



Meeting report

**WHO WORKING GROUP ON HIV INCIDENCE ASSAYS
Mexico City, 2 - 3 August 2008**

**Department of HIV/AIDS and Department of Essential Health
Technologies**

This report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WHO WORKING GROUP ON HIV INCIDENCE ASSAYS

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Content	page
1. Background	3
2. Meeting Objectives.....	4
3. New Data and Results	5
4. Applications of HIV Incidence Assays	9
5. Protocol Development for the Validation Pathway of HIV Incidence Assays	15
6. Recommendations and Next Steps for the WHO HIV Incidence Assay Working Group	19
7. Additional Information.....	23
Annexe 1: PROGRAMME OF WORK	<u>24</u>
Annexe 2: LIST OF PARTICIPANTS.....	<u>26</u>
Annex 3: Tasks and timelines recommended at the 1 st WHO(January 2008) working group on HIV incidence assays	<u>31</u>

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1. Background

Since 1997, there have been many research efforts by different institutions and international organizations to develop HIV assays in order to detect recently-acquired HIV infection. While there has been some progress in the area for detection of recent HIV infection at the individual clinical level, there remains more research to optimize assays for HIV incidence estimation at the population level. Several research groups have initiated development of assays based on different assay principles, but there remain some issues that require further clarification. Optimal methods and procedures to validate HIV incidence assays developed or under development must be clarified by consensus among technical experts before they can be used internationally to measure HIV incidence rates.

Following a series of formal and informal meetings during IAS 2006 (Toronto), CROI 2006 (Denver), and IAS 2007 (Sydney), WHO convened a meeting of experts during 28-30 January 2008 in Geneva. The main purpose of that meeting was to review approaches that have been used to validate HIV incidence assays and to seek agreement on the protocol for validation of existing and future HIV incidence assays. During the meeting, a comprehensive literature review on the calibration and validation of HIV incidence assay was presented, a draft protocol was discussed and amendments suggested and several other issues were raised for further clarification among the group. It was agreed to formalize the group of experts into a WHO working group on HIV incidence assay.

The WHO Working Group on HIV Incidence Assays listed a number of tasks and assigned lead persons to ensure their accomplishment for the 2nd WHO Working Group meeting to be held in Mexico City, 2 - 3 August 2008 (see Annex 3). Some of the tasks have already been accomplished while others are still undergoing. The Mexico City meeting assessed the progress on the different areas agreed by participants. But the main discussion was how to operationalize the protocol and move the research agenda forward. It was acknowledged that funding needed to be sought to enable the working group to function properly and move the agenda forward.

2. Meeting Objectives

The 2nd WHO Working Group meeting on HIV incidence assays was opened by representatives of World Health Organization (WHO) departments of HIV/AIDS and Essential Health Technologies. Participants attending the meeting included the core members as well as representatives from the Bill & Melinda Gates Foundation, Family Health International, the European Commission and Joint United Nations Programme on HIV/AIDS (UNAIDS). The main objective of the 2nd WHO Working Group meeting on HIV incidence assays was to develop an action plan for moving the HIV incidence issues forward.

Specific objectives included:

1. To agree on the outline of a proposal for funding (to the Bill & Melinda Gates Foundation) to secure the necessary resources to carry out the activities of the action plan;
2. To update the knowledge on HIV incidence assays by reviewing new data available from recent research studies;
3. To finalize the literature review of HIV incidence assays and discuss strategies for its publication and dissemination;
4. To review and finalize the different tasks (2, 3, 4, 5 and 8 from table 1) as agreed at the January 2008 WHO working group meeting; and
5. To seek agreement on the protocol for validation of existing and future HIV incidence assays, including identification and collection of specimens for assay validation.

The meeting participants described the three purposes for which HIV incidence assays can be used and the extent to which they are currently used.

HIV incidence assays may be applied to serve three purposes:

1. Identifying acute or recent HIV infection in individuals (for clinical use and prevention of secondary transmission through contact tracing)
2. Incidence estimation on a population level (for public health use to monitor the HIV epidemic and assess efficacy of prevention interventions)

3. Incidence estimation for assessment of outcomes in prevention or intervention trials, or to identify suitable populations in which to perform such clinical trials (e.g. vaccines, microbicides)

Specific questions for consideration by the Working Group included:

1. What are the needs of the above mentioned three distinct purposes, and how do these different applications affect the characteristics (specifications) of the "ideal" HIV incidence assay? Or alternatively, do the different applications demand slightly different assays and hence different assay development and validation pathways?
2. Is it possible to develop a unique HIV incidence assay that will fit all situations? It may be the case that one assay with two different cutoffs could be developed (one for recent infection detection and one for incidence projection). Or two different assays may be needed for the different applications (assuming that the required assay characteristics (specifications) for situation 2 and 3 are the same)? In addition, as these incidence assays will be used globally, does one need to factor in the practical conditions (needs) of resource-limited settings, if the assay is to be performed in those settings?

3. New Data and Results

Some new approaches used within the US blood bank setting were presented. A description was given of the desired composition of panels to characterize the evolving humoral immune response that HIV incidence assays discriminate, with use of initial seroconversion units from infected blood donors or interval seroconversion panels derived from source plasma donors or incidence cohort studies which include longitudinal specimens collected either prospectively or archived. A new assay approach was introduced: using the VITROS ECiQ Anti-HIV 1+2 (Ortho-Clinical Diagnostics), with either a pre-dilution and cutoff modification (detuned ECi) or an additional disruption step to exploit the avidity of the immune response (avidity ECi). The potential effects of misclassification (i.e., false recent rates) due to elite controllers and individuals on treatment were highlighted. The proportion of long-term survivors and elite controllers, i.e., the number of anti-HIV positive, but those with low or undetectable viral load RNA negative individuals with low antibody responses, may present a particular and possibly increasing problem for HIV incidence assays, when used in mature HIV epidemics. Recent data

estimate the percentage of elite controllers to be between 1% and 7% (see below); but it is possible that this will vary considerably by location, posing an additional challenge for the interpretation of HIV incidence assay results, possibly requiring that RNA detection be incorporated into incidence testing algorithms.

Canadian scientists (University of Toronto) have assessed the potential of archived specimens to be included in serologic panels to characterize the immune response in HIV incidence assays. Archived specimens from HIV diagnostic clinics in Ontario, covering a time period of several years and specimens from some 29,000 individuals were assessed. It was found that although data were missing that could have been useful and limited volumes of critical post-seroconversion specimens existed, there appeared to be utility for use of these specimens in incidence assay validation projects as for many individuals there were serial specimens from the first year post infection as well as beyond the first year (providing data to assess false recent infection rates).

Data gleaned from the Rakai cohort (Uganda) and populations in Baltimore (USA) were presented. In these studies, acute HIV infection was monitored with antibody and nucleic acid testing performed on all specimens. It was found that the rate of elite controllers was up to 3% (in US blood donors and Baltimore ER populations) but could be even higher in African cohorts (7% in Rakai, Uganda). In both settings a subset of elite controllers had low titer and low avidity antibody responses, resulting in their potential misclassifications as recent infections by incidence assays. If there are a large proportion of elite controllers (viral suppressors) in a particular study population, then these are individuals who will live longer and may therefore outnumber the number of new infections. This may effect calculations of incidence significantly and hence the proportion of elite controllers needs to be taken into the equation as incidence is projected, possibly by excluding RNA-negative subjects from the incidence calculation.

Possible approaches can be considered to account for false recent infection results due to unknown numbers of people on treatment and/or elite controllers. A relative practical and feasible approach could be the use of a battery of different types of assays to identify and

eliminate the false recent results provided they have similar mean window period. An algorithm was proposed to determine HIV incidence based on application of multiple assays in a specific order, i.e. the incidence assay with the highest sensitivity first, then those assays that have lower rates of misclassification of recent infections (higher specificity). However, this algorithm makes the assumption that the rate of death = rate of new infection which may or may not be the case.

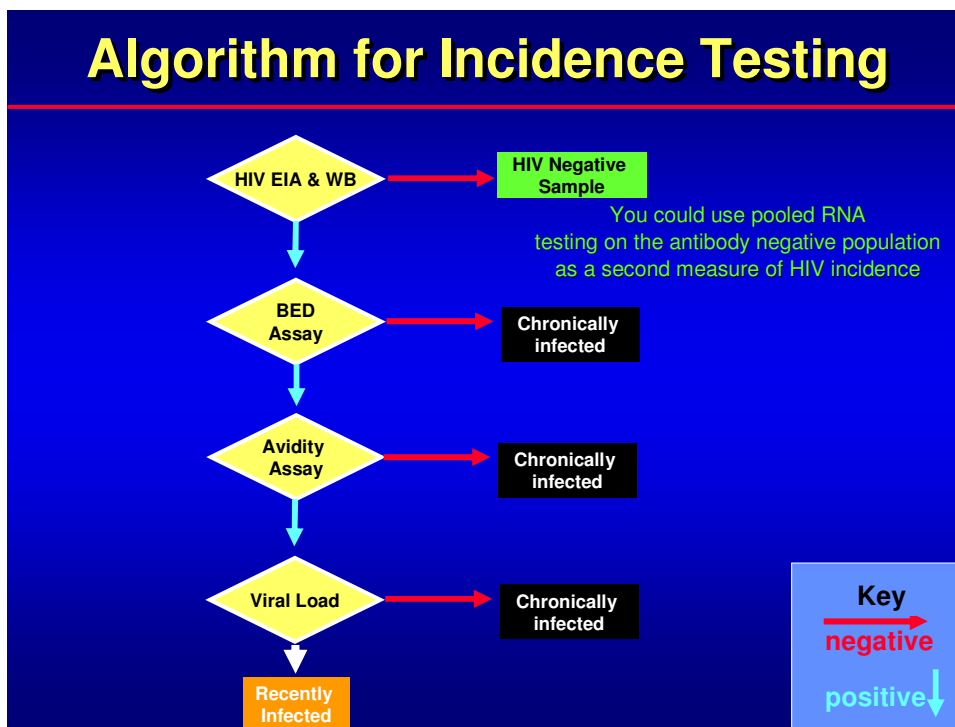


Figure 1 - Testing Algorithm for HIV Incidence Testing proposed by Johns Hopkins University

One comment made about this approach was the use of a relatively small sample size; it will be crucial to generate substantially more data to allow for derivation of confidence intervals for HIV incidence calculations based on an algorithmic approach. The level of uncertainty may become great for the incidence estimate if the lower limit of the confidence interval goes below zero.

Another comment focused on the applicability of HIV incidence assays for infants and children, i.e., from 2 to 13 years of age, for which essentially no data currently exist on performance of incidence assays (although new HIV infections in these age groups are believed to be very rare).

At the present time, there is also not sufficient data generated outside of the United States; for example, in the WHO Working Group literature review, up to 75% of the data was drawn from the US on subtype B, and primarily within blood and plasma donor or very high risk populations, which introduces bias. Another aspect is cost, the more assays in the algorithm the more expensive the exercise will be.

Data using the anti-p24 IgG3 immunoassay within a population from Melbourne were generated at the request of the Department of Human Services in Australia. Four groups of sourced archived specimens were tested to assess if there is a specific IgG3 antibody response to the p24 antigen that may be useful as a marker of recent HIV infection. To date, the number of specimens tested using this methodology has been limited, but the results are promising (high sensitivity and high specificity compared to BED and avidity approaches). The assay appears to work for different HIV subtypes, but some misclassification occurs for individuals on ART treatment. The suggestion was made to evaluate the anti-p24 IgG3 immunoassay as part of an algorithmic approach, i.e. in combination with BED, detuned or avidity assays.

A modification of a simple and inexpensive particle agglutination assay (Serodia HIV-1 PA, Fujirebio) was presented (published by Neil Constantine – [add reference](#)). The approach adapted the sensitive/less sensitive methodology (i.e., pre-dilutions) to distinguish recent from established HIV-1 infection. To date this approach has only been rigorously validated against clade B subtypes, but non-B clades from India (subtype C) and Nigeria (subtype G and A/G) have been collected and most of the testing has been performed; preliminary analysis of the clade G and A/G recombinants indicates that this approach was able to correctly classify 92% of samples with recent and established infections.

One comment was made about the need for a lower cost assay that is appropriate for use in resource-limited settings. Indeed, many companies that may be interested in commercialising HIV incidence assays require an assurance that they have a market for high throughput screening with the incidence assay modified protocol performed as reflex application on seropositive specimens for recent infection detection.

In summary, several new approaches towards HIV incidence estimation were presented, including data on new HIV incidence assays and combinations of HIV incidence assays. The

challenge of false recent HIV infection classifications due to individuals on ART remain with these newer assays, and may be further complicated by the varying proportions of elite controllers in different populations:

There was general consensus that one HIV incidence assay does not seem to be up to the task; therefore testing strategies using a combination of different assays, either different HIV incidence assays or HIV incidence assays combined with HIV RNA tests or CD4 tests may bring a solution. It was suggested that the group should also look at assays using other markers than the traditional HIV antibody response, including plasma chemokines and cytokines that are elevated during primary HIV infection. It was remarked that the HIV antigen/antibody response is very well known and stable. In addition serum/plasma specimens are widely available and accessible and serological assays can be performed globally.

4. Applications of HIV Incidence Assays

The key principles of incidence estimation and their challenges were reiterated. In order to improve the performance of HIV incidence assays, refinements may be introduced such as use of two assays (sequential testing algorithm), adjusting for test inaccuracies (Satten, Parekh, etc) and adjusting for long-term false-incident misclassifications (McDougal & Hargrove). The presenter highlighted the importance of understanding the different settings in which HIV seropositive individuals are captured and consequent biases resulting from differential rates of presentation during acute and infection stages, e.g. STD diagnostics settings, blood donation, contact-tracing, sero-surveillance surveys. Due to variables such as anti-retroviral therapy, clinical diagnosis of AIDS-related illnesses, etc., the specificity of HIV incidence assays must be high. Other limitations of incidence estimates include: low power of many studies (due to small sample sizes), lack of representativeness of tested sample sets to general populations, and selection bias (time).

There is the potential need for different window period estimations for different subtypes. This implies that incidence assays may need to be calibrated in different clade settings and the proportions of clades in study populations may need to be known. It is important that the intended uses of the assays are well defined, especially if regulatory approval for use of the

assays in diagnostic settings is a goal. It may be that one user of the assay may desire better sensitivity at the expense of specificity, and vice versa.

The next speaker presented a practical application of HIV incidence assays in the South African context. The determinants of HIV transmission are complex and difficult to influence; this makes HIV incidence assays crucial to assisting epidemiologists in monitoring the nature of the HIV epidemic. Two national household surveys (one in 2005 and one currently being conducted in 2008) have applied anonymous testing using dried blood spots (DBS) for serological determination of HIV infection and other parameters, including estimations of HIV incidence with the BED assay. The McDougal sensitivity/specificity adjustment was used in the 2005 analysis to provide annualized HIV incidence rates, expressed as the number of new infections per year per 100 persons at risk. The same formula-based adjustment approach will be also used for the 2008 incidence analysis.

Data from 2005 indicated that one-third of all new infections occurred in youth aged 15-24 years. Based on the BED incidence estimates, females accounted for 90% of the recent HIV infections among youth aged 15-24 years. It was found to be extremely useful to add HIV incidence testing to the national HIV surveys given that biological data could then be correlated with recent behaviours or recent behavioural changes. For example, the 2005 analysis identified high levels of disproportionate risk among widowed persons, pregnant women and among young people engaged in unprotected sex.

In order to examine the plausibility of the HIV incidence estimates the investigators compared the adjusted BED estimates with estimates derived from mathematical modelling, using the ASSA AIDS and Demographic model (Figure 2). Overall, the estimates were similar between the two methods used, which was reassuring. HIV incidence estimates by sex differed especially for young males where the model appears to overestimate HIV incidence compared to the population-based estimate.

BED HIV incidence vs ASSA model (estimates for 2005)

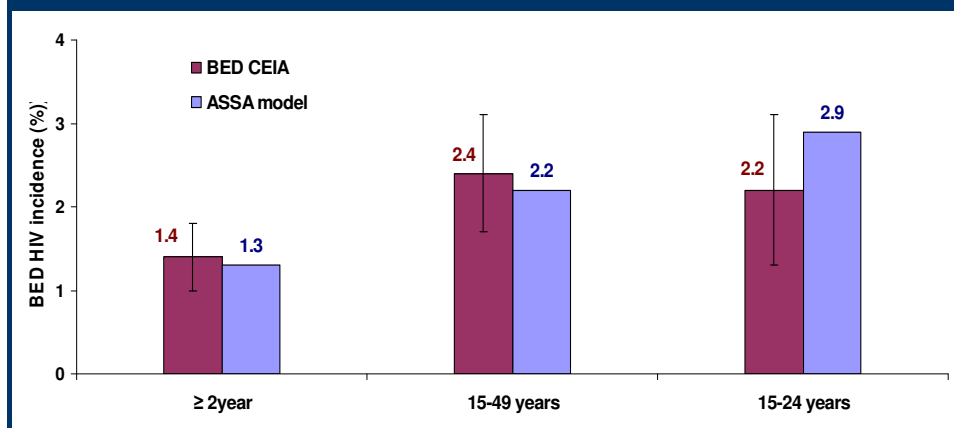


Figure 2 Comparing adjusted BED HIV incidence rates with ASSA model estimates (South Africa 2005)

Antiretroviral therapy (ART) has now increasingly been introduced into the South African population while the impact of ART was negligible in the BED HIV incidence analysis for 2005; this is not the case for 2008 when approximately 500,000 individuals are estimated to be receiving ART in South Africa at the time the survey is carried out. The study investigators were not able to consider that information given verbally by study participants to be accurate (there is much misreporting). Due to the prevailing stigma linked to HIV in South Africa, it is difficult for individuals to disclose their known HIV status or admit ARV usage. In order to correct for misclassification due to ART, High-Performance Liquid Chromatography (HPLC) coupled to Tandem Mass Spectrometry will be employed using DBS to qualitatively determine if HIV positive individuals have been exposed to ART.

The current South African approach is adjusting in the annualized incidence calculation for long-term false-positives (i.e. 5.7% of total positives, including long-term non-progressors or elite controllers). However, given the dynamics of the maturing South African epidemic, it would be desirable to estimate the percentage of elite controllers within the population.

It is intended to further explore a two step sequential testing algorithm (i.e., BED, followed by a modified antibody avidity testing method, B.Parekh) using samples of the 2008 survey.

Next, a mathematical approach to the estimation of incidence estimation using serological assays was presented. A cut-off on some immunological response, presumed to grow over time post-infection, is set, to generate 2 categories of infected persons – those under and those over the threshold. Below threshold is a naïve ‘recently infected’ category, but the question of ‘misclassification’ is handled systematically by precise concepts at the level of the model, which however does not capture the full physiological complexity. The cut-off used is essentially arbitrary, but should be chosen so that 1) the typical time spent below threshold is long enough to make it easy to count people in this category in reasonably sized surveys. Several months are far preferable to several days or weeks and 2) the vast majority of individuals do at least reach the threshold in a reasonable time like 1 year.

All current methods of incidence estimation, premised on a calibration that takes place separately from the cross sectional incidence measurement, make the problematic assumption that those who don’t progress on the immunological response relevant to the assay (a class to be distinguished from, but possibly overlapping with disease non-progressors such as elite controllers) have the same survival distribution, post infection, as those who do progress on the assay. There is also no systematic approach to handling those individuals who regress below the threshold set on the assay, having once passed above it.

For tests with problematic long term ‘misclassification’ rates, such as BED, the model can readily be extended to handle a ‘test for recent infection’ which consists of multiple assays.

New data, from the Arica Centre in the Hlabisa district in KwaZulu-Natal, was also presented, showing that a locally valid estimation of the BED non-progression rate is needed in order to obtain consistency between cross sectionally estimated incidence and incidence measured by follow up. The two are not reliably consistent if using ‘package insert’ estimates for this parameter.

A simpler use of cross sectional data for incidence estimation is possible if we are only interested in trends i.e. if wanting to compare a seroprevalence survey in 2003 vs. 2007. The mean 'window period' parameter drops out of the analysis for a direct comparison such as testing a null hypothesis that the incidence has not changed. Absolute incidence estimates of course still require an estimate for the window period.

The following key question was raised: "What is an acceptable error around an incidence estimate, and for what assay performance characteristics can such sufficiently accurate incidence estimates be obtained?" During discussion, it was noted that the following all impact the quality of an incidence estimate: performance characteristics of an assay, the accuracy with which these performance characteristics have been estimated, the sample size, the particular embedding demographic and epidemiological scenario where the test is to be used.

In summary, it was remarked that there is still some controversy about the correct inference formulas, some key assumptions should be refined to obtain a practically useful separation of biological versus epidemiological data (to separate 'calibration data' as cleanly as possible from 'incidence measurement survey data'), the uncertainty estimates are non-trivial and somewhat discouraging, calibration is problematic for current candidate assays such as BED (not trivially transferable from one time and place to another), and more robust (consistently higher) 'long term specificity' is crucial.

The next speaker presented another mathematical analysis of the approach used for HIV incidence assay.

The window period is a random variable related to the evolving virologic or immunological response in early HIV infection. The incidence assays are developed based on the premise that the immunological response to HIV infection increases for a number of months after infection is acquired, and that it is possible to routinely measure some aspect of this evolution and thereby determine whether an infection has been recently acquired or not.

The window period W and its distribution depend on the incidence assay used to measure $Y(t)$. For a chosen assay, the window period W and its distribution depend on the threshold value θ . Once the assay and the threshold value are both given, the distribution of W may depend of

the subtype of HIV-1 infection. In general, W is intrinsic, driven by the evolution of the virologic or immunologic responses within an infected individual.

The presenter reviewed the principles and conditions in the HIV-1 incidence estimation formulae based in Brookmeyer and Quinn (1995)

$I = P/\mu$, based on the principle “Prevalence = Incidence \times Duration”.

It was highlighted that Incidence is highly sensitive to the estimated mean window period used as the denominator. The presenter described the different variations and corrections made by some authors (Parekh, Kennedy, et al. , 2002; Mc- Dougal, Parekh, et al. 2006)

Although these formulae only involve the mean window period as a single parameter, their validity demand certain statistical properties for the window period distribution, such as its shape and its tail properties. If there are a positive proportion of individuals whose immune response will never increase to the threshold value, the observed "mean" window period is no longer a valid parameter to be used in these formulae. Another hidden assumption is the "constant incidence". To make the incidence formulae valid, it is assumed that HIV incidence must remain constant for duration up to the maximum possible value of the window period distribution. Clearly, a window period with a long tail is not desirable.

It was argued that of all these variations and corrections, little emphasis has been given to the distribution of the window period W except for its mean, and sensitivity and specificity are treated as constant parameters that maybe calibrated from other laboratory studies using some gold standard. The presenter then address three issues:

1. the necessary conditions for the core (1) to be applicable involve other properties of the distribution function of W than just its mean value;
2. both sensitivity and specificity depend on the interface between the distribution function of the window period W and the distribution of the testing time T from seroconversion;
3. comments on existing statistical methods seen in the literature in relation to the window period distribution estimation.

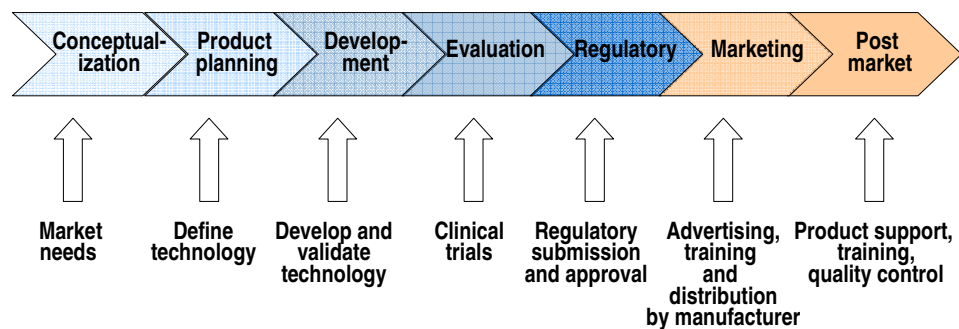
The presenter finalized making some general comments with pros and cons on statistical methods used for estimating HIV incidence that has been published in the literature. First addressed the non-parametric survival analysis approach and secondly the linear regression and imputation approach. This talk challenged this belief and presented two mathematical formulae showing that sensitivity and specificity were driven by the convolution of two processes: (1) a process intrinsic to the evolution of the immune response within infected individuals; (2) the duration between infection time and the testing (sampling) time, which is extrinsic to the evolution of the immune response, but depends on the stage of the epidemic in the population. Therefore, the probability of misclassification depends on time, place and age of the population being tested, that cannot be obtained in designed experiments, where the distribution of time-since-infection at testing was determined by the individuals in a cohort.

The presentation finish pointing out that that more statistical work should be done in this area.

5. Protocol Development for the Validation Pathway of HIV Incidence Assays

Historically, several groups have developed HIV incidence assays with varying success, most focused on modifying existing HIV antibody assays. In an environment where technological improvements result in assay replacement by new generation tests (on average every 5 years), this is a very vulnerable situation. One of the reasons why it has been so difficult to develop an HIV incidence assay or an HIV incidence algorithm using different assays, is that the basic step-wise approach for assay development (see here below) has not been followed. A systematic back-to-basics approach, as outlined in figure 3 below, may assist assay-developers to producing a product that meets the predefined requirements and therefore is marketable in the long-term.

Development pathway of an assay



Diagnostics and Laboratory Technology, Mexico 020808

Figure 3- Conceptual pathway for development of a diagnostic assay

As a first step, it is important to have an understanding of the applications of the assay(s), how and by whom they are to be used. What is the demand, in terms of volumes of tests required and periodicity of testing (e.g. are there annual or bi-annual peaks in testing [surveillance testing] or is demand continuous?). In other words, a market analysis is critical. A second step is to agree on a suitable technology and format for the assay(s), considering the conditions in both technologically advanced and resource-limited settings. The avenue of using existing assays with modified procedures for the HIV incidence determination should continue to be explored. Suitable companies could be contacted regarding their interest in commercializing a candidate HIV incidence assay. A realistic business plan would need to assess future risks and balance perceived incentives to encourage manufacturing of HIV incidence assays at low cost. The purpose, specific applications and characteristics of assays (specifications) need to be defined and mapped out before the performance of potential of HIV incidence assays are challenged, as described in a validation protocol. There will be a need for HIV incidence assays to undergo WHO Prequalification of Diagnostics status, if WHO is to make any recommendations about specific assays to WHO Member States. Prequalification may only be possible if an assay is commercialised and manufactured under ISO13485. At the same time the utility of the HIV incidence assay(s) will need to be advocated to countries and programs.

Finally, attention will need to be given to appropriate training and quality control before wide scale roll out of HIV incidence testing can be recommended at a global level.

The critical pathway for HIV incidence assay validation was presented as one of the accomplished tasks, as recommended in the 1st WHO Working Group meeting.

The following operational characteristics were deemed important:

1. accuracy in distinguishing recent from long-term infection, i.e. minimum false recent rate;
2. differential performance of the assay in the context of clade variability;
3. availability of assay components and equipment (transferability to resource-limited settings);
4. cost (it should be affordable).

The pathway may include:

- Primary assay development: initial calibration and characterization of the assay's performance carried out in-house by the developer/manufacturer; (independent minimum standards need to be set, calibration = development of the cut off value/ formula used to estimate incidence based on the rate of detection of recent infections and window period associated with the incidence assay cutoff);
- Incidence reference specimen panel: this panel would be provided to the developer/manufacturer of the assay in a blinded manner for verification of the cut off setting with possible fine tuning based on interaction with the study group;
- Repository of archived seroconverter and false recent specimen panels: these panels will be used for comparative evaluations of the assay(s) by a Central Laboratory (minimum standards need to be developed);
- Field Studies (only for assays that have passed the previous steps): further assessment of the performance of the assay(s) in the setting of intended use.

The critical pathway for an HIV incidence assay must be transferable (able to be performed in all settings) and the results must be comparable in some way to other methods for estimating incidence. It is not likely that the manufacturers will have the necessary biological material to perform the calibration, it may be required that this type of material be made available to diagnostic companies showing interest in developing HIV incidence assays.

The latest version of the Protocol for Validation of Existing and Future HIV Incidence Assays (here after referred to as the 'Protocol') was presented. The aim of the Protocol is to provide guidance on standard procedures for validating existing and future HIV incidence assays and algorithms for estimating incidence from cross-sectional surveys.

Some additions in the new version (added since January 2008) include:

- Table with characteristics of existing HIV incidence assays.
- Characteristics of seroconversion panels for window period estimation.
- Field validation: Alternative method for quantifying rate of false-recent using cross-sectional design – the epsilon study, including sample size calculations.
- Characteristics of specimen panels for assay validation, including sample size calculations.
- Critical pathway for HIV incidence assays

The group expressed the utility and advantages of considering a central document repository, such as a WHO HIV incidence assay website, for this protocol and other documents such as the literature review, to ensure easy access and enabling updates as required. Additional comments on the validation protocol pertained to the fact that Epsilon will influence the precision of the incidence assays. If we are going to move forward with an algorithm-based approach then Epsilon will have to be calculated in each setting as one can not take the estimation of epsilon in one setting and transfer to another. There was consensus that the estimation of an epsilon correction factor derived from a large representative sample in a specific HIV subtype region is very well transferable to similar study settings with the same HIV subtype predominance. There is doubt about how feasible this would be for public health programmes but as there appear to be few other options at this time, it can not be discounted.

At present HIV incidence assays may be used by any program that is interested in monitoring and evaluating prevention initiatives or interventions. It is important to understand the problems associated with specificity in some of the current assays as it will affect the sample size and window period estimation, thus impacting the accuracy of the assay. Perhaps if a country has completed two or more population-based prevalence study, then they maybe in a position to use the BED assay (with adjustment formula/ factor) and compare the two time points to see an increase or decrease in the trend of incidence, even if there are errors in the actual incidence rates. This may only work in at national level and not in small populations.

6. Recommendations and Next Steps for the WHO HIV Incidence Assay Working Group

The meeting reviewed the status of the task as outlined in Annex 3, and prepared an overview of new tasks and timelines which are summarized in tables 2 and 3 here below..

Table 2- Status of WHO HIV Working Group on HIV Incidence Assays Publications

What?	Who is coordinating?	When due by?
Literature Review	John Kaldor, Rebecca Guy (NCHER, Australia)	To be finalized and published as soon as possible
Two Publications: 1. The accuracy of HIV incidence assays in estimating the population rate of new infections: A systematic review 2. Sensitivity and Specificity of HIV Incidence Assays: A Systematic Review	John Kaldor, Rebecca Guy (NCHER, Australia)	Comments to NCHER and WHO by 31 August 08
Validation Protocol: Methodological guidance for validation of existing and future HIV incidence assays	Andrea Kim (CDC, USA)	More comments to be sought
Funding Proposal to support on-going work of WHO HIV Incidence Assay Working Group	Tim Mastro (Family Health International, USA)	Beginning of September

Table 3 - List of tasks to be included in a proposal for funding.

Tasks	When?
1. Perform a scoping exercise to understand the market for HIV incidence assays and to talk to as many different consumers of incidence assays, i.e. national health programs, small study populations, clinical trials, diagnostic labs.	2009
2. Define what HIV incidence assays should be able to do in the context of different applications and test settings.	

3.	Support the development of existing/pipeline/new assays that may circumnavigate the problems associated with BED assay i.e. IDU/V3 loop assay, IgG3 assay.	
4.	Formalise the process of assay comparison (maybe a separate WG subgroup), i.e. the criteria for head-to-head evaluation of all existing/pipeline assays. Has the gold standard been defined (maybe against a longitudinal observational cohort)?	
5.	Define the role of an HIV incidence assay algorithm approach. There needs to be a way to compare individual assays vis a vis algorithms, i.e. specific criteria for their validation.	
6.	Assemble panels of specimens that can be used for calibration of window periods, validation, determination of false incidence rates, evaluation/comparison of individual tests and algorithms, etc. The specific characteristics of the panels must be set. A mechanism for gaining access to the panels must be established (collaboration or funding). How and where will they be stored? Will it be a centralized repository, and if so how will they shipped and who will pay for shipping and maintenance of the panels? Panels for QC/QA measures will need to be established at a later stage, should one think about collecting those types of panels as well?	
7.	Laboratory for validating assays in an independent manner.	
8.	Statistical and modeling aspects related to incidence inference.	
9.	Further research and development of assays for new biomarkers for incidence inference.	
10.	All comments on the current draft of the validation protocol (including the assay pathway)	To A Kim by end of August 2008
11.	Website to be instituted to house all technical information i.e. validation protocol, literature review, peer-reviewed publications, etc.	WHO, 1th Q 2009
12.	WHO/UNAIDS statement on value of HIV incidence testing.	WHO, 1th Q 2009

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It was decided that many of the above mentioned tasks were difficult to carry out with the current membership of the WHO Working Group, as conflict of interests do occur when those actively involved in assay development are also involved in the assessment/evaluation component. Therefore; it was suggested that a smaller core group under the lead of FHI would prepare a proposal outlining the planned activities. FHI was suggested as the potential primary funding recipient, with a core steering group (project implementation group) to ensure oversight and transparency of the process. The following figure was produced as an outline for a possible reconfiguration of this project implementation group. The WHO working group on HIV incidence assays (potentially with sub-groups for specific technical areas) would play an important role in overall technical guidance and feedback on specific deliverables of the project. The functioning of the WHO working group on HIV incidence (i.e., 2 meetings per year) would be supported by the project proposal).

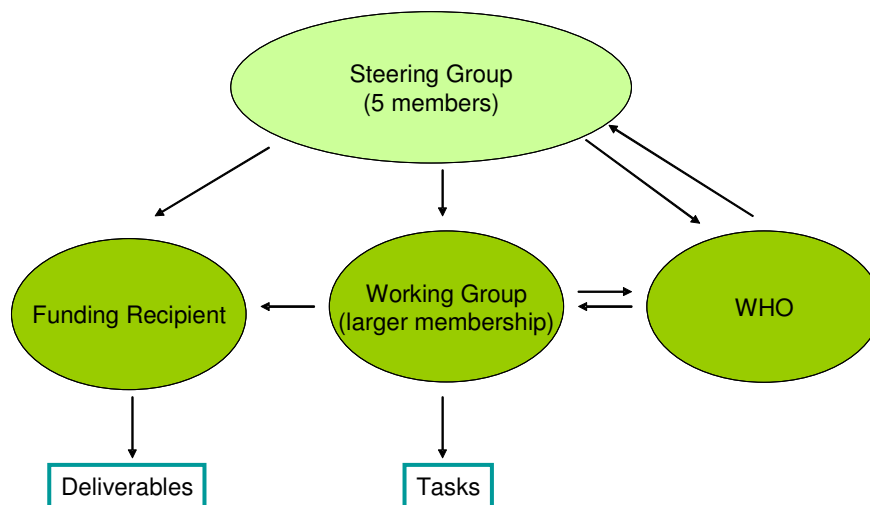


Figure 4 - Suggested organigram for to further develop and fulfill activities around HIV Incidence Assays

Formation of a Project Implementation Group (Steering Group) on HIV Incidence Assays
 Proposed membership of Project Implementation Group on HIV Incidence Assays

- FHI (funding recipient ;Tim Maestro and Connie Sextopn)
- John Kaldor
- Thomas Rehle
- Mike Busch
- WHO (TxemaGarcia-Calleja and Gaby Vercauteren)

The representatives of the Bill and Melinda Gates Foundation outlined some specific deliverables they would be interested to see the WHO Working Group on HIV Incidence Assays work on.

Table 5- Specific Deliverables Desired by BMGF

<p>1. A technical guidance document for individuals interested in performing HIV incidence testing right now with the currently available assays and with the existing methods for analysis.</p> <p>For example, if you are interested in observing for a 30% decrease or a 50% decrease in incidence, then this is what you do. In different sample sizes, here are the assays you could use and the methods to be used to analyse the data.</p> <ul style="list-style-type: none"> ○ 1,000 to 5,000 population size ○ 5,000 to 15,000 - 20,000 population size ○ 20,000 and over population size
<p>2. Technical guidance document for assay developers. This would be rather close to the current assay validation document. But could be broken down into the 3 suggested sample sizes aforementioned. The document would detail the methods for calibration of window period, validation of the assay, how to compare assays head-to-head, where to get the specimens, etc.</p>
<p>3. The specimen repository, including prospective collection as required. This repository could be used by assay developers and other parties for comparison studies and algorithm development and validation.</p>
<p>4. A MRD (market requirements document) which would detail the universe of individuals who would want to study incidence and the exact nature of the different sample sizes and population sizes.</p>
<p>5. A SPRD (specific product requirement document) including provision for large vs. small study populations. It may be the case that a smaller population would need to have best specificity, while a large population</p>

could compromise on specificity to a degree.
6. Some specific research studies including statistical modeling studies. BMGF could support some of these studies but with a lesser priority to the above-stated deliverables.
7. The website for all technical information, publications, literature reviews, etc to be housed.
8. Research into other potential biomarkers for estimation of HIV incidence.
9. WHO to formalize a functional WHO Working Group on HIV Incidence Assays (with 3 - 5 years of funding)

7. Additional Information

Other publications

The European network will be publishing a special issue on HIV Incidence Assays in September 2008 which will include a literature review that has been completed by their group. These publications are available at this link : <http://www.eurosurveillance.org/Default.aspx>

Next meeting

It was proposed to schedule the 3rd meeting of the WHO Working Group on HIV incidence assays for 12-13 February 2009, as an adjunct to the CROI 2009 Meeting that will be held on 8-11 February 2009 in Montreal, Canada, with the objective to review progress made and move tasks forward.

Annexe 1: PROGRAMME OF WORK

Day 1: Saturday 2 August 2008

<i>Session 1</i>			
	<i>New Data and Results</i>	Presenter	Chair
08:30 - 09:00	Registration of participants		
09:00 - 09:15	Introduction to objectives and expected results for the meeting Review agenda	WHO JM Garcia-Calleja G Vercauteren	
09:15 - 10:00	Framework and platform for validation and development of HIV incidence assay	J Kaldor	G Vercauteren
10:00 - 11:30	New data or approaches on HIV incidence assays	M Busch	
	Results of HIV cross incidence testing	O Laeyendecker	
	A comparison of incidence assay performance	K Wilson	
	Particle agglutination assay using seroconversion panels from Nigeria	N Constantine	
11:30 - 11:45	<i>Coffee break</i>		
<i>Session 2</i>			
	<i>Applications of HIV Incidence Assays</i>	Presenter	Chair
12:00 - 13:00	Principles and uses of HIV incidence estimation from recent infection testing	S Le Vu	JM Garcia-Calleja
	Practical application of HIV incidence testing: the 2008 national HIV survey for South Africa	T Rehle	
13:00 - 14:30	<i>Lunch</i>		
<i>Session 3</i>			
	<i>Protocol Development</i>		
14:30 - 15:30	Issues on HIV assays specifications	G Vercauteren	J Kaldor
	Define assay assessment pathway	G Murphy	
15:30 - 16:00	<i>Coffee break</i>		
16:00 - 17:00	Measuring HIV incidence from survey and validating HIV	A Welte	

	incidence assay	
	A few statistical issues	P Yan
	New revised validation protocol	A Kim
17:00 - 18:00	General Discussion	

Day 2: Sunday 3 August 2008

Session 4	General Discussion on Next Steps	Presenter	Chair
09:00 - 09:30	Summary of main issues raised the previous day	G Vercauteren JM Garcia-Calleja	J Kaldor
9:00 - 10:30	General discussion Next steps, Outline for a proposal, steps, timeline		
10:30 - 10:45	<i>Coffee break</i>		
10:45 - 12:30	General discussion Next steps, Consensus in milestones and products		
13:00 - 14:00	<i>Lunch</i>		

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Annex 3: Tasks and timelines recommended at the 1st WHO(January 2008) working group on HIV incidence assays

Task	Time-line	Completed
1. Draft project planning and time line (Gantt chart) into the long term (approx. 3 years)	End of April 2008	Partially
2. Desirable characteristics of HIV incidence assays	End of March 2008	Partially
3. Potential uses and challenges of HIV incidence assays for different applications	March 2008	Partially
4. Finalize literature review <ul style="list-style-type: none"> • Disseminate and publish • Two separate publications to be prepared 	RG to send a revised version in 7 working days (11 Feb 08) and then 2 weeks to submit final comments (25 Feb 08)	Yes
5. Define assay assessment pathway	April 2008	Partially
6. Finalize validation protocol <ul style="list-style-type: none"> • Incorporation of comments from meeting • Circulate by email for additional comments 	May 2008	Partially
7. Organize working group on statistical approaches	June 2008	Postponed due to GMS go-live
8. Finalize window period estimation protocol (statistics)	May 2008	Partially
9. Define characteristics of specimens consistent with the needs of the assessment pathway	April 2008	Partially
10. Submit joint funding request for a WG meeting in Mexico City (WHO)	March 2008	Yes
11. Brief meeting report to be prepared	August 2008	Yes
12. Develop funding proposal to support further work Sub-group to be convened to further discuss	August 2008	Partially