MEETING REPORT
ANNUAL MEETING OF THE WHO TECHNICAL WORKING GROUP ON HIV INCIDENCE ASSAYS

26–27 SEPTEMBER 2012 GENEVA, SWITZERLAND
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1. BACKGROUND

The most recent meeting of the steering committee of the WHO Technical Working Group on HIV Incidence Assays took place on 26–27 September 2012 at WHO headquarters in Geneva, Switzerland. This meeting follows the August 2011 Working Group meeting that took place in Atlanta, Georgia, USA and addresses the key outcomes of that meeting (1). This report documents the meeting’s objectives, outcomes, main discussion points and next steps for the Working Group meeting that is proposed to take place in about 12 months.

In 2008, WHO established a Technical Working Group on HIV Incidence Assays to examine the issues and challenges involved in assay-based estimation of HIV incidence. This group has worked to standardize terms in the areas of assay calibration, validation and use for estimating incidence. Several meetings to advance this agenda have been held, and copies of reports are available on the Working Group web page (http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en). These meetings have been successful in bringing together a wider group of assay users, especially those from countries affected by the epidemic who may consider using HIV incidence assays in the future together with key experts on applying laboratory-based methods for estimating HIV incidence. The importance of HIV incidence as a key indicator of national programme success or failure has been highlighted, and health ministries clearly need to be aware of the complexities of producing estimates based on data generated by the currently available assays.

The UNAIDS and WHO five-year strategies for 2011–2015 aim to significantly reduce the incidence of HIV infection, and even though there is no clear consensus on how to measure incidence in all countries, tests for recently acquired infection provide one method for estimating HIV incidence and can be especially useful in countries with a high burden of HIV infection.

In 2010, the Gates Foundation awarded a grant to the United Kingdom Health Protection Agency and the Blood Systems Research Institute to develop a specimen repository and evaluate existing HIV incidence assays. The approved proposal has five main objectives and aims to validate the performance of existing and future HIV incidence assays when used with different samples from the sample repository created and to identify the key parameters to enable the laboratory results to be correctly interpreted. The grant is for two years, and progress on the work was presented at the 19th International AIDS Conference in Washington, DC in July 2012. Information on the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) is available at http://www.incidence-estimation.com/page/cephia-overview.
In collaboration with the United States Centers for Disease Control and Prevention (US CDC), the Working Group produced a guidance document on when and how to use assays for recently acquired infection to estimate HIV incidence at a population level, published in 2011 in English and French (http://www.who.int/diagnostics_laboratory/110906_guidance_hiv_incidence.pdf). Further, the South African Centre for Epidemiological Modelling and Analysis (SACEMA) organized a training course on how to use HIV incidence assays for estimating HIV incidence. The course was conducted on 9–11 May 2012 at Stellenbosch University in South Africa.

The August 2011 meeting report [1] highlighted the key deliverables for the Working Group: (1) a protocol for field validation of assay-based estimation of HIV incidence and (2) a pathway for development, calibration and validation of HIV incidence assays. The Working Group agreed that progress on these documents should be discussed during the course of the year and finalized at the following Working Group meeting.

The following objectives and expected deliverables were developed for the September 2012 meeting.
2. **OBJECTIVES OF THE MEETING**

The objectives of the meeting were:

- to update progress on the draft protocol for field validation of assay-based estimation of HIV incidence (Annex 1);
- to present a draft guidance document for estimating HIV incidence in case-based surveillance systems in high-income countries;
- to present a concept note on estimating the level of and risk factors for recently acquired infection in cross-sectional settings;
- to provide an update on the grant proposal by the United Kingdom Health Protection Agency and Blood Systems Research Institute and coordination and synergy with the Gates Foundation grant;
- to review the recommendations made and progress since the last meeting held in Atlanta in August 2011 [1]; and
- to outline the work for 2012–2013.

2.1 **METHODS OF WORK AND EXPECTED OUTCOMES**

Annex 3 presents the agenda and presenters for the meeting. The participants were provided with the 2011 meeting report and current scientific articles related to estimating HIV incidence written by various Working Group members. Group discussion following presentations led to consensus for the next steps for future Working Group work.

The expected outcomes were:

- to discuss substantive issues remaining to complete the field validation protocol;
- to agree on an outline of guidance for estimating HIV incidence in HIV case-based surveillance systems in high-income countries;
- to agree on a concept for estimating the level of and risk factors for recent HIV infection in cross-sectional settings;
- to discuss lessons learned on training and implementing guidance on when and how to use HIV incidence assays to estimate the HIV incidence in a population;
- to share progress with participants on the various activities undertaken; and
- to decide on the next steps needed for validating existing HIV incidence assays.
2.2 PROGRESS SINCE 2011

Although there are now scientifically supported guidelines and recommendations for how to use HIV incidence assays and estimate the HIV incidence at population level, it was emphasized that there still is not a current gold standard of assay-based estimation of HIV incidence in the field. Promising new approaches to detecting whether people have recently acquired HIV infection using biomarkers and molecular diversity are currently being developed.

The sessions and posters applicable to HIV incidence estimation that were presented at the 19th International AIDS Conference in 2012 were described. Several country experiences and multiple approaches were presented for the Working Group to consider. The most appropriate approaches for different settings need to be discussed: in particular, national AIDS programme requirements for evaluation, the need to assess changes in incidence over time and the development of appropriate incidence assays or algorithms to help meet these needs should be addressed.

2.3 SUMMARY OF OUTSTANDING ISSUES

Although the Working Group has made significant progress, several outstanding issues need to be addressed: specifically, the approaches for estimating the mean duration of recency and false-recent rate (false-recent rate), such as:

- consideration of the new definition of “T” – time;
- the frequency and time of data collection;
- determination of the false-recent rate for multi-assay algorithms; and
- details in designing separate studies to estimate the false-recent rate.

Because of statistical considerations related to behavioural surveillance survey sampling (such as respondent-driven sampling) or other complex survey sampling methods, the suggestion was made to form another group to make recommendations about analysing complex survey data for estimating the HIV incidence.

Recommendations from the Statistical Consultation meeting held in Atlanta on 18–19 August 2011 were summarized: (1) SACEMA’s online tool for calculating HIV incidence and sample size would be updated and simulation panels would be produced to test the validity of assumptions and (2) issues regarding estimation of the mean duration of recency needed to be address. In addition, CDC tools should be available online for access by projects supported by the United States President’s Emergency Plan for AIDS Relief. As mentioned above, challenges still to be resolved include analysing survey data collected using respondent-driven sampling, such as among key populations at higher risk of HIV infection, as well as the need for incidence assays with longer durations of recency and lower false-recent rates.
The Working Group expressed the need to look more carefully at how incidence was calculated in the respondent-driven sampling or integrated biological and behavioural surveillance surveys that are currently used in key populations before they could accurately comment on the methods. Confidence intervals will be wider because of the need to account for uncertainty in the parameters included in the incidence formula (such as sample size, mean duration of recency and false-recent rate). These parameters among key populations at higher risk may differ from the false-recent rate among the total population living with HIV and could therefore be difficult to determine.

Based on the update on the development of WHO guidance documents for HIV incidence assays, it was recommended that regional workshops be held and that clear guidelines for incidence studies among key populations at higher risk and interpreting the results be provided. Guidance is strongly needed in low- and medium-income countries, and this is currently being worked on in collaboration with the European Centre for Disease Prevention and Control.

Previously, WHO had suggested developing a form of criteria to validate the old and new assays embodied in the draft document on guidance on developing, validating and evaluating HIV incidence assays. This would be considered a bridging publication to provide confidence to implementers to change from the current method to the new method, providing guidance on how to validate an assay before implementation in a country. In addition, a second publication on guidance for validating assays and algorithms used for estimating HIV incidence, would bridge between the development stage and major field application; it would include easy-to-follow implementation steps and could be part of or an annex to the previous publication.

The main issues and recommendations regarding outstanding issues are as follows.

• The Working Group concurred that an additional issue should be addressed in reference to the last objective “to outline work for 2012–2013”. The progress of the assays themselves, particularly what (currently) needs to be done to fill the gaps, will be added to the outline of work for the coming year.

• The question was raised whether the Working Group had received any feedback from countries, particularly from the six countries involved in SACEMA. The workshop was evaluated at the end, and the feedback was very positive in general. Countries received the technical materials and were advised to contact SACEMA for any further assistance or questions. However, there is no information on how the countries are applying the new concepts learned.
The purpose of Session 1 was to provide an update on HIV incidence assays, including the requirements for in-house and field-testing validation, reviewing the progress made on the development of the draft document on guidance on developing, validating and evaluating HIV incidence assays and CEPHIA evaluation. The second half of Session 1 explored new directions in estimating HIV incidence as well as potential new laboratory tests for detecting recently acquired infection among individuals.

The first two presentations focused on the minimum requirements for validating assays and addressed:

- assay standardization;
- recency period – seroconversion panel;
- misclassification rate: specimen panels from individuals with long-standing HIV infection and from those receiving antiretroviral therapy;
- incidence estimates: cross-sectional application;
- different subtypes; and
- technology transfer and proficiency testing programme.

**REQUIREMENTS FOR VALIDATING HIV-1 INCIDENCE ASSAYS**

In assay standardization, data should demonstrate that an HIV incidence assay is well standardized and the critical parameters for validation, such as the window period and false-recent rate are well defined. In addition, assay testing should use specimens from populations of interest during both in-house and field validation.

In-house evaluation should include the following elements:

- assay standardization and reproducibility;
- performance in known seroconversion panels; and
- applied cross-sectionally (retrospectively) before the assay is taken to the field for validation.

In field validation, the process should include comparing results from multiple methods. Concordance or discordance with observed incidence used as a comparison is considered the gold standard, with the understanding that recruiting individuals in prospective follow-up studies has its own biases. Strong consideration should be given to evaluating the association of known risk factors in the key population with recent acquisition of HIV-1 infection. Comparing these elements enables one to better determine whether the assay is working or not to distinguish people who recently acquired HIV infection from those with long-standing HIV infection.

It was strongly emphasized that, before validation, the assay steps and conditions should be final, as should procedures for the quality control of test kits and reagents; no additional changes in production should need to be made during the validation process.
The mean recency period (?) varies from assay to assay and is determined by testing panels of seroconversion specimens collected longitudinally at frequent intervals. Sufficient specimens are needed to determine the mean recency period. Since these panels are difficult to collect and procure, coverage of key populations with targeted subtypes should be sufficient; the mean recency period does not need to be determined in every country or population for validation purposes.

The false-recent rate is the percentage of people with known long-standing HIV infection (more than one year) whom the assay misclassifies as acquiring HIV infection recently. A high false-recent rate can affect the accuracy of incidence estimates (falsely elevated incidence) and weaken the association of risk factors with recent HIV-1 infection. Studies of the false-recent rate require many specimens from people who have not received antiretroviral therapy and have known long-standing HIV infection. Overlying issues include the question of how often the false-recent rate should be studied and when the false-recent rate can be ignored (<2%). It was noted that a false-recent rate of 2% or less is desirable but may not necessarily be ignored in the formula for estimating incidence.

Elite controllers are people who are able to control viraemia naturally without antiretroviral therapy. They have a low viral load and therefore a weak immune response, and an incidence assay can therefore misclassify specimens from such people as recently acquired HIV infection. Although elite controllers make up a small fraction of the people living with HIV, this group has important implications for assays of recently acquired infection.

How antiretroviral therapy affects the estimation of HIV incidence is important to address. Antiretroviral therapy suppresses viral load, causing a decline in antibodies, resulting in misclassifying people with long-standing HIV infection who are receiving antiretroviral therapy as having recently acquired HIV infection. The false-recent rate among people receiving antiretroviral therapy varies with the duration of treatment and viral load suppression. Using a viral load exceeding 1000 copies per ml in an algorithm may help to address the issue of misclassification related to antiretroviral therapy and elite controllers. Incidence estimates can be compared with those of a retrospective cross-sectional cohort as a critical step before field validation takes place.

For an assay developed in house, a commercial kit should be available before further characterization, including field validation, is conducted. When a modified diagnostic assay is being used, the modification should be available as a supplemental kit, quality control specimens should be defined and included and a standardized data management tool should accompany the kit.

A hands-on training programme complete with training manual and algorithm (initial plus confirmatory testing) is needed as are a means of addressing quality assurance and quality control and a data management tool. A dedicated training panel to cover the range of biomarkers and a separate competence panel to document successful implementation of the assay in the laboratory should also be available. Quality control data should be periodically monitored, and a hotline for help should be available for participants after the training.

An established proficiency testing programme is part of the external quality assessment for laboratories, in which coded specimens are sent periodically to laboratories to assess their performance. Proficiency
testing helps to identify laboratory-specific issues that can be followed with corrective action and to identify kit lot problems or reagent issues. The programme requires dedicated specimens for proficiency testing and predefined values and classification (recent or long-standing). A widely used assay should be monitored using quality control data reviews and proficiency testing programmes.

Annex 2 presents a matrix summarizing the essential elements for assay validation, organized by objectives, specimen type, subtypes and status. Field validation studies should be used to complement the rigorous process of in-house validation. Field validation entails the real-time application of the assay in the field, where different challenges and issues may emerge that the in-house validation did not capture.

The main issues, questions and recommendations regarding the document on requirements for validation of HIV-1 incidence assays were as follows.

- Although there are several supported requirements for validating assay-derived estimations, it was emphasized that there still is not a current gold standard in the field because of inherent biases and the assumptions of other approaches (observed or model-based incidence). Comparison with observed incidence is still considered the gold standard for validation.
- As much in-house validation as possible should take place before piloting a new assay in the field.
- In-house validation should include all HIV-1 subtypes.
- Comparison with observed incidence or seroconversion specimen panels is still considered the gold standard for validation.
- The draft document on guidance on developing, validating and evaluating HIV incidence assays will be shared with the Working Group for review and comment.
- Guidelines and training materials have been developed for countries so that technical assistance in transitioning from the BED enzyme immunoassay to the limiting antigen (L.Ag) avidity enzyme immunoassay can be provided.

FIELD VALIDATION OF HIV-1 ASSAYS

The presentation provided an overview of field validation methods for the HIV-1 assay with their associated benefits, limitations and recommendations on their use: (1) comparison of assay-based estimates of HIV incidence with estimates derived from prospective or retrospective cohort studies, mathematical models or multiple assays and (2) analysis of factors associated with recently acquired infection to check the plausibility of results.

The comparison of assay-based estimates of incidence with results from prospective cohort studies was described as the optimal study design. The rate of HIV seroconversion (people acquiring HIV infection) is measured directly in a well-defined cohort of HIV-negative people followed longitudinally and tested at regular intervals. This design also allows the follow-up of the people determined to be living with HIV at the inception of the cohort and the subsequent seroconverters, which may be useful in designing misclassification studies. Its advantage is that it directly measures incidence in a sample of people at higher risk. The disadvantages of prospective cohort studies are that they are costly, logistically difficult
to implement and prone to biases, such as selection, intervention and attrition bias. Further, the number of seroconverters may be very small; assay-based estimates of incidence may therefore not be very precise if they are derived from testing a very small number of specimens for recently acquired infection. Recommendations for using this approach include: (1) identifying existing cohorts or stored specimens from cohort studies – a cohort study should not be conducted for the sole purpose of assay validation; and (2) ensuring that validation studies are sufficiently powered.

The second design described was the retrospective cohort study in which existing records of repeat HIV testing results are linked and retrospectively analysed. Stored HIV-positive specimens are tested using the incidence assay, and then assay-based incidence is compared with directly observed incidence in the retrospective cohort calculated by the conventional epidemiological method. In this design, a database of HIV testing records (results and dates) for a large population of individuals who are repeatedly tested for HIV infection (such as HIV testing clients, blood donors and military personnel) is required. Data from repeat testers with two or more linked HIV tests whose first test was HIV-negative are used to form a retrospective cohort for analysing the density of HIV incidence. The validation population ideally includes people who test frequently (such as every 3–6 months versus every 2 years) so that seroconversion dates can be estimated more precisely. The advantages of this approach are its relatively low cost compared with a longitudinal cohort study and the potential for assessing misclassification (that is, the false-recent rate). The disadvantage of this method is its dependence on existing high-quality specimens, sufficiently complete HIV testing history and linking information. Using this approach requires that the information that enables identification and linkage of data for repeat testers be of high quality, and the quality of linking information for those who remain HIV-seronegative must be similar to those who seroconvert to permit unbiased estimation of HIV incidence.

Another approach used in field validation is to compare assay-based estimates of incidence with estimates derived from mathematical modelling. We can construct mathematical models to infer incidence from HIV prevalence and mortality data, adjusting for how antiretroviral therapy affects survival. Examples of models used to estimate HIV incidence include EPP/Spectrum, modes of transmission, Asian epidemic model and the synthetic cohort (such as Hallett) method. The advantage is that modelling is relatively easy and inexpensive compared with conducting a cohort study. An example shown was the comparison of Kenya's 2007 national HIV incidence estimates by various methods: EPP/Spectrum, the Hallett method and assay (both BED and LAg) derived.

Another recommended step when validating an incidence assay is to analyse the association of testing for recent acquisition of HIV-1 infection with sociodemographic information and potential risk factors for acquiring HIV. An analysis of factors (such as demographic, behavioural and clinical characteristics and sexually transmitted infection status) among cross-sectional survey cases that tested for recent acquisition of HIV-1 infection may help assess the biological and epidemiological plausibility that an assay is able to distinguish people who have recently acquired HIV infection from those with long-standing HIV infection in the survey population. Although such analyses do not directly validate the assay, they provide additional information on current risk for acquiring HIV (as compared with assessing risk factors among people living with HIV that may have been acquired years ago). A limitation is that the lack of an association between testing recent and known HIV risk factors may be caused by limitations in the study design, sampling, bias or insufficient power rather than poor assay performance.
UPDATE ON THE FIELD VALIDATION PROTOCOL

An update of the subgroup’s progress on the draft document on field validation of assay-based incidence estimation was presented and the current draft described. Section 6, on sample size and statistical considerations, is of particular concern. At issue is the large sample sizes (50,000 for each comparison group) needed for statistical tests of equivalence. This substantive issue still remains after a statistical consultation was convened at US CDC headquarters in which no consensus was reached. There was much discussion among statisticians at the consultation on the “area of scientific indifference”. This area depends on how much bias one is willing to accept, and there are no set rules for choosing it. In some cases, a value within 20% may be considered acceptable. Alternatives to equivalency testing need to be identified. One suggestion made during the statistical consultation was to conduct one very large validation study in one country, such as comparing cross-sectional with longitudinal results, and then subsequent studies in other countries could be relatively smaller. Because the subgroup is uncertain about how to overcome the challenges presented by section 6, they will distribute the draft to the Working Group for feedback.

MAIN CONCLUSIONS AND RECOMMENDATIONS FROM THE DISCUSSION ON THE DRAFT DOCUMENT ON FIELD VALIDATION OF ASSAY-BASED INCIDENCE ESTIMATION

- A subgroup will distribute the draft document on field validation of assay-based incidence estimation to the Working Group for additional feedback.
- Strong concerns were expressed about recommending that every country validate each new assay for use. The field validation document may set high standards that will be difficult for many countries to meet. The Working Group needs to consider this issue. The document should therefore address what is reasonable for field validation and what country teams who want to use assays for estimating HIV incidence should require.
- As an alternative to equivalency testing for assay validation, mathematical modelling should not be recommended because results are frequently inconclusive.

Four presentations providing an overview of the current assay validation efforts in Italy, the United States and France discussed the key issues and challenges with the AxSYM/ARCHITECT avidity assay (Italy), Bio-Rad HIV-1/HIV-2 PLUS O EIA (US CDC), LAg-Avidity EIA, 2-well Al-EIA (US CDC) and the V3-IDE enzyme immunoassay for recently acquired infection (France).

UPDATE ON HIV ASSAYS

AXSYM/ARCHITECT AVIDITY ASSAY (ITALY)

The Instituto Superiore di Sanità has used ARCHITECT for about 6–7 years. The team evaluated the coefficient of variation with the ARCHITECT HIV Ag/Ab (Abbott Laboratories) on 10 serum samples (4 from people who recently acquired HIV infection and 6 from people with long-standing HIV infection), each one tested daily in triplicate for 10 days, according to a Clinical and Laboratory Standards Institute protocol. All samples showed a precision of <10%. This evaluation was based on a precision study with
ARCHITECT HIV Ag/Ab, with 231 serial samples from 68 people living with HIV with an estimated seroconversion date without antiretroviral therapy and 190 samples with AIDS or CD4 count exceeding 200 per m$^3$. The results on 421 samples with a 0.80 cut-off acceptability rate yielded 32 (7%) in a “grey zone”. The team based the results on receiver operating characteristic analysis for various avidity index (AI) cut-offs.

The team reached the following main conclusions.

- AI can be measured with different commercial methods, although with every new method it is important to define the variability (precision study) and the appropriate cut-off.
- Antiretroviral therapy slows the maturation of the AI in 25% of cases.
- HIV subtype B versus no subtype B showed no effect on the false-recent rate.

General challenges with ARCHITECT primarily involved looking for antigen and antibody and some problems of misclassification associated with ethnicity, risk group, age or sex. The question remains whether the type and time of initiation of antiretroviral therapy could affect the AI false-recent rate. The validation explored how antiretroviral therapy affected AI and found misclassification as a false-recent rate of 10% for people not receiving antiretroviral therapy versus a 28% false-recent rate for people receiving antiretroviral therapy. The limitation was noted that the assay is not as precise, especially for people with long-standing infection. There is follow-up planned regarding recently acquired versus long-standing infection with AI.

A main recommendation from this evaluation emphasized the importance of developing a very descriptive protocol and to assure that staff members follow the protocol very closely.

Two points of discussion were raised to the Working Group.

- Can the AI potentially be used to monitor the compliance with antiretroviral therapy among individuals recently acquiring HIV infection (review avidity assay for toxicity)?
- Can the Working Group expect to identify the perfect avidity assay for individuals recently acquiring HIV infection? If not, perhaps the Working Group should lower expectations and be satisfied with lower specificity and sensitivity?

BIO-RAD HIV-1/HIV-2 PLUS O EIA: US CDC

The presentation included a discussion regarding dried blood spots (DBS), since countries want to use this method with assays. After the Atlanta meeting in 2011 and the refiguring of the time ($T = 1$ year), the US CDC team reanalysed statistics for the mean recency estimates. The new numbers came from a large population: 1144 observations from 273 subjects and subtypes – B, A, CRF01 and CRF02. Analysing subtype B versus non-B, the mean duration of recency was compared at AI 30% cut-off. Several studies were reviewed ($n = 6$) for additional data in the statistical analysis that included four different cohorts from Kenya, Nigeria, Thailand and HIVNet.
The review of the estimation of the false-recent rate among individuals who have not received antiretroviral therapy and with long-standing HIV infection at various Al cut-off values \((n = 225)\) included many individuals who had been living with HIV for more than 14 years. It was shown that the time of initiation of treatment makes a huge difference – specifically earlier versus later.

In the protocol development and evaluation of Bio-Rad avidity and DBS, there were past samples from 1994, which were stored properly, monitored and used. The optimization study used paired plasma and DBS from Cameroon and the United States of America. The DBS optimization results showed a good correlation between Al values comparing plasma versus 1 spot \((n = 54\) pairs) and the plasma versus 1 punch \((n = 48\) pairs). The results showed a good correlation between plasma and spots.

The National HIV Behavioral Study pilot study, a self-reported behavioural study in the United States of America, was new to using DBS. The team therefore encountered some logistical challenges, since DBS and whole blood were collected in venues and handled at a research training centre before being sent to the US CDC. The plasma was obtained within 24 hours at room temperature and the DBS were shipped at room temperature with desiccants and humidity indicators. In addition, the plasma and DBS had to be stored at \(-20^\circ\text{C}\). The plasma versus DBS testing \((n = 116)\) included 71 people who had not received antiretroviral therapy and 45 who had.

The results found a good correlation of Al between plasma using a 6-mm punch. The correlation varied between sites: deviation of collection, handling, storage and misclassification at 30% cut-off was rare.

An update on Bio-Rad avidity mentioned that there has been progress on the manuscript draft, which will be sent to co-authors for comments within the next couple of weeks after the meeting. In addition, the training panel was distributed to several sites, including CEPHIA sites, and in Canada and Germany.

Currently the following activities are either planned, or are in progress:

- DBS-plasma validation;
- retest of samples from the United States of America used for estimating incidence (30 000 samples or more);
- mean duration of recency and false-recent rate for non-B (currently few panels for non-B) in Kenya, Thailand and Center for HIV-AIDS Vaccine Immunology (CHAVI); and
- the potential for Bio-Rad to market a modified assay.

The following conclusions were noted regarding the Bio-Rad assay.

- The assay format was finalized with a mean duration of recency that is primarily subtype B data, and a manuscript has been sent through for US CDC clearance.
- Preliminary data with DBS are promising, although the handling requirements need to be assessed further.
- More data from multiple sources will available from CEPHIA and training panels.

The challenges include receiving data from field studies and issues surrounding the commercial potential of these assays.
UPDATE ON THE LAG-AVIDITY ASSAY

An overview was given of the limiting antigen (LAG)-avidity enzyme immunoassay with an update on the characterization and validation comparing it with the BED assay. Key findings were: the assay has similar mean duration of recency (132 days to 143 days) in four subtypes or populations (subtypes A, B, C and D); the false-recent rate is low, being close to 1% or less in multiple populations tested (Côte d’Ivoire, Ghana, Thailand, the United States of America and Viet Nam); and the assay provided plausible incidence estimates, similar to observed estimates, in multiple cross-sectional population (Kenya, Swaziland and Zimbabwe). Two-peer reviewed articles have been published, and field validation data from Swaziland, with antiretroviral therapy coverage among the eligible population exceeding 60%, was presented at the 19th International AIDS Conference in Washington, DC in July 2012. The assay has the desired performance characteristics and met validation requirements.

The LAG-Avidity EIA is now manufactured as a commercial kit with standardized quality control process in place for lot release. The assay is available for purchase from the manufacturer (Sedia Biosciences, Portland, OR, USA; www.hivincidence.com) and will soon be available from another manufacturer (Maxim Biotech, Rockville, MD, USA). Currently the assay is optimized for serum or plasma, and a procedure for use with DBS will be coming in the near future.

The United States Food and Drug Administration has currently broadened the use of the LAG-avidity EIA kit with a label stating “for research use”, unlike the BED assay, which was “for surveillance use” only. This facilitates use of the assay beyond surveillance in identifying people recently acquiring HIV infection for recruitment in clinical trials, transmission of various subtypes and transmission of drug-resistant viruses, among others. The company (Sedia BioSciences) is exploring the possibility of filing an investigational device exemption with United States Food and Drug Administration for individual use, which can help with targeted counselling and prevention.

It will be important to monitor the LAG-avidity assay as countries begin to use the assay with different subtypes and in different settings for any confounding factors (such as expanding antiretroviral therapy use). Standardized training and a training manual have been developed for transferring the technology. The current US CDC proficiency testing programme will be expanded to include the LAG-avidity EIA as more laboratories begin to use the assay.

A laboratory and epidemiology manual has been developed to assist programme personnel funded by the United States President’s Emergency Plan for AIDS Relief to move forward in replacing the BED assay with the LAG-avidity assay. The US CDC plans follow-up to ensure that people are using the LAG-avidity EIA correctly. In addition, cross-assay comparison by groups such as CEPHIA will further provide critical data for the proper use of the LAG-avidity EIA and other assays.

The US CDC and the United States President’s Emergency Plan for AIDS Relief give priority to estimating the HIV incidence to assess the success of programmes, identify populations at higher risk, target resources and implement prevention programmes for reducing incidence. At this time, LAG is the best available tool, as demonstrated by extensive characterization and multiple validation studies.
EIA-RI (ALSO KNOWN AS IDE-V3): FRANCE

The presentation described the validation of the enzyme immunoassay for recently acquired infection (EIA-RI; IDE-V3 in France). This was an in-house assay developed in 2002. Initially, measures of antibody binding to the immune-dominant epitope of gp41 and the V3 region of gp120 were combined into an algorithm. Only measures of the normalized ratio of optical density (\(\text{OD}_{\text{specimen}} / \text{OD}_{\text{negative control}}\)) of the immune-dominant epitope are currently used for estimating incidence. The assay is used to classify people who recently acquired HIV infection and those with long-standing HIV infection and in surveillance of new diagnoses (people who have not received antiretroviral therapy).

The mean duration of recent infection has generally been estimated in various settings with homogeneous epidemics in terms of subtype (one region = one subtype). The team hypothesized that both the subtype and the “origin” of people (genes or environment) might play a role in serological markers, since the epidemic in France is heterogeneous in terms of subtype (32% of the people newly diagnosed with HIV infection were born in sub-Saharan Africa, 39% are infected by non-B subtypes and 24% of the people newly diagnosed with HIV infection born in France are infected by non-B subtypes).

A study sample of seroconverters was developed to disentangle the effects of subtype and origin. A linear mixed-effects model and survival analysis were used to estimate the mean duration of recent infection. Regarding the mean duration of recent infection across subtypes and people’s origins in using the mixed model, it was shown that the subtype parameter is significant, whereas the parameters for origin and interaction subtype origin were not significant. The survival analysis provided the same results. The team therefore concluded that mean duration of recent infection differs significantly (shorter) among people with subtype B HIV infection than among people with another subtype of HIV infection. Annex 2 provides the paper provided to the Working Group at the meeting discussing the effect of prolonged treatment on EIA-RI assay.

The first cross-sectional use of the assay among 886 men who have sex with men showed that 18% of those were living with HIV and 28 were EIA-RI positive. However, there is still a concern regarding treatment, as the study did not collect information about treatment.

The team understood that initiating antiretroviral therapy during the primary infection affected the EIA-RI assay results and therefore wanted to assess the effect of antiretroviral therapy when infection is already established (2).

The cohort included: 96 people with long-standing HIV infection receiving highly active antiretroviral therapy with undetectable viral load at two intervals three years apart; 143 people with long-standing HIV infection at the AIDS stage who had never received treatment; and 150 people with long-standing (at least two years) HIV infection not at the AIDS stage who had never received treatment. The study’s false-recent rate results showed: 22.9% of those receiving highly active antiretroviral therapy; 4.2% of those never treated at the AIDS stage; and 0.7% of those never treated and not at the AIDS stage.

The outcomes of the EIA-RI were used in a cross-sectional estimation for men who have sex with men. A total of 886 men who have sex with men were recruited at 14 commercial gay venues using a cross-sectional design, resulting in 157 (18%) testing HIV positive (and 28 testing EIA-RI positive).
The questionnaire did not ask about treatment. However, the team did analyse EIA-RI-positive samples using liquid chromatography and mass spectrometry.

The incidence estimation showed that, of the 157 people living with HIV, 15 were deemed to have recently acquired HIV infection, and the overall HIV incidence was estimated at 3.8% per person-year (95% confidence interval 1.5–6.2). It was emphasized that this was the first community-based survey in France to estimate the HIV incidence among men who have sex with men. Although the results cannot be generalized to men who have sex with men in Paris, given the factors, it is currently the best estimate so far for men who have sex with men attending gay venues in Paris (3).

The following issues were highlighted regarding validation of the EIA-RI.

- The results showed no influence by origin but influence by virus subtype (roughly B versus non-B).
- The testing algorithms for recently acquired infection could include serotyping results.
- The assay has not been commercialized but is carefully characterized (mean duration of recent infection and false-recent rate) for use in France.
- Should the team decide to continue with another testing algorithm for recently acquired infection, they would have to use another test that is not influenced by subtypes and other sources of error.

France does plan to continue to use EIA-RI. It was noted that, in comparison, the United States of America currently still uses the BED assay for domestic surveillance, and it was emphasized that in general it is complex to move from one assay to another as a standard.

**MAIN ISSUES AND CHALLENGES FROM THE UPDATE ON CURRENT ASSAY VALIDATION EFFORTS**

- The Working Group needs to consider its role in the recommendations or endorsement of the LAg-avidity assay in light of the US CDC recommendation that countries using money from the United States President’s Emergency Plan for AIDS Relief to purchase test kits should start using the LAg-avidity assay and no longer order BED assay test kits.
- Guidance will need to be developed for countries that have been using the BED assay on how to interpret trends after the transition has been made.
- The Working Group should consider where these endeavours towards validation are leading the Group, specifically regarding providing timely guidance and confidence for countries.
- The Working Group recommended that this body collaborate with the US CDC to convene an independent, external panel to review the existing data on the LAg-avidity assay and assess test kit performance to inform subsequent recommendations.
CEPHIA EVALUATION UPDATE

The presentation provided the background and objectives of CEPHIA and an update on CEPHIA progress on the global plan for the repository and testing of assays. During the presentation, various issues regarding the relationship between CEPHIA and the Working Group were raised, specifically concerning how to elevate and aid the Consortium’s progress, particularly given the validation results for the LAg-avidity assay.

CEPHIA provided an update in reference to their global plan, repository and testing. The results from their testing should help build the market for assays in collaboration with the Working Group. Currently, CEPHIA is behind in testing, since they are still getting specimens in place and identifying new problems as the process continues. As one of CEPHIA’s main objectives is to standardize the validation of assays, they have had a challenge in obtaining enough specimens for different HIV subtypes, people receiving antiretroviral therapy, elite controllers and people with AIDS. There was concern whether CEPHIA could successfully evaluate all the assays available, considering the various testing algorithms for recently acquired infection used for the HIV incidence assays.

In summary, CEPHIA continues to work on many fronts:

• adding to the library of documents on the WHO web site to address how people can use and access tools for evaluating HIV incidence;
• creating assay development and the steps required for validation;
• sharing validation by individual laboratories;
• communicating with manufacturers when there are assay problems;
• the need to select some assays only as some are not available in the quantities requires for evaluation;
• the consensus in testing is difficult; and
• a regulatory claim complicates testing.

There have been laboratory issues hampering the identification of the “ideal” incidence assay. CEPHIA is currently trying to identify these limitations and learn to work around them. It was suggested that the Working Group collaborate in adapting the current product profile to establish a more reasonable target.

It was noted that building a successful repository is challenging, as it is expensive, although it was agreed that it brought added value to the projects. The presentation provided graphics illustrating the types of samples needed for evaluating HIV incidence and an overview of the protocol of validation sets (such as qualification and evaluation). Once assay evaluation takes place, CEPHIA moves forward with targeted work on individual assays once it is confirmed that they work.

CEPHIA’s database includes demographic data. A complex graphic of the CEPHIA database management is available on the CEPHIA web site to any specific group interested. It was emphasized that the traceability of data is a strong feature of the database, as no data are lost or overwritten, and everything is recorded. A database will be built for CEPHIA II for non-plasma assays.
Some challenges with assay testing and the problems from some results focus on companies’ regulatory claims. This complicates testing for CEPHIA, because certain controls are needed, in accordance with what companies are obligated to do to get a licence for marketing their products. A summary of 16 potential assays that need to be evaluated, including information regarding which should be avoided and why, is underway.

An overview of testing issues highlighted that assays should not be tested until a quality assurance system is in place for the specific assay, training is done beforehand and all results are traceable. Current testing challenges include the following.

- The procedural aspects of testing assays need to be verified.
- The associated documentation needs to be correct. There have been mistakes within documents that developers should check beforehand and correct.
- The testing equipment available differs, and tests may require different ranges of readers.
- Some necessary assays and equipment are not available internationally, and CEPHIA must therefore find resourceful means of procuring them.

The current focus is on results from the evaluation between LAg and BED assays as well as the false-recent rate for specimens from people receiving treatment, elite controllers, people with AIDS patients and people with different subtypes of HIV infection. In addition, CEPHIA is involved with documenting the utility of assays for estimating incidence: whether an assay will work better under certain conditions.

CEPHIA stated that it is moving forward with testing assays. The Bio-Rad assay will be tested soon, and CEPHIA is testing the BED assay.

The increasing interest of the United States Food and Drug Administration in the LAg-avidity assay increased interaction between CEPHIA and the United States Food and Drug Administration. Concern was voiced regarding how the development of the LAg-avidity assay and endorsement by the US CDC may change the level of interest of companies. There is potential added value, since CEPHIA could receive additional funds for kits as companies move forward in commercializing kits. However, the US CDC pointed out the urgency of deploying incidence assays because of support of programmes and willingness to change to any other assay if it proves to be a better tool than the LAg assay.

### MAIN ISSUES AND RECOMMENDATIONS FROM THE DISCUSSION ON THE CEPHIA UPDATE

- CEPHIA continues to be as inclusive as possible with all partners, specifically the Working Group.
- There is concern about how CEPHIA can receive some regulatory support. It is important to have the endorsement of organizations such as WHO and the Working Group.
- The Bio-Rad assay will be retested soon, CEPHIA has completed testing the BED assay and the specimens for LAg–avidity and other assays in its repository. CEPHIA would like the Working Group involved in analysis so that a body of experts can endorse the work; this will give sufficient weight behind the recommendation that an assay should or should not be used.
The collaboration between the Working Group and CEPHIA is intended to ensure that the recommendations will be made accordingly. There is pressure in getting these results to the public.

- The data on certain assays might have to be shared before a proper endorsement or recommendation takes place.
- It was recommended that the Working Group meeting in 2013 focus on reviewing data together to help determine the questions to guide proper recommendations and define the next steps. The resulting information can be used to illustrate CEPHIA’s progress and approach funders.

DISCUSSION: ARE WE THERE YET?

The discussion following Session 1 focused on these topics:

- current successes and challenges for HIV incidence assays;
- promising approaches from current assays;
- the next steps; and
- the development of consensus and a possible time frame for projects.

Three major flows of activity were identified.

- There are investigator- and science-led activities regarding HIV incidence assays.
- There are activities funded by CEPHIA and the Gates Foundation that resulted from a 2009 subgrant to WHO and South Wales (2008–2010) and after a series of meetings convened in Chapel Hill, North Carolina and Cape Town, South Africa.
- The US CDC has the ability to disseminate guidance to the global community. CEPHIA or WHO have not endorsed the recommendation to use the LAg-avidity assay, but the US CDC pointed out the delay in testing and that the US CDC cannot continue to let the field use the BED assay given the better performance characteristics of the LAg-avidity assay.

Considering these activity flows, it was suggested that the Working Group clarify confusing issues surrounding the use of incidence assays worldwide. As such, the Working Group must consider how commercializing the BED and LAg-avidity assays will affect global and national programmes that may leave countries unsure of how to move from the BED assay to implementing the LAg-avidity assay. The Johns Hopkins University will present the results from multi-assay evaluation in 2013.

ROLE OF THE WORKING GROUP: MOVING FORWARD

It was recommended that the Working Group set guidelines for evaluating the assays CEPHIA currently uses. This would be a public demonstration that the Working Group commits to supporting CEPHIA endeavours, providing an endorsement by experts and lending credibility to the organization’s reports. Based on their previous recommendation, CEPHIA suggested that a document therefore be created with the aim of reviewing all assay evaluations with countries as the target audience and that the Working Group would endorse the document.
The question was raised regarding when assays (such as the LAg-avidity assay) will move from research to clinical use and what the recommendation from the Working Group will be at that point. Working Group should consider whether moving the concept of recommending an assay to recommending an algorithm would be more efficient. In reviewing algorithms, the Working Group can recommend certain types of surveys to be used. This should be deliberated soon as the commercialization of many assays may occur in the near future and within a small time frame. CEPHIA can comment on this because of their experience with the assay repository. However, this recommendation would probably be a rank ordering of the best assays to use under certain situations versus the “winner” of all assays. The suggestion was given that Working Group should organize a subgroup comprising the US CDC, WHO and key members to discuss LAg and recommendations on the way forward.

The question of whether WHO will plan to evaluate assays was raised. It was explained that, if WHO were to formally recommend an assay, it would need to have an independent evaluation of data (a step in the process of WHO prequalification). WHO could therefore not be the only evaluator. The data clearly show that the LAg-avidity assay is better. There are published facts on how the test is performing. Neither WHO nor the US CDC can endorse a specific company. However, the Working Group must consider how to proceed since there is only one company providing the assay commercially now. Nevertheless, one more company will have an LAg EIA available very soon. The approval is for the assay, which is licensed to more than one company (currently three). This approval is not for the company but for the product.

**ISSUES REGARDING THE REVIEW BY THE US CDC OF THE LAg-AVIDITY VALIDATION DATA**

When Working Group has agreed on the parameters for assay evaluation, the Working Group will come to a consensus and then distribute information publicly. There was strong concern that an independent data review from the US CDC was needed to inform any recommendations on the LAg-avidity assay. Since the US CDC developed the assay, there may be a perceived conflict of interest related to recommending the assay. The suggestion was made that two subsets of the Working Group could review the performance characteristics of the LAg-avidity assay, whether the company can produce an adequate number of kits and what qualifying body validates the performance of kits.

The Working Group should consider reviewing the US CDC data and decide on what parameters should be used for evaluation. Countries need a document or guidance that would unify the US CDC and WHO recommendations for a country to move forward with its decision-making in what assay to use. It was emphasized that this guidance would also be very important in influencing policy-makers to support testing at the national level.

The US CDC is currently standing in for that qualifying body and providing the technology transfer needed. It was emphasized that the US CDC has a great interest in ensuring that the use of the LAg-avidity assay is carried out correctly in the countries supported by the United States President’s Emergency Plan for AIDS Relief. CDC had an open invitation for any organization to review the data and outcomes of LAg–avidity assay validation before making this unilateral move to endorse the assay, although it was questioned whether the US CDC actively invited other groups to do this. It is important to note that the United States Global AIDS Program is moving towards the LAg-avidity assay, but the
domestic HIV incidence system in the United States of America continues to use the BED assay. To this end, a significant testing comparison may be conducted using past BED specimens with the LAg-avidity and Bio-Rad assays as mentioned previously.

The US CDC noted that United States President’s Emergency Plan for AIDS Relief is strongly interested in writing guidelines for the LAg-avidity assay. It was suggested that the Working Group consider this in its future work. The countries supported by the United States President’s Emergency Plan for AIDS Relief are also strongly pushing to know what assay to use for estimating HIV incidence.

OTHER ISSUES RELATED TO VALIDATING HIV ASSAYS

There was brief discussion regarding how countries and developers relate to the change in the concept of $T$ (mean recency period estimates $[T = 1]$ by avidity cut-off values). The Gates Foundation is working on phase I and perhaps will fund a phase II. The results are showing interesting results, and it is challenging to decide which study design should be used.

The long-term issues of the false-recent rate were also readdressed. If the false-recent rate is low, it is challenging to handle but researchers are clear and aware about knowing uncertainty. The Working Group should discuss this issue and develop a recommendation or guidance. There should therefore be a very open and transparent process of determining how the Working Group and CEPHIA would carry out a mass analysis plan. This work is in progress at SACEMA, with a modelling group reviewing data. This group should comment on the long-term issues with the false-recent rate; a recommendation would help influence CEPHIA to approve activities to move forward and complete the validation project.

CEPHIA is not comparing the in-house to the observed incidence evaluation. CEPHIA is collaborating with the John Hopkins University laboratory to identify which incidence assay should be used depending on the specific setting.

CEPHIA faces challenges in conducting cross-evaluation of assays because of their availability; getting enough assays to evaluate is difficult. CEPHIA is becoming restricted by assay availability and therefore becoming more dependent on laboratories in the United States of America, especially the US CDC.

Since the results from the BED assay are not linked, its primary use is for surveillance, created only for public health purposes (nonhuman subjects) and only used by the US CDC for laboratories to use domestically. The United States Food and Drug Administration therefore does not have to approve it. However, since the United States Food and Drug Administration does not have jurisdiction internationally, international programmes can use the BED assay widely. The approval for using assays follows a different protocol than diagnostics.

In 2009, FHI 360 (formerly Family Health International) conducted a market assessment for HIV incidence assays that included surveillance applications. This project revealed that there was a much larger market for an assay that could be used for both HIV diagnosis and determining HIV incidence. If the LAg-avidity assay could be used for diagnosis as well as recency, this could potentially open a
larger market for a single test to show whether a person is living with HIV and whether the HIV infection was acquired recently or is long-standing. The current work on the Bio-Rad assay could potentially be used for diagnostics.

THE NEXT STEPS FOR VALIDATING HIV INCIDENCE ASSAYS AND A POSSIBLE TIME FRAME FOR PROJECTS

The closing of Session 1 focused on the following issues:

• the direction of the Working Group;
• how to agree on the criteria for validation;
• scheduling a Working Group meeting to discuss a public statement or recommendations; and
• exchanging data and information with CEPHIA.

In the past, Working Group played a stronger role in providing guidance on assays. However, innovation in assays is progressing rapidly.

• Since the results from CEPHIA have arrived, it was suggested that the Working Group convene before or after the 20th Conference on Retroviruses and Opportunistic Infections on 3–6 March 2013 to discuss progress on reviewing data and to discuss whether there are gaps in the knowledge base and direction of research.
• It was suggested that the Working Group meet within the next six months to discuss these issues raised and that an update on the LAg-avidity assay be drafted before the meeting.
• The Working Group should organize a subgroup comprising the US CDC, WHO and key members to discuss the LAg-avidity assay and make recommendations on the way forward.
• It was recommended that the Working Group set parameters to be used in evaluating the assays CEPHIA currently uses.
• A very open and transparent process should be used for determining how the Working Group and CEPHIA would carry out a mass analysis plan.
• It was recommended for future Working Group and CEPHIA work that a document be created, with the aim of reviewing all assay evaluations with countries as the target audience and that the Working Group would endorse the document.
• The SACEMA modelling group and the Working Group should comment on the long-term issues with the false-recent rate; a recommendation would help to influence CEPHIA to approve activities to move forward with the Gates Foundation project.
• It was suggested that two subsets of this group could review (a) the performance characteristics of the LAg-avidity assay and (b) assay kits – whether the company can produce enough kits and what qualifying body validates the performance of kits.
• The Working Group needs to address issue of the countries supported by the United States President’s Emergency Plan for AIDS Relief requesting what assay to use to estimate incidence by developing guidance or recommendations. The US CDC has moved forward by endorsing the LAg-avidity assay in the countries supported by the United States President’s Emergency Plan for AIDS Relief.
NEW DIRECTIONS IN ESTIMATING HIV INCIDENCE

In the second half of Session 1, three presentations describing potential new directions in estimating HIV incidence covered the following topics:

- a genome-based HIV incidence assay (University of Southern California, Los Angeles, USA);
- a Luminex multimarker assay method (US CDC); and
- genetic diversity as a marker for timing the acquisition of infection among people living with HIV (National HIV & Retrovirology Laboratories, Public Health Agency of Canada).

The first presentation on new molecular methods to be used for estimating HIV incidence described a genome-based HIV incidence assay. The method looked at the HIV sequences sampled from each person living with HIV.

The laboratory team conducted a meta-analysis on the sequence samples of people living with HIV, reviewing HIV sequences from three publications covering five countries with different populations and subtype B and C. The sequence dataset consisted of 5596 full-envelope HIV-1 genes obtained from 182 people who had recently acquired HIV infection and 43 people who had long-standing HIV infection.

The HIV diversity was quantified using the Hamming distance method and analysing the Hamming distance distribution. The first measures looked at the mean and variance of the Hamming distance distribution. The measure of diversity was defined as the average Hamming distance among all pairs of sequences divided by the sequence length. The measure of variance was defined as the variance of Hamming distance among all possible pairs of sequences divided by the sequence length.

It was first shown that the measure of diversity or variance might misclassify the early stages of individuals whose infection started with multiple founder viruses as being long-standing infection. It was thereafter decided that diversity and variance were not good measures; the team therefore sought out alternatives. People who had recently acquired HIV infection showed a tangible number of similar, very closely related sequence strains, whereas those with long-standing HIV infection had a low rate of similar strains; most among people who recently acquired HIV infection.

The novel biomarker was described as the 10% decile of Hamming distance distribution ($Q_{10}$) and should be low, which indicates recently acquired HIV infection. A high value of $Q_{10}$ represents long-standing HIV infection. Receiver operating characteristic analysis showed remarkably high sensitivity (100%) and specificity (97.3%) of this genome-based assay. The sensitivity was defined as the proportion of people with recently acquired HIV infection correctly identified as incident. Specificity was defined as the proportion of people with long-standing HIV infection correctly identified as long-standing. By using the isocostline analysis, which maximizes the sum of sensitivity and specificity with equal consideration, the team was able to determine the optimal cut-off value.

The summary table of the estimation of incidence illustrated that, regardless of the length or location of the envelope gene, the sensitivity and specificity continue to be markedly high, greater than 95%. Further analysis demonstrated that the biomarker is not sensitive to changes in viral load or the clade
of the viral strain. The study team therefore concluded that the biomarker of the sequence similarity was robust to viral and host-specific factors.

The team compared the estimate incidence rates in three incidence trials (4–6) using the team’s modelling estimates (model 1 and 2) versus serological assay estimate. This was a simulation study based on reference incidence but not based on genetic material from clients. This model was described as an interesting new way to estimate HIV incidence at the molecular and genetic level versus immune system. This method could potentially be one direction for the future in this field. The study team is currently using the method of ultradepth pyrosequencing to obtain a massive number of sequences from many individuals, which allows many samples to be processed in parallel, thereby developing a cost-effective and highly accurate HIV-1 incidence assay.

**BIO-PLEX MULTI-ANALYTE ASSAY**

The presentation provided a description of the Bio-Plex multi-analyte assay (Bio-Rad HIV-1/HIV-2 PLUS O Avidity), which the US CDC validated. This evaluation is in accordance with the Johns Hopkins University and United States National Institute of Allergy and Infectious Diseases laboratory regarding variation and the current analogue being developed.

A proof-of-concept paper was developed in 2011 for this assay. Multiple analytes posed a challenge in establishing the optimal assay cut-off values because the immune response differed for each analyte.

The mean recency period estimates ($T = 1$) by avidity cut-off values were calculated for each analyte and combinations. The incidence calculation examined simulated populations using incidence modelling. The team thereafter split data and did remodelling with $T = 1$.

The team compared the mean duration of recency at AI 30% cut-off, examining both subtype B and non-B subtypes in four cohorts. They also found that the assay works with subtype C, although the laboratory definitely needs more subtype C samples. They included additional data for the statistical analysis from six sources.

The US CDC mentioned that the team was considering incorporating IgG3 in response to p24 of the analysis. This would be redone with Luminex versus plate. It was shown that reactivity drops drastically after 50 days after acquiring HIV infection.

An overview was given of the Bio-Rad false-recent rate results as well as protocol development and evaluation of Bio-Rad, avidity and DBS. The elution protocol was developed, and optimization was conducted with paired plasma and DBS from Cameroon and the United States of America. The DBS optimization results showed good correlation between AI values. Further, an optimized protocol with a National HIV Behavioral Surveillance pilot study of men who have sex with men in five cities in the United States of America was evaluated in 2011.

The results from the DBS validation showed that there was a good correlation of AI between plasma and DBS using one 6-mm punch and a correlation of DBS from the field lower than laboratory-made. There
was also a correlation that varied between sites due to following factors: deviation of DBS collection; handling; storage protocol; humidity; and freeze–thaw cycles. It was also shown that misclassification at the 30% cut-off was rare.

A manuscript has been drafted on the Bio-Rad avidity assay validation. It is in the process of receiving comments from the co-authors. The manuscript covers the following topics: assay method; mean duration of recency; limited false-recent rate for subtype B with no antiretroviral therapy; and the false-recent rate with antiretroviral therapy – one cohort.

A training panel for the Bio-Rad avidity assay has been distributed to several sites including CEPHIA sites, Canada (Toronto and Quebec) and Germany. DBS-plasma validation is planned as well as a large retesting of samples from the United States of America used for estimating incidence using the Bio-Rad assay. The mean duration of recency and false-recent rate for non-B subtypes is planned to be determined in Kenya, Thailand and CHAVI cohorts. The US CDC and United States Institute of Behavioral Research have announced the potential for Bio-Rad to market a modified assay for diagnostics in the near future. Countries can purchase this assay commercially but still perform it in-house.

GENETIC DIVERSITY AS A MARKER FOR TIMING THE ACQUISITION OF INFECTION AMONG PEOPLE LIVING WITH HIV: EVALUATION OF A 6-MONTH WINDOW AND COMPARISON WITH THE BED ASSAY

The data for this evaluation came from a national transmitted HIV drug resistance surveillance programme and through sharing of data from other laboratories around the world. In Canada, first-time HIV diagnostic specimens (from people who had never received antiretroviral therapy) are sent to the National HIV and Retrovirology Laboratories for drug resistance testing. The anonymized data collected for public health purposes can be then used for public health research. The genetic diversity of HIV has great potential, and the amount of HIV genetic diversity within an individual is significant. Viral diversity increases over time in response to host immune pressure; however, in late-stage infection, as an individual’s immune system becomes depleted, diversity decreases.

The team used Sanger sequencing (bulk sequencing) from a routine genotype. Using the sequencing string, the proportion of mixed bases is generated to try and predict the duration of infection. The definition of mixed base is when two bases are identified on the sequencing trace and the smaller peak is of sufficient magnitude to be considered above the noise threshold. In most cases, the secondary peak is considered significant if its area is at least 20% of that found in the dominant peak general signal of all viruses in the population.

The method of study was as follows.

- The sequencing string (FASTA) file was taken from a routine genotype. This is the baseline and is produced for other reasons.
- All of the positions at which there is a mixed base call were enumerated. This is to determine what part of the sequencing has ambiguity.
- The percentage of mixed bases found in the FASTA files that best distinguished long-standing infection from recently acquired infection was determined. The longer the duration since HIV
infection was acquired, the more mixed bases accumulate. The hypothesis is that the greater the number of ambiguities, then the more likely the FASTA was produced from more long-standing HIV infection.

The key findings included: (1) the correlation between number of mixed bases in sequence and the duration of infection, (2) the ability to derive a negative predictive for recently acquired infection (<1 year) of 98.7% and that mixed base percentage decreased after eight years since acquiring HIV infection but was higher among people who inject drugs.

The goals of the study were to focus on:
• drug resistance, with information only from a BED (plasma) data bank;
• a cut-off of 155 days to compare with the BED assay;
• examining the threshold of mixed base calls by sequencer to see whether it influenced the accuracy of the tool; and
• examining specific parts of the sequence to see whether specific locations were enriched for informative data: positions where base changes occurred, entropy changed, positively selected or associated with human leukocyte antigen.

The team calculated a percentage of mixed bases, and since the greater proportion of mixed bases was associated with recently acquired infection, these were predicted to be recently acquired. The National Laboratory is trying to encourage the provinces to consider validating this technology.

The limitations for the study included:
• determining the cut-off, since the team did not have control over the mixed base–call threshold among all datasets;
• using a heterogeneous test set from the real world (not cleaned), which included everyone who had acquired HIV infection from 14 days to 14 years previously (no information on the duration of infection other than that provided by the BED assay);
• classification was based on the BED assay; and
• misclassification occurred in both directions and was therefore not simply due to the false-recent rate.

The area under the curve was maximized using mixed-base data from high-entropy sites and was found to be the best of all models. The performance of the mixed-base classifier on the test set was as good (sensitivity 85.2%, specificity 83.5%, negative predictive value 0.86, positive predictive value 0.83) and required only 356/1354 bases (27% of the data). The mixed-base classifier, however, yielded was only moderately concordant with the BED assay data.

The conclusions drawn addressed the challenging 155-day cut-off used to parallel BED; the benefit of using mixed-base calls from HIV genotypes is that the data are “free”, since they are contained within the genotype. If the method can be refined, there is potentially no requirement for extra testing to determine how recently HIV infection was acquired. If the operating characteristics of the test cannot
be improved sufficiently to use it as a stand-alone test, this method could potentially have a role in multiassay algorithms or other algorithms.

Participants had several questions regarding this new method, such as whether it could quantify when the infection was acquired, including subtypes B and C, and whether there would be considerable misclassification. The authors had performed an additional study focused on the Aboriginal population, which is known to have a restricted human leukocyte antigen pattern, and among whom people who inject drugs primarily acquired HIV. Within this population, the virus was less divergent between the people living with HIV, and the performance of the mixed-base classifier may need to be reassessed in specific groups such as these. These technologies are still in the phase of addressing accuracy in genotype testing.

The genome-based methods are an expensive and complex method to use in the absence of routine baseline genotyping. It may potentially be ready for greater use in a number of years but would currently be difficult to implement in a low-resource country. Comparisons were made to current methods that pose challenges with sample quality and logistics, including antibody testing.

There was also a concern that information such as testing history (such as the last date tested for HIV and whether the person had received antiretroviral therapy) would not be included. The United States of America has a genotype system in 11 states; this method is unlikely to be incorporated for surveillance. Although not perfect, in certain contexts this new method could add value to what is strived to be accomplished in estimating incidence and potentially have a role in multiassay algorithms and other algorithms.

UPDATE ON CROSS-SECTIONAL HIV INCIDENCE TESTING

The presentation provided a detailed overview of a comparison of cross-sectional incidence testing to determine incidence using multiassay algorithms being carried out by the HPTN Network Laboratory Repository.

No samples from Asia and the Russian Federation were included in the Repository. The samples used to determine the multiassay algorithm were taken from the performance cohorts of HIVNET 001, MACS and ALIVE. They included men who have sex with men; people who inject drugs; and women and had 1782 samples from 709 individuals. The range of duration of HIV infection was from one month to more than eight years. The cohorts also included individuals with AIDS and with viral suppression and individuals that had been exposed to antiretroviral therapy.

None of the 500 samples from the individuals who had been living with HIV more than eight years (Johns Hopkins HIV Clinical Practice Cohort) were misclassified as having acquired HIV infection recently using the multi-assay algorithm. In addition, a sample of longitudinal cohorts (HIVNET 001 and HPTN 064) was used and annual incidence estimated using multiassay algorithms.

It was shown that there was a high misclassification in sites in southern and eastern Africa in the Partners in Prevention trial. There were 2900 subjects at the end of the trial with partners living with
HIV who were HIV-negative when the trial started. A detailed investigation therefore ensued that reviewed the misclassification arising from the BED capture enzyme immunoassay and the Bio-Rad avidity assay by subtype in Rakai, Uganda, specifically for subtypes A and D, tested using the BED assay and conducted sequencing for the Rakai data.

The CAPRISA 002/004 samples tested included:
• 97 subjects and 556 samples (mean 6; range 1–7 per subject);
• two subjects had more than three time points tested; and
• samples corresponding to the following months after HIV infection was acquired were requested (from <3 months to 48+ months).

The team analysed the percentage of CAPRISA samples that had acquired HIV infection recently by incidence assay and time from infection and examined the classification of subtypes A and C by time from seroconversion.

The high-resolution melting diversity assay was described briefly. High-resolution melting is a molecular method that can be used in determining HIV incidence. High-resolution melting scores are highly associated with diversity measures based on next-generation sequencing (7). The study compared high-resolution melting scores in various populations and also with long-standing and recently acquired HIV infection. High-resolution melting scores and sequenced-based diversity measures increase over time after infection is acquired. Data were from the Rakai Health Sciences Program and included 220 paired longitudinal serum samples from 110 individuals. The results from using the high-resolution melting diversity assay show that there were very low scores for acutely acquired and recently acquired HIV infection in all regions. In long-standing HIV infection, diversity patterns vary from person to person: the scores were typically high in at least one region, and in some regions the high scores independently predicted long-standing infection.

A graphic showing the difference between the current multiassay algorithms and next-generation multiassay algorithms illustrated the second step of the CD4 count being omitted, as it is logistically difficult, and examining the avidity assay versus BED assay. In addition, the high-resolution melting diversity assay replaces parameter of the viral load exceeding 400 copies per ml. This was described as a better algorithm and will identify recently acquired HIV infection. It was noted that the BED assay does add value in the multiassay algorithms, especially considering the false-recent rate.

UPDATE ON CEPHIA-II AND INDIVIDUAL STAGING

CEPHIA received a new award from the Gates Foundation for an additional three years of project work. The CEPHIA-II grant will continue to build the repository and will support emerging priorities in HIV incidence research: collecting needed multiple- specimen types (DBS, peripheral blood mononuclear cells, urine, stool and matched plasma). This will allow CEPHIA to create custom specimen sets and characterize them, which is a different component from the last grant. The organization and management structure of CEPHIA will remain the same. There is funding for new collaboration to support the collection of new specimen types.
CEPHIA-II priorities include a recent request for proposals on HIV incidence biomarker discovery and projects funded by the United States National Institute of Allergy and Infectious Diseases. Other emerging incidence measurement priorities will be identified by the Working Group and also from proposals directly received by CEPHIA.

The Gates Foundation Biomarker Discovery Grant highlights non-hypothesis-driven innovation in HIV incidence studies. Some interesting examples were presented from current projects involving:

- metabolites (urine and blood): urine specimens from CEPHIA among people with long-standing versus recently acquired HIV infection;
- validation experiment of metabolomics to distinguish cancer, viral versus bacterial infection, rabies and tuberculosis stages; and
- cell-associated viral load in specific T-cell subsets (peripheral blood mononuclear cells).

Specific emerging projects for measuring incidence would include:

- DBS versus blood plasma performance (laboratory tests);
- fresh whole blood versus stored plasma performance (rapid tests); and
- a prospective study of clinical staging accuracy.

The Working Group should begin considering individual staging, since changes are anticipated in the recommending tracking based on case definition for people with “stage 0” HIV infection in the United States of America because of targeted prevention efforts. The utility of characterizing recently acquired HIV infection for surveillance has also been demonstrated. This recommendation in the United States of America requires that health departments track people with recently acquired HIV infection, therefore resulting in a push to determine incidence. In 2012, a meeting on incidence was held in Washington, DC to examine the feasibility of generating data on a test to support an individual staging claim. However, measuring incidence was not considered. The United States Food and Drug Administration now encourages using CEPHIA data for claims for individual disease staging, which could amplify the important role of CEPHIA specimen sets in the future.

LABORATORY ASSAYS FOR INDIVIDUAL USE

An overview of new laboratory assays for individual use, specifically the Multispot and dual path platform (such as Chembio DPP® HIV 1/2 Assay and Bio-Rad Geenius™ HIV 1/2 Supplemental Assay) addressed the following points:

- whether they will work;
- whether they are useful;
- use of assays and algorithms;
- impact on transmission;
- accuracy and inaccuracy;
- benefits versus harm; and
- discussion with questions and answers.
A brief overview of supplemental tests for individual testing cited that Western blot outperforms the BED assay and Vironostika LS-EIA in classifying people as having acquired HIV infection in the past 90 days [8]. However, the window period or mean duration of recent infection is too short, is subject to interpretation and a lack of controls or calibrators and is very expensive. The current direction is towards rapid supplemental tests replacing immunofluorescence assay and Western blot.

New US CDC algorithms recommend supplemental (confirmatory) testing using the HIV 1/2 discriminatory rapid assay. The Multispot and dual path platform fall into this category. The DPP Immunoblot (approved in Brazil in 2009) proved to be less costly and easier to use. Bio-Rad hopes to receive approval for Geenius™ from the United States Food and Drug Administration and use it as an incidence test as well as diagnostic.

The dual path platform is different from lateral flow technology, since HIV-specific antibodies do not compete with non-specific antibodies. However, it is sensitive enough for multiple antigens on the same strip. The assay is also powered by AAA batteries, requires a printer and has a laptop interface. The Bio-Rad Geenius is bus powered but requires a laptop. A benefit is that it is free standing and mobile. The main difference between dual path platform and Western blot is that dual path platform has shown high clinical sensitivity and specificity in trials to date. In addition, it has a multiplex format, automated readout, recombinant antigens, internal protein A control and worldwide good laboratory practices manufacture and distribution.

The advantages of rapid tests for clinical staging and incidence measurement are that they:
- enable the diagnosis of acute HIV;
- assist in the routine reporting of “stage 0” HIV infections;
- facilitate the immediate linkage of patients to services;
- reduce the need for blood, a cold chain and supply testing to complete the multiassay algorithms;
- recalculate the collection of additional data on risk factors from people who recently acquired HIV infection;
- are potentially less costly than Western blot; and
- are currently going to clinical trials.

The downfalls of rapid tests are few. However, they do have poor control of specimen input (finger-stick blood and oral fluid), calibrators are lacking and substantial training is necessary.

A preliminary performance incidence and staging analysis of the dual path platform examined how often people who acquired HIV infection more than one year previously are misclassified. There were 146 unselected people living with HIV (234 specimens) with seroconversion date known within 45 days. The mean duration of recent infection was calculated by interpolating observed data and the false-recent rate estimate based on one-year cut-off.

A summary of dual path platform rapid tests mentioned that the test is already on the market and will be used worldwide. There has only been one complete evaluation of an existing dual path platform test configuration. It probably performs well enough to discriminate between newly diagnosed people who
recently acquired HIV infection and newly diagnosed people with long-standing HIV infection. Initial field-based screening of survey participants will be further evaluated for having acquired HIV infection recently. Recent infection test algorithms (multiassay algorithms) incorporating these or similar rapid tests should be evaluated. Further modifications (changes in antigen concentrations, dilution and/or avidity modifications) of these off-the-shelf tests should be explored. Given advancements in rapid nucleic acid amplification testing and CD4 technologies, all-rapid tests and in-the-field testing algorithms for recently acquired infection could offer substantial cost savings for HIV surveillance programmes.

Brazil is not collecting data on dual path platform. This is very different from what has transpired with assays elsewhere in the world: countries collect data first and try to commercialize after. Brazil commercialized the assay quickly, demonstrating their confidence that the diagnostic testing works.
The first set of presentations in Session 2 provided an overview of two country experiences in implementing the guidance on estimating HIV incidence. Then SACEMA described the lessons learned from the first training on guidance for estimating HIV incidence conducted in South Africa in 2012.

The Botswana-Harvard AIDS Institute Partnership provided an overview of the Botswana experience in estimating HIV incidence in the field. Currently, the team has some confidence that HIV prevalence is declining based on trends.

Brief statistics regarding the situation in Botswana described the country as having high prevalence, 18% in the general population and 31% among pregnant women, with the coverage of services to prevent the mother-to-child transmission of HIV exceeding 90%. The antiretroviral therapy coverage is more than 93% of those who are eligible. Various organizations are using different ways of examining incidence to illustrate how programmes are making an impact in the country.

The prevalence has increased in Botswana, considering the increasing number of people receiving treatment. Although incidence is a moving target, the Botswana team believes that they are getting closer in their ability to estimate.

A brief history of assays that have been used in Botswana since 2001 was provided:

- 2001–2003: serum/plasma detuned (Vironostika);
- Botswana AIDS Impact Survey II;
- 2005–2007: (BED and detuned);
- 2009: BED (DBS only);
- 2011: BED (DBS tested but serum also collected);
- 2012: special populations, ongoing studies that include key populations at higher risk, prisoners and sexual minorities – the protocol includes the BED assay and other assays; and
- 2013: Botswana AIDS Impact Survey III.

The guidelines assisted the team in implementing algorithms. Botswana is using the guidelines to estimate the incidence among pregnant women attending antenatal clinics (2011 sentinel surveillance). The team is uncertain whether it can collect sufficient data to distinguish between people who recently acquired HIV infection and people with long-standing infection. The team has confidence in the data, since programmes to prevent the mother-to-child transmission of HIV can collect information from women’s cards, which they are required to bring to appointments. Only a few forget the card, and there is also interview information available.
The issue was raised that self-reporting of antiretroviral therapy is not a good biomarker. South Africa has included the qualitative determination of antiretroviral drugs in DBS samples in the survey protocol of the 2008 and 2012 national HIV household surveys. Botswana is working with a South African laboratory on using this technology. Currently, Johns Hopkins University is also investigating a reliable inexpensive test to examine antiretroviral therapy for excluding long-standing infection. In adjusting for long-standing infection in the algorithm, the team believes it needs a more appropriate way of examining the data. Using the given algorithm might refine data more.

Applying the incidence calculation to the sample set showed that 2.97% of the individuals recently acquired HIV infection despite being classified as having acquired HIV infection more than 1 year previously using the BED assay. The team was therefore uncertain whether they should adjust for the false-recent rate. When adjusting for the false-recent rate, the team thought it was reasonable to assume that the false-recent rate is “0”. For 2011, the estimated incidence was calculated at 2.72% (95% confidence interval 1.99–3.44%).

In examining trends (unadjusted incidence), the team believes they are seeing trends, particularly regarding age: the number of younger people acquiring HIV infection was declining.

The team used Botswana AIDS Impact Survey III data and applied the false-recent rate. They do not look at the false-recent rate for pregnant women, since most are enrolled in services to prevent the mother-to-child transmission of HIV; finding women who are not exposed to antiretroviral medicines is difficult. The false-recent rate may be lower than what Botswana has found.

4.1 ISSUES FOR DISCUSSION

The Working Group should consider important changes in the profile of people receiving services to prevent the mother-to-child transmission of HIV, since an increasing number are receiving antiretroviral therapy, and this has implications for calculating incidence. Currently the proportion on antiretroviral therapy when they present is over 30%. Most participants knew their HIV status in their latest pregnancy (89%), and 64% of the attendees living with HIV had acquired HIV infection more than one year previously. Further, many attendees living with HIV presenting now have multiple pregnancies, and prevalence increases by parity.

For calculating the false-recent rate, the sample included 512 people who had never received antiretroviral therapy enrolled in observational cohorts who were followed up for 3–5 years (adults older than 18 years of age). The time points used were 18–24 months with the BED assay: 6.05% (4.15–8.48%) and AI: 5.57% (3.70–8.00%). For the BED and AI combined comparison (2.25%), the results showed no comparison. The team felt it was more encouraging to calculate the false-recent rate using multiassay algorithms: 1.43% when comparing the BED assay and LAg assay using multiassay algorithm 2.
Botswana is trying to get a sense of the various incidence estimates and what to use in their situation. It is hoped that the Working Group can review this soon and provide guidance. Several HIV incidence estimates from various sources have been applied to Botswana; it is unclear how some calculated the estimates, and confidence is lacking in some of the results. It is also hoped that guidelines will address more complex studies (such as weighted data). The danger is that some researchers may just plug in rates and run incidence formulas. It is important to triangulate data to better understand what is happening in the population.

The lessons learned from implementing the guidance in Botswana were:
- testing algorithms for recently acquired infection to improve incidence estimates;
- adjusting appropriately for the false-recent rate and biases;
- estimating the false-recent rate – it is encouraging, but more work is needed;
- guidance on adjusting for missing data is needed;
- the method needs to be changed from the BED to LAg assay, although it is good news that the samples stored well in the laboratory;
- need to address the changing profile of antenatal care attendees; and
- statistical issues with a complex survey (same size, weighting etc.).

Future plans in Botswana are to conduct more evaluations and studies. Studies of the false-recent rate are to continue, with the Bio-Rad avidity assay, BED assay, LAg assay and a second type of false-recent rate that assesses changes over time. There are also future plans to test samples from antenatal care attendees in 2005, 2006 and 2007 (serum/plasma) and in 2009 and 2011 (DBS and plasma).

Botswana also plans to conduct the Botswana AIDS Impact Survey III (DBS), and the Botswana AIDS Impact Survey IV currently has a budget for two assays (Bio-Rad avidity and BED assays). The results are expected soon, and the Botswanan president is expecting a report; the team cannot wait for more guidance to come out in 2014.

### 4.2 EXPERIENCE WITH HIV INCIDENCE TESTING IN GHANA

The presentation described the work done on HIV incidence testing by the Noguchi Memorial Institute for Medical Research in Ghana. The HIV prevalence rates in the general population are determined by the HIV sentinel surveillance system. The HIV sentinel surveillance system is a cross-sectional survey that targets women attending antenatal clinics in selected sites. The country is using the prevalence among people 15–24 years old as a proxy for incidence. The prevalence is 2.1%, ranging from 0% in rural sites to 9.6% in urban sites. In 2011, the estimated number of people living with HIV in Ghana was 217 428 and the annual number of people dying from AIDS-related causes was 14 330. There were 31 576 children living with HIV, and an estimated 2933 children were newly infected with HIV.
In 2010, the National AIDS Control Programme, Noguchi Memorial Institute for Medical Research and US CDC performed a false-recent rate study to estimate the misclassification rate in Ghana. A total of 1045 specimens were collected from eight clinical sites offering antiretroviral therapy services for HIV from February 2010 to December 2010. The target group was people living with known long-standing HIV infection aged 18 years or older receiving antiretroviral therapy. It excluded those receiving antiretroviral therapy and those with HIV-2 or HIV-1/2, an indeterminate result for HIV and those with incomplete data. There were 23 specimens with missing identification numbers and 46 HIV-1-negative specimens (HIV-2-positive or false-positive or indeterminate).

The study first determined the false-recent rate of the BED and the LAg assays. Initially, BED testing was conducted on 642 specimens at the Noguchi Memorial Institute for Medical Research. Overall, samples from 953 people living with HIV-1 were tested with the BED assay and 952 with LAg the assay at the US CDC in Atlanta. BED kits expired during specimen collection, and 357 specimens were therefore shipped to the US CDC in Atlanta for testing. During shipment, one specimen tested with the BED assay went missing.

The main characteristics of the study participants were presented. Most enrolled participants were women (74%). The predominant age group among the enrolled participants was 35–44 years (36%). About 70% of the enrolled participants had CD4 cell counts exceeding 200 cells per mm³.

The BED false-recent rate was estimated at 61 of 953 or 6.4% (95% confidence interval 4.9–7.9% with a coefficient of variation of 12.1%), whereas the LAg false-recent rate was estimated at 7 of 952 or 0.7% (95% confidence interval 0.21–1.3% with a coefficient of variation of 36.9%). The BED assay misclassified 6.4% as having recently acquired HIV infection, in contrast to the LAg-avidity assay, which misclassified only 0.7% as having recently acquired HIV infection.

Ghana is now considering assay-based HIV incidence surveillance. There is currently no information on pregnant women. There are plans to use the LAg assay on existing or prospective specimens to generate incidence estimates for female sex workers and men who have sex with men by looking at existing banks of samples (past studies by the US CDC and FHI 360); pregnant women within the annual HIV sentinel surveillance; and female porters (Kayayei).

There is an issue regarding the use of existing specimens, as few of the surveys collected previous information on antiretroviral therapy for study participants. Regarding the studies of men who have sex with men, it was explained that men who have sex with men were recruited using respondent-driven sampling with support from the University of California at San Francisco. The HIV-positive rate was 12% with a sample size of 2000. In addition, 4000 female sex workers were recruited for the study. The HIV prevalence is higher than among the heterosexual population. Initially, FHI 360 estimated the size of the population of men who have sex with men to be about 1000.
4.3 Lessons Learned from the First Training Session on Assays for Estimating HIV Incidence

The main lesson learned was that two days of training was not sufficient for participants to absorb the material and be able to apply the concepts. More examples needed to be provided as well as practice and a common language for the participants to relate to and communicate. The training strived to be interactive, although initially there were more didactic lectures to learn the concepts. Instructors have to re-evaluate how to structure the training so there will be enough time for work and practice.

Feedback from participants confirmed that they would have liked a mix of facilitators. It was suggested that the younger collaborators would be quite good as future facilitators. There should be more communication leading up to the course, so that participants are prepared when they arrive. Funding is needed to put forth effort in the training.

When the training was initially presented to the various countries, it was clearly presented that participants had to be self-funded. There were 25 participants from various countries, many of whom already work in incidence. Almost all participants were from African countries, except for a few representatives from Viet Nam. There were both francophone and anglophone participants. The training was conducted in English. It was highly suggested that the training have a limited number of participants and a waiting list.

There are follow-up plans to have a second training session, although funding is scarce. There currently is not enough funding to follow up countries to know what work they are doing in estimating incidence. However, SACEMA is willing to collaborate with countries if specific output is identified.

Various materials were distributed to participants (spreadsheets), and there were sufficient information and materials to review and use when participants returned to their respective countries.

There was not sufficient time to discuss correcting for bias and the false-recent rate; the training could not cover this much detail in the time provided. It was suggested that workshops be conducted in other regions. SACEMA has made the training materials available on its web site.
5. SESSION 3: DEVELOPING GUIDANCE FOR ESTIMATING HIV INCIDENCE IN HIV CASE–BASED SURVEILLANCE SYSTEMS

5.1 ESTIMATING HIV INCIDENCE USING TESTS FOR RECENTLY ACQUIRED INFECTION IN THE HIV SURVEILLANCE SYSTEM IN CATALONIA

Centre d’Estudis Epidemiològics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya (CEEISCAT) gave a presentation on estimating HIV incidence using tests for recently acquired infection in the HIV surveillance system in Catalonia. The context provided an overview of the rates of newly diagnosed HIV cases, distribution by risk group (ages 15–49 years old), number of HIV tests and positivity rate.

In 2009, the rate of newly diagnosed HIV cases in Catalonia was 9.8 per 100 000 population. HIV testing is free in primary health care centres, voluntary counselling and testing clinics and clinics for sexually transmitted infections. The results show that the numbers of HIV tests have been rising for the past decade and the positivity rate remained stable, showing a trend towards increasing in the past two years.

In 2001, the AERI study was designed aimed at assessing the viability of introducing a test for recently acquired HIV infection. Included was a network of 11 hospitals and 6 voluntary counselling and testing sites. From 2003 to 2005, the AERI study was implemented. In 2006, tests for recently acquired HIV infection were incorporated into the routine HIV surveillance system in Catalonia, which captures the voluntary sentinel surveillance network covering 50% of all new diagnoses. The reporting system does not include data from blood centres.

The inclusion criteria were:
  - everyone with newly diagnosed HIV infection at the participating centres;
  - serum samples obtained within the first six months after HIV infection was newly diagnosed; and
  - consenting and 16 years and older.

People diagnosed less than six months before samples were collected or diagnosed with HIV-2 were excluded. Samples with insufficient volume and duplicate samples were also excluded. The recently acquired infection testing algorithm was used from 2003 to 2005 and again from 2006 to 2008. Between January 2005 and May 2007, the team used Vironostika and, from June 2007 onwards, the BED assay.

When specimens were collected, aliquots were identified by a unique study number and no personal identifiers were used. A graphic of the testing algorithms for recently acquired infection used from 2003 to 3005 was presented, illustrating the next step in estimating incidence. The mean window period for
both techniques was 170 days for Vironostika and 155 days for the BED assay. The largest challenge for the Catalonian team was that they were unable to establish a denominator for negative HIV tests although the team attempted to collect information from the Catalonian HIV sentinel surveillance. The data were collected in a two-stage process.

Laboratory personnel identified newly diagnosed individuals and completed a form including CD4 counts, HIV viral load and the most recent HIV test result (to identify previous HIV diagnoses). Under the supervision of a physician, data on demographic, clinical and epidemiological variables at the time of diagnosis were collected in a second round. Catalonia does not systematically collect information from people newly diagnosed with HIV infection. Thereafter, HIV incidence was estimated for the subpopulation tested for HIV by combining data from the HIV surveillance system and the test for recently acquired HIV infection in Catalonia.

The team used the McWalters-Welte formula for statistical analysis. Since not all HIV-positive tests were tested with testing algorithms for recently acquired infection, the team rescaled the data on negative tests using the formula. Since 2007, two tests for recently acquired HIV infection were used and the average duration was 162.5 days. In the statistical analysis, the HIV incidence was compared between two time periods using a spreadsheet from SACEMA’s web site. Since the spreadsheet required a single value for the false-recent rate, an estimate was obtained by pooling data, using two testing algorithms for recently acquired infection, establishing a false-recent rate of 3.8% (coefficient of variation 15.7%).

The characteristics of testing algorithms for recently acquired HIV infection and testing people living with HIV were illustrated in table form. Highlighted was the new testing algorithms for recently acquired infection testing people living with HIV, yielding about 0.46% over time.

The team was unable to delete duplicates from the aggregate testers from the negative results; the data therefore had many duplicates. The Catalonian team questioned whether the results and increased estimates of HIV incidence were viable, since there were changes in the methods of the testing algorithms for recently acquired infection during the study period; however, the false-recent rate estimates of the testing algorithms for recently acquired infection were similar, and the tests for recently acquired infection used showed good agreement.

There was a demand for tests during the study period, possibly because of government policy changes in Catalonia and not necessarily repeat testers. However, men who have sex with men are more likely to be repeat testers (the median number of tests before diagnosis was four among men who have sex with men versus 1.5 among heterosexuals). Further, introducing rapid HIV testing in voluntary counselling and testing in 2007 may account for an increase in incidence.

The team used triangulation to support HIV incidence estimates. The HIV incidence in the general population was estimated at 0.1–0.2 per 1000 population using the EPP Package. Moreover, preliminary results for the ITACA Cohort, a prospective longitudinal study of HIV-negative men who have sex with men recruited among clients of a community-based centre detecting HIV and other sexually transmitted infections in Barcelona, showed that the directly measured HIV incidence was 3.4 per 100 population year, with a trend toward increase. However, several challenges were noted.
There was no information on individual testing behaviour, and the team could therefore not estimate the HIV incidence in the general population. The HIV incidence was limited to the testing sample. Data on negative tests had to come from alternate sources, which are reported in aggregated data by each centre, contain duplicates (repeat testers) and had no epidemiological information available. The points for discussion focused on in this situation were how the team could have improved the accuracy of the estimates considering sampling issues and using several mean durations of testing algorithms for recently acquired infection and false-recent rates when assessing HIV incidence trends. It was suggested that a random subsample of people with sexually transmitted infections or people who inject drugs be collected if possible, and then an estimate could be made. As an example in Canada, they have testing patterns for those who come in for testing (people who inject drugs and men who have sex with men) and use this data to estimate the denominator.

5.2 SURVEILLANCE AND ESTIMATION OF HIV INCIDENCE IN THE UNITED STATES OF AMERICA

The subgroup is currently working on the data needs chapter for the document on guidance for estimating HIV incidence in case-based surveillance. The presentation gave an overview of how HIV data are collected in the United States of America in a case-based surveillance system. By using a stratified extrapolation approach, an average weight is calculated for homogeneous group individuals who fall into a transmission category. The incidence is extrapolated to areas that did not provide data through HIV incidence surveillance (this is applied to AIDS case reporting).

HIV incidence surveillance is one part of HIV surveillance. HIV case surveillance should include an adult case report form with demographic, risk factor and clinical data, testing history information and the result for the serological testing algorithm for recent HIV seroconversion (STARHS) for the diagnostic specimen.

In describing the information flow for HIV case surveillance, it was emphasized that all states require practitioners and laboratories to report cases and to report names. Government public health staff (disease investigation specialists) track down this information (maintaining anonymity) at sites and from individuals. The information is then sent to the US CDC to analyse data.

Since surveillance is not a research activity, the name can therefore be reported. CD4 counts and viral load reporting to states is mandatory, and every state has jurisdiction to collect information. There is a method to statistically handle providers who do not report this information. There is training for providers to emphasize why testing provides important information to gather and report, how information is helpful in producing incidence data and to show providers that what they report has an impact.

The questions regarding testing history cover previous positive tests (less than 5% report a previous positive test) and previous negative HIV testing. In asking about antiretroviral therapy, information is often not complete and taken from charts, not asked directly. The purpose is to determine whether antiretroviral therapy received within 6 months of data collection for the BED assay, which might affect
the results. The motivation for a person to get HIV testing is also asked to help substantiate previous responses and to link with services.

When a diagnostic specimen is obtained, if it is new specimen, it is shipped, and if it is older, then it is not shipped. Private laboratories ship all Western blots to a STARHS laboratory, and only one laboratory can provide STARHS results.

Some main issues and challenges raised regarding the estimation of HIV incidence in a case-based surveillance system in the United States of America were:

- all information is self-reported;
- reporting is delayed or incomplete (for testing and treatment data) and data are therefore imputed;
- incomplete data on testing and treatment history, since some providers do not want to provide information;
- unavailable specimens for testing, since providers (in-kind) handle this at their own cost (pulling samples etc.); and
- future changes in the HIV testing algorithms.

A question was raised regarding when the United States of America will use the LAg assay and not the BED assay. As mentioned earlier, specimens tested using the BED assay will be retested using the Bio-Rad avidity assay developed by a domestic programme, but there are no plans to test them by the Lag assay. Currently, the US CDC is trying to secure funding to assess the Bio-Rad assay and the BED assay for estimating incidence. An explanation or guidance to countries should be provided when the change occurs. Potentially, this could be done in a training format. If a country does not collect case-based data, making comparisons is difficult.

5.3 POPULATION-BASED HIV INCIDENCE IN FRANCE

The main points raised regarding the HIV surveillance system in France for discussion were as follows.

- Surveillance is more centralized than in the United States of America, and there is uncertainty in estimating the population based on immigration status.
- Validation of HIV testing and whether any external factors could change HIV testing, starting with diagnosis.
- Testing patterns for people not at risk: how to better understand the situation of patients before entering the surveillance testing system.
- There are three different measures for estimate comparable to the system in the United States of America.
- Need to better know how to compare these different methods and estimates. Could incidence estimates be simulated on a population using these methods?
Currently in France, there are no data on antenatal clinics, no mandatory testing and no cross-sectional studies on incidence. It was suggested that, if there were a comparable large population study, (such as the United States National Health and Nutrition Examination Survey) the team could utilize national surveillance information, as it is important to get denominator.

HIV incidence evaluations in the United States of America do not use the United States National Health and Nutrition Examination Survey as the data source because there are low HIV numbers. In Canada, there is a move to integrate all national information to better calculate a denominator.

5.4 DRAFT DOCUMENT ON ESTIMATING HIV INCIDENCE USING HIV CASE SURVEILLANCE

An overview and summary of the current progress on the draft document on estimating HIV incidence using HIV case surveillance was presented. High-income countries also need to monitor HIV incidence, but they do not do this systematically. However, many of these countries have large databases of HIV test results but have little knowledge of how to estimate incidence from the data. This presents an opportunity with advantages (first diagnosis and not receiving antiretroviral therapy) and disadvantages (selection bias and incomplete data).

After the Working Group meeting in 2011 at which the recommendation to develop these guidelines was discussed and supported, nine Working Group members were identified to participate. The subgroup has developed an outline, and specific members have been allocated to each of the seven sections of the document.

The work the European Centre for Disease Prevention and Control has done so far on estimating HIV incidence was described. The European Centre for Disease Prevention and Control launched a project to develop a framework for HIV incidence studies in Europe in 2009. The project focused on strengthening HIV/AIDS surveillance with the European Union and European Free Trade Association to provide more reliable data on the HIV epidemic and obtaining estimates of HIV incidence in countries not using testing algorithms for recently acquired HIV infection to better target interventions and more efficiently allocate resources. The outputs included a literature review of HIV incidence studies in Europe, a framework document for implementing testing algorithms for recently acquired HIV infection and a protocol technical guidance document for implementing testing algorithms for recently acquired infection using surveillance data. In the future, there will be pilot incidence studies across Europe.

The aims and objectives of the technical guidance document were to originally prepare a protocol for a pilot study to implement testing algorithms for recently acquired infection in selected European countries. However, the focus changed and now the document provides general guidance on how to establish this type of surveillance system and analyse and interpret data.

The protocol outline includes epidemiological sections covering five main areas. In the fourth part of the guidance, the subsection on the pilot study is still not confirmed. The statistical section is to cover 10 subsections in detail. In the laboratory section, the subgroup mentioned that it will direct readers
towards the best data available at the CEPHIA web site. The next step for developing the guidance document is to work closely with the Working Group to complete it. Similar to the protocol for field validation of assay-based estimation of HIV incidence, they plan to have an E-tool available and need to address missing data and limitations.

5.5 DISCUSSING AND RESOLVING EXISTING ISSUES IN ESTIMATING HIV INCIDENCE IN CASE-BASED SURVEILLANCE SYSTEMS

USING TESTING ALGORITHMS FOR RECENTLY ACQUIRED HIV INFECTION TO MEASURE HIV INCIDENCE IN TORONTO

The presentation provided an overview of methodological issues related to testing algorithms for recently acquired HIV infection that arose from the experience in Toronto, Canada with measuring HIV incidence: specifically, how their team managed selection bias and used triangulation. Although testing algorithms for recently acquired infection can calculate incidence, validly determining incidence requires appropriate selection of a study population, high-quality data and careful interpretation and control of biases. The major challenges to validity are handling laboratory issues, data quality and completeness and selection bias.

The Toronto Laboratory Enhancement Program was described. The Laboratory Enhancement Program was originally an HIV laboratory collaboration and developed into a programme. It sends a questionnaire to physician when an HIV test is positive for the first time, for surveillance purposes. This helps to gather data regarding ethnicity, geography and immigration as well as previous testing history.

The Laboratory Enhancement Program has assisted in gathering better info on risk factors. In particular, there was difficulty in handling testers with no identified risks, since the risk factors for this group were incomplete. To assess testers with no identified risks, the team used weights from the Laboratory Enhancement Program. If the risk factors were inaccurate, they were reassigned using weights from the Laboratory Enhancement Program and, if the results of testing algorithms for recently acquired infection were unavailable, the team would assign based on the proportions among known testers.

The seroconversion effect – what the US CDC describes as “motivated testing” – was defined as the disproportionate proportion of people who recently acquired HIV infection who test soon after becoming infected (that is, testing algorithms for the recent acquisition of HIV infection window period) that can overestimate HIV incidence. Since people at higher risk are more likely to test, this effect can capture incidence (first infection). These individuals may have heightened awareness regarding HIV and are coming in to testing sites because they perceive seroconverted illness, isolated high-risk exposure or concomitant sexually transmitted infections. The team therefore developed a formula expressing the measured HIV incidence as a function of the true incidence and the seroconversion effect to adjust for this.

Issues of selection bias, representativeness and HIV testing and frequency were also addressed. Selection bias in using diagnostics to calculate HIV incidence means that people who test may not be representative of the entire population and that the testing frequency may vary with the risk of acquiring
HIV infection. HIV testing and frequency increases among the people with higher risk of acquiring HIV infection. This would overestimate HIV incidence, although simulation studies suggest that it is not a major problem.

With regard to representativeness, bias may be in either direction, since some populations do not test much because they perceive low risk (real or otherwise). Similarly, people at higher risk may not be prepared to accept the possibility of an HIV-positive result. The impact of this issue has not yet been quantitatively assessed.

It was concluded that the modelled HIV incidence fit well to the observed HIV incidence and that the adjusted incidence may be 50% lower than the crude incidence, although it varies by exposure category and region and over time. It is useful to enhance diagnostic data, since they are frequently missing and unrepresentative of risk factors and HIV test history data.

5.6 WHO WORKING GROUP SUBCOMMITTEE ON GUIDANCE FOR HIGH-INCOME COUNTRIES

The guidance for high-income countries is currently being drafted, and the Working Group needs to address several issues, mainly:

- how to best frame the scope of the document;
- which types of studies to include;
- approaches with and without denominators, especially to address this question applied to different country situations;
- coordination with the European Centre for Disease Prevention and Control;
- finalizing the work plan; and
- timelines and responsibilities.

The outline of the draft guidance covers seven areas.

1. Types of studies and databases to estimate incidence: a case-reporting system or laboratory databases that would complement and help us understand other populations.

2. Data needs: important sources of HIV test data including case-based surveillance, HIV testing history, laboratory markers and antiretroviral therapy history and measurement, especially considering pre-exposure prophylaxis.

3. Using laboratory assays to detect recently acquired infection: selecting testing algorithms for recently acquired infection assay and performance characteristics, laboratory testing methods and issues and obtaining appropriate samples for testing algorithms for recently acquired infection.
4. Data analysis: the suggestion was made to create a checklist to help countries in analysis, considering the components below:
   » data preparation, including checking the quality of the data;
   » treatment of missing values;
   » using multiple imputation;
   » method of estimating incidence;
   » analytical approach from the number and proportion of diagnoses determined to have recently acquired HIV infection (when denominators [number testing] are available and when they are not);
   » adjusting the false-recent rate with testing algorithms for recently acquired infection;
   » adjusting for selection bias; and
   » inferential analysis.

5. Statistical measurements include:
   » sample size;
   » estimating the uncertainty around HIV incidence estimates;
   » the need for independent validation of the analytical approach;
   » testing assumptions; and
   » data interpretation to compare populations and over time, and there is therefore a need for estimates of the population size to extrapolate the incidence rates and numbers to compare between groups.

6. Practical guidance: include criteria for selecting the population in which to estimate HIV incidence as well as a checklist of data requirements for estimates based on HIV case reporting.

Challenges and issues in developing a draft of the guidance for low- and middle-income countries focused on the following:

- whether the Working Group should consider changing testing algorithms for recently acquired infection assays as a stand-alone or whether this should be integrated into the analysis;
- when there is a simple cohort (need people who are HIV-negative and HIV-positive) or a virtual cohort created through collaboration with groups to make the data more powerful;
- providing an iterative spreadsheet using mortality, or should this come from empirical data; and
- the idea of triangulating data (reality tests to determine whether the incidence is really plausible).
MAIN POINTS OF DISCUSSION REGARDING UPDATING GUIDANCE DOCUMENTS AND RESOLVING EXISTING ISSUES IN CASE-BASED SURVEILLANCE SYSTEMS

• Concern was raised that there will be overlap and duplication of work at WHO and discussion on how this could be avoided.

• There is good, close coordination between the development of activities and Valerie Delpech from the United Kingdom Health Protection Agency with the subcommittee on low- and middle-income countries to better harmonize documents and strengthen cross-fertilization. The team will ensure that the document reflects their shared efforts.

• Having one combined document is possible. It was decided that there would be two documents: one produced by the European Centre for Disease Prevention and Control and one produced by WHO. WHO will endorse both documents.

• Experts are discussing parts of the document that can be finished and leaving the laboratory section to be changed later. Currently there is an outline for the document, and the group has committed to writing.

• It was suggested that the subcommittee consider narrowing the scope of the document, since it seems broad. Countries need specific and concise information so that they can apply this directly to their situation. The target audience for this document is any country with a disease reporting system, not necessarily just countries under the European Centre for Disease Prevention and Control. It was suggested that the title should be more concise to specify the audience for the guidance.

• It was strongly emphasized that there is demand for these types of documents. The Working Group and US CDC are recommended to develop this type of guidance document because of this demand.

• No global guidance is currently available on how to conduct HIV case reporting. The WHO regions are adapting these documents for their specific context.

• The document should not just be limited to case-based reporting. The subgroup also considered databases where diagnostic data are available.

• It was suggested that the draft be circulated to the Working Group for review and comment.
6. SESSION 4. DEVELOPMENT OF GUIDANCE ON ESTIMATING THE FREQUENCY OF AND RISK FACTORS FOR RECENTLY ACQUIRED INFECTION

6.1 COUNTRY PRESENTATION ON ESTIMATING THE FREQUENCY OF AND RISK FACTORS FOR RECENTLY ACQUIRED INFECTION: KENYA

The main focus of the analysis presented was to compare people living with HIV who tested as having recently acquired HIV infection with HIV-negative people to assess risk factors for testing as having recently acquired HIV infection. Testing as having recently acquired HIV infection in this context is a proxy for having recently acquired HIV. The Kenya study used the LAg-avidity assay to determine the proportion of the HIV-positive survey specimens identified as recently acquired.

The presentation gave an in-depth overview of the survey design of the Kenya AIDS Indicator Survey 2007. Currently Kenya is in the third round of the Kenya AIDS Indicator Survey and will be finished in 2013. The Kenya AIDS Indicator Survey 2007 was a nationally representative household survey conducted in eight provinces in both urban and rural areas. It included men and women aged 15–64 years and obtained informed consent for interviews, drawing blood, testing for HIV and sexually transmitted infections and storing specimens for future testing.

Of 17,940 participants, 87% provided blood for testing and storage for future testing. All available HIV-positive specimens were tested with the BED assay in 2008 and the LAg-avidity assay in 2011. For the estimation of HIV incidence, a proxy false-recent rate (1.6%) was estimated by testing a subsample of specimens from Kenya AIDS Indicator Survey respondents with known long-standing infection and excluding a few specimens for people who self-reported receiving antiretroviral therapy or whose CD4 count was less than 400 cells per mm³.

There has been confusion on the use of information about antiretroviral therapy use, either based on self-report or by directly testing specimens for antiretroviral medicine. Excluding specimens based on assessed antiretroviral therapy status is potentially misleading and misreads the guidance. Current guidance does not recommend that people receiving antiretroviral therapy be excluded from analysis but rather that specimens be classified as long-standing if subjects are apparently receiving antiretroviral therapy. Depending on the immunological or virological assays being used, this may substantially improve (lower) false-recent rates with little harm to (reduction in) the mean duration of recent infection. A key point is the importance of consistency: if a false-recent rate is derived from a study on people who have never received antiretroviral therapy but a survey is performed in a population including people receiving antiretroviral therapy and evidence indicates that using antiretroviral therapy predicts a tendency to produce false-recent results, then the false-recent rate in the survey context is likely to be higher than the estimate at hand.
Classifying specimens from people receiving antiretroviral therapy as non-recently acquired is desirable. Ideally, the performance of the combination of primary assay and antiretroviral therapy status assessment would be benchmarked, because of the subtleties that:

- some people initiate antiretroviral therapy early, and this, in principle, in certain contexts, somewhat changes the mean duration of recent infection; and
- the antiretroviral therapy assessment is not necessarily perfect.

When additional criteria such as an assessment of antiretroviral therapy status are invoked to “improve” test performance, this creates a new test (algorithm for recently acquired infection testing) and both the mean duration of recent infection and the false-recent rate should be estimated as well as possible for this particular algorithm, without assuming that these properties are simply the same as previously estimated for one part of the test.

The study found several independent risk factors associated with testing having recently acquired HIV-1 infection on the LAg-avidity assay. Among women, age older than 30 years, current marriage, having incomplete or no primary education and herpes simplex virus-2 infection were independently associated with testing having recently acquired HIV-1 infection. Among men, current marriage, Rift Valley residence, more than one sex partner in the past year and the presence of a genital ulcer were independently associated with testing having recently acquired HIV-1 infection.

In summary, although a few (n = 61) of specimens testing having recently acquired HIV-1 infection, the analysis showed differences in demographics and risk behaviour between those with recently acquired and long-standing HIV infection, highlighting the usefulness of analysing data on recently acquired infection beyond estimating HIV incidence. Countries are considering these methods where the sample sizes required for accurately estimating incidence cannot be achieved. Consensus and clear guidance on appropriate analysis are needed.

6.2 MAIN ISSUES AND DISCUSSION ON ESTIMATING THE FREQUENCY OF AND RISK FACTORS FOR RECENTLY ACQUIRED HIV INFECTION

- If countries conduct surveys that yield a limited number of HIV-positive specimens, can an estimate be calculated? Many countries are challenged by requirements for sample sizes large enough to reliably estimate assay-based HIV incidence.
- An important issue that needs clarification and consensus concerns estimating the proportion testing having recently acquired HIV infection (versus an incidence rate) and analysing the characteristics associated with testing having recently acquired HIV infection. This is especially important in a variety of settings (such as Central America and countries with small populations that are at very high risk of acquiring HIV infection). Guidance on statistical considerations and sample size requirements is in high demand but not yet available.
Although policy-makers may want a point estimate or an answer about whether an intervention has resulted in a reduction in incidence or a declining trend, small numbers may result in confidence intervals so wide that caution must be exercised in interpreting the results. Investigators need to clearly communicate results to policy-makers and those making programmatic decisions, especially when there is potential for misinterpretation or lack of confidence in the results. Countries may need guidance in communicating the results from studies that are not sufficiently powered to answer the questions being asked.

In addressing concerns that a study might not have sufficient power to identify differences in incidence rates, it was further explained that the Kenya study team analysed risk factors for testing having recently acquired HIV infection. They used a different approach to describe the epidemic and identify the characteristics of the proportion of the people living with HIV who tested having recently acquired HIV infection and, in so doing, identified areas or subgroups in which the risk for testing having recently acquired HIV infection was significantly greater. Case-based reporting in the United States of America was cited as an example of providing current information to help explain where outbreaks are currently occurring so that timely public health interventions can be implemented.

This type of analysis can assist HIV prevention programming by providing real-time data to implement interventions targeting those at higher risk for acquiring HIV infection.

Although this approach might not yield precise results or an incidence estimate, it provides valuable information about how people acquire recently acquired HIV infection.

The recommendation that consistency be using in calculating and applying the false-recent rate must be underscored. Exclusions used in the false-recent rate study (such as based on antiretroviral therapy use, CD4 count or severity of HIV disease) should be the same as those applied in the incidence estimation study.
After the objectives of the meeting and Working Group were briefly reviewed, the following lessons gleaned from the various presentations were summarized: the main issues that influence the future role and work of the Working Group.

There is urgency, since the global community is awaiting clearer guidance on incidence assays in estimating incidence. Great interest in new progress on the development of assays was illustrated by the recent statement by the United States Secretary of Health and Human Services during the 19th International AIDS Conference in Washington, DC in July 2012 regarding the promising results of the LAg-avidity assay. It was discussed that the Working Group should develop a statement clarifying the current state of the science. This would be followed by a thoughtful, independent review of the data on the LAg-avidity assay.

CEPHIA has more results available and prepared an abstract for the 20th Conference on Retroviruses and Opportunistic Infections on 3–6 March 2013.

PRESENTATIONS ON NEW TESTING METHODS: MOLECULAR METHODS, GENETIC DIVERSITY

The Luminex multimarker method and the high-resolution melting diversity assay updated new innovations in the field. Although the potential of new biomarkers seems to be far in the future, consideration should be given to the possibility that low- and middle-income countries could use these methods and how to transfer this new technology and knowledge to the field.

The feedback on the first training session on guidance for HIV incidence estimation was very positive. There are plans to do a second workshop with changes based on the participant feedback. WHO will seek funding to support the workshop. Regional workshops were recommended. It was suggested that potential funding via the United States National Institutes of Health through grant mechanism R25 could support workshops and should be explored.

The Working Group discussed at length the difference between the various guidance documents that are being developed. It was emphasized that clear guidelines are needed for incidence studies among key populations at higher risk, for complex surveys and for interpreting results.

There was a recommendation that the draft document on guidance for validating assays and algorithms used for estimating HIV incidence would work as a bridge between development and large field application, would be easy to follow during implementation and could be part of or an annex to the draft document on guidance on developing, validating and evaluating HIV incidence assays. It is an important document, and the Working Group should support finishing it. The subcommittee will share the draft with the Working Group for more comments.
The Working Group will need to consider the significance of treatment as prevention in estimating HIV incidence as HIV testing increases. There are new possibilities for laboratory tests for individual use (self-testing) that have been or will be commercialized in the near future.

Issues regarding the false-recent rate in estimating incidence and statistical testing using HIV case-reporting data pose a great challenge. It is still uncertain whether more false-recent rate studies should be conducted again to address various stages in the epidemic and assays. An overlying issue is how often a false-recent rate study should be conducted and when the false-recent rate can be ignored (<2%). The importance of consistency in calculating the false-recent rate was emphasized. The calculation used for the field should be the same for the false-recent rate study or calculation. A Working Group recommendation on the long-term issues with the false-recent rate would provide guidance to countries that are uncertain how to calculate the false-recent rate based on specific settings.

The experience of other countries estimating HIV incidence in Botswana, Ghana, Kenya, Uganda and South Africa provided very useful feedback and information for the Working Group. It was suggested that there be a database repository for this type of information.

**WHAT SHOULD BE THE ROLE AND AGENDA OF THE WORKING GROUP IN THIS NEW CONTEXT FOR THE COMING YEAR?**

- **Guidance for developers:** it was a concern that this is not possible at this time, should be postponed and it would be better to concentrate on completing the validation for field document, since it is close to completion.

- **Statement or fact sheet on LAg:** the Working Group noted interest in the CEPHIA project now that the specimen repository is well stocked. It was suggested that a technical update be developed and attached to this report. This brief document could be posted on the web site for regions. Working Group members recently drafted the update for the LAg-avidity assay following the meeting. Annex 3 provides the draft document of this report.

- **Meeting of the US CDC and WHO:** the US CDC hosted a meeting associated with the timing of the 20th Conference on Retroviruses and Opportunistic Infections on 3–6 March 2013. It was suggested that CEPHIA share the performance indicators with the Working Group in advance to review. Proposed title: Consultation – Data Review of Incidence Assays (CEPHIA and US CDC). Reference group people should participate to better support the recommendations.

- **Estimating HIV incidence using HIV case-reporting surveillance:** the Working Group agreed that it was still important to follow this direction of work.

- **Timelines**
  - A document on guidance for estimating HIV incidence using HIV case surveillance to be complete by the end of 2013.
  - CEPHIA released more data in early 2013.
» The US CDC hosted a consultation meeting in early 2013 associated with the timing of the 20th Conference on Retroviruses and Opportunistic Infections on 3–6 March 2013.
» The next Working Group meeting is proposed to be held between July and end September 2013.

NEXT STEPS

1. Working Group members completed a recent draft statement on the LAg-avidity assay following the meeting (Annex 3). The Working Group should review and comment on the document.

2. The subcommittee developing the draft document on guidance for validating assays and algorithms used for estimating HIV incidence will share a draft with the Working Group for comment.

3. The subcommittee developing the draft document on guidance for low- and middle-income countries will share an outline of the document with the Working Group for review and comment.

4. The US CDC secured dates to host a consultation meeting in early 2013 associated with the timing of the 20th Conference on Retroviruses and Opportunistic Infections on 3–6 March 2013.

5. CEPHIA shared the performance indicators with the Working Group before the spring 2013 US CDC and WHO meeting. The reference group was to be invited.

6. Dates, location and funding should be secured for the next Working Group meeting after June 2013 and before October 2013.

7. Plans should be secured for SACEMA to conduct a second workshop. WHO will seek funding to support the workshop.
8. REFERENCES


## ANNEX 1. ESSENTIAL ELEMENTS OF ASSAY VALIDATION

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<th>Subtypes</th>
<th>Status</th>
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<td>Optimization and commercial kit</td>
<td>Serum, plasma and/or DBS</td>
<td>A</td>
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<tr>
<td>Mean window determination</td>
<td>Seroconversion panels</td>
<td>B</td>
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<tr>
<td>Determination of false-recent rate</td>
<td>&gt;1 year (not receiving antiretroviral therapy) + AIDS</td>
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<tr>
<td>False-recent rate (at least one cohort)</td>
<td>Elite controllers</td>
<td>D</td>
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<td>Impact of antiretroviral therapy (at least one cohort)</td>
<td>Individuals receiving antiretroviral therapy</td>
<td>AE</td>
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<td>Incidence estimates (at least 2–3 cohorts)</td>
<td>Cross-sectional cohort</td>
<td>CRF</td>
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<td>Subcategory risk analysis</td>
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<td>Training and implementation</td>
<td>Well-defined training panel</td>
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<td>Quality control and proficiency testing programme</td>
<td>Quality control specimens and proficiency testing panel</td>
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<th>Speaker or facilitator</th>
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<td>08:30–08:45</td>
<td>Welcome remarks</td>
<td>Gottfried Hirnschall</td>
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<td>08:45–09:15</td>
<td>Introduction, objectives and expected outcome, review agenda and outstanding issues</td>
<td>Jesus M. (Txema) Garcia Calleja, Gaby Vercauteren</td>
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<tr>
<td></td>
<td>Session 1 Update on HIV incidence assays</td>
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<td>09:15–10:00</td>
<td>Minimum requirements for assay validation</td>
<td>Bharat Parekh, Joyce Neal</td>
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<tr>
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<td>• Assay standardization, quality control</td>
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<td>• Recency period – seroconversion panels</td>
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<td>• Misclassification rate – long-standing panels and from individuals receiving antiretroviral therapy</td>
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<td>• Incidence estimates – cross-sectional application</td>
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<td>• Different subtypes</td>
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<td>• Technology transfer and proficiency testing programme</td>
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<td>• Discussion with questions and answers</td>
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<td>10:00–10:15</td>
<td>Break</td>
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<td>10:15–11:15</td>
<td>Update on current assay validation efforts</td>
<td>Barbara Suligoi, Michele Owen, Bharat Parekh, Stéphane Le Vu</td>
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<td>• AxSYM/ARCHITECT avidity assay</td>
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<td>• Bio-Rad HIV-1/HIV-2 PLUS D EIA</td>
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<td>• LaG-avidity EIA, two-well Al-EIA</td>
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<td>• V3-IDE EIA</td>
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<td>11:15–11:45</td>
<td>CEPHIA evaluation update</td>
<td>Gary Murphy</td>
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<td>• Comparative performance of multiple assays</td>
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<td>11:45–12:15</td>
<td>Discussion: Are we there yet?</td>
<td>Joyce Neal</td>
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<td>• Success and challenges</td>
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<td>• Promising approaches from current assays</td>
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<td>• Developing consensus; possible time frame</td>
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<td>New Directions</td>
<td>Ha Youn Lee, Michele Owen, James Brooks</td>
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<td>Chris Pilcher, Oliver Laeyendecker</td>
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<td>Lessons learned on training and implementing the guidance in countries</td>
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<td>Bharat Parekh</td>
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<td>William Ampofo</td>
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**DAY 2: 27TH SEPTEMBER 2012**

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<td>• Draft guide for estimating HIV incidence using HIV case surveillance</td>
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<td>• Discussion and resolution of existing issues in estimating HIV incidence in case-based surveillance systems</td>
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<td>Anna Esteve</td>
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<td>Buzz Prejean</td>
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<td>Stéphane Le Vu</td>
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<td>Adamma Aghaizu</td>
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<td>Robert Remis</td>
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<tr>
<td>Session 4</td>
<td>Development of guidance on estimating the frequency of and risk factors for recently acquired infection</td>
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<td>• Country presentation on estimating level or and risk factors for recently acquired infection: Kenya</td>
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<td>• Discussion of existing issues on estimating level or and risk factors for recently acquired infection</td>
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<td>• Role and tasks of the WHO Technical Working Group on HIV Incidence Assays</td>
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<td>• Review of the 2011 meeting report recommendations</td>
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<td>17:00</td>
<td>Meeting adjourned</td>
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## ANNEX 3. LIST OF PARTICIPANTS

<table>
<thead>
<tr>
<th>Participants</th>
<th>Institution/Role</th>
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<tbody>
<tr>
<td>Adamma Aghaizu</td>
<td>Senior Scientist (Epidemiology) HIV/STI Surveillance &amp; Prevention Health Protection Services, Health Protection Agency London United Kingdom</td>
</tr>
<tr>
<td>Anna Esteve</td>
<td>Centre d’Estudis Epidemiològics sobre les Infeccions de Transmissió Sexual i Sida de Catalunya (CEEIISCAT) Barcelona Spain</td>
</tr>
<tr>
<td>Tim Mastro</td>
<td>FHI 360 Research Triangle Park, NC USA</td>
</tr>
<tr>
<td>Gary Murphy</td>
<td>Virus Reference Department Centre for Infections Health Protection Agency London United Kingdom</td>
</tr>
<tr>
<td>Ray Shiraishi</td>
<td>Statistics Team Epidemiology and Strategic Information Branch Division of Global HIV/AIDS United States Centers for Disease Control and Prevention Atlanta, GA USA</td>
</tr>
<tr>
<td>Ruiguang (Rick) Song</td>
<td>Statistics Team Epidemiology and Strategic Information Branch Division of Global HIV/AIDS United States Centers for Disease Control and Prevention Atlanta, GA USA</td>
</tr>
<tr>
<td>Thomas Rehle</td>
<td>Director &amp; Senior Program Advisor Human Sciences Research Council Cape Town South Africa</td>
</tr>
<tr>
<td>Alex Welte</td>
<td>SACEMA University of Stellenbosch Stellenbosch, Cape Province South Africa</td>
</tr>
<tr>
<td>Wolfgang Hladik</td>
<td>Lead, Population Surveillance Team Epidemiology and Strategic Information Branch Division of Global HIV/AIDS United States Centers for Disease Control and Prevention Atlanta, GA USA</td>
</tr>
<tr>
<td>Michele Owen</td>
<td>Laboratory Diagnostics United States Centers for Disease Control and Prevention Atlanta, GA USA</td>
</tr>
<tr>
<td>Bharat Parekh</td>
<td>Chief, Serology/Incidence and Diagnostics Team GAP International Laboratory Branch United States Centers for Disease Control and Prevention Atlanta, GA USA</td>
</tr>
<tr>
<td>Robert Remis</td>
<td>University of Toronto Toronto, Ontario Canada</td>
</tr>
<tr>
<td>Name</td>
<td>Title and Affiliation</td>
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<tr>
<td>Oliver Laeyendecker</td>
<td>Senior Research Associate, National Institute of Allergy and Infectious Diseases,</td>
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<tr>
<td></td>
<td>National Institutes of Health, Instructor, SOM, Johns Hopkins University, Baltimore,</td>
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<tr>
<td></td>
<td>MD, USA</td>
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<tr>
<td>Stéphane Le Vu</td>
<td>Institut de veille sanitaire, Département des maladies infectieuses, Saint-Maurice</td>
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<tr>
<td></td>
<td>Cedex, France</td>
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<tr>
<td>John Kaldor</td>
<td>Professor of Epidemiology and NHMRC Senior Principal Research Fellow, Public Health</td>
</tr>
<tr>
<td></td>
<td>Interventions Research Group, Kirby Institute, University of New South Wales, Sydney,</td>
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<tr>
<td></td>
<td>Australia</td>
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<tr>
<td>Joyce Neal</td>
<td>Epidemiologist, Surveillance Team, Epidemiology and Strategic Information Branch,</td>
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<tr>
<td></td>
<td>Division of Global HIV/AIDS, United States Centers for Disease Control and Prevention</td>
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<tr>
<td></td>
<td>Atlanta, GA, USA</td>
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<tr>
<td>Buzz Prejean</td>
<td>Incidence and Viral Resistance Team Supervisor, HIV Incidence and Case Surveillance</td>
</tr>
<tr>
<td></td>
<td>Branch, Division of HIV/AIDS Prevention, United States Centers for Disease Control</td>
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<tr>
<td></td>
<td>and Prevention, Atlanta, GA, USA</td>
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<tr>
<td>Barbara Suligoi</td>
<td>Instituto Superiore di Sanità, Rome, Italy</td>
</tr>
<tr>
<td>Sikhulile Moyo</td>
<td>Ministry of Health, Gaborone, Botswana</td>
</tr>
<tr>
<td>Chris Pilcher</td>
<td>Blood Systems Research Institute, San Francisco, CA, USA</td>
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<tr>
<td>Ha Youn Lee</td>
<td>Associate Professor, Department of Molecular Microbiology and Immunology, Keck School</td>
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<tr>
<td></td>
<td>of Medicine, University of Southern California, Los Angeles, CA, USA</td>
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<tr>
<td>William Ampofo</td>
<td>Senior Research Fellow, Noguchi Memorial Institute for Medical Research, University</td>
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<td></td>
<td>of Ghana, Accra, Ghana</td>
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<tr>
<td>Adrian Puren</td>
<td>Deputy Director, National Institute for Communicable Diseases, Division of the</td>
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<tr>
<td></td>
<td>National Health Laboratory Service, South Africa</td>
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<tr>
<td>WHO</td>
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<tr>
<td>Gaby Vercauteren</td>
<td>Diagnostics and Laboratory Technology, Department of Essential Health Technologies,</td>
</tr>
<tr>
<td></td>
<td>Health Systems and Services</td>
</tr>
<tr>
<td>Jesus M. (Txema) Garcia</td>
<td>Strategic Information Team, Department of HIV/AIDS</td>
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<tr>
<td>Calleja</td>
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<tr>
<td>Nita Bellare</td>
<td>HELP Design Group, Rapporteur</td>
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