35 years, and 211 (59%) females with a median age of 30 years. An overall prevalence of 11% was calculated from the 728 seropositive out of the 6,415 subjects screened in this clinic for the study period. Figure 1 describes how the study subjects were selected.

**Figure 1**

*Flow chart on criteria for study subjects selection. A total of 6,415 subjects visited KNH-VCT between 2002 and 2003. Of these, 728 tested HIV-1 positive and were asked to join the study. Only 357 consented and were enrolled in the study. Further confirmation of sera samples for HIV-1 by ELISA revealed that there were two discordant samples leaving 355 samples for further detuning*



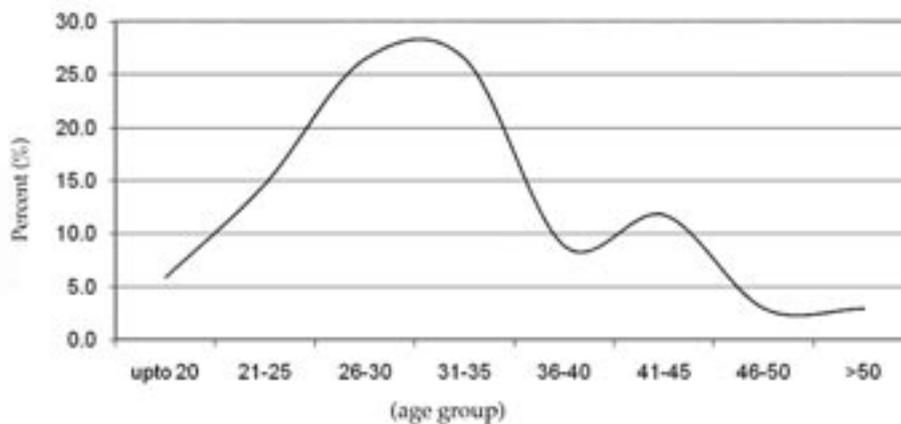
*Incident HIV-1 infections as described by STARHS:* Out of 355 plasma samples confirmed HIV-1 positive, 47 (13.2%) had an SOD of less than 2.00, and of these, 34 (9.6%) were classified using the detune screen assay as being from individuals with recent infection. An incidence of 1.3 infections per 100 person-years (PY) with a 95% CI of 0.872% to 1.728% is thus obtained, indicating a low rate of new HIV-1 infections in this population. Trends of recent HIV-1 infection among different age groups in this population showed a bias towards young adults aged less than 26 years (Figure 2). Beyond this age, the number of new infections declined significantly: Most people over 26 testing positive had longstanding infections. There were clear differences between males and females in rate of new HIV infections (Figure 3). Fifty-nine per cent of the new infections occurred in females, with the

remaining 41% occurring in males. Females acquired HIV-1 infection much earlier in life than did males. The peak age for recent HIV infection in females was 26 years while for males it was 31 years. An interesting observation in this study was that, although there were hardly any new infections in older women (>35 years) a few new infections were seen in men in their early 40s and below 50 years.

*Validation of detuned assay:* One of the limitations of this study was lack of enough HIV incidence samples for validation in this population. However, based on the available samples obtained from this population, the detuned assay had a positive predictive value (PPV) and negative predictive value (NPV) of 85.7% and 92.3% respectively. These results suggest that this assay may be used for estimating HIV-1 incidence at population level.

**Figure 2**

*Trends of recent HIV-1 infection among different age groups in this population. There was a rapid increase in new HIV-1 infections among young adults aged less than 26 years. Beyond the age of 26 years most people appeared to have longstanding infection resulting in a decline in the number of new infections*



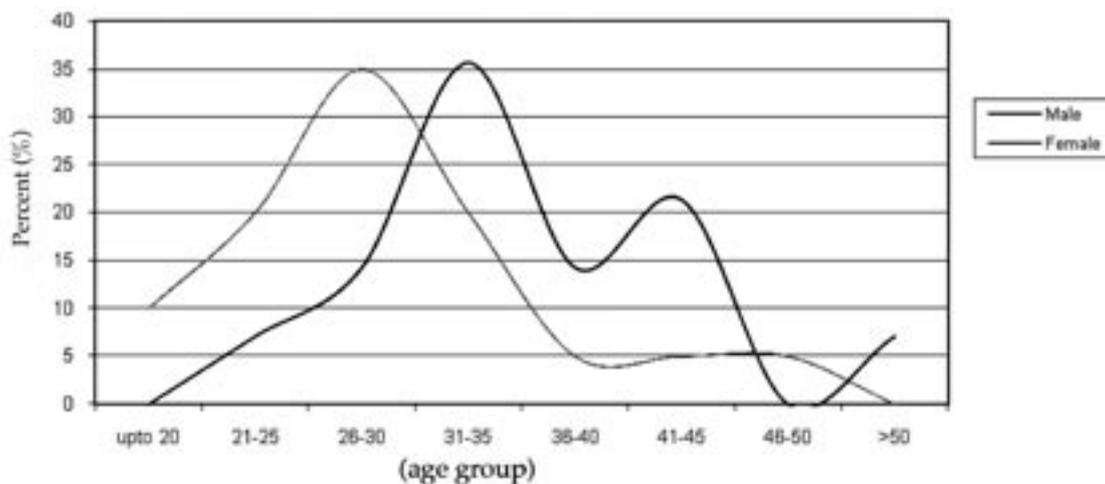
**Table 1**

*Validation of detuned assay using sera from Kenyan subjects. A total of 40 serum samples were used for this study. Fourteen were classified as seroconversion since the first HIV positive test results and the last HIV-1 negative results were less than 170 days. The remaining 26 samples were classified as longstanding infection as they had been infected with HIV for more than 170 days. These samples were used to validate detuned assay.*

HIV-1 infection for more than 170 days	HIV-1 Infection as determined by Detuned assay.		
	Recent	Longstanding	Total
Recent	12	2	14
Longstanding	2	24	26
Total	14	26	40

**Figure 3**

Shows clear differences between males and females in terms of new HIV infections in different age groups. Females in this population acquired HIV-1 infection much earlier in life than males. This is shown by the curve for females shifting to the left of the graph compared to that of males on the right. The peak age for recent HIV infection in females was 26 years while for males it was 31 years. Fifty nine percent of the new infections occurred in females with the remaining 41% of them occurring in males. An interesting observation in this study was that, although there were hardly any new infections in older women (>35 years) a few new infections were seen in men in early 40s and after 50 years. This shows that a few men continue with high risk sexual behaviour into their 50s



## DISCUSSION

HIV-1 incidence in adults visiting Kenyatta National Hospital VCT centre in Nairobi was estimated using STARHS, an approach for detecting recent infections. At the time of this study, the HIV-1 prevalence in this population was within the national (6-9%) prevalence (9). The estimated incidence was generally low (1.3/100 person years). However, similar incidence data have been observed in a population similar to those visiting this facility. Rutherford *et al.* (10) reported an estimated HIV incidence of 1.1 per 100 person years among subjects attending anonymous testing sites in San Francisco, USA. In another study, a cohort of men in Bujumbura, Burundi had annual HIV-1 incidence of between 1.5 and 2.3% (11). While a detuned assay was developed and validated for use in identifying recent infections in subjects with infections to HIV-1 clade B viruses, this assay has been used to validate BED HIV-1 Capture EIA Assay, another assay used for estimating HIV incidence from subjects infected with HIV-1 non-clade B virus (12).

Our study found that women became HIV-infected more frequently, and at an earlier age than the male counterparts. There are a number of explanations for these differences. The first explanation is lack of economic empowerment in young women. In many developing countries, young women start having sexual relationships at an early age, often with men who are much older. Many of these relationships are transactional or the result of coercion. A study done in Uganda, observed that new HIV infections were

highest in young women who had sex with wealthy mature men in exchange of material gifts (13). The second reason for the high HIV incidence in young women than men could be due to the biological or anatomical differences between the two sexes. Because HIV is mainly transmitted via heterosexual route in this population, the vaginal track of women are likely to harbor the virus for a longer time upon sexual intercourse with an infected person than the genitalia of men. This long residence time in the vaginal track may give the virus enough time to find its target cells and cause infection. Additionally, circumcision in men may reduce their ability to get infected with HIV upon sexual intercourse. A recent study by Auvert *et al.* (14) showed that circumcision can reduce HIV transmission in men by about 60%. While circumcision has such high protective role in men, the same intervention is not available for women. The rate of new HIV infections in young men peaks in their late 20s and early 30s. One possible explanation for this finding is that this is the age at which young men marry or start sexual relations with young women, some of whom had been infected with HIV earlier. Similar trends have been seen in other African countries (15).

The temporal relationship of age of infection with HIV-1 and HIV incidence seen in our study is in line with previous findings. Gouws *et al.* (12), using a mathematical model and STARHS, showed that incidence and prevalence of HIV-1 infection increases rapidly in women aged between 15 and 20, peaks among women aged 24 years, and declines among older women.

The concentration of new HIV-1 infections among younger women may indicate a more advanced epidemic. In early stages of an epidemic incidence of HIV-1 infection will be spread broadly over all age groups, particularly where similar proportions of women of all ages have multiple partners, or spouses with causal or concurrent partners. As the epidemic progress, fewer women at high risk will survive uninfected into older ages, with the result that the age-distribution of HIV-1 incidence will become less dispersed and increasingly skewed towards younger ages (1, 16, 17).

Our study has demonstrated that in men, the peak at which most men are infected occur much later (31 years) in life than in women. Similar results have been obtained from a European study (19), where increase in incidence was strongly skewed towards older men ( $\geq 34$  years). In Kenya this fits well with age at which most men are getting married to probably girls who are already infected with HIV.

The use of the STARHS to detect new infections has a number of shortcomings that may affect incidence estimation (19, 20) in Kenya. Detuned assay has been validated for use in Europe and North America where the most prevalent HIV clade is B. As such, validation of this assay was done using serum from the Kenyan population. However, even though the sample size used for validation was small, it picked most of the incidence samples. This study demonstrates that age-specific HIV-1 sero-incidence is important for the interpretation of cross-section sero-incidence surveys. For this reason age should be one of the key variables to be collected in HIV sentinel studies. However, it must also be remembered that trends in HIV incidence among people seeking anonymous testing may be influenced by changes in test-seeking behaviour as well as changes in underlying incidence in the target population (21).

Finally, a larger sample size of HIV incidence sample is needed to validate the use of detuned assay in this population. By identifying HIV infected persons during acute stage of the infection this can help the healthcare giver to educate the infected person on ways preventing infection of the other sexual partners. Additionally, with the availability of free anti-retroviral drugs, such persons can be put on treatment depending on the treatment guidelines. Treatment of such persons may lead to dramatic reduction in viral load and low viral load is associated with reduced HIV-1 transmission. Use of detuned assay can also help in identifying HIV core transmitter groups. Although most HIV-1 core transmitter groups such as commercial sex workers are well known, this study has identified young women and men in their mid forties as other groups that needs targeting for intervention. By educating girls especially those who have not reached 18 years of age on the risks of acquisition of HIV-1, many of the new infections

among this group could be reduced. This can help in reducing the rate of HIV transmission among acutely infected persons to the susceptible persons in the population. Additionally, detuned assay will provide a tool for determining trends in HIV epidemic as well as identifying HIV high risk groups. Since VCTs are found in all parts of the country and those visiting these centres are people of different demographic backgrounds, these centres offer excellent facilities for studying the trends of new infections within the country.

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