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

13–15 OCTOBER 2014
BARCELONA, SPAIN
WHO/UNAIDS
ANNUAL MEETING OF THE TECHNICAL WORKING GROUP ON HIV INCIDENCE ASSAYS

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<tr>
<td>AI</td>
<td>Avidity index</td>
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<tr>
<td>ANC</td>
<td>antenatal care</td>
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<tr>
<td>ART</td>
<td>antiretroviral therapy</td>
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<td>ARV</td>
<td>antiretroviral</td>
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<td>BED</td>
<td>B, E and D serotypes</td>
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<tr>
<td>BMGF</td>
<td>Bill and Melinda Gates Foundation</td>
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<tr>
<td>BSRI</td>
<td>Blood Systems Research Institute</td>
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<td>CDC</td>
<td>Centers for Disease Prevention and Control</td>
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<td>CEIA</td>
<td>capture enzyme immunoassay</td>
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<td>CEPHIA</td>
<td>Consortium for the Evaluation and Performance of HIV Incidence Assays</td>
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<tr>
<td>CoV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>CROI</td>
<td>Conference on Retroviruses and Opportunistic Infections</td>
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<td>DBS</td>
<td>dry blood spot</td>
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<td>DEFF</td>
<td>design effect</td>
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<tr>
<td>EIA</td>
<td>enzyme immunoassay</td>
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<tr>
<td>EQA</td>
<td>external quality assessment</td>
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<td>EQAPOL</td>
<td>External Quality Assurance Program Oversight Laboratory</td>
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<td>FIND</td>
<td>Foundation for Innovative New Diagnostics</td>
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<td>FRR</td>
<td>false recent rates</td>
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<tr>
<td>HIA</td>
<td>HIV impact assessment</td>
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<tr>
<td>HRM</td>
<td>higher resolution-melting</td>
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<tr>
<td>IDU</td>
<td>injecting drug user</td>
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<tr>
<td>JHU/HPTN</td>
<td>Johns Hopkins University/HIV Prevention Trials Network</td>
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<td>KAIS</td>
<td>Kenya AIDS Indicator Survey</td>
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<tr>
<td>LAg</td>
<td>Limiting Antigen</td>
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<tr>
<td>MAA</td>
<td>multi-assay algorithm</td>
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<td>MDRI</td>
<td>mean duration of recent infection</td>
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<td>MRM</td>
<td>multiple reaction monitoring</td>
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<td>MSF</td>
<td>Médecins Sans Frontières</td>
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<tr>
<td>MSM</td>
<td>men who have sex with men</td>
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<tr>
<td>NAAT</td>
<td>nucleic acid amplification test</td>
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<tr>
<td>NHBS</td>
<td>national HIV behavioural system</td>
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<tr>
<td>NIAID</td>
<td>National Institute of Allergy and Infectious Diseases</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<tr>
<td>OD-n</td>
<td>optical density</td>
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<tr>
<td>PHE</td>
<td>Public Health England</td>
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<tr>
<td>RITA</td>
<td>recent infection testing algorithm</td>
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<tr>
<td>SACEMA</td>
<td>South African Centre for Epidemiological Modelling and Analysis</td>
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<tr>
<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>TPP</td>
<td>target product profiles</td>
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<tr>
<td>TRI</td>
<td>test for recent infection</td>
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<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>VL</td>
<td>viral load</td>
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<tr>
<td>WB</td>
<td>western blot</td>
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HIV incidence is a key indicator of the impact of interventions by national HIV programmes to control the HIV epidemic. Although the first assay for HIV incidence was developed in 1997, approaches to measuring incidence using such assays have remained complex. In 2008, WHO established a Working Group on HIV Incidence Assays to examine the issues and challenges involved. This group – comprising epidemiologists, laboratory specialists and public health officials from numerous countries – has worked towards standardizing terminology in assay calibration and validation. Several meetings have been held to advance the agenda and share best practices. These meetings have been successful in convening a wide group of current and future assay users, and key experts in applying laboratory-based methods for incidence estimation (1). In 2010, in collaboration with the US Centers for Disease Prevention and Control (CDC), the Working Group produced a guidance document for estimating HIV incidence at population level in cross-sectional studies (2). In addition, a training course on how to use those assays to estimate incidence at population level has been conducted twice by the South African Centre for Epidemiological Modelling and Analysis (SACEMA) at Stellenbosch University.

In 2010, the Bill and Melinda Gates Foundation (BMGF) adjudicated a grant proposal to Public Health England (PHE) and the Blood Systems Research Institute (BSRI) in the United States of America (USA) to establish the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA1). The aim of CEPHIA was to develop a specimen repository in order to validate existing and future HIV Incidence Assays, compare results from existing assays with direct incidence measurements, and identify the key parameters that enable correct interpretation of results. CEPHIA’s findings were presented to a panel of experts at a symposium held during the Conference on Retroviruses and Opportunistic Infections on 3 March 2013 in Atlanta, USA. This was followed by an expert consultation meeting convened by the CDC on the Limiting Antigen (LAg) Avidity Enzyme Immunoassay (EIA); at that meeting, CEPHIA, the CDC and others presented further results. A technical update was subsequently published by the Joint United Nations Programme on HIV/AIDS (UNAIDS)/WHO based on these findings.2 Since then, CEPHIA has published their evaluation of five HIV Incidence Assays currently used to detect recency of infection at population level (3).

A meeting of the Working Group was held in October 2013 in Rome, Italy; however, several members were unable to attend,3 so the discussions could not be deemed to represent consensus. The most recent meeting was held on 13–15 October 2014 in Barcelona, Spain, with the aim of obtaining consensus on the next technical update to be produced by UNAIDS/WHO, and on any required updates for the revised WHO/UNAIDS population-based surveillance guidance. In addition, some developed countries with robust case-based HIV surveillance systems have successfully integrated routine testing for recent infection, and derived incidence estimates using these data. A second document providing technical guidance on assay-based HIV incidence estimation using case-reporting data is in draft.

The meeting was hosted by the Agencia de Salud Publica of the Generalitat de Catalunya in Barcelona. Meeting costs were shared between WHO, the CDC and CEPHIA.

1. BACKGROUND

1 http://www.incidence-estimation.org/page/CEPHIA
2 http://www.who.int/HIV/pub/me/tech_update_0513/en/
3 Due to the USA government shutdown at short notice
2. OBJECTIVES OF THE MEETING

The main objectives of the meeting were to:

- provide an update on assay validation results from CEPHIA;
- revise the technical update issued by UNAIDS/WHO in 2013;
- review recommendations for testing algorithms to estimate HIV incidence in cross-sectional surveys; and
- present the guidance document for HIV incidence estimation using case-based surveillance systems in countries with robust HIV case-reporting systems.

2.1. Methods of work and expected outcomes

The agenda and presenters of the meeting are included in Annex 1. In advance of the meeting, participants were provided with current scientific articles related to estimating HIV incidence, and the previous WHO/UNAIDS 2013 technical update. Sessions were arranged according to six key topical areas: results from the evaluation of HIV Incidence Assays; statistical issues specific to the recent infection testing algorithm (RITA); the incorporation of RITAs into population-based surveys; country experiences of using HIV Incidence Assays; new biomarkers for incident infection; and the draft guidance document for estimating incidence using HIV case-based surveillance data. Descriptions of the presentations made in these sessions form most of the remainder of this report.

The main outcome expected from the meeting was consensus on the technical updates needed, based on the findings of the evaluation of HIV Incidence Assays. More specifically, the expected outcomes were to:

- provide an update on the use of different HIV Incidence Assays in countries;
- provide an update on HIV incidence assay evaluations by CEPHIA and CDC;
- revise the 2013 UNAIDS technical update and guidance for estimating HIV incidence in population-based surveys;
- review the draft document and provide recommendations on the use of RITA and HIV case-based surveillance data to estimate incidence;
- discuss substantive issues regarding field validation, the development of a field protocol and training in the application of assays for recent infection to estimate population-level incidence;
- discuss and develop recommendations for addressing challenges related to assay-based incidence estimation (e.g. Sample size requirements, approaches for estimating incidence in key populations and detecting differences in incidence over two or more consecutive surveys); and
- provide an update on HIV incidence assay development.
3. HIV INCIDENCE ASSAYS AND EVALUATIONS

3.1 Update on CEPHIA 1: final objectives

In collaboration with several public health and research institutions, CEPHIA has collated residual specimens to develop a well-characterized repository for the evaluation of current and future HIV Incidence Assays.

CEPHIA started with four main objectives, which were to:

• create a repository comprising large panels of HIV-positive specimens for the assessments of RITA assays and algorithms;
• fully evaluate 10 candidate assays for their ability to correctly identify recent HIV infection;
• build collaborations and consensus around the characterization and deployment of RITAs in the field; and
• derive and deploy improved methodology to estimate RITA duration and false recent rates (FRRs), and determine algorithms that may be constructed from available components or tests (or both).

To date, outputs of CEPHIA have been:

• development of a quality system for assay evaluations
• development of target product profiles (TPP) and evaluation of assays against these
• development of a database of specimens that can be shared with other groups
• creation of a system to access specimens (researchers can now request these);
• creation of an assay development pathway
• provision of analytical tools (e.g. Spreadsheets for sample size calculations)
• development of an external quality assessment (EQA) programme
• definition and refinement of nomenclature
• provision of support training for laboratory and statistical aspects
• recommendations.

CEPHIA has evaluated the following assays:

• LAg-Avidity EIA;
• Bed-capture enzyme immunoassay (CEIA);
• Ortho Clinical Diagnostics VITROS Anti-HIV 1+2 assay (less-sensitive methodology developed by BSRI, known as LS-Vitros);
• Ortho Clinical Diagnostics VITROS anti-HIV 1+2 assay (avidity methodology developed by BSRI, known as BSRI-Vitros-Avidity); and
• Bio-Rad Genetic Systems HIV-1/HIV-2+ O (avidity methodology developed by CDC, known as CDC-Bio-Rad-Avidity).

At present, the LAg EIA (developed by the CDC) is the only assay that is commercially available as an assay for HIV incidence. The CEPHIA group found that this assay performed well, with an FRR <2% in all evaluations, not including specimens from patients on antiretroviral (ARV) therapy and elite controllers. However, the assay is sensitive to ARVs, and misclassification issues remain for those starting treatment immediately after diagnosis. In addition, there is variability between individual immune response and HIV subtype; in particular, subtype D. Testing algorithms will need to be validated using ARV and viral load (VL) information, and the use of dry blood spot (DBS) specimens. Sample size calculations will indicate to what accuracy incidence can be estimated.

For the TPPs developed thus far, acceptable and ideal performance of incidence assays depend on the purpose of the testing and the target population. These assays can be used to measure incidence over time in cross-sectional surveys or case-based surveillance, or for individual disease staging. The TPPs specify the intended use, target population, FRR, mean duration of recent infection (MDRI), algorithm, analyte, sample type and volume, infrastructure requirements, storage and shipping conditions, incubation temperature, shelf life, training needs and regulatory pathway. EQA schemes are currently in development.

Future objectives of CEPHIA are to:

• complete final evaluations;
• release ‘blue books’ (i.e. evaluation reports of new
diagnostic assays, including technical requirements, reproducibility and the availability of EQAs; • publish findings in peer-reviewed journals; • develop standard operating procedures, EQAs and TPPs; • encourage wider access to the database of specimens and data sharing; and • support the Working Group in developing guidance documents.

3.2 CEPHIA 2: progress on new biomarkers

After the first 2 years of CEPHIA 1, CEPHIA 2 was created to support the infrastructure and process for ongoing assay development. Such development includes biomarker discovery, proof of concept and evaluation, EQA, and other related research; it involved the use of prefabricated project-specific panels. CEPHIA 2 also made available alternative specimen types (e.g. DBS, serum, oral fluid, urine and faeces), and provided statistical support in conjunction with SACEMA.

The archives created contain 6000 specimens, 4000 of which are available in quantities >10 ml. This includes 3000 specimens from treatment-naive patients (2000 of which are from persons infected for >1 year), 120 from elite controllers infected >1 year, and 1100 from persons WHO have received ARVs >1 year and have suppressed viral loads (<50 copies/mL). The repository contains specimens with subtypes C and D. Most specimens are from within 3 years of seroconversion.

Important gaps raised were the need for more specimens from persons with subtypes D, AE and G; from persons infected >3 years and still treatment naive; and from those on ARV infected <2 years, both virally suppressed and non-suppressed. These specimens will be harder to source with time, particularly from long-term treatment-naive patients, because treatment guidelines now recommend earlier initiation of antiretroviral therapy (ART); hence, few patients have those characteristics.

Additional specimens are being sourced from the San Francisco Men’s Health Study and the Centre for the AIDS programme of research in South Africa (CAPRISA) and HIV pathogenesis programmes, which have contributed samples from long-term clade C infected patients. A network has been established to prospectively collect 1550 new specimen sets by August 2015.

The repository is used for hypothesis-driven assay concepts, non-hypothesis-driven biomarker discoveries, and proven concepts without kits or final protocols. Hypothesis-driven concepts start with a biomarker that can discriminate between recent and non-recent specimens, followed by the proof of concept stage examining whether a threshold can be devised for a plausible MDRI (between 4 and 24 months based on 100 specimens). If it is within range, the FRR is determined (using 100 naive and 50 elite controllers). For non-hypothesis-driven studies, larger numbers of specimens are needed.

All panels can be provided on request up front to groups for exploratory work. Once a biomarker has progressed through these steps, the qualification panel from CEPHIA 1 can be used for further independent evaluation.

The Geenius HIV-1/HIV-2 confirmatory assay was developed and optimized with a set of criteria to be a rapid diagnostic test similar to the western blot (WB) assay, and to give quantitative readings on multiple band intensities. A recency index or intensity score, and a threshold value associated with recency, were established by CEPHIA.

A second example presented was nuclear magnetic resonance (NMR) metabolite profiling, which is a novel concept for potentially identifying recent HIV infection. This approach was based on previous work on tuberculosis (TB) and rabies. Metabolite profiles change over time and may therefore be associated with recency. NMR spectroscopy is used to measure metabolites in the blood plasma and principal component analyses based on pattern recognition, performed to determine associations with recent infection. Researchers from University of California Davis studied different specimen types, established an MDRI (211 days) and an FRR (3.7%), and are now testing blinded specimens. Results have shown no relation to ARV use or elite controllers; also, NMRs can detect the presence of ARVs. Using CEPHIA 2, this process took only 2 years from the initial concept to the use of the CEPHIA 1 qualification panel.

Currently, there are many other CEPHIA users worldwide; the challenge will be to continue the ongoing availability of specimens.

3.3 Review of CEPHIA results for five candidate assays

A summary of the recently published CEPHIA results (1) was presented. The assays evaluated were LAg, BED, LS-Vitros, Vitros Avidity and Bio-Rad Avidity. The CEPHIA repository used to construct the evaluation panel contained specimens from Brazil, Kenya, Rwanda, South African, Uganda, the USA and Zambia, and contained subtypes B, C, A1, D and others. The MDRI and FRR (based on a post-infection time cut-off [T] of 2 years) were examined and analyses were conducted stratified by treatment history, viral load, CD4 cell count, time from infection to specimen draw and HIV subtype. The data were enriched with elite controllers and analyses conducted among these separately. For the estimation of infection times, infection was defined as WB seroconversion. Input data were
dates of the last negative and first positive HIV tests (not longer than 120 days apart), the diagnostic assays used, the reported average durations of Fiebig stages, and unambiguous acute retroviral syndrome symptom onset dates.

There was a sizeable difference between LAg and BED for the evolution and distribution of assay readings. The proportion of subjects infected for greater than 2 years with an optical density (OD-n) reading below the recent cut-off (the FRR) was considerably smaller for the LAg assay. The Bio-Rad Avidity (using methodology developed by CDC) had a low FRR and relatively long MDRI, which is favourable for use in surveillance. There was variation by subtype; however, it was observed that OD-n readings for all assays failed to decline with increasing time since infection for A1 and D specimens. Among all assays evaluated, LAg had the shortest MDRI (188 days) and also the lowest FRR (1.3%). LAg also had the lowest FRR among elite controllers. All assays were extremely sensitive to the presence of ARVs and low viral load, thereby producing high FRRs for those patients (FRR on ARVs [LAG] 58.8%, FRR with viral load <75 copies/mL [LAG] 47.1%). The variation in test properties by subtype (MDRIs and FRRs) will need to be considered when interpreting incidence estimates.

### 3.4 Incorporation of viral load results into incidence assay results

Unpublished CEPHIA data were presented on the ARCHITECT HIV Ag/Ab Combo and Geenius HIV-1/HIV-2 confirmatory assay, incorporating information on viral load data. The cumulative distribution of HIV viral load among persons infected for <2 years from time since infection (T=2) was shown. Approximately 2% of patients had a viral load of <75 copies/mL after being infected for 2 years, and 11% had <1000 copies/mL. Low viral load may be an indicator of ART usage (or recent cessation of ART) or an elite controller, and the FRRs were considerably lower when persons with low viral load were excluded. It was recommended to include viral load information in RITA. A viral load threshold is typically used for DBS specimens (1000 copies/mL) and treatment failure (3000 copies/mL), and it was suggested to standardize the cut-off to one of these, because the data are likely to be more readily available. A low threshold is preferred because higher viral load thresholds result in lower MDRIs; also, the low threshold has little impact on the magnitude of the FRR.

There is scarce information on the distribution of viral load, particularly among persons who have started treatment early, discontinued treatment or taken a treatment break. The SACEMA group clarified that they would not propose viral load cut-offs. They intend to provide tools that will allow users to calculate the FRR and MDRI based on chosen viral load thresholds. Further details of the impact of incorporating viral load in RITAs will be published by CEPHIA in due course.

### 3.5 Comparison of CEPHIA/CDC versus JHU/HPTN Bio-Rad Avidity

At present, two protocols exist for the Bio-Rad avidity assay (Genetic Systems 1/2+ O Elisa): one developed by CEPHIA/CDC and the other by Johns Hopkins University/HIV Prevention Trials Network (JHU/HPTN). A comparison of cross-sectional incidence methods and analyses using the two was presented. The differences in the protocols were that CDC/CEPHIA used:
- double the incubation time (1 hour vs 30 minutes)
- washing buffer instead of deionised water
- a sealer rather than a lid to cover the plate
- six wash cycles as opposed to five
- a 10-second longer soak time (40 vs 30 seconds)
- a 615 to 630 filter (3–6).

Results were compared for the HIVNET 001 study using 832 samples from 104 individuals, including 240 samples taken more than 2 years after seroconversion. The MDRIs and FRRs at various avidity index (AI) cut-offs between 20% and 80% were compared. It was found that the CDC protocol had a larger MDRI and FRR than the JHU/HPTN protocol; for example, MDRI at 20% CDC/CEPHIA: 172 (95% CI: 152–192) days vs JHU/HPTN: 24 (95% CI: 12–39) days, and AI at 80% FRR CDC/CEPHIA: 25.8% (95% CI: 20.4–31.9%) FRR JHU/HPTN: 6.7% (95% CI: 3.9–10.6%).

An update on the LAg assay and algorithms, and the impact of increasing T on the MDRI for different testing algorithms, was presented. The MDRIs were presented for subtype C, varying T from 1–4 years using first only the LAg 1.5 OD-n cut-off, second the 1.5 OD-n cut-off and viral load >1000 copies/mL, and third a multi-assay algorithm (MAA) with two assays (LAg 2.9 OD-n cut-off and Bio-Rad AI 85% and viral load >400 copies/mL and CD4 cell count >50 cells/µl). The lowest MDRI was obtained using LAg 1.5 OD-n threshold and viral load >1000 copies/mL, and the highest using the MAA. Of note was that the increase in MDRI seemed to plateau after 2 years when taking viral load into account. For subtype B, there was little change in the MDRI with increasing T using the MAA, compared to using only the 1.5 OD-n cut-off for the LAg, where MDRI increased with increasing T.

### 3.6 Development of incidence assay quality assessment programmes

The development of EQA programmes for HIV Incidence Assays was presented; until recently, this had only been available for assays produced by CDC. BSRI has worked closely with CDC to develop the concept of a proficiency programme
for HIV Incidence Assays, based on its existing one (7). The External Quality Assurance Program Oversight Laboratory (EQAPOL), established 4 years ago and funded by the National Institute of Allergy and Infectious Diseases (NIAID), has a repository of diverse specimens and is collaborating to create the EQA for HIV Incidence Assays.

The EQA timelines were presented for some of the programmes already undertaken by EQAPOL showing how shipments, data collection and report analyses were conducted up to 2–3 times a year. The EQA process was shown in detail using a site undertaking proficiency testing for LumineX. The site had expressed interest in the programme and created a profile online that allowed it to anonymously see the performances of other sites taking part in the programme. Specimen panels were sent to the site with temperature recorders in case of any thawing during shipment. The site was tasked with logging the date of receipt and completing a questionnaire on testing. The data were analysed promptly by a statistician and fed back via a report online with a rating based on timeliness, protocol adherence and performance, compared to other participating sites.

For the HIV incidence assay EQA programme, some of the tasks to be undertaken are to:

- identify appropriate specimens for use as large volume control material;
- establish EQA for leading candidate assays being deployed for incidence estimation – particularly the Bio-Rad Avidity assay given the widespread roll-out by the President’s Emergency Plan For AIDS Relief (PEPFAR) and other programmes; and
- provide EQA panels that are appropriate for other incidence assays or RITAs.

To date, specimens have been evaluated and benchmarked for a range of assays from different sites. The EQA panel has been constructed to include three blinded samples for each control sample, representing low, medium and high values for the assays; it also includes HIV negative and positive specimens for characterization. All are to be run in triplicate, totalling 45 determinations per panel.

The objectives for 2014–2015 are to create aliquots and ship specimens. There will be 2–3 shipments per year, and before shipping, EQAPOL and the BSRI will retest the EQA panel on primary assays (LAg and Bio-Rad Avidity). In 2016, it is anticipated that the panel will be rolled out to 20–30 domestic and international laboratories using the assays Bio-Rad Avidity (CDC, JHU), Architect Avidity, LS-Vitros/Avidity Vitros and Bio-Rad Dilution Avidity (Scotland).

It was questioned whether there was a concern that the number of laboratories able to access panels will be limited because of cost constraints. It was stated that the incremental costs for accessing the panels are minimal, and that non-USA-funded laboratories will be expected to cover shipping costs. Separate funding may be available from the European Centre for Disease Prevention and Control for laboratories in Europe.

Through the EQA programme, it is envisioned that relationships with laboratories will enable the collection of samples from incident cases for viral diversity studies.

### 3.7 HIV incidence assay project coordination: Foundation for Innovative New Diagnostics

The Foundation for Innovative New Diagnostics (FIND), established 10 years ago, focuses on diagnostics for diseases in lower- and middle-income countries. Diagnostics are needed to target interventions, identify outbreaks and monitor progress towards disease elimination and control. The goal of FIND is to catalyse the development of assays by supporting manufacturers’ programmes, developing guidelines and policy, and accelerating access and shaping the agenda. A network of global experts structures the organization, with headquarters in Geneva and country offices in the Dominican Republic, India, South Africa and Uganda.

Past achievements of FIND include:

- enabling drug susceptibility testing for TB patients within 2 hours at district hospitals (previously this took 120 days and was available only at national reference laboratories);
- developing a rapid diagnostic test for sleeping sickness;
- developing a FIND/WHO quality assurance model for rapid malaria tests, which has increased the proportion of quality products in use from 15% to 75%.

FIND has applied to the BMGF to help manage, coordinate and advance the work of CEPHIA. A grant proposal has been developed to fund the management of assay evaluations, the development of TPPs and a biomarker evaluation process for CEPHIA 1; and to develop a repository of specimens, analyse new biomarkers and also develop a biomarker evaluation process for CEPHIA 2. In addition, FIND will assist with management of new biomarker BMGF Discovery Grants. At present, a contract is in place to support elements of this work, including initiating stakeholder engagement activities and TPP development.

FIND supports advocacy and policy development for normative guidelines, collaborating with countries and policy-makers. It is conducting long-term financial planning for incidence assays, searching for co-funding to support activities beyond 2 years.
4. Obtaining Consensus on RITA-Specific Statistical Issues

4.1 Implications of moving from a post-infection time cut-off (T) of 1 to 2 years

The issue of the consideration of a post-infection time cut-off (T) with regards to defining the FRR was presented. The MDRI is represented by the area under the distribution curve up to the time point T; any assays that fall beyond T but are still labelled as recent by an assay will contribute to the FRR. By definition, a smaller T will generate a larger FRR, because a higher proportion of the non-recently infected population will fall beyond that point. It was stated that T should be where the FRR is smallest. Data from CEPHIA indicate T=2 years is appropriate, because it maximizes the MDRI while minimizing the FRR. There was consensus that 12 months is too short, and consequent FRRs too high.

It was raised that those estimating HIV incidence are mainly concerned with the number of infections within a 1-year period, and assays with a T value far beyond this may complicate the analysis and interpretation of results. In addition, it was discussed whether the choice of T would influence how studies are conducted in the field. Increasing T will generate higher numbers of recent cases, reducing required sample sizes. This would increase precision but also introduce additional bias. In relation to these points, it was clarified that most of the weighting for the recent cases is within the early part of the function (i.e. The first year); thus, problems may arise if only a few recent cases are observed, because the weighting will be more heavily influenced by the area after time T, which in turn influences the FRR. It was agreed that there is a need to look for tests with T closer to 1 year. At present, the primary recommendation is for countries that are using the LAg and other assays validated by CEPHIA to use T=2 years, with the caveat that this may be revised in future and may need further consideration for other assays and in different settings.

A major challenge identified during these discussions was the need to account for flexibility in the FRR, especially due to increasing ART coverage. It will also become increasingly difficult to conduct assessments for an appropriate T on specimens that accurately reflect the populations in which the incidence estimates are to be conducted, because of the increasing adoption of test-and-treat policies by countries.

4.2 Interpretation of recent infection for population-level incidence estimates versus individual diagnosis

This session did not have a formal presenter. Instead, invited participants discussed whether HIV Incidence Assays needed to have clinical utility to attract commercial interest and investment. Clinicians may be interested in using these for individual-level disease staging and for prioritising partner notification among persons with incident infection. Different thresholds and calibrations could be used to increase sensitivity and specificity. It was considered that the clinical use of these assays might not be an issue of the test, but rather of the correct interpretation of results, considering caveats pertinent to the application.

To date, no interventions or clinical recommendations exist for persons with primary infection. It was suggested that these had not been developed because technologies are not sufficiently accurate to diagnose incident infection at patient level. It is known that persons are more infectious during the early stages of infection than during chronic infection, even with low viral loads. In addition, earlier initiation of ART lowers the reservoir; in particular, the first 3 weeks are crucial. Ideally, recent infection could be detected with assays that can be used at point-of-care. The Geenius assay – a confirmatory test that can discriminate recency – was presented as an example. Currently, the Geenius assay is not licensed for individual-level use, but it has been submitted for Food and Drug Administration (FDA) approval.

To date, the United Kingdom of Great Britain and Northern Ireland (United Kingdom) is the only country that returns “test for recent infection” (TRI) results to clinicians and patients. A survey undertaken among HIV clinicians indicated they perceived the results to be useful, and had not experienced any adverse events when relaying these to patients. Some included the use of these in their partner notification and contact tracing protocols. This information is used for programmatic purposes but not for surveillance.

4.3 Consensus on definition and methods for MDRI determination

A comparison between different MDRI and cut-off values of the following four algorithms was presented (two by Brookmeyer et al. (8) and two by Konikoff (9):
- BED<1.6+AI<85%+VL>400+CD4>50
- BED<1.5+AI<40%+VL>400
- AI40%+LAg2.8
- AI85%+LAg2.9+ VL>400+CD4>50.

MDRI estimates were generated by the method of Kassanjee (10), and the window periods were taken from previously published estimates. By definition, a window period has no FRR as the distribution curve does not converge. For the MDRI, a cut-off time T is chosen, after which observations are classified as false recent. Results showed that the window period and MDRI estimates of these four algorithms were similar. It was therefore concluded that the window period and MDRI are the same, provided the FRR is close to 0, which is likely for algorithms that include multiple markers for recent infection, such as viral load and CD4 count.

The MDRIs were also compared for subtypes A, B, C and D for the LAg and BED assays using estimates from CEPHIA (based on methods from Kassanjee (3)) and JHU/HPTN (based on the method of Kassanjee (10)). These showed that, for LAg, JHU/HPTN estimates were slightly lower than CEPHIA’s, except for subtype D. For BED, the HPTN estimates were slightly higher, except for subtype C.

The variance at the beginning of time T (detecting infection) was discussed. Rapid tests and third and fourth generation assays are able to detect infection after approximately 20 days. The reference to the MDRI is therefore actually the mean duration since seroconversion, rather than infection. The CEPHIA group highlighted that the time between infection and detectable RNA is poorly established. There are few data on people with well-defined episodes of exposure, and consequently few data points to estimate the mean duration since infection. The type of test to diagnose HIV infection will need to be taken into account because the difference to the MDRI could be up to 1 month. CEPHIA has attempted to estimate these periods for the seroconversion panel specimens, and assign a date of viraemia and seroconversion to each subject. It was concluded that the methods to account for this needed further discussion; however, the difference between time since infection and since seroconversion should be emphasized.

### 4.4 FRR in context of increasing ART coverage and the effect of epidemic settings

The FRR of the LAg and BED assays were compared by subtypes A, B, C and D using the CEPHIA algorithm (3), which excludes all persons on ART and elite controllers, and by the JHU/HPTN algorithm, which excludes all persons with a viral load <400 copies/mL. For the LAg assay using the CEPHIA algorithm, lower FRRs were obtained for subtypes B and C compared to using the JHU/HPTN assay (Table 1). However, the HPTN algorithm had lower FRRs for subtypes A and D. Of note was that, for both algorithms, the FRR for subtype D was above 5%; both also generated higher FRRs for BED compared to LAg (Table 2).

<table>
<thead>
<tr>
<th>HIV</th>
<th>Subjects (samples)</th>
<th>FRR (95% CI)</th>
<th>Subjects (samples)</th>
<th>FRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype A</td>
<td>37 (106)</td>
<td>2.7 (0.1–14.2)</td>
<td>290 (1352)</td>
<td>0.52 (0.21–1.06)</td>
</tr>
<tr>
<td>Subtype B</td>
<td>190 (338)</td>
<td>0.5 (0.0–2.9)</td>
<td>637 (1016)</td>
<td>1.67 (0.98–2.67)</td>
</tr>
<tr>
<td>Subtype C</td>
<td>75 (144)</td>
<td>1.3 (0.0–7.2)</td>
<td>451 (547)</td>
<td>2.38 (1.27–4.03)</td>
</tr>
<tr>
<td>Subtype D</td>
<td>11 (18)</td>
<td>9.1 (0.2–41.3)</td>
<td>328 (705)</td>
<td>6.24 (4.57–8.29)</td>
</tr>
</tbody>
</table>

**Table 1. LAg FRR by subtype**

**CEPHIA** Excludes all subjects on ART and elite suppressors (Kassanjee (7), Table 2)

**HPTN** Excludes all subjects with low VL (<400 copies/mL)
A simulation exercise was undertaken examining FRRs in different epidemic settings. HIV incidence was assumed to be 1.3% in a stable epidemic, 4.2% in an emerging epidemic and 0.2% in a waning epidemic. Data showed the FRRs were higher for any algorithm in low incidence settings.

<table>
<thead>
<tr>
<th>HIV</th>
<th>Subjects (samples)</th>
<th>FRR (95% CI)</th>
<th>Subjects (samples)</th>
<th>FRR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype A</td>
<td>37 (106)</td>
<td>18.9 (8.0–35.2)</td>
<td>290 (1352)</td>
<td>13.3 (11.5–15.2)</td>
</tr>
<tr>
<td>Subtype B</td>
<td>190 (338)</td>
<td>4.7 (2.2–8.8)</td>
<td>637 (1016)</td>
<td>8.86 (7.18–0.8)</td>
</tr>
<tr>
<td>Subtype C</td>
<td>75 (144)</td>
<td>7.3 (2.6–15.7)</td>
<td>451 (547)</td>
<td>6.95 (4.96–9.41)</td>
</tr>
<tr>
<td>Subtype D</td>
<td>11 (18)</td>
<td>18.2 (2.3–51.8)</td>
<td>328 (705)</td>
<td>13.6 (11.2–16.4)</td>
</tr>
</tbody>
</table>

Table 2. BED-CEIA FRR by subtype

CEPHIA Excludes all subjects on ART and elite suppressors (Kassanjee (1), Table 2)

HPTN Excludes all subjects with low VL (<400 copies/mL)

A simulation exercise was undertaken examining FRRs in different epidemic settings. HIV incidence was assumed to be 1.3% in a stable epidemic, 4.2% in an emerging epidemic and 0.2% in a waning epidemic. Data showed the FRRs were higher for any algorithm in low incidence settings.
5. INCORPORATING RITAS INTO POPULATION-BASED SURVEYS

5.1 Update on USA government-supported HIV impact assessments and strategies for including RITAs

An update on USA-led HIV impact assessment (HIA) surveys was presented. The Kenya AIDS Indicator Survey (KAIS) 2012 was described, which CDC used to measure progress and as the basis for the HIA model. The KAIS collected data using electronic tablets, enabling real-time data entry and daily data transmissions to a central location. Blood specimens were collected for HIV testing, CD4 count and viral load testing at a central reference laboratory. HIV testing and CD4 were offered at point-of-care for positive persons referred for care. HIV prevalence estimates from the study were 5.6% (95% CI: 4.9–6.3%) for adults (aged 15–64 years) and 0.9% (95% CI: 0.5–1.3%) for children (aged 18 months–14 years). The HIV incidence estimate for adults was 0.5% (95% CI: 0.2–0.9%). This was lower than UNAIDS estimates for new infections in 2012 (85 000, 95% CI: 80 000–96 000).

The 2014 PEPFAR Country Operational Plan guidance states all long-term strategy countries with generalized epidemics are expected to support HIAs as a means for monitoring epidemics and the impact of HIV programmes. The design of future HIAs will be general population household-based, multistage cluster surveys measuring HIV programme impact at population level. Sample sizes will be between 15 000 and 30 000 households. Biomarker data will be collected in HIV serology (including CD4 count, viral load and HIV recency) to measure HIV prevalence and incidence (using LAg and viral load), the distribution of CD4 counts and the population viral load, and to develop a repository for future testing (e.g. For ARV metabolites). Data will also be collected on exposure to and uptake of services and interventions to determine the care cascade. A publicly accessible data warehouse will be created.

The Demographic Health Survey+ (DHS+), AIDS Indicator Survey (AIS) and HIAs are similar in that they are population-based household surveys, include household and individual questionnaires, obtain national and subnational HIV prevalence estimates, and have similar sample sizes. They are different in that HIA will collect biomarker data, generate national incidence estimates, and refer patients to care and treatment services as part of the survey. Limitations in the HIA approach include the high cost, the lack of feasibility of conducting these surveys in countries with <5% HIV prevalence, and the fact that some of the data are self-reported.

Due to regional differences in HIV prevalence and programme effectiveness, it is no longer considered adequate to collect data only for national-level estimates. Malawi was presented as an example, where HIV prevalence was higher in the south of the country than the north. However, there is a trade-off between desired granularity and required sample sizes or cost. In countries with a prevalence >5%, the proposed focus is national; where prevalence is <5%, the focus may be to conduct the survey in certain regions or states.

Currently planned HIA activities will commence in 2015/2016 in 12 long-term strategy countries. Standard methods will be applied across the surveys, including template protocols and questionnaires, which will be adapted and expanded for each country. There will be interagency coordination through the Office of Global AIDS Coordination Surveys and Surveillance Technical Working Group in collaboration with other partners.

The surveys will be powered for precision of the primary objectives, which are national HIV incidence and subnational viral load suppression. An example was presented for Malawi, where the estimated HIV prevalence is 10.6% and incidence 0.77%, and the relative standard error is <30% (95% CI: 0.28–1.25%). This would require 15 000 households and 19 850 adults (aged 15–49 years) to participate. For the Democratic Republic of the Congo, the estimated prevalence is 1% and incidence 0.1%, and the relative standard error is <30%; here, the estimated sample size required was 1–2 million adults, which was not considered feasible.

It was concluded that HIV incidence estimates can achieve reasonable precision at the national level, but may not be feasible at subnational and subpopulation levels, especially in lower prevalence countries. Also, HIAs will only be able to detect relatively large changes in incidence over time (e.g. 50%). However, measuring the changes in incidence is not the main objective; instead, what matters is to assess progress towards the UNAIDS 90–90–90 targets and continuum of care.

5.2 Sample size tools for powering population-based surveys

The TPP for inclusion of incidence assays in population-based surveillance was shown (11), illustrating the ideal and acceptable performance characteristics for:
• FRR (acceptable <2% across populations, clades, epidemic phases and ART coverage)
• MDRI (acceptable 120 days, ideal 365 days)
• the algorithm (acceptable: included in a RITA, ideally none required)
• the sample type (acceptable: frozen serum or plasma, ideal: frozen serum or plasma or DBS).

Data were presented from the CEPHIA publication (3) showing the MDRIs for the five evaluated assays, ranging from 188 days for the LAg (1.5 OD-n) to 333 days for the Bio-Rad Avidity (40% AI). The FRR for these assays ranged from 1.3% for the LAg to 9.7% for the LS-Vitros. The FRR for treated patients was high for all assays, ranging from 50% for the Bio-Rad Avidity to 76% for the LS-Vitros (3). The MDRIs and FRRs varied by subtype for all assays, and were highest for A1 and D specimens (e.g. for LAg, the FRR for subtype A was 2.7% and for subtype D 9.1%). The CEPHIA evaluations showed that no single assay was close to the TPP in populations including a significant proportion on ART. However, an FRR of 0.8% could be obtained using the LAg assay in combination with viral load data (>1000 copies/mL), a post-infection cut-off time T=2 years and an MDRI of approximately 200 days. In this case, 30% of patients were assumed to be on ART.

The practical implications of assay characteristics on the sample sizes needed to reliably detect a decrease in incidence over two consecutive surveys were demonstrated. The sample size needed is influenced by the MDRI and FRR, baseline prevalence and incidence, variability of assay performance and desired statistical power. Data presented showed that, in high incidence and prevalence settings, relatively small sample sizes had a high probability of inferring a reduction in incidence. In Lesotho, for example, the estimated incidence was 4% and prevalence 25%, which required a sample size of 2500 to obtain 80% probability of correctly inferring a reduction in incidence. In Kenya, where the incidence was assumed to be 0.5% and the prevalence 5%, a sample size of 20 000 was required to reach the same probability.

Further examples were presented examining required sample sizes for either a 30% or 50% reduction in incidence using an optimistic assay with T=2, an MDRI of 365 days, an FRR of 1%, 80% power and 10% significance. A sample size cap of 30 000 was considered feasible, which excluded numerous countries to observe either a 30% or 50% reduction (including Chad, DRC, Côte d’Ivoire, Haiti, Rwanda and Togo), and where in some instances sample sizes of over 100 000 were necessary.

Using more realistic parameter values with an MDRI of 200 days, many more countries surpassed the 30 000 threshold, leaving only Lesotho, South Africa and Swaziland below this threshold. In addition, the prevalence of subtypes varies by country. It is therefore unlikely that a 1% FRR is attainable in some of these settings. An online tool has been developed by SACEMA to calculate the power to detect a difference in incidence based on the parameters T, FRR, reference incidence and prevalence estimates, and the expected reduction. However, these do not account for the design effect of multistage clustering and uncertainty around the FRR. This needs to be further explored by statisticians.

Issues regarding the measurement of viral load from DBS specimens were also considered in this session. Using an extraction method (e.g. bioMeriuex, Roche or in-house) that extracts both RNA and DNA may lead to a signal or copy number reading for both combined. This can be avoided using a detection method that is selective for RNA only. There are published studies reporting the indicated category of specificity and sensitivity with DBS compared to plasma using a viral load threshold of 1000 copies/mL and various extraction systems (12). This is particularly a problem when persons are on ART and have low viral load.

The discussion on sample sizes was considered to be key. It was proposed this should form part of the guidelines so that intervention groups understand what is needed to observe an impact of efforts.

5.3 Recommendations for handling design effects in complex surveys

Issues concerning the use of complex surveys to estimate incidence with biomarker data were presented, including finding an expression for the relative standard error or coefficient of variation (CoV) and estimating the design effect (DEFF). The effect this has on survey planning and sampling considerations was discussed, together with ways to improve efficiency.

The formula to estimate HIV incidence in cross-sectional studies published by WHO (3) was briefly shown; it incorporates the number of negative tests, number recently infected, FRR and MDRI. This was re-parameterized to a function of four independent random variables for which the variances were derived using delta methods (first order Taylor series expansion). This generated new parameters (covariances) not present in simple random sampling.

HIV incidence and the CoV in complex surveys were calculated using a statistics package (SAS) macro, which included the

6 www.incidence-estimation.com/page/tools
mean window period, FRR, number of negative tests, number of positive tests, number of recent cases, variance of the number of negative tests, variance of the number of tests for recent infection, covariance of the number of tests for recent infection and the number of negative tests, variance of MDRI, and variance of the FRR. Estimates of CoV and DEFF were shown on hypothetical data: first, using multinomial variance and covariance, which, as expected, resulted in the same CoV as for simple random sampling (DEFF=1); and second, inflating the variances and covariances, which increased the overall CoV and DEFF. Examples were shown using KAIS 2007 data with LAg and BED assays; CoVs between complex and simple random sampling survey designs were compared, showing higher CoVs for surveys than simple random sampling (e.g. For LAg, estimated incidence was 1.2%, CoV for complex survey design 21.2 and simple random sampling 15.1, Generating a DEFF of 2.1; For BED, incidence was 1.9%, and CoVs were 14.7 and 12.2 For the complex and simple random sampling survey designs, respectively, generating a DEFF of 1.42). Of note was that the DEFF for BED was lower than for LAg. These estimates and the relationship between the covariances and the DEFF may be useful for planning impact assessment surveys.

The different MDRIs and FRRs were shown for varying AI thresholds alongside the CoVs for the MDRIs and FRRs. The recency threshold and MDRI had a linear relationship ($R^2=0.988$) Suggesting the FRR for a 1.5 OD-n FRR to be 1.3%, Which agrees with the latest estimate from SACEMA.

Survey efficiency was measured in terms of the required sample size at various MDRIs. Using data from Zimbabwe, for LAg, the required sample size was $\sim5500$ for an MDRI of 130 days, corresponding to a 1.5 OD-n cut-off. Increasing the cut-off and consequently the MDRI reduced sample size needs. The optimal MDRI was considered to be 216 days, corresponding to a 2.3 OD-n cut-off, after which the effect of a reduction in sample size levelled off. This reduced the sample size need by 25% ($n=4000$) compared to using a 1.5 Cut-off. Similarly, using data from Malawi, there was a considerable gain in a reduction in the sample size needed using a higher cut-off; the optimal MDRI here was considered to be 219 days, requiring a sample size of 4351, which was a 28% reduction compared the 1.5 Cut-off (sample size $n=6200$). It was concluded that gains in efficiency could be achieved free of cost by considering the variation in needed sample sizes at different recency thresholds of the assays.

5.4 Incorporating results of HIV incidence surveys into HIV national estimates: integration and implications

UNAIDS supports over 150 countries globally to develop annual estimates and projections of the HIV epidemic over time. Most countries produce estimates using the UNAIDS-supported modelling software tool, Spectrum, and a component model, EPP, developed by Futures Group and the East-West Centre. Country estimates are validated by UNAIDS, WHO and UNICEF, and published in various media, including Global Reports.

An overview of the modelling software was given, showing how countries input available HIV surveillance and population survey data. Most countries with generalized epidemics obtain HIV prevalence estimates from pregnant women attending antenatal care (ANC) clinics. Where national, population-based surveys have been conducted, HIV prevalence estimates may be obtained from these. In countries with non-generalized epidemics, estimates of HIV prevalence among key population groups can be used. The EPP fits possible prevalence curves to the surveillance and survey data using one of three models selected by the user (EPP classic, R-trend or R-spline). Recommendations on which model to use have been made by UNAIDS; they depend on the type of data available and historical trends. Annual Bayesian medians are selected from the curves to produce trends in prevalence. Incidence is estimated similarly, taking into account treatment levels and AIDS-related mortality. The curves are subsequently adjusted, based on biases in the data, such as differences in ANC and population prevalence. UNAIDS is currently proposing changes to the Spectrum/EPP tool to incorporate HIV/AIDS case-reporting, mortality and assay-based incidence data into the curve-fitting process.

The impact of incidence assay data within the EPP framework on prevalence and incidence estimates and changes of these over time under different scenarios was presented. The EPP model produces for any given time T:

- $y(t)$, the size of the infected population
- $Z(t)$, the size of the uninfected population, and consequently
- $p(t)$, the prevalence rate
- $I(t)$, the infection rate.

New inputs into EPP related to the inclusion of incidence in the model include:

- $N_s(t)$, the number of HIV-negative individuals
- $N_g(t)$, the number of persons with recently acquired HIV
- $N_{nr}(t)$, the number of non-recently infected HIV-positive individuals.

The approach considers the sampling scheme of incidence testing to be independent of other data (e.g. ANC); thus, these can be added as an additional part of the likelihood function.
Following the work of the SACEMA group, $N_S(t)$, $N_R(t)$ and $N_{NS}(t)$ were assumed to follow a trinomial distribution. The log-likelihood of these was derived, incorporating the FRR and the MDRI, using $T=450$ days, and added to the original EPP log-likelihood function. Synthetic data were used from three countries to examine the impact of including assay data varying the sample size, FRR, MDRI and $R$, the ratio between the incidence rate simulated from incidence assays and the incidence rate estimated by the EPP/Spectrum without assay data. Posterior outputs of the simulations showed the disparity of estimates with and without the incidence data. Results simulated for 2015 on data from rural Kenya varying the sample size from 5000 to 50 000 showed prevalence rates remained stable with increasing sample size, and credible intervals became narrower with the addition of the assay data. In contrast, incidence estimates increased slightly with increasing sample size. The probability of observing lower rates including assay data was stable between 0.3 and 0.35 for HIV prevalence, and decreased from 0.25 to 0.05 for incidence with increasing sample size. Variation of $R$ from 0 to 5 showed a slight increase in prevalence estimates with increase in $R$. For incidence, as expected, there was an increase in estimates with increase in $R$. The probability of lower estimates with assay data was 0 at $R=3$. Varying the FRR from 0.5% to 5% showed no change in prevalence, whereas incidence decreased slightly. Similarly, for changes in the MDRI from 90 to 190 days, no changes were observed for prevalence, whereas incidence increased marginally.

Further simulations were undertaken using incidence assay data for estimates in 2012; here, the focus was to compare the probability that prevalence and incidence rates were lower in 2007 than in 2012 for EPPs with and without assay data. The sample size was varied from 5000 to 50 000 and $R$ from 0.5 to 5. Unlike the simulation for 2015, outputs of the model showed prevalence estimates changed with sample size; by increasing the sample size to 45 000, the probability that 2007 prevalence estimates were lower than 2012 was close to 1. Similar trends were observed for the incidence estimates. Prevalence and incidence rates increased with increasing $R$. However, these trends were not true for all countries; data from South Africa showed a slight upward trend in the probability of prevalence estimates being lower in 2007 than in 2012 with increasing sample size but a stable trend in incidence. Similarly, with variation of $R$, this also increased for prevalence estimates and remained stable for incidence.

Running the EPP model under different scenarios is time consuming, and other means to find a reasonable approximation for including incidence assay data were suggested. It was questioned whether the methods to estimate incidence were independent of previous EPP estimates and whether, if there was a large discrepancy, the data could be combined. It was stated that discrepant estimates were to be expected to some extent because one source of data is a cross-sectional survey and the other is estimating incidence from prevalence using time trends. The ultimate aim is to incorporate the assay data into the fitting process and for estimates to be triangulated.
**6. COUNTRY EXPERIENCE IN THE USE OF HIV INCIDENCE ASSAYS**

**6.1 Kenya AIDS Indicator Survey (KAIS) and HIV incidence, 2012**

The use of LAg in KAIS was presented. The KAIS methods were presented previously (see Section 5.1); KAIS is a national, population-based, cross-sectional household survey with a two-stage cluster sample design. The study population includes men, women and children aged 18 months to 64 years in selected households. Informed consent was obtained for interviews and blood draw for home-based testing and counselling, and for laboratory testing for HIV, CD4 cell count, viral load and HIV incidence. Only specimens from persons aged 15–64 years with laboratory-confirmed HIV infection were tested for incident infection; testing was done at the National HIV Reference Laboratory in Nairobi using LAg on DBS specimens retrieved from storage (–80 °C). The data were analysed using the following four case definitions:

i. recent with LAg and ART naive (based on self-report);

ii. recent with LAg, ART naive and not virally suppressed (>1000 copies/mL);

iii. recent with LAg and not virally suppressed; and

iv. recent with LAg, not virally suppressed and ART naive (two persons excluded for whom viral load data were missing).

The CDC’s HIV incidence calculator (v.2.7) Was used, applying the 2013 WHO-recommended formula for estimating incidence in cross-sectional studies (where T=1). Confidence intervals were derived using a delta method approximation, which included the error associated with the calibration parameters (FRR and MDRI). Estimates were weighted to account for study design and non-response.

An FRR was calculated among a subsample of persons infected >1 year previously (n=234) – defined as those WHO reported to have had a positive diagnosis >1 year earlier (n=131) or history of ART (n=103) – and was weighted to account for survey design and non-response. With LAg only, the FRR was 17.6% (95% CI: 11.6–23.6%).

Using the recent case definitions (numbered i–iv) above:

i. Of 569 specimens tested, 72 were recent, of which 37 were on ART, reducing the number of recent cases by 51% to 35 (Fig. 1).

**Fig. 1 KAIS 2012 incidence algorithm – recent case definition 1 (LAg+ART)**

- Number HIV+ specimens tested with LAg: 569
- Number testing recent with LAg: 72
- 51% reduction in recent cases by excluding persons on ART
- Number on ART = 37
- Number meeting recent infection case definition: 35
ii. An additional 12 cases were reclassified based on viral load <1000 copies/mL reducing recent cases by 68% to 23 (Fig. 2).

Fig. 2 KAIS 2012 incidence algorithm – recent case definition 2 (LAg+ART+VL)

Number HIV+ specimens tested with LAg: 569

Number testing recent with LAg: 72

68% reduction in recent cases by excluding persons on ART and virally suppressed

Number on ART = 37

Number not on ART with VL <1000 = 12

Number meeting recent infection case definition: 35

iii. Recent cases were reduced by 61% (n=43, viral load data missing for two) to 27 (Fig. 3).

Fig. 3 KAIS 2012 incidence algorithm – recent case definition 2 (LAg+VL)

Number HIV+ specimens tested with LAg: 569

Number testing recent with LAg: 72

61% reduction in recent cases by excluding persons who are virally suppressed

Number with missing VL = 2

Number with VL <1000 = 43

Number meeting recent infection case definition: 27

iv. Recent cases were reduced by 69% (viral load missing for two), resulting in 22 recent cases (Fig. 4).

Fig. 4 KAIS 2012 incidence algorithm – recent case definition 2 (LAg+VL+ART)

Number HIV+ specimens tested with LAg: 569

Number testing recent with LAg: 72

69% reduction in recent cases by excluding cases that were virally suppressed and on ART

Number with missing VL = 2

Number with VL <1000 = 43

Number with VL ≥1000 and on ART = 5

Number meeting recent infection case definition: 27

The following FRRs and incidence estimates for varying case definitions were shown, also as numbered i–iv above:

i. 37/40 False recent cases were reclassified because on ART, resulting in a weighted FRR of 1.47% (3/234, 95% CI: 0–3.2%). Incidence was estimated to be 0.87% (95% CI: 0.44–1.31%) (Fig. 5).
Number known long-term HIV+ specimens tested with LAg: 234

Number testing recent with LAg: 40

Number on ART = 37

Number meeting recent infection case definition: 3

Weighted FRR: $3/234 = 1.47\%$ (95% CI: 0–3.21%)

ii. 39/40 False recent cases reclassified (additional two due to viral load <1000 copies/mL) resulting in a weighted FRR of 0.61% (1/234, 95% CI: 0–1.81%). Incidence was estimated to be 0.61% (95% CI: 0.29–0.92%) (Fig. 6).

Number known long-term HIV+ specimens tested with LAg: 234

Number testing recent with LAg: 40

Number meeting recent infection case definition: 6

Weighted FRR: $6/234 = 2.39\%$ (95% CI: 0.44–4.35)

iii 34/40 False recent cases were reclassified resulting in a weighted FRR of 2.39% (95% CI: 0.44–4.35%). Incidence was estimated to be 0.45% (95% CI: 0–0.90%) (Fig. 7).

Number known long-term HIV+ specimens tested with LAg: 234

Number testing recent with LAg: 40

Number on ART = 37

Number not on ART and with VL $\geqslant$ 1000 = 2

Number meeting recent infection case definition: 1

Weighted FRR: $1/234 = 1/234 = 0.61\%$ (95% CI: 0–1.81%)

Number with VL <1000 = 34

iv. 39/40 False recent cases were reclassified, 34 with viral load <1000 copies/mL and five additional on ART, resulting in a weighted FRR of 0.61% (1/234, 95% CI: 0.0–1.81%). Incidence was estimated to be 0.59% (95% CI: 0.28–0.90%) (Fig. 8).

Number known long-term HIV+ specimens tested with LAg: 234

Number testing recent with LAg: 40

Number meeting recent infection case definition: 6

Weighted FRR: $6/234 = 2.39\%$ (95% CI: 0.44–4.35)
For comparison, modelled incidence using Spectrum among persons aged 15–49 years was 0.44% (95% CI: 0.34–0.56%).

Using recent case definition iv, estimated HIV prevalence was 5.6%, ART coverage 63%, 75.4% were virally suppressed and the transmission risk (defined as [HIV incidence/HIV prevalence] * 100) was 10%. This compares to a transmission risk of 4% in the USA in 2010.

In conclusion, case definitions ii and iv generated similar results for FRR and incidence. The incidence estimates were considered plausible at 10% of prevalence and within the range of the modelled estimate using Spectrum. It was reiterated that, despite the large sample sizes of population-based surveys, reliable measures of incidence in subpopulations were challenging to obtain in settings with moderate HIV prevalence, such as are found in Kenya. Of note is that the addition of viral load to the algorithm reduced the number of false recent cases by 87%; however, 13% of persons not virally suppressed were on ART. A limitation of this study was that ART use was self-reported; 30% of HIV-positive persons aware of their infection reported no ART history despite being virally suppressed, suggesting that some were on ART but reported otherwise.

6.2 HIV incidence testing, South Africa, 2012

In 2012, a National HIV Household Survey was undertaken in South Africa among persons of all ages; 38,431 persons were interviewed and 28,997 tested for HIV. DBS specimens were tested for HIV antibody, and polymerase chain reaction (PCR) was used for infants. HIV-positive specimens among persons aged >2 years were tested for recent infection. Testing for antiretroviral drugs (ARVs) included all age groups. The HIV testing algorithm incorporated the Vironostika HIV Uni-Form II Plus O, Advia Centaur Xp and Roche Elecs 2010 HIV Combi assays. All specimens were tested with the first two assays; discrepant specimens were tested with the third assay and classified according to the result of that assay. High performance liquid chromatography (HPCL) coupled Tandem Mass Spectrometry for qualitative determination of ARVs (NRTI, NNRTI and PI) was used to test for ARVs. In 2012, prevalent ARVs were Atazanavir, Darunavir, Efavirenz, Lopinavir, Nevirapine and Zidovudine.

Results from the survey indicated that there were 6,422,000 people living with HIV of which 2,002,000, 31.2% (95% CI 28.1%-34.5%), were on antiretroviral treatment (ART). ART exposure was higher among women, 34.7% (95% CI 31.4-38.2%), than among men, 25.7% (21.2%-30.8%). HIV prevalence varied over the surveys conducted in 2002, 2005, 2008 and 2012; among persons aged 15-24 years, the prevalence increased from 9.3% in 2002 to 10.3% in 2005 and decreased to 7.1% in 2012. Among adults (>25years) HIV prevalence increased from 15.5% to 19.9% over this period.

HIV incidence was measured in two ways, firstly directly using an incidence testing algorithm, and secondly, indirectly using mathematical models (EPP/Spectrum; Thembisa) and the synthetic cohort approach with prevalence data collected from serial population-based surveys. The RITA used the LAg assay (cut-off 1.5, MDRI 130 days, optimised for DBS, Maxim Biomedical Inc., Rockville MD, USA), reclassifying specimens as long-standing if positive on ARV testing (Phenomonex Lunda 5 PFP column; API 4000 tandem mass spectrometer in the Multiple Reaction Monitoring (MRM) detection mode) or viral loads <1000 copies/mL (tested with Abbott m2000 HIV Real-Time System, Abbott Molecular Inc, Des Plaines, IL, USA.). An FRR of 0% was applied. Of 2758 persons HIV positive aged >2 years, 195 were recent on the LAg assay of which 96 tested positive for ARVs. An additional 10 had viral loads <1000 copies/mL resulting in 89 classified as recently infected. HIV incidence rates in the population 2 years and older were estimated to be 1.07% (95% CI: 0.87%-1.27%) overall and higher in women (1.46%, 95% CI: 1.18%-1.84%) than in men (0.71%, 95% CI: 0.57%-0.85%). (Table 3) resulting in 89 classified as recently infected. HIV incidence rates were estimated to be 1.07% (95% CI: 0.87–1.27%) overall and higher in women (1.46%, 95% CI: 1.18–1.84%) than in men for all age groups (0.71%, 95% CI: 0.57–0.85%) (Table 3).
### Table 3. HIV incidence rates by age group, South Africa, 2012

<table>
<thead>
<tr>
<th>Age groups</th>
<th>HIV incidence % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age ≥2 years</strong></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.07 (0.87–1.27)</td>
</tr>
<tr>
<td>Male</td>
<td>0.71 (0.57–0.85)</td>
</tr>
<tr>
<td>Female</td>
<td>1.46 (1.18–1.84)</td>
</tr>
<tr>
<td><strong>Age 15–24 years</strong></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.49 (1.21–1.88)</td>
</tr>
<tr>
<td>Male</td>
<td>0.55 (0.45–0.65)</td>
</tr>
<tr>
<td>Female</td>
<td>2.54 (2.04–3.04)</td>
</tr>
<tr>
<td><strong>Age 15–49 years</strong></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.72 (1.38–2.06)</td>
</tr>
<tr>
<td>Male</td>
<td>1.21 (0.97–1.45)</td>
</tr>
<tr>
<td>Female</td>
<td>2.28 (1.84–2.74)</td>
</tr>
</tbody>
</table>

HIV incidence was higher among single than married persons, higher among those reporting >1 partner than those reporting one partner, and highest among black-African females aged 20–34 years (Table 4).

### Table 4. HIV incidence rates by behavioural and socio-demographic variables, South Africa, 2012

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV incidence % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Marital status (15–49 years)</strong></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>0.55 (0.45–0.65)</td>
</tr>
<tr>
<td>Single</td>
<td>2.28 (1.82–2.74)</td>
</tr>
<tr>
<td><strong>Number of sexual partners in the past 12 months (15–49 years)</strong></td>
<td></td>
</tr>
<tr>
<td>One partner</td>
<td>1.67 (1.33–2.01)</td>
</tr>
<tr>
<td>More than one partner</td>
<td>2.43 (1.95–2.91)</td>
</tr>
<tr>
<td><strong>Selected at-risk populations</strong></td>
<td></td>
</tr>
<tr>
<td>Females 15–24 years</td>
<td>2.54 (2.02–3.04)</td>
</tr>
<tr>
<td>Sexually active females 15–24 years</td>
<td>3.53 (2.83–4.23)</td>
</tr>
<tr>
<td>Black-African females 20–34 years</td>
<td>4.54 (3.64–5.44)</td>
</tr>
</tbody>
</table>
The HIV incidence estimate for the 15-49 age group was compared with outputs from mathematical models which showed it was slightly higher than the estimate obtained using Spectrum and lower than that using the synthetic cohort approach (Table 5).

### Table 5. Comparison of incidence estimates using the LAg assay and mathematical models

<table>
<thead>
<tr>
<th>Method</th>
<th>HIV incidence (95% CI) (15–49 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAg/ARV/VL</td>
<td>1.72 (1.38–2.06)</td>
</tr>
<tr>
<td>Synthetic cohort</td>
<td>1.9 (0.8–3.1)</td>
</tr>
<tr>
<td>EPP/Spectrum</td>
<td>1.52 (1.43–1.62)</td>
</tr>
</tbody>
</table>

HIV incidence estimates were further examined by the different components of the algorithm; rates were estimated for LAg+ARV (1.98%), LAg+VL (2.24%) and LAg only (3.58%). A correction factor was calculated for each of the algorithm components to attain the value of 1.72% (Table 6). It was highlighted that if recent infection had been classified using only the assay (here, the LAg), the correction factor would have been 3.1%. It was recommended that LAg not be used in a single assay format.

### Table 6. HIV incidence and FRR by testing component, South Africa, 2012

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>HIV incidence (15–49 years)</th>
<th>FRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAg/ARV/VL</td>
<td>1.72%</td>
<td>–</td>
</tr>
<tr>
<td>LAg/ARV</td>
<td>1.98%</td>
<td>0.40%*</td>
</tr>
<tr>
<td>LAg/VL</td>
<td>2.24%</td>
<td>0.86%*</td>
</tr>
<tr>
<td>LAg only</td>
<td>3.58%</td>
<td>3.10%*</td>
</tr>
</tbody>
</table>

*FRR to reproduce LAg/arv/vl incidence estimates

In summary, the addition of ARV and viral load data reduced the number of LAg recent cases by 54.4%. Of note was that 15.7% of LAg+ARV specimens had a viral load >1000 copies/mL, and HIV incidence among persons aged 15–49 years was 9.1% of the HIV prevalence. The incidence estimates were highly sensitive to the MDRI: using an MDRI of 85 days (9) would have increased the estimate to 2.63%; Increasing it to 177 days and applying a 1.3% FRR would have reduced it to 0.66% (CEPHIA evaluation for subtype C) (1). Issues to be considered were whether all ARV+ specimens were long-term infections; for example, there may be recent seroconverters among pregnant women entering prevention of mother to child transmission (PMTCT) programmes or persons on pre- or post-exposure prophylaxis (PrEP or PEP). In addition, it has not been well established what proportion of recent infections may be ARV negative and have a viral load <1000 copies/mL (elite controllers). Data from SACEMA indicated this could be up to 11%. Lastly, this approach misses true recent cases because the testing algorithm is antibody-based, and is therefore unable to detect those HIV RNA who are antigen positive and antibody negative. Data from Swaziland were quoted, where this situation was found to apply to 0.1% of all persons tested.

### 6.3 Methods and tools to estimate HIV incidence in Catalonia

The application of tests for recent infection to estimate HIV incidence in Catalonia was presented, alongside an assessment of whether it was a worthwhile undertaking for this region of Spain. In 2012, the HIV prevalence rate in Catalonia was 0.46%, with 33,000 estimated to be living with HIV. Each year, 700–800 new HIV cases are notified to the register. Since 1993, the number of HIV tests has increased every year, reaching 225,000 tests in hospitals and 100,000 in primary care in 2011. In recent years, rapid testing in community-based centres has been introduced, with the tests being confirmed in a hospital setting.
increased the testing rate per population from 5 per 1000 in 1993 to 45 per 1000 in 2011.

The approaches available to estimate HIV incidence in Catalonia are to measure it in a cohort of HIV-negative men who have sex with men (MSM) recruited from testing and counselling centres (the ITACA cohort); to measure it indirectly through modelling using Spectrum and prevalence estimates from survey data; and to use HIV Incidence Assays. HIV incidence estimates from the ITACA cohort of 2200 HIV-negative MSM over 3500 person years with 85 seroconversions were estimated to be 3.84% between 2010 and 2011. This was an increase on 2.41% estimated between 2008 and 2009. The estimate for all persons aged 15–49 years in Catalonia using Spectrum was 0.02%.

In 2001, the feasibility of introducing incidence testing in Catalonia was assessed. Financial support was granted in 2002 and the study was implemented between 2003 and 2005. In 2006, tris were incorporated into routine HIV surveillance within the voluntary sentinel surveillance system, which now covers 50% of all new diagnoses. Samples were collected from 11 hospitals and six voluntary counselling and testing (VCT) services, and laboratories that volunteered to participate. Inclusion criteria were for patients to be newly diagnosed, be aged >16 years and have provided written informed consent, and for specimens to have been obtained within 6 months of the diagnosis. Specimens were excluded if there was insufficient sample volume, there were duplicates, or the infection was HIV-2. The residual diagnostic specimens were sent to a central laboratory where aliquots were identified by a unique study number. The Vironostika-LS (MDRI 170 days) assay was used from January 2003 to May 2007, and the BED-CEIA (MDRI 155 days) from 2007 onwards. The WHO-recommended algorithm was applied; recent cases were reclassified if the CD4 count was <200 cells/mm$^3$, there was evidence of ARV use or the person had symptoms of AIDS. The data were collected in two stages. First, laboratories identified newly diagnosed individuals and completed information on CD4 counts, viral load and the most recent serological test result to identify if persons had been diagnosed previously. Second, data on demographic and epidemiological information were collected by a physician. Information on testing practices was obtained through an enhanced surveillance questionnaire, to collect the date of the last negative HIV test. The proportion of recent infection was 25% in 2008 and 30% in 2009. The proportions were higher among MSM (28%) than heterosexuals (15%), and higher among those aged <30 years (27%) than among those aged >50 years (7%).

Currently, testing for recent infection has been discontinued. Data protection laws have changed since initiation of the programme, and the consent form is currently undergoing a new approval process. In addition, the complicated data collection pathways and difficulty in obtaining testing history data were barriers. It was questioned whether TRIs are worthwhile in this small population with a highly concentrated epidemic and whether there is value in other incidence estimation approaches.

### 6.4 USA country update: bridging USA HIV incidence surveillance to a new recency assay

The USA domestic HIV surveillance system was presented, along with work currently undertaken to bridge BED and Bio-Rad Avidity assay data. The USA HIV incidence surveillance system is a component of the National HIV Surveillance System, which is case-based and collects HIV testing, treatment and recency data. Currently, three reports have been published on HIV incidence in the USA using the BED assay (13-15). Data requirements for HIV incidence surveillance are case reports of HIV diagnoses including demographic data, risk information and clinical or laboratory information (e.g. CD4 counts, viral loads), testing (including date of the last negative and first positive HIV test, and testing frequency) and ARV data, and HIV incidence result from the diagnostic specimen or other related test within 3 months of diagnosis.

Bio-Rad Avidity was selected for recency determination based on performance characteristics and implementation factors; using an FDA-approved test modified for determining recent infection provided consistent availability and uniformity of reagents. In the USA, testing is conducted in different laboratories, including private and public hospitals; hence, transitioning to Bio-Rad Avidity is a huge logistical effort. Currently, remnant HIV diagnostic specimens of persons diagnosed from 2014 onwards are being tested with Bio-Rad at a central laboratory. Laboratory personnel were trained on performing Bio-Rad Avidity tests and passed proficiency testing, and to date no issues have been identified. It has been challenging for the laboratory to conduct testing with both assays (i.e. Using the BED assay for cases diagnosed in 2013 and earlier). From January 2015, only Bio-Rad Avidity will be used.

A study determining the effect of assay change on incidence estimates was presented. Remnant specimens of adults and adolescents diagnosed with HIV in 2010 with available BED results were tested with LAg and Bio-Rad Avidity. HIV incidence estimates were compared for each of these, using the sample survey approach (16). Of 12 893 persons diagnosed in 2010 with available BED results, 42% (5423) had sufficient specimen for testing with LAg and Bio-Rad. HIV incidence calculations are currently underway.
In parallel, CDC consulted experts to re-examine the sample survey method for estimating incidence—reviewing mathematical assumptions and other sources of potential bias, and the ability to monitor trends. Briefly, the current estimation approach stratifies population-based incidence by sex, age group, ethnicity, transmission category and testing history. This is conducted in states funded for HIV incidence surveillance, and results are extrapolated to non-funded states based on the observed number of new HIV diagnoses (funded states represent ~75% of new diagnoses). The survey sample approach divides the number of recent infections by the probability of being detected as recent, and does this separately for new and repeat testers. Issues with the data are reporting delay (not all newly diagnosed cases are reported by the end of the reporting period), duplicates, missing data (not all are tested for recent infection and some have missing testing history information) and the limited coverage in states that conduct the surveillance.

The assumptions examined were:

- Data missing at random: the results suggested that missing data were dependent on many variables. Based on simulation, the multiple imputation model worked well. The effect of additional variables was explored, and the source of testing-history data and variables for jurisdictions with the largest number of cases were identified as having an effect. These will be included in the imputation model.

- Accuracy of testing information (T) (interval between the last negative and first positive HIV test; post-infection time cut-off): the observed and expected recent cases for different Ts were compared, and were considered to be similar. Expected numbers were based on an accurate T, a correct recency period distribution for BED, assuming the infection date is uniformly distributed in the interval (T).

- FRR of the BED assay: a significant proportion may be misclassified as recent when HIV is diagnosed at late stage but before AIDS diagnosis. This bias is reduced by identifying late-stage diagnoses and classifying these as long-standing infections, irrespective of their BED results. In addition, the BED window may vary by age and other variables, which could introduce bias in estimates for age-specific groups. However, any bias in the overall estimate was considered to be small.

- Distribution of HIV testing before AIDS diagnosis (the testing hazard) is constant: motivation for testing is hard to define and collect data on; however, available data suggest there is no significant effect on frequent testers, but some effect on first-time Testers, resulting in more BED recent cases.

- Sensitivity of changing the testing hazard can be modelled as a mixture of different testing patterns or by competing events: biases in estimating HIV incidence depend on model parameters, such as the shape and scale of the distribution. Limited data are available to determine the hazard function.

- Testing behaviour has not changed over several years: other sources of behavioural data were examined, such as information on ever tested and time from last negative to first positive test. The data showed a slight increase in the proportions tested.

In addition, quality issues around the performance of BED testing were investigated, with no problems identified or differences regarding the specimen source. With respect to reporting delay, all infections will eventually be diagnosed through testing or death. Based on this consultation, additional covariates will be included in the multiple imputation model.

Next steps are to update the 2007–2010 estimates and produce initial 2011–2012 estimates, to revise the MDRI estimation, and to finalize transitioning to the Bio-Rad Avidity assay and analyses of the bridging study.

### 6.5 Testing for recent infection as part of the national HIV Behavioural Surveillance System

The USA National HIV Behavioural System (NHBS) was described in conjunction with recency testing pilot studies among MSM in five Cities in 2011, and injecting drug users (IDUs) in 19 Cities in 2012. The NHBS was implemented in 2003 to monitor prevalence and trends in HIV infection, risk behaviours, HIV testing and the use of prevention services. It is conducted annually among MSM, IDUs and heterosexuals at increased risk of HIV infection, in turn for each risk group, in 20 metropolitan, statistical areas. Sampling methods differed for MSM and heterosexuals. For MSM, venue-based and time-space sampling was undertaken, recruiting participants from randomly selected venues. For IDUs and heterosexuals, respondent-driven sampling was conducted, identifying and recruiting initial ‘seeds’ and distribution of up to five coupons for peer recruitment. The target sample size was approximately 10 000 per cycle. Anonymous interviews, HIV testing and supplemental activities were undertaken to determine recent infections. HIV recency testing was performed with Bio-Rad Avidity, optimized for DBS.

Results from the MSM survey between 2008 and 2011 showed that the proportion tested for HIV in the previous 12 months had increased by 12%, as had the proportion on ART after adjusting for other factors. Recency testing
was undertaken in Baltimore, Denver, Los Angeles, Miami and Washington DC. All specimens were screened with a fourth-generation immunoassay, and reactive specimens were tested with the Bio-Rad Multispot. A nucleic acid amplification test (NAAT) was performed to resolve discordant results and to detect acute infection among all those screened negative. All infections classified as recent were tested for viral load; recent infections were defined as AI <30%, viral load >150 copies/mL and no reported ARV use. For risk factor analyses, recent and acute infections were combined, and established infections excluded. Overall, a total of 937 specimens were tested, of which 243 (26%) were HIV positive and 22 AI <30%. In addition, two acute infections were identified, one of which was serology negative. Among the 22 with AI <30%, nine reported use of ARVs and three had viral loads <150 copies/mL, leaving 10 classified as recent and two as acute infections, totalling 12 incident infections. The proportion with early HIV infection was found to be higher in MSM of black ethnicity (3.9%), and from Baltimore (3.5%) compared to other cities (1.0%). There was not much variation by age. Multivariate analyses adjusted for city, race, partner race, receptive sex and internet use found a black last partner (adjusted odds ratio [AOR] 4.6, 95% CI: 1.2–17.3), Receptive sex at last sex (AOR 4.3, 95% CI: 1.2–15.0) and having used the internet to find sex partners more than once a day (3.3, 1.1–9.7) Was significantly associated with increased relative risk of infection.

The protocol for recency testing using DBS was optimized in the laboratory that had matching plasma stored. The DBS involved a 50 µl spot of whole blood on Whatman cards dried overnight at ambient temperature in the presence of desiccants and humidity indicators in bags, and stored frozen. DBS and plasma specimens were tested according to the modified avidity-based protocol and analysed by linear regression of the AI. There was strong correlation between the AIs of both specimens ($R^2$=0.92) and discrimination of recent and long-term samples. However, this was conducted under optimal conditions; an additional 116 specimens from the field were compared, and showed a lower correlation than in the laboratory ($R^2$=0.84). The agreement varied by site. Suboptimal processing of specimens was identified, such as having left specimens to dry for longer periods.

DBS and plasma pairs (n=81) were also examined from three sites from the IDU survey round in 2012. Overall, there was good agreement ($R^2$=0.91); However, this also varied by site. One site that had made DBS from tubes had misclassified specimens. This may have been due to specimens being incorrectly handled at the site or being exposed to humidity, or the sample being from a person exposed to ARVs. The effect of ARV on assay performance may be greater using DBS because of dilution of the sample.

In the survey conducted among IDUs, HIV testing followed local algorithms, whereby screening was conducted with a rapid HIV test and reactive specimens tested with WB. DBS specimens were prepared in the field and sent to CDC for recency testing (Bio-Rad Avidity). Here, recent infection was defined as AI <30%, no reported use of ARVs, and the time since diagnosis being <9 months. Viral load data were not available. A total of 9557 participants were tested, of whom 901 (9%) were positive and 82 had AI <30%; 33 reported ARV use, leaving 45 classified as recently infected. The prevalence of recent infection was found to be lower than among MSM; however, numbers were small.

Limitations of the surveys are possible misclassification of those on ART, small sample sizes and limited generalizability (data were not weighted). However, recency testing allowed for the evaluation of factors related to incident infection. The next steps were to liaise with JHU to examine the potential for using DBS for ART and viral load testing, to estimate incidence rates using the Bio-Rad Avidity window period (as the correlation with DBS was good), and to strengthen DBS collection and specimen transport.

### 6.6 Estimating HIV incidence in the United Kingdom using biomarker data

An overview of work towards estimating HIV incidence in the United Kingdom was presented, including a description of the case-based surveillance system and RITA testing in the United Kingdom.

Three systems are used to ascertain the number of new HIV diagnoses:

- the HIV, AIDS and deaths case-reporting system, which receives two independent reports by clinics and laboratories for each person diagnosed with HIV or AIDS;
- an annual, cross-sectional survey of persons accessing HIV-related care, which collects information on viral load, treatment and CD4 count data; and
- a CD4 surveillance scheme that collects results of any CD4 count test nationwide and links these to reports of new HIV diagnoses.

Information on attendances at sexual health clinics is captured through a separate system referred to as the genitourinary medicine clinic activity dataset (GUMCAD), which collects patient-level data quarterly, including information on the number of sexually transmitted infection (STI) screens and diagnoses and socio-demographic data.

In the United Kingdom, the HIV epidemic affects mainly MSM and black-African heterosexuals. Latest data show...
there were 6000 new diagnoses in 2013, half of which were among MSM. Over the past decade, the number of new diagnoses has decreased, mainly due to a decrease in the number of diagnoses among persons born abroad.

Testing for recent infection with HIV has been conducted at PHE for over a decade, originally in sentinel sites using the BED assay. From 2009, the AxSym avidity assay was used, and testing was offered to clinics and hospitals throughout the country. Recency testing is undertaken centrally at PHE, where results are linked to reports of new HIV diagnoses. In 2013, approximately 50% of new HIV diagnoses were linked to a recent infection result. Coverage of testing was examined by demographic characteristics and geography, and was found to be similar across all groups. To date, the WHO algorithm has been used with recent cases defined as AI <80%, a CD4 cell count ≥200 cells/mm³, no ARV use or evidence of AIDS. Overall, 12–15% were classified as recently infected each year.

Two methods are being used to estimate HIV incidence: the cross-sectional method, which is applied to attendees of sexual health clinics, and the survey sample approach for the missing data and produce stratified incidence estimates. For the survey sample approach, PHE found low completion rates of testing history data. In 2013, a follow-up exercise was undertaken to collect these data from clinics submitting HIV diagnosis data. Data completion for other inputs of the model was relatively high and similar across groups. Once the algorithm and FRR were finalized, the next steps were to undertake the multiple imputation for the missing data and produce stratified incidence estimates.

Regarding the clinical utility of TRIs, the United Kingdom is the first country to feed back recency results to clinicians. These results are relayed to the patient at the clinician’s discretion. PHE is also currently assessing the utility of RITA as an ‘outbreak investigation’ tool. An enhanced behavioural surveillance pilot study is currently underway among MSM in London with evidence of recent infection.

6.7 Incidence and cascade of care: HIV impact in population surveys

Results were presented from the surveys in three countries conducted by Médecins Sans Frontières (MSF) to assess HIV incidence, prevalence and cascade of care in settings identified for intervention initiatives. Three cross-sectional surveys were undertaken in 2012 among persons aged 15–59 years using cluster sampling, targeting a random selection of houses in the districts KwaZulu-Natal (South Africa), Ndhiwa (Kenya) and Chiradzulu (Malawi). These areas have around 150 000 to 300 000 inhabitants. Methods included a questionnaire, HIV testing using a rapid finger prick test, blood draw, administration of a second questionnaire among positive persons for information on HIV and ART, point-of-care CD4 count testing in some settings, and (among negative specimens) testing for acute infection using NAAATs. Recency testing was conducted with two assays, LAg and Bio-Rad Avidity.

Overall, of the 21 782 eligible persons identified, 19 006 (87.5%) were included and tested for HIV, 6076/6823 (89%) in Kenya, 5649/6688 (85%) in South Africa and 7269/8271 (88%) in Malawi. In total, 4117 were HIV positive and 96% had information available on viral load and CD4 cell count. In all three countries, current national guidelines for the initiation of ART are CD4 <350 cells/mm³; the proportion eligible were 60%, 69% and 80% in Kenya, South Africa and Malawi, respectively.

In Ndhiwa in Kenya (subcounty of 172 000 inhabitants), the operational objectives of MSF were to decide whether to intervene, and to generate baseline data for future studies. The prevalence in this region was 24.1–19.8% Among men and 26.8% Among women. The care cascade showed that among diagnosed persons 40% of the population had viral loads <1000 copies/mL, indicating effective treatment. However, 40% of those positive (10% overall) were undiagnosed, indicating that HIV testing efforts could benefit from MSF support. Once diagnosed, linkage to and retention in care was high. Recent cases determined by the assay and acute cases were combined to obtain the number of incident cases. For the LAg assay, the same algorithm was used: OD-n 1.5, MDRI 130 days, no ARV use and viral load ≥300 copies/mL. As NAAAT testing was undertaken, an additional 28 days were added, resulting in an MDRI of 158 days. Of 6076 sample tested, 1457 were positive for HIV, of which 1357 had complete information; 31 were classified as recent by the algorithm and 11 were acute. The FRR (calculated among persons WHO reported a first positive test >1 year previously) was estimated to be 0.5% (95% CI: 0–1%). Estimated HIV incidence was 1.9% (95% CI: 1.1–2.7%). Overall, it was higher among women (2.5%), 95% CI: 1.4–3.6%) Than men (1.1%, 95% CI: 0.2–1.9%), and highest among those aged 15–29 years (2.0%, 95% CI: 1.1–2.9%).

In Malawi, one of the first countries in which MSF introduced a programme for HIV (in 1997), ART was available as early as 2001. No universal testing has been undertaken; however, in early 2013, 27 000 patients were on ART. HIV prevalence was found to be 17% (95% CI: 16.1–17.8%), and here also was higher among women (19.6%, 95% CI: 16.1–17.9%) Than men (13.2%, 95% CI:
12.0–14.4%). The cascade of care showed 80% of positive persons were diagnosed and 60% virally suppressed with viral loads <1000 copies/mL, which compares to care cascades in France and the United Kingdom, which have strong public health systems. Because a high proportion were on ART, incidence testing was only undertaken among those with viral load ≥300 copies and not on ART. The MDRI applied was 130 days; of 7274 tested 1224 were positive, 1184 had complete information and 13 were recent on the LAg. An FRR was not calculated for this population and was assumed to be 0.5%. HIV incidence was estimated to be 0.4% Per 100 person years.
7. NEW BIOMARKERS

7.1 Novel biomarkers and future directions for HIV incidence assay development

In 2010, a programme announcement was made by NIAID for research to develop new biomarkers for HIV Incidence Assays. This programme announcement was extended to 2015. A list of grants was presented and the following work streams discussed in detail:

- **Distinguishing chronic from recent HIV infection using genomic information.** This research uses a single genome sequence to distinguish recently HIV infected patients from those chronically infected. The whole genome is used and diversity markers developed to identify recent infections by measuring an ambiguity index (defined as the total number of bases that are ambiguous divided by the total number of bases that are sequenced). These studies are being conducted among 100 recent and 96 chronic patients; it was found that most recent patients could be distinguished with a sensitivity of 74.5% and specificity of 87.2%. This was improved by incorporating information on CD4 count. The sequences of those persons being false recent were investigated and comparisons made between subtypes. Panels from the CEPHIA group have also been requested to evaluate the assay.

- **HIV diversity as a biomarker using MAAs.** A group is developing assays that use HIV diversity as a biomarker in MAAs. MAAs are being validated using a higher resolution-melting (HRM) assay as a diversity assay. The principle underlying this is to isolate and reverse transcribe viral RNA using PCR, to tag the DNA with a fluorescent tag and to subsequently subject this to high temperatures to release the tag. The fluorescence is measured under a microscope at the temperature at which DNA melting begins. Smaller peaks are observed among DNA with fewer mutations, and wider peaks among those with chronic infection. This HRM technology was developed to detect new patients without sequencing the dna. to date, the laboratory has tested numerous cohort samples, and eight regions of the virus have been used to validate the MAAs. The differing patterns of HRM scores of patients with recent (<1 year) and chronic infection were shown (using clade B and C specimens), and were comparable across cohorts. For one of the maas, the CD4 cell count was replaced with the HRM score and similar results were found. The algorithm that included HRM had an MDRI of 154 days and an FRR of 0%. The incidence estimates were nearly identical to that of the cohort follow-up. While results were only for subtype B, it was concluded that the MAAs evaluated do not require CD4 cell count data; use less expensive high-throughput serological assays first, minimizing costs and efforts; and allow the process to be performed with stored serum or plasma samples. The MAAs with HRM have window periods comparable to MAAs that include CD4 count, and provide accurate incidence estimates in low, medium or high-incidence settings.

- **Deep sequencing among recent cases.** Deep sequencing is being performed among known recent cases to assess viral diversity; a meta-analysis on 182 incident and 43 chronic samples showed higher diversity among chronic subjects. The classification based on the virus sequencing platform showed 100% sensitivity and 96% specificity among 12 incident and 24 chronic cases in this study. There was no statistically significant difference in the biomarkers between 10 AIDS patients and 14 non-AIDS patients. The high-throughput platform for genomic incidence assays has been designed and the biomarker characterizing the proportion of closely related sequences is robust. Currently, further specimens are being sought from CDC and CEPHIA, including samples from drug resistant mutation screening.

- **Amplicot assay.** A brief update was given on the Amplicot assay which measures the diversity and complexity of the virus. It was developed in-house and uses both the HRM technique to study viral diversity and the Amplicot to sequence the HIV envelope. A bar-coding system allows sequencing of up to 96 specimens in a single run, and diversity and complexity analysis with an in-house developed analytical pipeline and bioinformatics. This has a large dynamic range and is likely to be less affected by multiple founder viruses.

Points of discussion were possible problems with ARV-treated patients WHO initiated treatment early – these individuals may have limited sequence diversity, despite having been infected for a long time, which would imply having a false recent result using this biomarker. Super-infection and re-infection are also issues.

It was highlighted that different use cases have potentially very different TPPs. TPPs for some use cases had been reached with LAg and viral load data for some settings. It is not clear which use cases the new biomarkers in development meet.
In addition, five grants are funding the following concepts:

- **Exploring micro-RNAs, which can control several downstream RNA and transcription cascades.** This is a non-hypothesis-driven, complete, high-throughput screening process, screening plasma for non-cell-associated micro-RNA. Preliminary data show an association with HIV and recent HIV infection. A proof of concept MDRI is expected by December 2015.

- **Gut inflammation biomarkers, and investigating plasma and stool specimens for HIV antigen specific immunoglobulin G (IGg) subclasses and RNA expression arrays.** It is known that HIV depletes gut-associated lymphoid tissue over the first year, the timing needed for an incidence marker. This is irreversible, and the issues seen with an FRR are not expected. A preliminary MDRI will be available in the first quarter of 2015.

- **Scanning of different antibody classes and association with specific antigens and cell-associated viral loads in different cell subtypes over time.** An association has been found in primate models. This will be confirmed in human specimens in the first quarter of 2015.

- **Metabolites of HIV recency.** Metabolites have mostly been associated with general nutritional deficiencies and intestinal health. Metabolites in urine and blood are being studied to examine whether an association with HIV exists (non-hypothesis-driven). A preliminary MDRI has been obtained within the TPP and an FRR of 3.7%. This will be completed by the end of 2014.

- **Shapoid libraries, which are peptoid mimetics currently used for cancer diagnostics and infectious diseases.** This is a method to screen antibodies, mapping temporal patterns. A preliminary MDRI will be known in the first quarter of 2015.

### 7.2 An update on the Bio-Plex multi-analyte assay

The CDC-developed Bio-Plex multi-analyte assay was presented, the principle of which is to establish the profile of antibody reactivity, and to measure antibody and Avidity in the same assay. A basic schematic of the assay was shown explaining the process for which proof of concept studies have been published (18).

The Bio-Plex multi-analyte assay was evaluated using a simulated population with a 1% HIV incidence, comparing incidence estimates generated using individual analytes. Several promising algorithms were assessed, which improved observed estimates that were slightly overestimated with the individual analytes. One four-analyte algorithm obtained an incidence of exactly 1%.

Testing was compared between participating laboratories to establish whether variation could lead to potential misclassification. Results showed that the results were consistent. In addition, a DBS protocol has been developed, and high correlation achieved between DBS and plasma specimens. Further work is being undertaken to develop the assay for non-B subtypes using new biomarkers. Results have shown potential, and the next steps are larger scale kit production and evaluation. Cost of the platform and ART use are potential issues.

### 7.3 Input and consensus on the technical update for the use of HIV Incidence Assays

There was consensus that the technical update on HIV Incidence Assays should recommend incorporation of viral load data into MDRI and LAg algorithms. The viral load threshold to use may be guided by the availability of information (e.g. Threshold for treatment failure 400 copies/mL). A low threshold is preferred because higher viral load thresholds result in lower MDRI; also, the low threshold has little impact on the magnitude of the FRR.

It was debated whether ARV testing of specimens should be included in the algorithm to reduce false recent cases. This was considered to be dependent on the prevalence of ARV use, and it may be appropriate for some surveys; however, it should not form part of the general document, because testing for ARVs is not yet a widespread technology. In addition, no data are available on MDRIIs incorporating persons on ARV not virally suppressed. A comment concerning these issues should be included in the update, informing readers that work is underway to collate and review evidence on the value of self-reported ARV use, and of the benefits and limitations of ARV testing.

One of the key recommendations agreed was to use 2 years for the post-infection time cut-off, T. Data from CEPHIA indicate T=2 years is appropriate, because it maximizes the MDRI while minimizing the FRR. There was consensus that 12 months is too short, and consequent FRRs too high.

The need for a protocol and validation of DBS specimens was raised. There has been some progress using LAg with DBS specimens, and one of the commercialized kits has a protocol for DBS. Studies are still underway and more data will be available in the near future. As viral load data are to be incorporated into algorithms, viral load testing will need to be validated to minimize the number of false positives from virally suppressed patients. WHO is currently evaluating platforms for viral load testing using DBS and will be able to provide more information shortly. It was suggested to include a comment with regard to assays potentially not distinguishing RNA from DNA when using DBS.
Lastly, the feasibility of measuring incidence and differences in incidence over time using population-based surveys was discussed. It was agreed that recommendations needed to be provided indicating which countries are likely to achieve required sample sizes to observe changes over time as this depends on the epidemic type and on the prevalence and expected incidence. Based on calculations by FIND, it was suggested a threshold be put forward by the SACEMA group. However, further work is needed to account for uncertainty around the FRR and the DEFF. Both the international branch of CDC and SACEMA are providing training, tools and technical assistance for incidence estimation.

7.4 Discussion on training results and needs based on the September SACEMA course

Two training courses have been hosted by SACEMA; one in May 2012, and another in September 2014, which was slightly oversubscribed. There was a difference in the groups that attended the first and second courses; attendees of the first course were mostly new to many of the concepts, and attendees of the second were more knowledgeable, many with specific work in progress. The course was considered a success and may be repeated in the future.

The content of the course was closely aligned to the electronic tools developed by SACEMA, using these with dummy data. It was felt participants benefited from the concentrated exposure to the complex material and from the many opportunities for free discussion and interaction.

PEPFAR-funded countries using the LAg assay will receive laboratory training from CDC. It was stated it would be beneficial to synchronize CDC and SACEMA training to ensure consistency, such as recommending and using the same tools and parameters.
8. DRAFT GUIDE FOR ESTIMATING HIV INCIDENCE USING HIV CASE SURVEILLANCE

8.1 Development of guidance for estimating HIV incidence in HIV case-based surveillance systems; general discussion and next steps for the surveillance guidelines

An overview of the development of guidance for estimating HIV incidence with RITA using case-based surveillance data was presented. This document provides information on how to incorporate recent infection testing into established systems, monitoring numbers of new infections at national or regional levels. The audience intended for this document are countries with comprehensive voluntary or compulsory reporting of new HIV diagnoses that could benefit from using RITA for incidence estimation.

The document is divided into three sections:

- describing the contextual difference between this approach and cross-sectional HIV incidence estimation methods, and other methods such as cohort studies, back calculations and projections;
- introducing the incidence estimation model using RITA and case-based surveillance data with detailed steps of the calculation methods; and
- describing what additional information is needed, such as HIV testing history.

The model to estimate incidence is described as a stratified extrapolation approach (15, 16) where the overall annual number of new infections (diagnosed and undiagnosed) is a function of the number of recent infection diagnoses, and the probability of being diagnosed and detected in the recent stage of infection. Key steps include the imputation of key missing data (e.g. RITA result, transmission risk group and other stratification variables), stratification, sample size consideration and the computation of sampling probabilities. It is based on a range of assumptions: data are missing at random; testing information is accurate; the duration of recent infection is well characterized; HIV incidence and testing behaviours are stable over the previous 2 years; for first time Testers, the likelihood to testing prior to AIDS is constant; and testing is uniformly distributed in the interval between the last negative test and diagnosis.

Considerations regarding RITA are highlighted and references made to CEPHIA publications, signposting towards the latest available information on assay characteristics and estimations of the MDRI and FRRs, and reducing misclassification with data on ART, viral load and AIDS diagnoses. Further, information is provided on the effect of sample size on the precision of estimates.

Members of the Working Group suggested having simulated results to see how sensitive incidence estimates are to missing data. The USA imputed a large proportion of the TRI results but had otherwise high data completion, as did France. It was proposed that surveillance systems be benchmarked prior to embarking on this. Countries with small numbers of new diagnoses will not be able to stratify estimates.

There was consensus that this document should continue to be developed, as case-reporting is becoming increasingly common in middle and lower income countries.
9. REVIEW OF 2013 MEETING REPORT RECOMMENDATIONS

9.1 Summary of the proposed areas for inclusion in the WHO/UNAIDS technical update, next steps and products

Proposed areas for inclusion in the WHO technical update are listed in Section 7.3.

The development of guidance for RITA using case-based surveillance will continue, and feedback is awaited on the circulated draft from other members of the Working Group. Once final, it will be shared with two countries to assess its usefulness and establish whether areas require more detail or clarity.

The draft report of the incidence meeting is to be shared by the first trimester of 2015 (2014) for review and feedback from the group. In addition, the technical report will be prepared by WHO/UNAIDS with input from CEPHIA, CDC and SACEMA.

The WHO/UNAIDS general population-based survey guidance will be revised to include a component on HIV incidence estimation. This section should be consistent with the technical update and the report, and will also be shared with the group for review and feedback.

A global HIV surveillance meeting will be held in May 2015 with the aim of setting the agenda for HIV surveillance in the context of increasing ARV treatment. As the end of AIDS is approaching, methods to measure epidemics will need to be technically grounded. The meeting will be held over four days in Bangkok.
REFERENCES


## ANNEX 1. MEETING PROGRAMME

**Day 1**  Monday, October 13, 2014  

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<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>8:00–9:00</td>
<td>Participant registration</td>
<td>Txema Calleja, WHO</td>
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<tr>
<td>9:00–9:15</td>
<td>Welcome remarks</td>
<td>Antoni Mateu, Public Health Secretary, Agencia de Salut Publica de Catalunya</td>
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<tr>
<td>9:15–9:45</td>
<td>Introduction, agenda, objectives and expected outcomes</td>
<td>Txema Calleja, WHO</td>
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### Session 2: HIV Incidence Assays and evaluations – presentation. Facilitator: Christine Rousseau, Gates Foundation

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<thead>
<tr>
<th>Time</th>
<th>Activity</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>9:45–10:15</td>
<td>Update on CEPHIA 1: Final objectives</td>
<td>Gary Murphy, CEPHIA</td>
</tr>
<tr>
<td>10:15–10:45</td>
<td>CEPHIA 2: Progress on new biomarkers</td>
<td>Chris Pilcher, SFGH</td>
</tr>
<tr>
<td>10:45–11:15</td>
<td>Review of CEPHIA results</td>
<td>Alex Welte, SACEMA</td>
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<tr>
<td>11:15–11:45</td>
<td>Coffee break</td>
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<tr>
<td>11:45–12:15</td>
<td>Incorporation of viral load into incidence assay results</td>
<td>Alex Welte, SACEMA</td>
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<tr>
<td>12:15–12:30</td>
<td>Comparison of CEPHIA/CDC and JHU/HPTN Bio-Rad Avidity</td>
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<tr>
<td>12:30–12:45</td>
<td>Development of incidence assay quality assurance programme</td>
<td>Mike Busch, BSRI</td>
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<tr>
<td>12:45–13:15</td>
<td>LAg and beyond: using assays to detect recent infection effectively</td>
<td>Bharat Parekh, CDC</td>
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<tr>
<td>13:30–14:30</td>
<td>Lunch</td>
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### Session 3: Obtaining consensus on RITA-specific statistical issues – panel discussion. Facilitator: David Burns

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<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>14:30–14:50</td>
<td>Implications of moving from a post-infection time cut-off (T) of 1 to 2 years</td>
<td>Alex Welte, SACEMA</td>
</tr>
<tr>
<td>14:50–15:10</td>
<td>Interpretation of recent infection for population-level incidence estimates versus individual diagnosis</td>
<td>Alex Welte, SACEMA, Rick Song, CDC, Anindya De, CDC</td>
</tr>
<tr>
<td>15:10–15:30</td>
<td>Consensus definition and methods for MDRI determination</td>
<td>Gary Murphy, CEPHIA, Mike Bush, BSRI, Bharat Parekh, CDC, Oliver Laeyendecker, JHU</td>
</tr>
<tr>
<td>15:30–16:00</td>
<td>Relevance of FRR studies in the context of increasing ART coverage</td>
<td>Gary Murphy, CEPHIA, Mike Nusch, BSRI, Bharat Parekh, CDC, Oliver Laeyendecker, JHU</td>
</tr>
<tr>
<td>16:00–16:30</td>
<td>Coffee break</td>
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### Day 2 | Tuesday, October 14, 2014

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<th>Session</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>9:00–9:30</td>
<td>Recap of the first day’s discussions</td>
<td>Txema Calleja, WHO</td>
</tr>
<tr>
<td>9:30–9:50</td>
<td>Update on USG-supported HIV Impact Assessments and strategies for including RITAs</td>
<td>Joyce Neal, CDC</td>
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<tr>
<td>9:50–10:10</td>
<td>Sample size tool for powering population-based surveys</td>
<td>Neil Parkin, FIND</td>
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<tr>
<td>10:10–10:30</td>
<td>Recommendations for handling design effects in complex surveys</td>
<td>Anindya De, CDC</td>
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<tr>
<td>10:30–11:00</td>
<td>Incorporating results of HIV incidence surveys into national estimates: integration and implications</td>
<td>Kim Marsh, UNAIDS, Jingyo Ye, PSU</td>
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<tr>
<td>11:00–11:30</td>
<td>Coffee break</td>
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<tr>
<td>11:30–11:50</td>
<td>Kenya AIDS Indicator Survey and HIV incidence, 2013</td>
<td>Joyce Neal, CDC</td>
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<tr>
<td>11:50–12:10</td>
<td>HIV incidence testing, South Africa, 2012</td>
<td>Thomas Rehle, HSRC</td>
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<tr>
<td>12:10–12:30</td>
<td>Methods and tools to estimate HIV incidence in Catalonia</td>
<td>Colin Campbell, CEEISCAT</td>
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<tr>
<td>12:50–13:10</td>
<td>Testing for recent infection as part of the National HIV Behavioural Surveillance System</td>
<td>Gabriela Paz-Bailey, CDC</td>
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<tr>
<td>Time</td>
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<tr>
<td>13:30–14:30</td>
<td>Lunch</td>
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**Session 5: New biomarkers.**
**Facilitator: Gary Murphy**

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<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
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<tbody>
<tr>
<td>15:30–15:50</td>
<td>Novel biomarkers and future directions for HIV incidence assay development</td>
<td>Usha Sharma, NIAID, Christine Rousseau, BMGF</td>
</tr>
<tr>
<td>15:50–16:15</td>
<td>Coffee break</td>
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<tr>
<td>16:15–16:30</td>
<td>An update in the Bio-Plex multi-analyte assay</td>
<td>Kelly Curtis, CDC</td>
</tr>
<tr>
<td>16:30–17:00</td>
<td>Discussion on training results and needs based on SACEMA September course</td>
<td>Alex Welte, SACEMA</td>
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</table>

**Day 3  Wednesday, October 15, 2014**
**Presenter**

**Session 6: Draft guide for estimating HIV incidence using HIV case surveillance.**
**Facilitator: Keith Sabin**

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<thead>
<tr>
<th>Time</th>
<th>Topic</th>
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<tbody>
<tr>
<td>9:00–9:45</td>
<td>Development of guidance for estimating HIV incidence in HIV case-based surveillance systems</td>
<td>Stéphane Le Vu, INVS</td>
</tr>
<tr>
<td>9:45–11:00</td>
<td>General discussion an next steps for HIV case surveillance guidelines</td>
<td>Txema Calleja, WHO</td>
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<td>11:00–11:30</td>
<td>Coffee break</td>
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**Session 8: Wrap-up**

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<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
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<tr>
<td>11:30–13:00</td>
<td>Review of 2013 meeting report recommendation, summary of the proposed areas for inclusion in the WHO/UNAIDS Technical Update 2014, next steps and products</td>
<td>Txema Calleja, WHO</td>
</tr>
</tbody>
</table>
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