



WHO Technical Working Group on HIV Incidence Assays

Meeting Report

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16 and 17 July 2009

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Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AIS	AIDS indicator Survey
ART	Antiretroviral Therapy
ASSA	Actuarial Society of South Africa
CD4+	CD4+ T lymphocyte
CDC	Centers for Disease Control and Prevention
CEIA	Capture Enzyme Immunoassay
CHAVI	Center for HIV/AIDS Vaccine Immunology (USA)
CoV	Coefficient of Variance
DBS	Dried Blood Spot
DHS	Demography and Health Survey
EIA	Enzyme Immunoassay
EPP	Estimation & Projection Package
FHI	Family Health International
FRR	False Recent Rate
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HPTN	HIV Prevention Trials Network
HSV-2	Herpes Simplex Virus Type Two
KAIS	Kenyan AIDS Indicator Survey
NAT	Nucleic Acid Test
PBS	Phosphate-Buffered Saline
RITA	Recent Infection Testing Algorithm/Assay
STARHS	Serological Testing Algorithm for Recent HIV Seroconversion
WHO	World Health Organization

1. Background

HIV incidence is the number of HIV infections per unit of person-time at risk, usually expressed as a rate per 100 person-years. It is measured by national HIV/AIDS control programmes in order to monitor the epidemic and assess the impact of prevention, treatment and care programmes. Conventionally, costly longitudinal cohort studies are employed to directly observe the incidence of HIV infection in a defined population over time. However, the provision of HIV prevention interventions to those in the study limits the generalizability of the measured incidence. Alternatively, serial measures of HIV prevalence can be used to estimate incidence; however, such estimates require years to produce and are often not accurate enough for practical purposes. More recently, surveillance efforts have been expanded as a number of countries have added testing of biological specimens to existing demography and health surveys (DHS). Development of novel laboratory methods to detect recent HIV infection would allow for enhanced ability to determine HIV incidence in a more simple, cost-effective manner using specimens collected in cross-sectional surveys.

Assays for detection of recent HIV infection may have one of three testing objectives: 1) estimation of HIV incidence at a population level; 2) use in clinical intervention trial settings (e.g. identifying suitable study populations and monitoring trends throughout the study); and 3) for detection of recent HIV infection at the individual level to prioritize contact tracing and appropriate care. For the most part, this report will focus on the first testing objective of establishing estimates of HIV incidence at the population level.

World Health Organization (WHO) coordinates the WHO Technical Working Group on HIV Incidence Assays (hereafter referred to as the Working Group) to further the dialogue between assay developers, assay users and policy makers and to accelerate progress towards the far-reaching use of accurate laboratory methods to detect recent HIV infection. It is clear that an increasing number of countries have conducted studies to assess HIV incidence in order to monitor their epidemics without complete technical knowledge. Since 2006, a series of formal and informal meetings have taken place to discuss guidance on use of assays to detect recent HIV infection and to provide estimates of HIV incidence. A meeting of the "WHO Technical Working Group on Statistical Approaches for Development and Validation of HIV Incidence Assays" was held in April 2009 and Family Health International (FHI) / WHO / HIV Prevention Trials Network (HPTN) / Center for HIV/AIDS Vaccine Immunology (CHAVI) / Bill and Melinda Gates Foundation convened a "Meeting on the Development of Assays to Estimate HIV Incidence" in May 2009. Following these two meetings, several information and operational guidance documents are in preparation on HIV incidence assays and their application.

In July 2009, a meeting of the Working Group was held in Cape Town, South Africa. This meeting was jointly organized by WHO departments of Essential Health Technologies and HIV/AIDS and was hosted by the Human Sciences Research Council, South Africa. The aim of this particular meeting of the Working Group was to accommodate a wider audience for review and discussion of these Working Group documents.

The primary meeting objective was to disseminate technical information about methods to estimate HIV incidence using laboratory-based techniques. The specific meeting objectives were:

1. To review the outcome of the FHI market analysis for HIV incidence assays and finalize the assay requirements for each of the testing objectives of HIV incidence assays.
2. To review and finalize the statistical methods for the estimation of HIV incidence using assays for recent infection.
3. To review and finalize the statistical approaches required for each step of the assay development pathway and for field validation of HIV incidence assays for each testing objective.
4. To review and discuss guidance documents and information that will be disseminated on the WHO web pages.

The meeting was attended by 24 participants, including country representatives as the users of assays for recent HIV infection and subsequent use in methods to estimate HIV incidence. It was intended that information on the technical aspects would be shared and input would be received from the predicted users of assays for recent infection, in particular in countries most affected by the epidemic.

2. Proceedings

2.1. Country Experiences: Use of Assays for Recent HIV Infection and HIV Incidence Estimates

The country perspective was invited in this session, several of the meeting participants presented an overview of their national programme for HIV incidence estimation and some data of recently conducted studies.

South Africa

Three National HIV Household Surveys (2002, 2005, 2008) have been conducted over the past six years. These surveys collected data not only on the HIV status but also information on socio-demographic and behavioural determinants which greatly enhanced the analysis and interpretation of the observed trends in HIV infection. HIV prevalence in the total population of South Africa has stabilized at around 11%. HIV prevalence remains disproportionately high for females in comparison to males, and peaks in the 25-29 year age group where one in three (32.7%) were found to be HIV positive in 2008. HIV prevalence among males peaks in the 30-34 year age group where 25.8% were HIV positive.

HIV incidence estimation is based on a combination of methods including:

- Modelling: Estimation & Projection Package (EPP), Spectrum, Actuarial Society of South Africa AIDS Model (ASSA)
- HIV incidence from single year prevalence in 15-20 year age group
- HIV incidence from repeated cross-sectional measures of HIV prevalence
- Laboratory-based methods: tests for recent HIV infection (BED capture enzyme immunoassay [CEIA])

Indirect HIV incidence estimates were mathematically derived from prevalence in young people using prevalence data by single year of age and assuming that HIV prevalence differences between the age strata represent incident HIV infections. This method is best applicable in younger age groups when the effect of AIDS-related mortality on HIV prevalence levels is still minimal. The derived HIV incidence profile of the 15-20 year olds showed a substantial drop in incidence for the 2008 survey year compared with the incidence figures calculated for the 2002 and 2005 survey years, especially for the single year age groups of 15, 16, 17, 18, and 19 years. The epidemiological HIV incidence estimation is currently extended to the entire population 15 years and older, using a recently proposed method that infers population level HIV incidence from prevalence obtained in two cross-sectional serosurveys (see end of section 2.7)

One of the novelties of the 2008 survey was the addition of antiretroviral drug (ARVs) testing into the survey protocol which enabled the analysis of ART (antiretroviral therapy) exposure in the study population by age and sex. The presence of ARVs in the blood specimens was assessed by High Performance Liquid Chromatography coupled to Tandem Mass Spectrometry (HPLC – MS/MS). The analysis of the impact of ART on HIV prevalence levels and the proportion of ARV positive specimens misclassified as recent infections in the BED CEIA was still ongoing at the time of this meeting.

It is now well established that a number of individuals are misclassified as recently infected by the BED CEIA, with the main sources reputed to be individuals with one or more of the following characteristics: 1) late stage HIV infection (including AIDS diagnoses and low CD4+ T cell count); 2) use of ART; and 3) elite controllers (disease non-progressors, often associated with low HIV viral load). In order to address these challenges the following laboratory-based adjustment for HIV incidence estimation has been proposed (Figure 1):

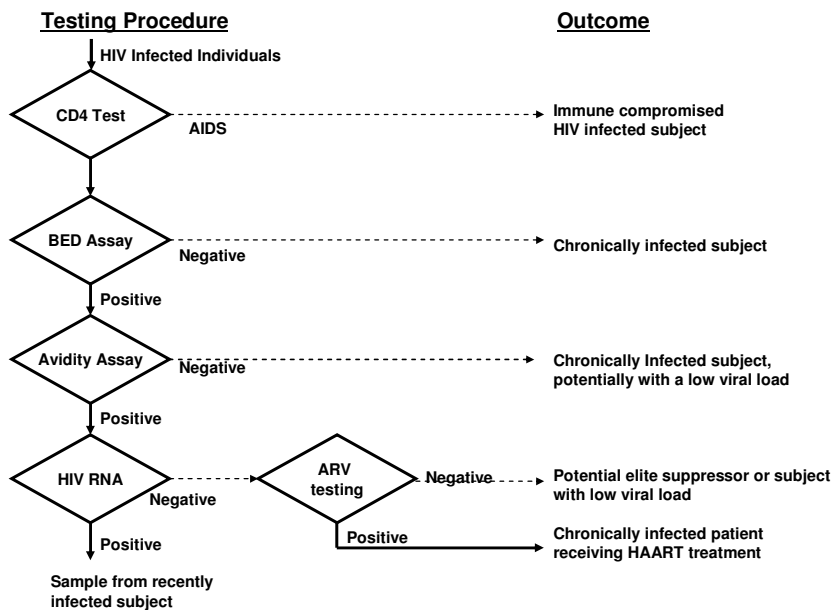


Figure 1 Proposed testing algorithm for recent HIV infection

The impact of each of these components on the overall effectiveness of the proposed algorithmic approach is currently the subject of collaborative evaluations.

Kenya

The 2007 Kenyan AIDS Indicator Survey (KAIS) was carried out on 18,000 individuals aged 15-64 years old mostly living in rural areas. Survey participants underwent HIV testing and counseling and were given their results in the same visit. An additional specimen was taken from those identified as HIV seropositive. HIV prevalence was consistently higher in females with the exception of the 50 to 54 year age group where the prevalence was equal. The female 30 to 34 year age group had the highest prevalence (13%) Laboratory-based methods were then used to estimate HIV incidence. Specimens that were identified as recently infected by BED CEIA were then subjected to case-based exclusion based on the following criteria:

- Currently on ART (method for determination not specified)
- Last HIV positive test >1 year ago (self-reported)
- CD4 T-lymphocyte count <500 cells/ml

Of the 1,073 individuals identified as HIV seropositive in survey, 181 (16.9%) were initially identified by BED CEIA as recent¹. Of these 181 individuals, 21 were on ART, 41 had a CD4 count <500 and one individual self-reported HIV infection >1 year. This left 118 individuals classified as recently infected or approximately 11% of all the HIV seropositive individuals identified in the survey. Males aged 25-35 years old showed the highest percentage of recent HIV infection (47%). The age group with the largest discrepancy in recent infection between males and females was the 15-24 year old age group (36% in females vs. 13% in males). Nyanza and Rift Valley were the provinces with the highest distribution of recent HIV infection, these are parts of the country where male circumcision is little practiced, although other social determinants could contribute to this observation. HSV-2 was also found to be associated with higher levels of recent infection, using a multivariate model for risk factors. Next steps would be

¹ See Preliminary Report of the Kenya AIDS Indicator Survey (KAIS) 2007, accessed on 28th August 2009 http://www.kanco.org/FW266/html/pfd/KAIS%20-%20Preliminary%20Report_July%2029.pdf

to apply a dual HIV incidence assay algorithm consisting of BED CEIA plus avidity protocol on the AxSym HIV-1/2 random access analyzer.

United States

A relatively complex method is used for HIV incidence surveillance within the domestic United States setting through case-based surveillance with additional laboratory testing. At the time of HIV diagnosis, a number of demographic data are collected as well as information about prior testing. Within the public sector, state/local health departments are obliged to report all HIV/AIDS diagnoses and send a specimen to the single designated CDC STARHS laboratory. This one laboratory is contracted by CDC to perform all testing for recent infection within the United States and is currently the only laboratory permitted to purchase the BED CEIA test kits and test specimens. In reality, this referral of specimens is not always performed and in spite of CDC pre-paying for shipment costs. A similar system of information/specimen flow should also occur within the private sector. The number of recent HIV infections is determined by excluding those on ART and those with clinically-defined AIDS and then testing using the BED CEIA.

The method for estimating HIV incidence relies on the stratified extrapolation approach where:

Target population, new infections in a year = N

Observed sample, those diagnosed as recent by BED CEIA = R

Probability of being observed = P

Sample weight, where $W_t = 1/P$

Population size, where $N=R/P$

The probability that a new infection is classified as BED recent is calculated as $P=p_1 \cdot p_{1w}$.

Where:

p_1 = probability of being tested within 1 year after infection

p_{1w} = probability of having a BED-recent result if the test is one year

New infection = individuals without AIDS within 6 months after their HIV diagnosis

The probability of repeat testers was determined by testing frequency, assuming the infection date is uniformly distributed in the inter-test interval (T) and is calculated as

$P_{1rep} = 12mo/\max(12mo, T)$.

The probability of new testers was estimated from a competing events model: HIV test vs. AIDS diagnosis, assuming HIV testing hazard is constant after HIV infection until AIDS diagnosis. Given $P_{1_{new}}$ = probability of being detected in the state of recency.

Sampling weights are assigned to each person identified as recently infected ($W_t=1/P$). The inverse of the probability that a seroconverted person with similar demographic and risk characteristics was identified as HIV seropositive and was identified as having recently infected by BED CEIA.

Using the extrapolated approach, the estimated number of new HIV infections among adults and adolescents in the United States for 2006 was 56,3000 new infections (95% CI 48,200 - 64,500). The key assumptions were: information about the previous test (T) was accurate, BED mean RITA duration ('state of recency') was well-defined, timing of HIV testing was independent of HIV infection, incidence was relatively constant, testing behaviour was not significantly changed and that observed variables such as previous test results and BED results were missing at random.

False-recent BED results could be due to: unconfirmed EIA reactive specimens being sent for incidence testing before confirmatory testing was performed, inconsistent specimen handling, and presence of any one of the following: chronic infections, inflammation, hypergammaglobulinemia, subtype heterogeneity, AIDS, and ART use.

2.2. Overview of Terminology for Estimation of HIV Incidence

There is a plethora of terms used to describe assays and methods to estimate HIV incidence. A subgroup of the WHO Technical Working Group on HIV Incidence Assays met in April 2009 to discuss statistical approaches to HIV incidence estimation and to propose and discuss consensus on nomenclature. The following Table 2 outlines the proposals made by the subgroup on new and improved terminology that is believed by the experts to more accurately describe the concepts. These terms were taken from p.7 of Meeting Report of the WHO Technical Working Group on Statistical Approaches for Development and Validation of HIV Incidence Assays, held in Geneva on 22 to 24 April 2009.

Table 2 - Terms to estimate HIV incidence

<u>Recent Infection Testing Algorithm (RITA)</u>	<p>A combination of specific laboratory and/or other methods that is intended to classify individuals as recent or not recent for the state of 'recent infection', for the purposes of estimating HIV incidence. The assay would usually only be performed on specimens from individuals who have been classified as HIV positive by conventional serology.</p> <p>An 'ideal' RITA is one which has the property that all individuals transition from the RITA-recent to the RITA-non-recent state, and remain RITA-non-recent until death.</p>
<u>Recent infection</u>	<p>A transient period soon after HIV exposure, the rate at which the susceptible population enters this transient state is the incidence of HIV infection. Its duration varies across individuals and depends on the method used for detection.</p> <p>In the absence of an ideal testing algorithm, the state of recent infection may be unobservable even though the number of individuals who are recently infected can be systematically estimated.</p>
<u>Incidence</u>	<p>The incidence of a disease is the number of new cases occurring in a population per person-time at risk, usually expressed as a rate. The natural units for expressing incidence are "probability of an infection occurring, per person, per unit time".</p>
<u>Annual risk of infection</u>	<p>An alternative way of expressing incidence – the probability that an individual will become infected if subject to a constant incidence for a period of one year.</p>
<u>Assay for recent HIV infection</u>	<p>A laboratory method or assay (used either alone or as part of a testing algorithm) to classify HIV infection as recent, for the purposes of estimating incidence.</p>
<u>RITA non-progressors</u>	<p>Individuals that perpetually stay RITA-recent. It appears that for most proposed recent infection assays, there are subpopulations of individuals who never develop the RITA-non-recent biomarkers. Some time after infection, members of this anomalous subpopulation may be regarded as being 'falsely' RITA-recent. These individuals may not necessarily experience disease non-progression..</p>
<u>RITA regressors</u>	<p>Individuals that transition from RITA-recent to RITA non-recent as</p>

	expected, but then revert to being RITA-recent at some later time, after which they may be regarded as being 'falsely' RITA-recent.
<u>RITA false recent rate (FRR)</u>	The fraction of non-recent infections that are falsely classified as RITA recents as a result of RITA-non-progression and RITA-regression. Under specific assumptions, this may be an intrinsic property of the RITA, but in general, it is a property like the positive predictive value of a diagnostic test, which depends on a combination of factors like assay characteristics and the population on which the assay is used, and hence may vary depending on characteristics of the population sampled.
<u>Mean RITA duration</u>	The mean duration of the 'state of recency', which critically depends on the specific RITA being used. In the non-ideal case, where there are either RITA-non-progressors or RITA-regressors, or both, this must be carefully defined, and attempts to measure it at the population level, in 'calibration studies', must take the precise definition into account. It must essentially be defined as the mean time spent in the first sojourn in the RITA-recent state, for individuals who in fact progress to the RITA-non-recent state.
<u>Assay threshold</u>	A critical value, chosen for an assay which yields a quantitative result, to produce a categorical value for use in a RITA. For example, normalised optical density below a critical value can be used to turn the BED CEIA into a stand-alone RITA. In the past, RITA has only been used on specimens that have already been identified as HIV seropositive using the usual serological methods. In the future, an assay for recent infection may include both objectives of testing.

The new term RITA (replacing STARHS) was introduced to be more inclusive of all possible scenarios including non-serological laboratory methods e.g. possible future novel biomarkers. Much discussion centered on the appropriateness of the terms RITA-recent vs. RITA-positive vs. RITA-reactive and it is anticipated that further dialogue will be required before a consensus can be reached.

The RITA concept was transferred to discussions on the window period which currently is a confusing term as it is also used to denote the diagnostic window period i.e. the period of time when an individual's immune response is still undetectable by any commercially available assay for the purposes of diagnosis. The term mean RITA-duration was agreed to denote the mean

duration of the 'state of recency'. The use of the term 'post-seroconversion window period' was discouraged because in general assays for recent infection have little to do with seroconversion.

There was much discussion related to the terms of calibration, specificity and sensitivity. In particular, the term calibration has different means for assay developers/laboratory specialists, mathematicians and epidemiologists. With regards to calibrating an assay for recent infection, the term 'RITA-assay calibration' would be restricted to defining the RITA-duration and determining the false recent rate (FRR). It was further suggested that all technical guidance documents be updated to reflect the agreed upon terminology.

There are other terms that are often used in conjunction with HIV incidence that are confusing and do not directly relate to the concepts of estimating HIV incidence, these are outlined in Table 3.

Table 3 - Non-incidence related terms

<u>Acute/primary infection</u>	Is a clinical term that refers to the period immediately after HIV infection when the individual is highly infectious and may or may not show clinical symptoms. This is often clinically important at the individual level. The individual may display RNA or p24 antigen reactivity/positivity, with or without appearance of serological markers, i.e. antibodies to HIV. To eliminate confusion, this term should not be used when referring to recent infection for the purpose of determining estimates of incidence.
<u>Established infection</u>	Infection that lasts for more than the mean RITA duration. It is greater than approximately 6 to 12 months post HIV infection, and may also include long-standing infection. To eliminate confusion, this term should not be used when referring to estimates of incidence.

2.3. Update on HIV Incidence Assays

Current Laboratory Methods for Detection of Recent HIV Infection

Most laboratory methods to detect recent HIV infection exploit the maturation of the host's humoral response to HIV infection and seek to identify differences in the antibody response during the period of recent infection. This is achieved through a variety of different methods outlined here.

Detuned assay protocol

A typical commercially available 2nd generation indirect enzyme immunoassay (EIA) is "detuned" or made less sensitive by increasing the specimen dilution (1:20,000) and shortening incubation times. The detuned method identifies recent infection by detection of differential antibody titers in recent versus non recent infection. However, false recent diagnoses can be due to decreased antibody titers both late in infection and during ART as well as variability among HIV-1 subtypes and the assay's ability to measure these antibodies. Specimens from a recently infected individual would be reactive on the sensitive (unmodified assay protocol) and non-reactive on the less-sensitive protocol. The original assay of choice for the detuned method, the Abbott 3A11 (Abbott Laboratories) has been discontinued and it is uncertain if the most commonly-used detuned assay: the Vironostika HIV-1 Plus O microelisa system (bioMérieux, France) is still commercially available. Apparently, the rights for sale and distribution of the assay have been bought by a company called Avioq Inc (Rockville, USA) and the assay has been re-named as Avioq HIV-1 microelisa system. It is also thought that SeraCare Inc (USA) will now produce the calibrators and controls for use with this assay (these were previously distributed by CDC). Other assays have been adapted for a detuned protocol, see later section 3.4 on future assays.

BED CEIA

The BED assay incorporates peptides from HIV-1 subtypes B, E and D into an IgG capture EIA (CEIA) that measures the proportion of total IgG that is HIV-specific. The assay is commercially available through Calypte Biomedical Corporation but the assay is currently difficult to procure in many countries for a variety of reasons. One major disadvantage of the BED CEIA is the high false recency rate. This factor makes the assay unsuited to some uses and certain mathematical adjustment formulas (e.g. McDougal and Hargrove formulae) have been developed to adjust for this error with varying degrees of success. There are some subtleties in defining and estimating the false recent rates for the BED CEIA. However in principle, it appears to vary by region and stage of epidemic thus posing a fundamental problem to correct estimation of all the parameters required for incidence inference.

Avidity assay protocol

The test protocol for the AxSym HIV-1/2 gO random access analyzer (Abbott Laboratories) has been adapted to include an avidity step that takes advantage of the maturation of the avidity of the humoral immune response early in infection i.e. quality of the antibodies produced not quantity. In brief, an aliquot is treated with a dissociative agent (e.g. urea, guanidine or potassium thiocyanate, low pH, etc) and subjected to the normal assay procedure, a control aliquot

that does not receive the dissociative agent is also run in the same assay on the analyzer. The ratio of the two sample/cutoff values is calculated to provide an avidity index. Antibodies produced early in infection are known to be of lower avidity and are more susceptible to dissociative agents. In principle, there is no reason why the avidity protocol can not be adapted to other commercially available 3rd generation immunoassays, see later section 3.4 on future assays.

IDE-V3 assay

The humoral immune response is assessed towards a specially-constructed peptide (gp41 epitope and gp120-V3 loop) in an in-house EIA format assay. As early antibody responses tend to be directed toward the V3 loop and gp41, responses to these peptides, compared to other HIV proteins, is more common during recent infection.

p24 IgG3 assay

This in-house EIA format assay exploits the transient IgG3 response towards the p24 antigen that is observed early in infection only.

INNO-LIA HIV-1/2 Score assay

The INNO-LIA HIV-1/2 Score (Innogenetics, Belgium) is a commercially available line-immunoassay that is most commonly used as a confirmatory assay. The protocol for result interpretation has been adapted to take into account the intensity of the five antigen bands and emergence of specific banding patterns. In resource-rich countries such confirmatory assays are routinely performed and the results recorded. Thus an advantage exists as an additional test need not be performed but rather the results be used in a manner to support population-level surveillance efforts.

Future Assays and New Developments

To date, none of the assays described above have been found to be completely ideal because of high false recency rates, nevertheless, they are used in a variety of settings to aid estimation of HIV incidence. In the future, multi-assay testing algorithms for recent infection may provide the best solution. Issues related to poor RITA-specificity of certain assays could be mitigated by the introduction of a second assay with better RITA-specificity characteristics. Discussion ensued as a number of research groups have already suggested possible RITA algorithms, for example see figure 1. Further consideration and laboratory validation is required.

To guide new assay development, there are a number of desirable performance and operational characteristics for an assay(s) to detect recent HIV infection, listed in Table 4.

Table 4 - Characteristics of an assay for recent HIV infection

Desirable characteristics	<ul style="list-style-type: none"> • Well-defined mean duration of the 'state of recency' (previously known as window period) • Relatively long duration of the 'state of recency' • Small variance between individuals • Minimally affected by virus subtype, mode of transmission, ART, opportunistic infections, age, sex, race, pregnancy, co-infections, IgG levels
Operational characteristics	<ul style="list-style-type: none"> • Easy to perform & interpret • Different specimen types with small specimen volume • Minimal need for complex equipment • Generally available, commercially or otherwise • Exportable, transferable • Simple storage conditions • Quality Assurance (QA) amenable • Low cost

A further useful proposal was to develop an assay with pluripotency i.e. an assay that can achieve diagnosis and a test for recency within the one test. If such an assay were to exist, the market demand will be much higher than that of a test for recency alone.

New antigen:

CDC has developed a new multi-subtype antigen called rIDR-M that could be used as a replacement for antigens currently used in a variety of methods including: two-well avidity index EIA (AI-EIA), one-well limiting antigen avidity EIA (Lag-avidity EIA), gp41-LS (detuned) EIA and rapid incidence-prevalence (Rapid I-P) assay. Investigations are preliminary but appear promising to date with proof-of-concept developed and some limited studies completed using small seroincidence panels. Furthermore, CDC has used this antigen in a rapid I-P assay that would be of immunochromatographic (lateral flow) format with 3 lines: one positive control (confirms presence of human IgG), the rIDR-M protein applied at low concentration and the same protein applied at high concentration. Thus a single positive control line indicates a valid test with no infection, two lines indicating non recent infection and three lines indicating a recent infection.

New detuned adaptations:

A detuned protocol has been developed for the Vitros ECI HIV-1 + 2 random access analyzer (Ortho Clinical Diagnostics) with the dilution component performed off-board (there is still capacity for on-board dilution). Blood Systems Research Institute and Ortho Clinical Diagnostics worked together to identify the specimen dilution that best correlated with the results of the detuned Vironostika HIV-1 Plus O microelisa system (found to be a 1:400 dilution) and then tested a series of blood donor HIV positive sample sets. The mean duration of 'state of recency' was determined by seroconversion panels (407 longitudinal observations from 70 individuals) using a range of assay cutoff values (S/Co values as determined by the automated Vitros instrument using manufacturer-supplied controls) between 5 and 30. A cutoff value of 15 gave a mean duration of 'state of recency' of approximately 180.1 days (95% CI of 128.0 - 232.1).

New avidity adaptations:

Genetic Systems HIV-1/HIV-2 plus O EIA (BioRad Laboratories) has been adapted for an avidity assay for HIV incidence estimation (RITA). In total, three seroconversion cohorts (from Atlanta, Canada and Uganda) were evaluated to determine the mean duration of 'state of recency' for a range of different avidity cutoff values (20 to 50%), the effect of different avidity cutoffs was on the false-recent rate was also assessed. For the Atlanta cohort, it appeared that avidity was not affected by late stage disease, using CD4 T-lymphocyte count as an indicator. For the Canadian cohort, there were less false-recent infections detected with the avidity protocol (compared to BED CEIA). Further studies using specimens from the Johns Hopkins University Moore Clinic and the Rakai cohort in Uganda also confirmed that the avidity adapted protocol detected fewer false-recent infections in individuals with longstanding infection and known ART exposure than BED CEIA alone.

An avidity protocol for the Anti-HIV-1 + 2 assay run on the Vitros ECI Immunodiagnostic System (Ortho Clinical Diagnostics) takes the ratio of a 1:10 specimen dilution with 1M guanidine to a 1:10 specimen dilution with phosphate-buffered saline (PBS). The mean duration of 'state of recency' was determined by seroconversion panels (407 longitudinal observations from 70 individuals) using a range of assay cutoff values between 0.5 and 0.8. A cutoff value of 0.5 gave a mean duration 'state of recency' of approximately 140.1 days (95% CI of 101.7-177.5). A Pearson correlation was used to measure the strength of dependence between the different assays that have been this far adapted for avidity (e.g. Vironostika and Genetic Systems) against the BED CEIA.

Comparison of available assays

A Pearson correlation analysis was conducted by CDC to measure the strength of dependence (correlations) of classification of recent infection between the different assays that have been thus far adapted for avidity (i.e. Genetic Systems [BioRad Laboratories] and Vitros [Ortho Clinical Diagnostics] assays) against the BED CEIA [Calypte Biomedical] and the detuned Vironostika [bioMérieux, France] and Virtos [Ortho Clinical Diagnostics] RITA assays. These results showed reasonable correlations but also discordancies, suggesting the need for further head-to-head comparative studies on common specimens panels and consideration of combining assays into testing algorithms for optimal RITA performance.

Luminex-Based RITA System

Microbeads can be coated with specific HIV or other antigens and then assayed using the Luminex platform that allows simultaneous detection of multiple parameter (certain humoral immune responses) that are indicative of recent infection, for example both avidity and antibody titer against multiple HIV antigens can be measured in the same system. Small specimen volume is required (1µl). The principle is outlined below, figure 2

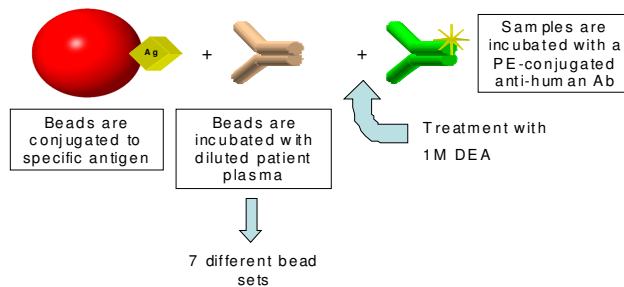


Figure 2- The Luminex assay for detection of antibody

The data can be analysed as the amount of direct binding of HIV-specific antibody to the beads (ratio of specimen to calibrator) and as avidity of the antibodies to each antigen after treatment of specimens with DEA (avidity index). This approach looks promising based on limited preliminary studies and is in the process of being tested on the three large seroconversion panels previously described in this section (new avidity adaptations).

2.4. HIV Incidence Assay Development

To maximize efficient development of assays for recent HIV infection, a critical pathway approach can be employed to ensure that all candidate assays are developed in a sustainable manner that will ensure continued availability. A major obstacle for incidence assay development has been the inability to consolidate a globally relevant specimen panel that would be suitable for assay developers to calibrate assays and then later for assays evaluators to compare performance of assays. A three-step pathway would ensure that candidate assays are triaged through the development process so that only well-performing assays would be eligible to progress through to the next stage, see figure 3. RITA false-recent rates and precision would be determined by the assay developers in the first stage and submitted to the Core Committee for review. The Core Committee would control the availability of specimen panels and assess assay performance on each panel to decide if the assay will progress to the next stage. The Core Committee would need to be composed of a group of experts that should not have a conflict of interest with assay development.

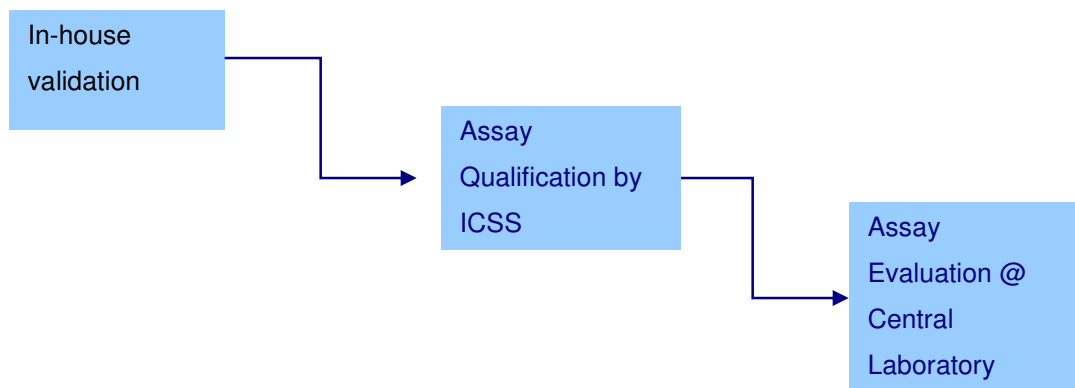


Figure 3 - Critical pathway for assay validation

The most precious sets of biological specimens can be conserved for the later stages of assay qualification after the assay has proved performance in the hands of the assay developer. The assay qualification by incidence core specimen set (ICSS) would consist of a standardized specimen set sent to the assay developer to test on their assay, if performance is good then the assay would progress to the final stage. The independent assay evaluation is important for end-users as the process will assess assay performance, transferability of laboratory methodology and inter-assay comparison between new and existing assays for recent HIV infection.

2.5. Progress Reports of Products from the FHI Meeting (held in May 2009)

Results of Market Analysis Performed by FHI

Family Health International (FHI) solicited the opinion of a wide range of scientists, public health experts and incidence assay users to estimate the global demand for HIV incidence assays for the coming five years. A market research company (bioStrategies Group) was engaged to perform a market assessment through qualitative telephone interviews of key informants. The interviews described which assays are currently used and the perceived strengths and limitations of these assays. Desired attributes were collated with accuracy prioritized over ease of use and cost. The main demand drivers were improved accuracy and reproducibility and the need for HIV incidence estimates as a measure of impact of many programmes. Demand would be linked to application e.g. public health surveillance/monitoring or programme evaluation and research. The four key areas of application would be:

- population-based surveys
 - AIDS indicator surveys (AIS), demography and health surveys (DHS), etc
- case-based surveillance
 - on all HIV positive cases reported
- sentinel surveillance
 - most commonly pregnant women presenting to antenatal clinics (ANC), injecting drug users (IDU), sex workers (SW), blood donors, etc
- special studies
 - includes research, etc

The next step was to make a qualitative estimation of current and projected demand for HIV incidence assays based on the following three scenarios: 1) the current assays available; 2) improved assays (e.g. a better FRR); and 3) the 'game changer', where a novel assay would be developed that would detect both seropositivity and recency. This area of work is on-going and will be finalized in the coming months.

Review of Virtual Cohorts/Banks of Specimens Suitable for Assay Validation

As outlined in section 3.5, the incidence assay research and development community is in urgent need of well-characterized biological specimens suited for calibrating, validating and evaluation of assays for recent infection. A virtual database of available specimens would be the first step towards establishment of a global repository of specimens for use by assay developers and assay evaluators. In brief, a literature search of peer-reviewed publications was conducted to identify cohort studies (completed, on-going, planned). Two types of cohorts were of interest: 1) seronegative cohorts, where seronegative individuals are followed over time to capture the

seroconversion event and 2) acute cohorts, where p24 antigen and/or HIV RNA positive but HIV antibody negative individuals present with symptoms and are sampled.

To date, 43 seronegative cohorts have been identified, located mainly in Africa and the Americas. The study investigators will be contacted to obtain further details and determine their willingness to contribute volume of specimens. Study investigators will be asked to provide details of available specimens, such as specimen volume, storage location and available time points. This information will be compiled into a database. The long term objective is to centralize the location of available specimens and provide access for development and validation of assays for recent HIV infection.

Outline of WHO Guidelines for Field Application of Assays for Recent HIV Infection to Estimate HIV Incidence

Consumers/users of laboratory methods for estimation of HIV incidence have a multitude of options available to them including choice of assays for recent infection and various cross sectional study methodologies. There is a need for global guidance for the use and interpretation of data obtained from assays for recent HIV infection.

A chapter outline has been elaborated and preliminary work has begun on the writing of a guidance document on the field application of assays for recent infection to estimate HIV incidence at a population level. The writing of this document will continue in the coming months. The consensus on new terminology (see section 3.2) will be introduced to a wider audience through the document as will an overview of existing incidence assays and recommended algorithms for determining recent infection. Methods for estimating incidence, including formulae, will be incorporated to aid calculation of incidence estimates, confidence intervals, etc. Appropriate sampling frames for incidence estimation from cross sectional studies will be outlined, in addition to sample size requirements. Best practice methods for specimen storage and transport will be included in this document as well as any other necessary logistics concerns.

2.6. Dissemination of Guidance and Documentation for WHO Web Pages

The newly launched section of the WHO website gives an overview of HIV incidence and the challenges associated with determination of estimates of HIV incidence. The Working Group and its objectives are introduced. The web pages can be accessed at the following link:

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

Suggestion and comments were invited from the group and the wider membership of the Working Group. A number of products are yet to be finalized and these are instead located on the members-only EzCollab site. In addition, to documents for review by the Working Group, this restricted site contains all the information relevant to the meetings including presentations, agendas, meeting reports. It acts as a useful forum to enable more extensive and manageable review of documents.

2.7. How to Estimate HIV Incidence at Population Level: Epidemiological and HIV Incidence Testing Approaches

Reliability of Estimation of HIV Incidence from Cross-sectional Surveys

Assays for recent HIV infection are generally applied to a cohort of interest when HIV negative and HIV positive individuals can be identified. HIV positive individuals would then be tested for a biomarker that indicates recent HIV infection i.e. classifies individuals as RITA-recent. Incidence estimators rest on the probability of remaining classified as recent as seen in figure 4.

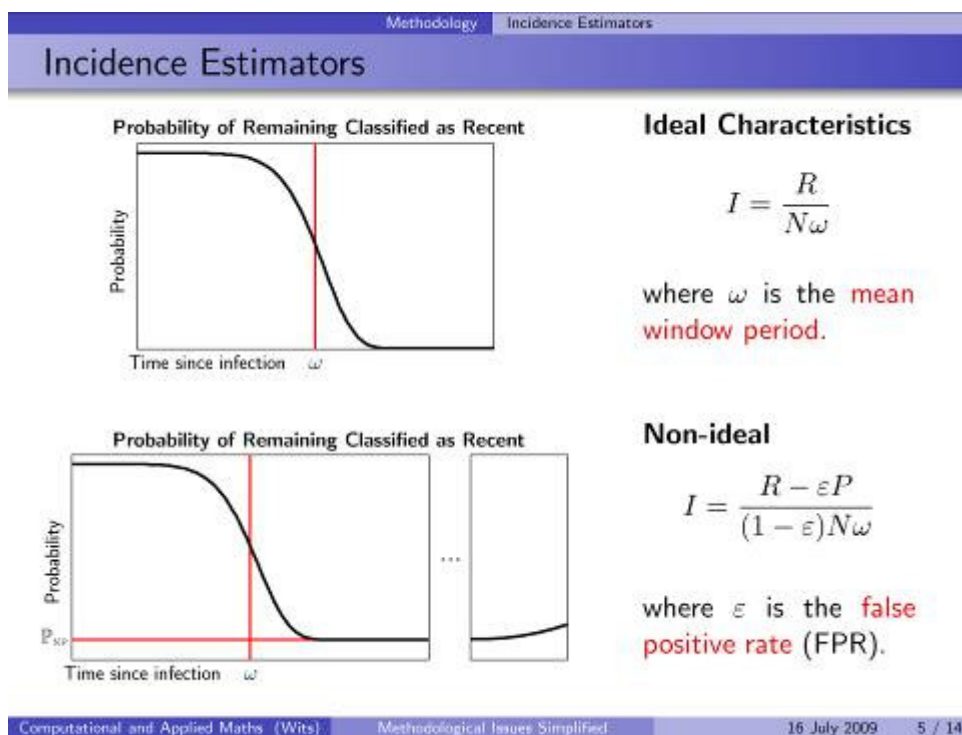


Figure 4 - Ideal and non-ideal characteristics of incidence estimators

In the field, there is considerable confusion about which assay for recent infection is robust and accurate enough for this intended use and which estimation formula to use (e.g. McDougal or Hargrove adjustments). A new incidence estimator has been derived that requires two RITA-

specific parameters (RITA-duration and RITA-FRR), and gives an estimate of HIV incidence with confidence intervals.

The coefficient of variation (C_v) for the estimator can be derived using the delta method. The 95% confidence intervals are computed as $I \pm 1.96 \times C_v I$. Sample sizes can be computed to ensure significance of estimates, for example by assuming a steady state epidemic or a particular combination of incidence and prevalence. . As a function of incidence and RITA-FRR, the sample size contour plots for coefficient of variation (CoV)s of 25% and 15% are provided in figures 5 and 6 below. The assumed parameters are RITA-duration of 153 days and mean post infection survival of 11 years

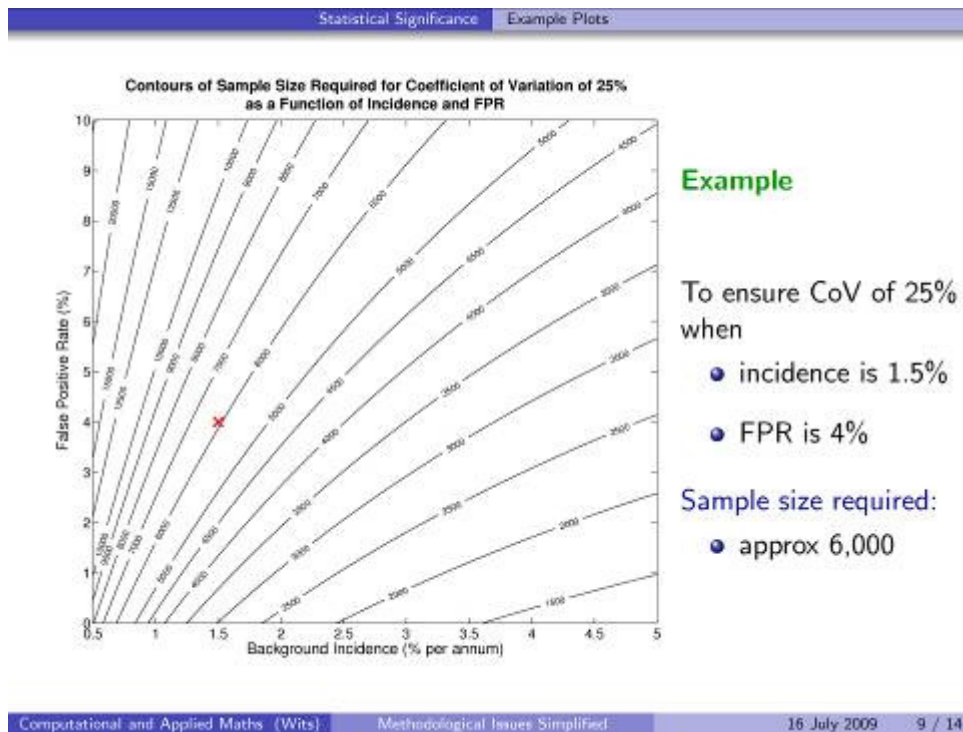


Figure 5 - Sample size requirements for coefficient of variation (CoV) of 25% as a function of incidence and FRR

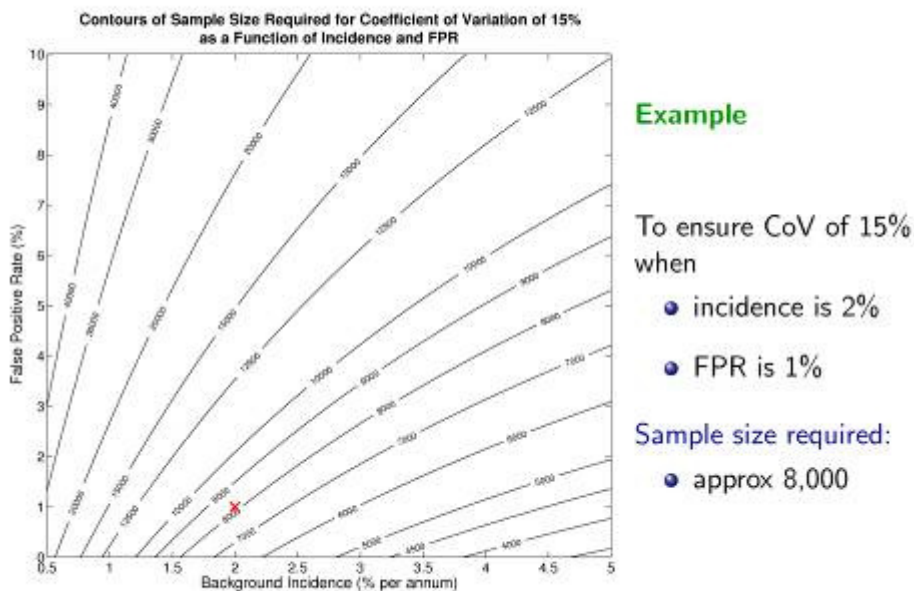


Figure 6 - Sample size requirements for CoV of 15% as a function of incidence and FRR

A number of spreadsheets have been elaborated to enable Assay Based Incidence Estimation (ABIE). These are available online at:

<http://www0.sun.ac.za/sacema/collaboration/ABIE/index.html>

The following worksheets are available:

- Incidence estimates with confidence intervals
- Hazard ratios with confidence intervals
- P-value for a change in incidence between two surveys
- Approximate sample size required to ensure statistical power
- Statistical power to detect a change in incidence using two surveys of a specified size

A number of inputs are required to provide the necessary outputs, these inputs will vary from sheet to sheet. There still exists a considerable tradeoff to obtain sufficient statistical power as a long RITA duration is required as well as a low RITA-FRR.

Epidemiological Ways to Estimate HIV Incidence

Some countries are now fortunate enough to have completed population-based surveillance surveys on more than one occasion (Niger, Zambia, Kenya, Mali, South Africa, etc). If these are

nationally representative, the data from well-performed repeated cross-sectional surveys may be used to derive HIV incidence estimates using one of the following options.

Option 1: a model that describes prevalence as a function of incidence, fitted to data. A method that is simple to formulate but hard to solve and computationally demanding.

Option 2: comparing prevalence in different age-groups to infer incidence occurring in that interval. A simpler method but prone to confounding effects.

Option 3: using cross-sectional distribution in two surveys, which partly reduces the confounding variables of age and time, see figure 7 below.

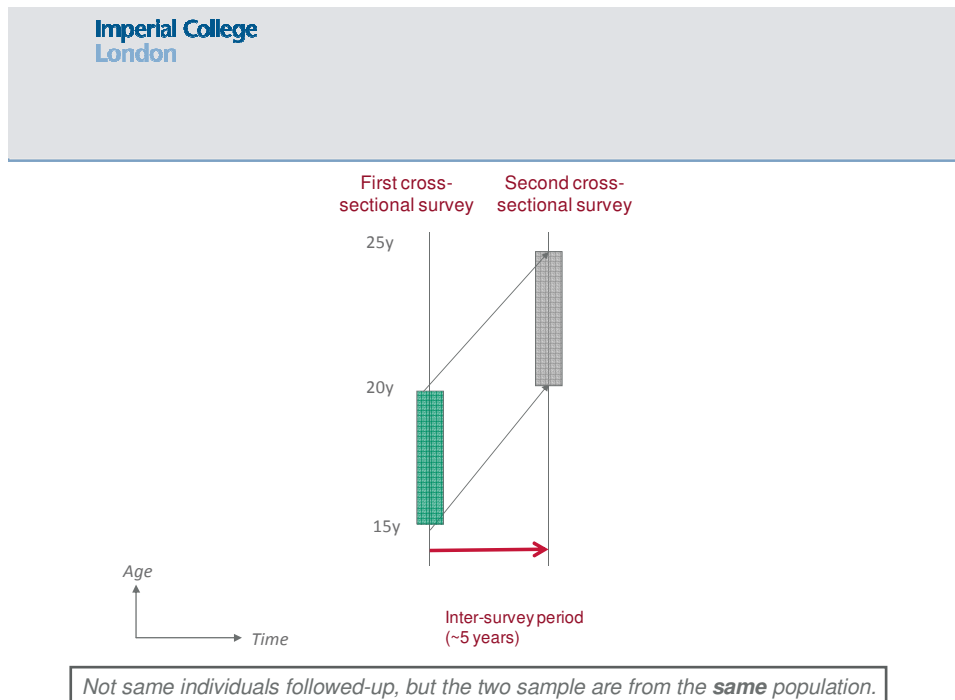


Figure 7 - Repeated household-based cross-sectional national surveys to estimate incidence

The fraction of HIV positive individuals that survive between the two samples (surveys) can be defined using one of two approaches. Method 1 uses observed mortality rates within the cohort among those infected, while Method 2 calculates the expected mortality rates using the distribution of survival after HIV infection. These methods have been tested with simulated data and real data (from Manicaland, Masaka and Kisesa cohort studies) with good success. For each age-group in each site each time, the estimates computed by method 1 and Method 2 are with 95% confidence interval of the measurement. These methods take into account changes in the cohorts due to new infections and mortality among infected and uninfected individuals but are still

imperfect. More information is needed about prevalence, ART usage and survival distribution in other settings.

3. Recommendations

The meeting came with the following recommendations about the Working Group products:

Table of Contents - The proliferation of a number of useful documents now requires an index where all the guidance information developed by the Working Group can be catalogued and more easily found by interested parties.

Incidence Terminology - This document would introduce a set of nomenclature (discussed at the meeting held in April 2009 in Geneva and this meeting) that could be to more accurately describe the concepts related to HIV incidence estimation. This would be placed in the public domain and could be used as a reference point for future assay developers and study investigators.

Guidance for Incidence Assay Users - This document would be for assay users, national programme managers and policy-makers for making decisions about the production of incidence estimates with laboratory-based methods. This document should be suited for high prevalence generalized epidemics as well as concentrated and low-level epidemics. In particular, other methods of sampling for surveillance studies should be represented e.g. respondent-driven sampling (RDS) for hard-to-reach populations in concentrated epidemics and other situations where household surveys are inappropriate e.g. low-level epidemics.

The guidance should document: 1) the implications of particular RITA (including RITA stand-alone assays and RITA algorithms, 2) the advantages and/or disadvantages of each method/assay, 3) practical examples and case studies of the use of laboratory-based methods and how to interpret results, 4) consideration of the epidemiological use of assays for recent infection and 5) how to produce HIV incidence estimates in-country i.e. how to calculate annual incidence rate from percentage of recent infections detected.

Statistical Approaches for Application of HIV Incidence Assays for HIV Incidence Estimates - An important component of the above document for assay users, is the statistical power that must be generated in order to make incidence estimates robust. A brief overview of the different statistical approaches for employing the results of laboratory-based methods for incidence estimates should be written that takes into account the deficiencies of the current assays e.g.

high RITA-FRR and that would be applicable when better performing assays become commercially available. At the meeting of the WHO Technical Working Group on Statistical Approaches for Development and Validation of HIV Incidence Assays (held 22 to 24 April 2009 in Geneva) a draft technical briefing note was developed that related specifically to statistical issues. This could serve as the foundation for a formal document issued by the Working Group.

Validation Protocol - The protocol (Methodologic Guidance for Validation of Existing and Future HIV Incidence Assays, draft version 5.0, January 2009) is still a valued document for assay developers but too large and cumbersome in the current format. It was suggested that certain sections be cut out into a set of smaller documents, specifically one document for assays developers (emphasizing calibration and the RITA duration) and other sections can be incorporated into the guidance document for users, described above

Web pages - The current format and content of the WHO-hosted website on the Working Group http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html are dynamic and any comment and suggestions would be invited. The EzCollab site has proved a useful method for disseminating documents and information within the Working Group as there is a great body of work that is not yet finalized for distribution. This allows for members of the Working Group to review this information and provide comments as well as acts as a repository for all the information pertaining to the meetings including presentations, etc.

4. Conclusions

The meeting was successful in bringing a wider group of assay users, in particular from countries affected by the epidemic who may consider in the future using HIV incidence assays, together with key experts in the field of applying laboratory-based methods for estimates of HIV incidence. The importance of HIV incidence as a key indicator of national programme success or failure was highlighted and it was clear that Ministries of Health need to be aware of the complexities of producing estimates based on data generated by the currently available assays. Certain deficiencies in some assays may necessitate the adoption of an algorithm-based approach rather than the use of a single assay alone. Presently, many research groups are working towards evaluating this approach.

The timing of the next meeting of the WHO Working Group on HIV Incidence Assays is yet to be determined. All information pertaining to this meeting (meeting presentations, etc) is available on the members-only EzCollab website.

Annex 1: PROGRAMME OF WORK

Day 1	16 July 2009	
08:30 - 12:30	Registration of participants	
12:30 - 13:30	Lunch	All
13:30 - 13:45	Welcome remarks	T Rehle
13:45 - 14:00	Introduction to objectives and expected outcomes	JM Garcia Calleja
	Review agenda	G Vercauteren
Session 1	Country experiences: use of HIV incidence assays and HIV incidence estimates	
14:00 - 15:00	South Africa	T Rehle
	Kenya	A Barsigo
	USA	B Prejean
15:00 - 15:15	Discussion	All
15:15 - 15:30	Tea/Coffee Break	
15:30 - 15:45	Outline of WHO guidance document: how to use HIV incidence assays	J Micallef
15:45 - 16:00	Discussion	All
Session 2	How to estimate HIV incidence at population level: epidemiological and HIV incidence testing approaches	
16:00 - 16:30	Epidemiological approaches	T Hallet
	Reliability in measuring HIV incidence: methodological issues	T McWalter
16:30 - 16:55	Discussion	All
16:55 - 17:00	Review of the day	
Day 2	17 July 2009	
Session 3	Progress reports of products from the FHI meeting (held in May 2009)	
08:00 - 08:30	Results of market analysis performed by FHI	T Mastro

08:30 - 09:00	Discussion	All
09:00 - 09:30	Review of virtual cohorts/banks of specimens suitable for assay validation	J Micallef
09:30 - 10:00	Discussion	All
10:00 - 10:30	Tea/Coffee Break	
Session 4	Update on HIV incidence assays	
10:30 - 11:30	Overview of HIV incidence assays	G Murphy
	New modifications of existing assays	M Busch
11:30 - 12:00	Discussion	All
12:00 - 12:15	Overview of terminology	A Sands
12:15 - 12:30	Discussion	All
12:30 - 13:30	Lunch	
Session 5	HIV incidence assay development	
13:30 - 14:15	Assay critical pathway	A Sands
14:15 - 15:00	Discussion	All
15:00 - 15:30	Tea/Coffee Break	
15:30 - 16:00	Technical guidance and documentation for WHO web pages	JM Garcia Calleja G Vercauteren
16:00 - 16:45	Recommendations	
16:45 - 17:00	Closing Remarks	T Rehle

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