HIV SURVEILLANCE

MEETING REPORT

WHO WORKING GROUP ON

HIV INCIDENCE MEASUREMENT AND DATA USE

3-4 MARCH 2018, BOSTON, MA, USA

World Health Organization

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WHO/CDS/HIV/18.3

This publication contains the meeting report of the WHO Working Group on HIV incidence measurement and data use (3-4 March 2018, Boston, MA, USA) and does not necessarily represent the decisions or policies of WHO.

CONTENTS

ABBREVIATIONS AND ACRONYMS

ACTG	AIDS Clinical Trials Group
AEIDRP	The Acute Infection and Early Disease Research Program
AGYW	Adolescent Girls and Young Women
ANC	Antenatal Care
ART	Anti-Retroviral Therapy
ARV	Antiretrovirals
CDC	United States Centers for Disease Control and Prevention
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CEPHIA	Consortium for the Performance and Evaluation of HIV Incidence Assays
DBS	Dried Blood Spot
ECT	Epidemic Control Team, within PEPFAR
EDDI	Estimated Date of Detectable Infection
ELISA	Enzyme-Linked Immunosorbent Assay
EQAPOL	The External Quality Assurance Program Oversight Laboratory
FIND	The Foundation for Innovative Diagnostics
FRR	False Recent Ratio
GUMCAD	Genitourinary Medicine Clinic Activity Dataset
HARS	HIV/AIDS Reporting System
HIITE	HIV-1 Incidence and Infection Time Estimator
IVD	In Vitro Diagnostic
JHU	Johns Hopkins University
LAg	HIV-1 Limiting Antigen Avidity Assay
MDRI	Mean Duration of Recent Infection
MeSH	Measurement and Surveillance of HIV Epidemics, a Consortium
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	United States National Institutes of Health
ODn	Normalised Optical Density
PEPFAR	U.S. President's Emergency Plan for AIDS Relief
PHIA	Population-based HIV Impact Assessments (surveys in PEPFAR countries)
PMTCT	Prevention of Mother-to-Child Transmission
PrEP	Pre-Exposure Prophylaxis
QVOA	Quantitative Viral Outgrowth Assay
RCCS	Rakai Community Cohort Study
RDS	Respondent-Driven Sampling
RITA	Recent Infection Testing Algorithms
SACEMA	South African Centre for Epidemiological Modelling and Analysis
SBIR	Small Business Innovation Research (R44 NIH grant program)
STAR	Strategic Technical Alignment for Results, PEPFAR
STI	Sexually Transmitted Infection
TDF/FTC	emtricitabine/ tenofovir disoproxil fumarate (Truvada®, used for PrEP)
TPP	Target Product Profile
UCSF	University of California, San Francisco
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VICITS	Spanish acronym for STI Surveillance, Prevention, and Control Strategy
WHO	World Health Organisation

1. BACKGROUND

In 2008 the World Health Organisation (WHO) established a Working Group on HIV Incidence to consider the issues and challenges involved in assay-based HIV incidence estimation methods. The working group is comprised of experts from governmental and non-governmental organizations and donor partners, including epidemiologists, laboratory specialists, and public health officials.

Since its creation, the working group has developed:

[1] standardised definitions of key concepts;

[2] standardised methods of calibrating tests for recent infection (including multi-assay recent infection testing algorithms, or RITAs);

[3] consensus on methodological approaches to interpreting assay and/or RITA results in cross-sectional population surveys; and

[4] quality control and laboratory procedures.

Since the creation of the group, several important milestones have been met, which include the publication of the 2011 guidance on when and how to use assays for recent infection to estimate HIV incidence at the population level (UNAIDS/WHO, 2011), and 2015 guidance on monitoring the impact of the HIV epidemic using population-based surveys (UNAIDS/WHO, 2015). In addition, WHO and UNAIDS have published technical updates on the application of assays for public health surveillance in 2013, 2015, and 2018 (WHO, UNAIDS, 2013; UNAIDS, WHO, 2015; Global HIV Strategic Information Working Group, 2018) that stem from annual meetings and relevant published literature. With input from the working group, in 2017, an updated set of target product profiles (TPPs) and a market assessment also has been published (FIND, 2017; Morrison et al., 2017). The 2018 technical update also included links to updated incidence estimation tools using a bootstrapping approach, in the "inctools" R package (SACEMA, 2018).

Despite these advances, challenges remain in the application of RITAs for public health surveillance purposes. For example, issues related to subtype differences in assay performance are not yet fully described. It has become increasingly clear that as the world moves toward routine, sustainable data reporting systems – including HIV case surveillance and DHIS-2 programme and patient-level monitoring (HISP, 2018) – data among key populations in low-level and high-burden epidemics are needed, to monitor new infections and antiretroviral therapy (ART) coverage. RITAs offer significant potential to contribute to these opportunities for enhanced epidemic monitoring.

The working group's primary objective - to consider the issues and challenges involved in assay-based HIV incidence estimation methods - remains relevant today, especially to guide implementers and users of these methods (such as national departments of health, research organisations and global public health agencies) on best practices and appropriate uses. It was suggested during the 2016 working group meeting in Seattle, USA that the working group's terms of reference should be revisited to reflect some of these more recent challenges.

After discussion, the expanded list now includes:

[1] Collect evidence on the application of assays for recent infection and methods in HIV surveillance, e.g. HIV case-based surveillance and contact tracing;

[2] Review methods for estimating incidence, especially in key populations, including statistical methods appropriate to respondent-driven sampling techniques;

[3] Review program activities like HIV prevention interventions and impact assessments using recency testing information;

[4] Review validations and evaluations of new biomarkers/assays for recent HIV infection;

[5] Maintain a library of publications on HIV incidence estimation methods; and

[6] Link with other WHO/UNAIDS working groups, e.g. the Working Group on Global HIV/AIDS and STI Surveillance and the HIV Modelling Consortium in the topics of HIV incidence measurement.

With this revised charge, on its tenth anniversary the working group convened in Boston, USA in March 2018, to continue its work.

2. OBJECTIVES, METHODS OF WORK AND EXPECTED OUTCOMES

At the end of the previous working group meeting in Seattle (February 2017), a number of research gaps were identified that members determined should be the focus of upcoming meetings, including:

- context-adapted false recency ratios estimates;
- the impact of early ART initiation, discontinued treatment, treatment interruptions, and incomplete viral suppression on the performance of assays for recent infection;
- the performance of assays for recent infection in populations infected with HIV subtypes insufficiently represented in current calibration data (e.g. CRF02_AG, CRF01_AE) to estimate subtype-specific mean duration of recent infection (MDRI) and false recent ratio (FRR);
- the increasing difficulty of estimating assay performance characteristics, owing to increasing ART coverage, adoption of treat-all strategies, the impact of pre-exposure prophylaxis (PrEP) use, etc.;
- review of validation and evaluation of new and proof-of-concept biomarkers/assays, including test performance characteristics, validation using larger specimen sets, etc.;
- the impact of the changing data sources to estimate incidence, such as the expansion of HIV case-based surveillance; and
- use of recent infection results at individual level for prevention and program improvement.

Given this, the specific objectives of this meeting in Boston were identified as:

[1] To provide an update in the development and validation of new assays to distinguish recent from longstanding HIV infection ("HIV recency assays");

[2] To share experiences and recommendations in the national population surveys using HIV recency assays;

[3] To review results from the use of HIV recency assays among different populations like key population and pregnant women;

[4] To review different methods for estimating HIV incidence; and

[5] To identify additional counterparts for future working group participation.

The expected outcome of the meeting was a revised Technical Update in methods to improve incidence measurement, or potentially a larger update to WHO's 2011 guidance on the use of recent infection assays to estimate HIV incidence at the population level.

3. CURRENT METHODS AND ADVANCES IN HIV RECENCY ASSAYS

The purpose of this session was to identify recent advances in the use of HIV recency assays and RITAs to estimate population-level incidence. In recent years RITAs have begun to be applied to population-based surveys in numerous countries worldwide; this requires improved guidance from WHO/UNAIDS to ensure RITAs are appropriately used for calculations in the field.

There are a number of specific items on the research agenda for RITAs, which this session was intended to discuss, including:

[1] The need for additional tools to support appropriate contextual adaptation of MDRI and FRR for HIV recency assays used in the field;

[2] Further investigation of test performance characteristics in populations with less prevalent HIV subtypes (e.g. CRF02_AG or CRF01_AE);

[3] Improved understanding of the impact of recent developments in prevention and treatment strategies and outcomes, including PrEP, ART soon after diagnosis, disengagement and treatment interruptions, and incomplete viral suppression;

[4] Statistical methods for estimating incidence and uncertainty in population-based surveys with low or zero case counts of recent infections; and

[5] Assessment of RITA performance among pregnant women and other key populations.

Alex Welte and Eduard Grebe (SACEMA) shared updates on methods of estimating MDRI and FRR as well as results of analyses of pooled data from Johns Hopkins University (JHU) and the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA), using these methods to examine assay performance by subtype. Oliver Laeyendecker (JHU) reported on experience from the field in Rakai, Uganda in the use of HIV recency assays. Anita Sands (WHO) provided updates about WHO recommendations for post-market surveillance of in vitro diagnostics. Thomas Rehle (University of Cape Town) and Bharat Parekh (CDC) each presented a case for the inclusion of ARV measurements in RITA calculations. Finally, Gary Murphy (Public Health England) presented an update on CEPHIA.

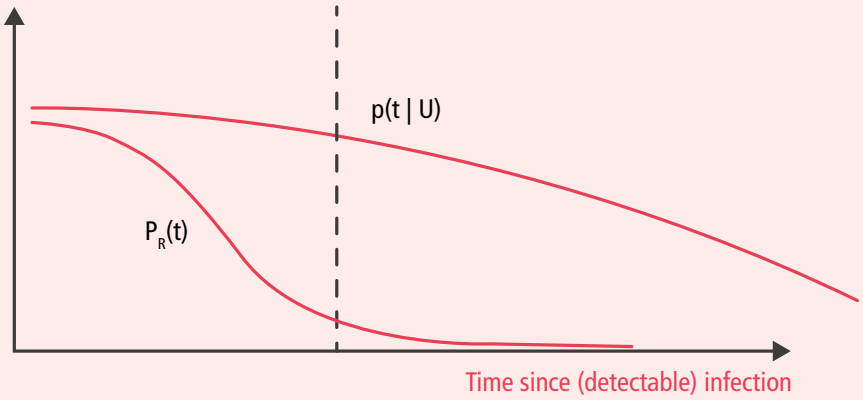
Updates on methods for MDRI and FRR estimation

Even if a global decision is made about the definition of "recent" infection, assay performance for determination of recency is affected by many contextual factors in population-level surveys, including incidence, prevalence, sample size, treatment coverage, distribution of times since infection among subjects, subtype, and case definitions for being HIV positive. For these reasons, MDRI in a published paper for a particular assay may be quite different from MDRI of that assay when used in the field.

To estimate context-specific FRR, one must assign a distribution of untreated times since infection. To calculate the FRR for untreated individuals, one must average the product of two curves: the distribution of untreated infection times, and the probability of testing recent (see Figure 1).

A recent paper (Kassanjee, et al. 2017) provides detailed methods for former MDRI calculation in cross-sectional surveys. These methods include resampling data at the subject rather than the data point level, addressing the problem of repeat visits for testing with the same subject being treated as independent observations, which is true in black box MDRI calculation methods.

Figure 1. FRR amongst untreated individuals



p(t | U) - Distribution of untreated infection times (indirect data)

$P_R(t)$ - Probability of testing recent (highly data driven)

Note different vertical scales (normalisation, alignment to Incidence)

Averaging product of curves, over time, gives untreated FRR

An important concept for MDRI calculation is the determination of "big T," otherwise known as the threshold for recent vs. longstanding infection. To choose a threshold, one must first optimize performance of an assay. Increasing the threshold of big T comes with a trade-off of some downside, typically an increased FRR. The only way to determine where to draw the threshold is to choose the point with the smallest error bar for the test, balancing the MDRI against the FRR. MDRI is then estimated by a cut-off of big T, and a decision of recent/non-recent based on the value of the assay at that point.

Most actual MDRI estimates are based on cohort studies with substantial subject follow-up, biasing the estimates (as data points should not be treated as independent observations). A more ideal variation would be a cohort with a single follow-up at exactly big T since negative enrolment, which is of course unrealistic in practice. A third potential study design would involve large amounts of data from seroconverting blood donors; however, in these cases there is often a large interval between last negative and first positive donation, leading to a highly uncertain estimated date of infection.

Test properties must also be considered during incidence estimation, but it is difficult to include them as random variables at the same level of survey data, as they are mainly understood based on "external" estimates, and have likely already influenced many of the other variables being included in analysis of the survey data (i.e. the impact of the test properties is being recycled). One option to consider would be to provide sensitivity analyses for MDRI and FRR, rather than a single computed confidence interval.

As a final point, the presentation considered the definition of "recent." Notably, "recent" is not a chosen time to which assays are tweaked. Though it is useful to compare varying results, recency thresholds must also be considered to be context-dependent and not as fixed, sacred thresholds used in all contexts. It is also critical to consider the definition of "sufficiently virally supressed," as this concept may be different in various contexts (i.e. treatment monitoring, treatment as prevention, a technique for FRR reduction in incidence calculations, and when considering what platform to use for a recency assay). The question was posed to the group: Could "recent" mean "not on treatment?" Is this a valid and logical way to proceed with incidence estimation, given the changing landscape of ART worldwide?

Analysis of pooled data from JHU and CEPHIA, to examine assay performance by subtype

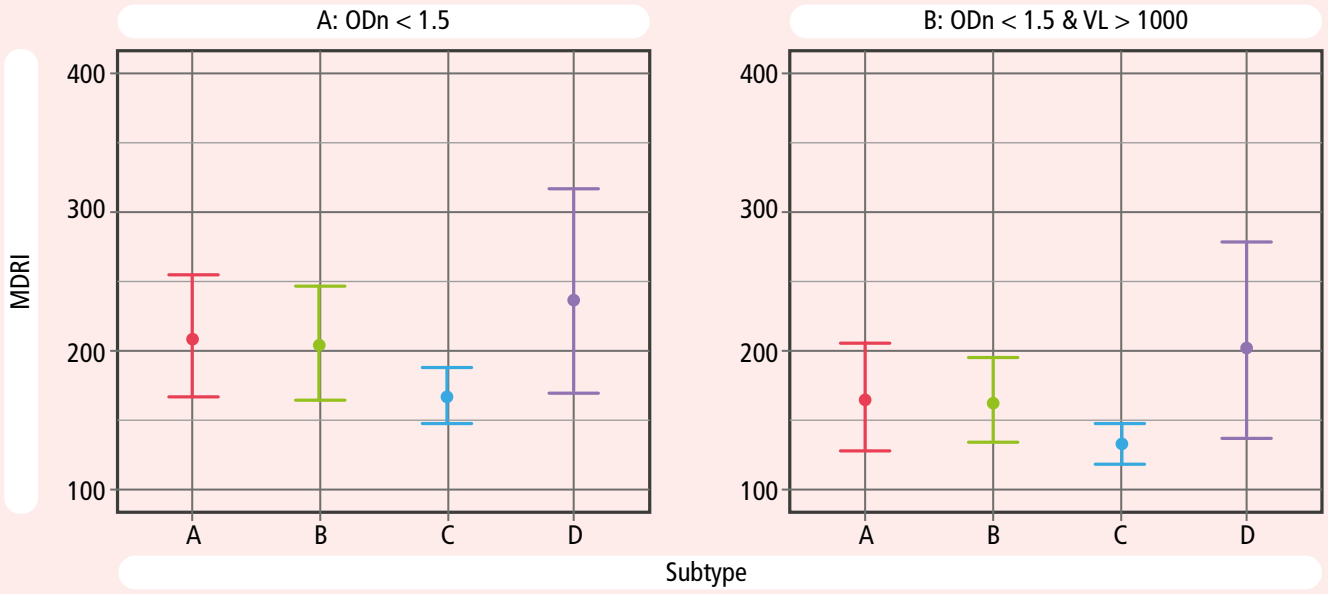
This presentation focused on findings from an analysis of pooled data from JHU and CEPHIA, with specimens tested using the Sedia™ HIV-1 LAg-Avidity EIA (Sedia BioSciences Corporation, USA). The analysis included 10,322 specimens from 2,297 unique subjects across 17 cohort studies. Using a range of ODn thresholds and LAg results alone and in combination with HIV-1 viral load results, infection timing needed to be standardized across the datasets. To do this, testing histories of subjects were used, in combination with known "diagnostic delays" of the various diagnostic assays. These delays were referenced to 1 copy/ml viral load assays (i.e. the diagnostic delay for an assay is the mean number of days it takes this assay to detect HIV after the day a quantitative viral load assay with a 1 copy/ml level of detection would have detected virus). Using this method, estimation of the date of detectable infection (EDDI) is dependent on the most sensitive last negative test and least sensitive

first positive test in a subject's diagnostic history. An online tool is now available to assist researchers with EDDI calculations for subjects in their dataset: tools.incidence-estimation.org/idt. Using these methods, 3,197 specimens from 1,084 subjects in the dataset were considered suitable for MDRI estimation (i.e. they had a reasonably well-estimated date of detectable infection).

There was an extremely high correspondence between optical density (ODn) values obtained on specimens tested by both CEPHIA and JHU. However, JHU specimens tended to have a higher ODn than CEPHIA specimens within the same categories of time since infection. Upon further examination, this appears to be the result of a strong sex effect – particularly for subtype C specimens ($p = 0.021$) – whereby females consistently have higher ODn than males at the same time since infection; the JHU specimen set had a substantially higher proportion of females than the CEPHIA specimen set did.

While some subtype differences can be seen in the data (see Figure 2), they are not very clear except in the case of subtype D, which had a much higher MDRI than other subtypes.

Figure 2. MDRI by subtype and supplemental viral load threshold (ODn < 1.5)



No statistically significant evidence of difference by pregnancy was found; however, this does not mean that there is no actual difference in ODn by pregnancy status. In this dataset the sample size was small, the data may be incomplete (i.e. pregnancy status may be assumed or unknown for some subjects), and some non-pregnant women may be post-partum, affecting the results. No analysis has yet been done by age, geography, or other risk factors.

Ultimately in a mixed-subtype population, a weighted average of subtype-specific MDRIs could be used. Potentially the same type of method could be used to account for sex distribution. Estimating context-specific FRR is also necessary, though more complicated. SACEMA hopes to release an update to inctools (github.com/SACEMA/inctools) in the near future to aid in these types of MDRI and FRR calculations.

Later on Day 1, Bharat Parekh (CDC) also shared some data about RITA performance characteristics among pregnant women in Zimbabwe, tested in the Zvitambo study. In this study, women were tested for HIV and enrolled in the study within 96 hours after delivery. The study found that antibody kinetics are faster in women during the first year post-partum compared to the second year, and that this has implications for MDRI and incidence estimation (Hargrove, et al., 2017). In summary, physiological state may affect antibody kinetics; estimation of incidence by laboratory methods should not be viewed as absolute but is useful to help identify hot spots in a region and to assess relative differences between populations.

Experience from the field in Rakai, Uganda

Calculation of HIV incidence using plasma specimens tested through the Rakai Community Cohort Study (RCCS), a population-based HIV incidence cohort in 50 communities in Rakai district, Uganda, presents a useful example of the types of decisions required to determine context-dependent MDRI in the field. Multiple rounds of RCCS enrolment over many years has allowed for point estimates of incidence compared to observed incidence, as well as a comparison of incidence in fishing villages vs. farming and trading villages.

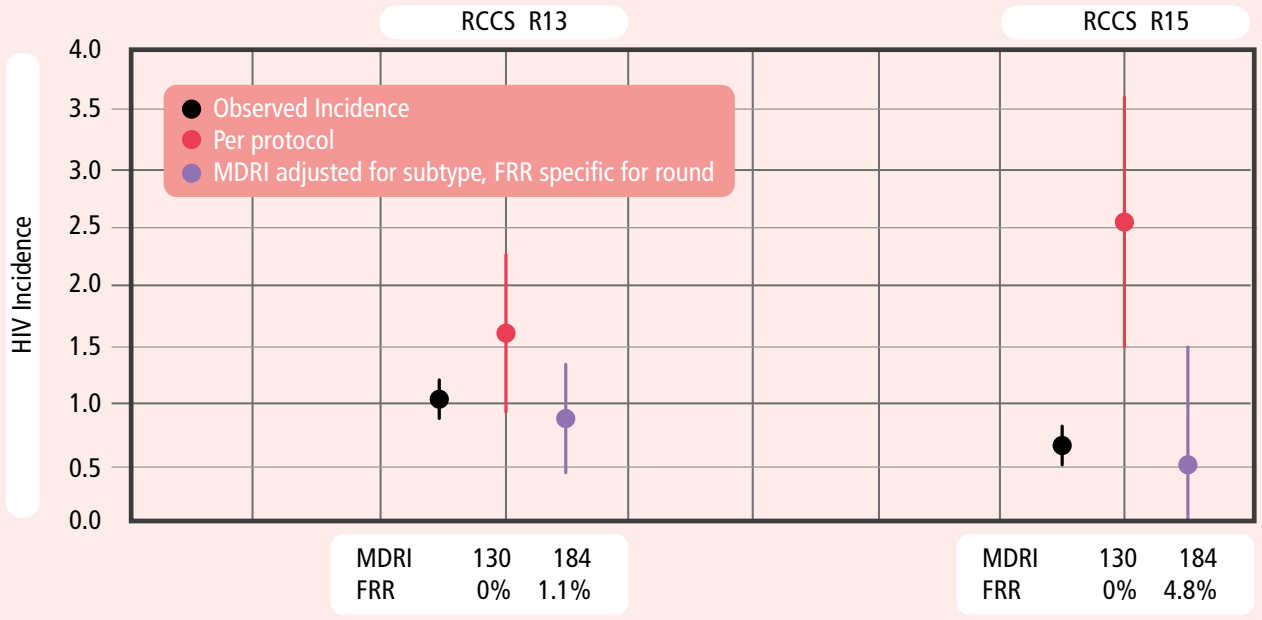
Given the mix of 45% subtype A and 55% subtype D in the sample, RCCS researchers used the mixed MDRI of 187 days on the Sedia™ HIV-1 LAg-Avidity EIA, using the weighted average method shared in the prior presentation (Grebe). With this MDRI, only 6 of 544 specimens were misclassified (FRR 2.2%, CI 0.4, 2.4%).

In summary, per protocol (Sedia™ HIV-1 LAg-Avidity EIA with HIV-1 viral load, assuming MDRI of 130 days and FRR of 0.0%) greatly overestimated HIV incidence, compared to this adjusted MDRI and FRR model (nearly a 4-fold excess incidence in the Round 15 RCCS survey). The adjusted MDRI and FRR resulted in an incidence estimate very close to the observed incidence (see Figure 3); in actuality, the different FRR in each model had the greatest effect on findings.

It can be seen in Figure 3 that the subtype-adjusted FRR differed significantly between Round 13 and Round 15 of RCCS. Calculations of FRR excluded people with known ARV usage, and therefore it is possible that the increase in FRR between the two time periods was related to increased use of ART in Rakai.

During discussion of this session, the group discussed the importance of considering the impact of repeat subjects in multiple rounds of a longitudinal cohort study, as well as the impact of ARVs – particularly for people who are partially adherent and partially suppressed – on MDRI calculations. Assumptions regarding subtype based on geography may also be outdated, with the most recent global subtype distribution mapping being completed in 2006.

Figure 3. Impact of inappropriate MDRI/FRR on RCCS incidence estimates



WHO guidance on post-market surveillance of in vitro diagnostics

Most "recency assays" were not actually developed with the intention for this usage (i.e. they were developed for screening/diagnostic purposes). Manufacturers develop and validate their assays for an intended use. Their post-market obligation is to ensure the assay continues to meet requirements as specified when registered for that intended use. Therefore, the manufacturer is not legally required to respond to issues when used off-label. Recency assays are not currently regulated; however, there are established systems for post-market surveillance of in vitro diagnostics (IVDs), and off-label use of these products is one of the main ways that interesting applications have been realized for purposes of estimating incidence.

WHO post-market surveillance of IVDs involves both proactive strategies (e.g. lot verification testing, evaluation of quality control data), and reactive strategies (e.g. investigation following a complaint). Both can result in a field safety corrective action, and in some cases, issuance of a

Field Safety Notice. When complaints are received by WHO, they are characterized according to seriousness;

Once an investigation has begun WHO expects the following response from a manufacturer:

[1] Root cause analysis (how/why did this happen?)

[2] Analysis regarding related areas (is this same issue occurring elsewhere?)

[3] Correction with completion dates (fix immediately)

[4] Corrective action with planned completion dates (to prevent recurrence)

Manufacturers take field safety corrective actions to reduce the risk, once there is manufacturer-defined unacceptable increase in risk associated with use of an IVD. These could include change to labelling or instructions for use, recall (return or destruction), and/or exchange (swap-out). More information about WHO's post-market surveillance guidance is available in a 2015 report (WHO, 2015) and online at their website: www.who.int/diagnostics_laboratory/postmarket/en.

The main question for the working group is whether more proactive post-market surveillance could be implemented for assays used in RITAs. The main proactive strategy used is lot verification testing, though this is typically not done for HIV assays. It is very expensive and potentially interrupts supply. Other ways to proactively conduct surveillance of assay performance include quality control programs or external quality assessment schemes, though many programmes do not have well-functioning quality management systems. Quality control programs for HIV incidence testing would uncover shifts and trends in assay performance, like lot-to-lot variation. Is it possible to come up with quality control material that could be used systematically, worldwide? The suggestion was made that the EQAPOL lab quality assurance program could be extended to include the LAg Avidity assays. Many people in the field are using assays that they don't really understand, and wouldn't realize when something is unexpected about the assay performance. Addressing this problem requires education about expectations and screening for problems, and also potentially an independently sourced quality control panel that could be run with every test run.

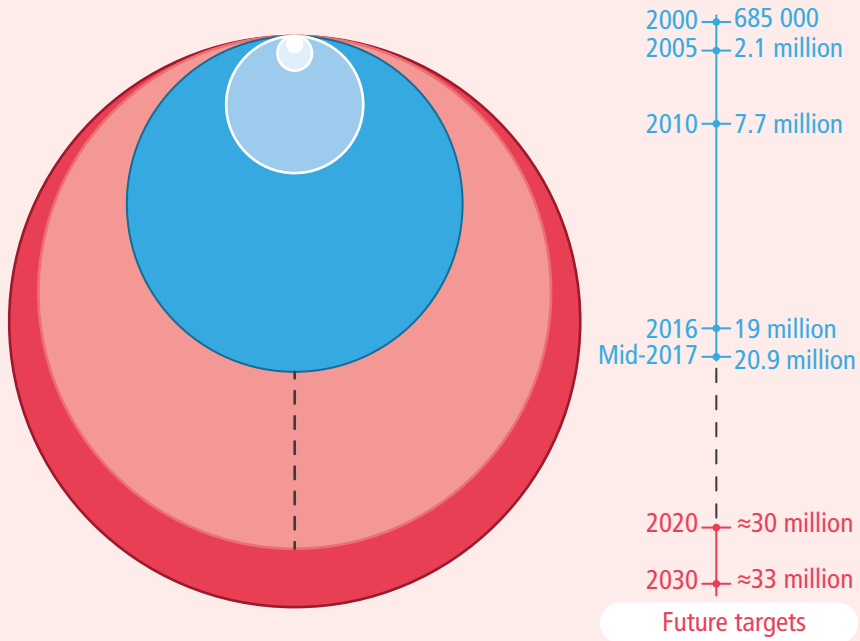
One direct example that was raised in the group was the difference in performance between Sedia vs. Maxim versions of the LAg Avidity assay; CEPHIA has found that subtype C MDRI is 240 days for the Maxim HIV-1 Limiting Antigen Avidity (LAg-Avidity) EIA Kit (Maxim Biomedical Inc, USA), and 168 days for the Sedia™ HIV-1 LAg-Avidity EIA, with a p-value of .0002. These differences between LAg Avidity assay versions underscore the importance of properly identifying the assay manufacturer when reporting results from LAg testing.

Inclusion of ARV measurements in RITA calculations

The context of ART use has changed considerably in the last 15-20 years (see Figure 4). While the FRR of most commonly-used HIV recency assays is fairly low for specimen sets where elite controllers and people using ARVs have been excluded, multiple examples were shared that demonstrate that when assays are used with people receiving ARVs (particularly those who are early treated), FRR rises substantially. As ART coverage continues to increase globally, methods for incidence estimation must find ways to properly account for ARV use, especially for those initiating ART within 6 months of infection.

Since both elite controllers and those on ART are characterized by low (typically undetectable) viral load, inclusion of viral load in a RITA can help identify persons who are misclassified due to ART. For this reason, WHO, CDC, and CEPHIA have all recommended a recent infection testing algorithm that incorporates HIV-1 viral load as a second line assay for incidence calculations. However, individuals on ART who are not virally suppressed may still be included as falsely-recent on a conventional RITA. This includes adolescents who may have started ARVs during infancy, and people on ARVs who have poor adherence or emerging drug resistance.

Figure 4. UNAIDS/WHO estimates of the number of people receiving ART



In one strategy, DBS specimens were tested for ARVs (specifically Nevirapine, Efavirenz, Lopinavir, Atazanavir, and Darunavir) using High Performance Liquid Chromatography coupled to Tandem Mass Spectrometry, with a selected limit of detection of 0.02 µg/ml. People with detectable ARVs were excluded from incidence analyses, except for those on PrEP (TDF/FTC).

CDC's Global AIDS Program is now recommending that all HIV-positive specimens be tested with LAg-Avidity EIA (either of the two brands), HIV-1 viral load, and for ARVs before a determination is made. This is not required within PEPFAR for routine surveys, unless the intent is to measure progress toward 90-90-90 goals. The proposed testing algorithm is serial, to lower cost and time required to implement. Data from four countries was presented that demonstrated the actual impact of ART in RITAs, where the addition of ARV testing drove down the estimated incidence in all cases, though not always statistically significantly.

There was further discussion about the validity of viral load testing using DBS specimens, as is commonly done for RITA.

Concerns were raised regarding whether:

[1] a threshold of 1000 copies/mL is the appropriate lower limit of detection;

[2] false undetectable results pose a larger problem than originally thought (e.g. a recent NICD study found that 30% of HIV-infected individuals not on ART had an undetectable viral load, which is highly unlikely to be due only to elite controllers); and

[3] there could be potential overestimation of viral load due to cell-associated RNA.

These issues are becoming more clinically significant because many clinicians assume that someone with non-acute infection who has a high viral load or low CD4 count must have longstanding infection; however, recent research from ACTG/AEIDRP have demonstrated much more rapid disease progression than expected (median of 18 months) for untreated patients. This means that globally there may be a greater number of people starting ARVs much earlier after infection than previously thought. Expanded use of PrEP clearly amplifies these challenges.

The working group had a lengthy discussion about the opportunities and challenges of including ARV testing into standard RITA recommendations. In some cases, it has been shown to improve the accuracy of incidence estimates, but it requires a specialized laboratory, so the impact of including a third step should not be overstated. The general feeling was that more validation of this idea was required before it could be included in more general recommendations regarding the use of RITAs.

CEPHIA Update

CEPHIA began in 2011, with four aims: to build collaborations, evaluate assays used to estimate HIV incidence, build a specimen repository for evaluation of new and established assays (commercially available or otherwise), and develop statistical methods related to incidence estimation and assay performance. Today, CEPHIA is an unfunded initiative; however, the repository contains more than 18,000 very well-catalogued specimens from all over the world. Twelve assays have been evaluated using a 2500-specimen CEPHIA Evaluation Panel, with a greater and more diverse representation of clade and false-recent "challenge" specimens than in the smaller CEPHIA Developmental and Qualification Panels. The summary of these assay evaluations is shown in Figure 5.

CEPHIA has already released a series of papers on these evaluations (Kassanjee, et al. 2014; Kassanjee, et al., 2016; Keating, et al. 2016; Grebe, et al., 2017), policy recommendations (Murphy, et al. 2016), and other uses of recency assays (Keating, et al., 2017; Schlusser, et al., 2017; Seaton, et al., 2017). Forthcoming papers will deal with methods for infection staging and dating, further lab-based papers, and joint papers with other groups, combining CEPHIA data with other datasets for analysis. CEPHIA also aims to complete further formal reports of assay evaluations, and is in search of funding to sustain future efforts.

Figure 5. UNAIDS/WHO estimates of the number of people receiving ART

Assay	MDRI	FRR	Potential modification to improve usefulness	Limitations
Sedia LAg	188	1%	Yes	
Maxim LAg	248	2.91%	Yes	
Glasgow BioRad Analyte	88	1%	Yes	Commercial assay - modified for purpose
CDC-BioRad	333	6%	Yes	Commercial assay - modified for purpose
Geenius	179	6.06%	Yes	Modified commercial - need access to software
Architect Avidity	128	1.5%	Yes	Expensive automated plataform
Ortho Avidity	285	7%	Poss	Expensive automated plataform
IDE-V3	216	5.17%	No	In-house
Ortho Less sensitive	306	10%	No	Expensive automated plataform
BED	302	7%	No	Confidence
Architect diagnostic	209	3.4%	Yes	Expensive automated plataform

4. USING HIV RECENCY ASSAYS AT POINT OF CARE AND FOR PROGRAMMATIC USE

The purpose of this session was to review findings from studies using assays for recent HIV infection in routine testing settings, including at or near to point-of-care and planned expansion of HIV incidence testing within PEPFAR-supported programmes in 2019.

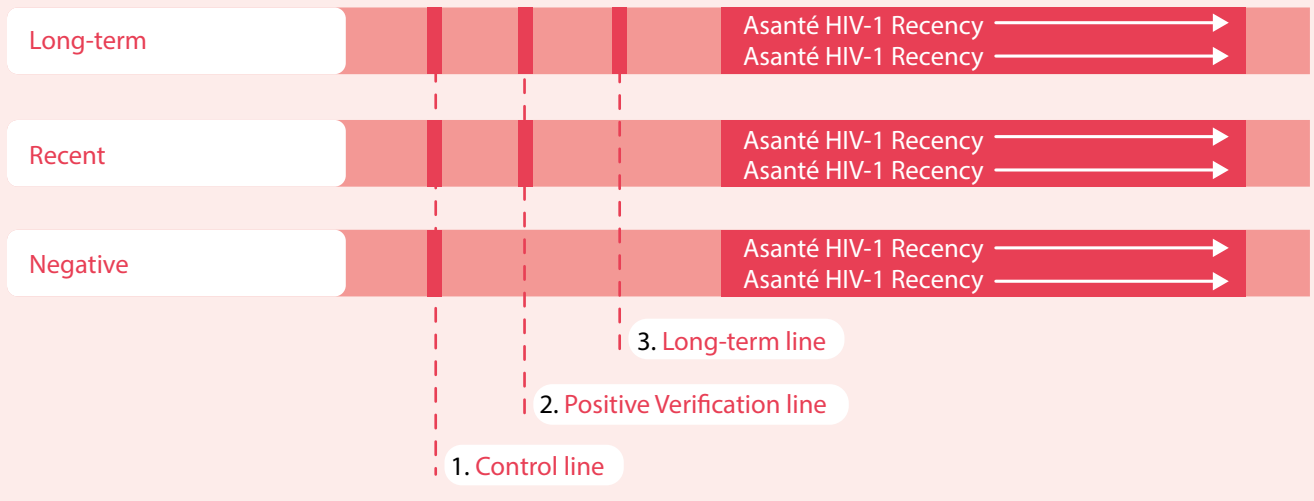
Elfriede Agyemang (CDC) shared preliminary findings from a pilot study in Malawi that incorporated testing for recent HIV infection among adolescent girls and young women. Sanny Northbrook (CDC) presented preliminary findings from the integration of the Asanté™ HIV-1 Rapid Recency™ Assay (Sedia Biosciences, USA), a rapid diagnostic test in immunochromatographic format that can be used at or near to point-of-care, into HIV testing services in Central America, and implications for case-based surveillance and partner notification activities. Katie Curran (CDC) shared an update on PEPFAR's planned expansion of rapid HIV recency assays among newly diagnosed individuals, starting in 2019. Brian Rice (London School of Hygiene & Tropical Medicine) shared findings from the MeSH consortium regarding integration of a RITA into routine testing services in three programmatic settings. And finally, Jeff Eaton (Imperial College London) discussed the incorporation of incidence measurements from population surveys into model-based epidemic estimates.

Experiences from the field: Adolescents and young women in Malawi

Malawi has experienced a significant increase in HIV infections in young women between ages 20-29.

To determine the proportion of recent HIV infection among pregnant adolescent girls and young women (AGYW) attending their first ANC visit in public facilities in 4 high-HIV prevalence districts in Malawi, the Malawi HIV Recency Study is using the following RITA with return of results to participants: Firstly, the Sedia™ HIV-1 LAg-Avidity EIA is used for any girls or women newly diagnosed as HIV-positive; HIV viral load is measured for any individuals identified as recent by the LAg Avidity EIA. The study also incorporates validation of the Asanté™ HIV-1 Rapid Recency™ Assay, an immunochromatographic (lateral flow) rapid diagnostic test that provides a control line, HIV-diagnosis ("positive verification") line, and long-term infection line (i.e. if the control line and HIV-diagnosis lines are reactive but the long-term infection line is non-reactive, the subject is considered to be recently infected) (see Figure 6).

Figure 6. Asante Visual Interpretation



In this validation study, only one specimen that tested recent on the Asanté™ HIV-1 Rapid Recency™ Assay; about 7 that tested as recently infected by LAg Avidity EIA tested as long-standing infection by the Asanté™ HIV-1 Rapid Recency™ Assay. The assay can be read either visually by eye or with a quantitative reader; there was 96.7% agreement between visual reading vs. quantitative reader for the assay.

When identified as recently infected by the RITA, participants were told that the recency results suggest they may have been exposed to HIV infection within the past 12 months. There is no nuance in messaging depending on the ODn (i.e. no difference between messaging for a LAg result of 0.1 or 0.5). The study has a standard operating procedure and guidelines and a script to guide staff in messaging results.

Key messages related to return of results include that:

[1] Standard of care for new diagnoses should not differ by recency status;

[2] HIV post-test counselling regarding available psychosocial support resources, ART adherence and partner disclosure is critical, as per national guidelines; and

[3] Counselling messages must emphasize that history of ART use can result in a false-recent result.

Anecdotally, the return of RITA results has been well-received by subjects (they are not reporting concerns about test validity, or fears of domestic violence, etc.); however, as of the meeting only 49 results had been returned so these findings are preliminary. Thus far the mean number of days between enrolment and ANC receipt of is 24.8 days.

Implementation of the Asanté™ HIV-1 Rapid Recency™ Assay in PEPFAR Central America

PEPFAR Central America includes 6 countries, from Guatemala to Panama. These countries conduct sentinel surveillance of STIs through VICITS clinics, who are Ministry of Health clinics providing comprehensive prevention and wellness services for key populations (focused on men who have sex with men and sex workers), beyond HIV testing. In these settings, PEPFAR has worked to integrate the Asanté™ HIV-1 Rapid Recency™ Assay into routine HIV testing services using a second specimen obtained during the same visit; they have found that acceptance of assays for recent HIV infection has ultimately been <90%, though obtaining the second specimen has been difficult given that many patients are not ready to navigate to care immediately following their HIV diagnosis. At the clinic level it takes about 30 minutes for the counsellor to complete all procedures, then 20 minutes for the venous whole blood specimen, then sometimes there is a further wait for the navigator to return and disclose the result. Results are not currently being interpreted or disclosed in this context of other clinical data; for this reason, when recency results are provided to patients they are notified that the results indicate they may have been infected in the past 12 months, but more testing (including viral load) is necessary to confirm recent HIV infection.

The Asanté™ HIV-1 Rapid Recency™ Assay produced recent results on 33% of specimens in Guatemala, and 15% of specimens in Nicaragua. Of those testing recent, 83% in Guatemala and 66% in Nicaragua had a HIV-1 viral load ≥1000 copies/ml. So far there have been two false negative results on the Asanté™ HIV-1 Rapid Recency™ Assay, when compared to the national testing algorithm. There has been low to moderate levels of acceptance of assisted partner services when offered, and it resulted in substantially increased counselling time. 46% of patients accepted assisted partner services; 59% of partners were tested, with a 30% HIV-positivity result (n=9), none of whom were recent.

Main challenges included collection and transfer of data. HIV recency information was not originally included on the HIV notification form, making data collection extremely difficult. There have also been some challenges with transferring technology to the MOH's centralized information system.

Planned expansion of HIV incidence activities in PEPFAR, 2019

PEPFAR's goal is to reach epidemic control and 95-95-95 targets in all PEPFAR countries. Last fall there was a new directive for establishment of epidemic control teams (ECTs) within country teams. PEPFAR-supported countries are categorized as ECT I (at attainment), II (>70% ART coverage for at least one population), III (<70% ART coverage), or IV (key population-focused and STAR countries) based on progress towards epidemic control, including ART coverage. ECTs are tasks to identify priority barriers and high-impact, efficient solutions to reaching epidemic control, appropriate to ECT level.

In 2017 and 2018, PEPFAR evaluated the Asanté™ HIV-1 Rapid Recency™ Assay in Vietnam, Rwanda, and Nigeria. They also integrated the assay into HIV testing services in Guatemala, Nicaragua, Panama, and El Salvador. CDC has found that these pilot programs have shown promising results, and that rapid HIV recency testing may improve surveillance and enhance program activities, without altering routine HIV care. For these reasons, PEPFAR plans to expand use of recency testing in 2019, to establish an HIV recent infection surveillance system in routine HIV testing services to detect, characterize, monitor, and intervene on recent HIV infection among newly-diagnosed cases.

Planned methods include integrating a recency test (usually the Asanté™ HIV-1 Rapid Recency™ Assay) into routine HIV surveillance as supplemental test for clients who are HIV-positive per their national testing algorithm. Results are returned to clients, who then receive counselling and confirmation via viral load; results are then reported routine monitoring and epidemiological analyses.

During discussion, the group suggested that this may be best initiated in high-priority populations, program settings, and/or geographic areas before bringing to

scale. There was also discussion about the importance of understanding the sensitivity and other performance characteristics of assays being used in each country's testing algorithm, if the results are going to be used as a measure of incidence (e.g. if recency testing is only run on people who have been diagnosed using 3rd generation serological assays, this will have an impact on incidence calculations).

Integration of the RITA into programmatic settings, MeSH Consortium

The MeSH (Measurement and Surveillance of HIV Epidemics) Consortium operates with the hypothesis that strengthening the collection, analysis, and use of routine HIV data, and developing and informing prevention and treatment monitoring and targeting tools, will sustainably transform the insights we have into tracking HIV incidence in sub-Saharan Africa, thus accelerating its decline. MeSH is in the process of conducting three pilots, designed to assess feasibility of integrating RITAs into routine service delivery, and to develop, adapt, and pilot approaches to consent, results dissemination, and counselling to use it in prevention applications.

Pilot 1 involves the use of a RITA in clinics providing HIV testing linked to prevention of mother-to-child transmission (PMTCT) services. Conducted in Western Kenya, HIV prevalence in this study site was 18% in 2014, with HIV incidence exceeding 2% in some age groups. Pilot 2 uses a RITA within an outreach programme for female sex workers in Zimbabwe. They identified sociodemographic risk factors for recent infection through secondary analysis of RDS survey data across 18 sites in Zimbabwe using a RITA that incorporates HIV-1 viral load. A protocol was developed for the use of the RITA among female sex workers without a history of previously testing HIV-positive. The RITA will be piloted within 5 fixed sites. Finally, Pilot 3 involves surveillance of recent infection in routine HIV testing and counselling clinics in Nairobi, Kenya. This pilot will include on-site CD4 and viral load testing, with outreach workers that link and follow-up with patients.

Each of these pilots are designed to assess feasibility of the routine use of a RITA for all people confirmed to be HIV-infected and ART is offered to those with recent infections, who otherwise may have delayed treatment initiation due to resource constraints. Pilots will also measure feasibility and acceptability among survey test counsellors, outreach workers, and health care providers.

Incorporation of incidence measurements into model-based epidemic estimates

Among the most commonly used methods to derive HIV incidence is the UNAIDS/WHO recommended Estimation and Projection Package (EPP) (Brown, et al., 2008). This presentation focused on updates to the EPP model using design-based HIV incidence estimates from household survey data. Incidence estimates from population-based surveys must account for complex sampling design (i.e. weighting and clustering); ideally there would be a consistent approach for handling both HIV incidence and HIV prevalence calculations.

Incidence and prevalence estimated from the same survey are correlated, which must be captured in modelling, which uses both estimates. Given this, there is a desire for the EPP model to capture uncertainty in test characteristics in a consistent way, as primary survey analyses. To do this, a standard design-based survey estimator yields estimates of population totals and covariance accounting for weighting and clustering:

N_N: number HIV-negative

N_R: number HIV+, recently infected

N_{NR}: number HIV+, not recently infected

$$\Sigma_N = \mathrm{Cov}(\{N_N, N_R, N_{NR}\})$$

The Delta method of approximation or parametric bootstrapping is then used to estimate the standard error of the incidence estimate. This is the approach recommended by Kassanjee et. al (Kassanjee, De Angelis et al. 2017) and implemented within SACEMA and CDC incidence estimation tools, including the web tool designed for this purpose: https://incidence.shinyapps.io/incidence_calculator.

An example was shared incorporating a model of basic transmission dynamics, combining survey, health facility, and RITA data to generate an estimate of HIV infection by district, using the estimated HIV transmission rate for untreated results x HIV prevalence at time t x ART coverage at time t in each district. This model can be used for identifying transmission hotspots, by additionally allowing for calculation of the amount that HIV incidence is greater or lower than 'expected' in a particular district, based on the HIV prevalence and ART coverage. As the number of estimated recent infections increases, there is more power to detect hot spots using this method.

This model can be further enhanced by incorporating covariates, particularly those that may be correlated to areas of excess transmission (e.g. presence of a main roadway, key population sizes, or percent of sexually active unmarried women). In some cases, it would make sense to explicitly build out specific risk group structures (transmission dynamics and covariates) into the spatial models.

Suggestions for research priorities for HIV estimates included improvements in:

[1] Models for more granular, precise, and timely estimates of changes in incidence;

[2] Interpretation of assays for recent HIV infection among pregnant women, related to assay performance characteristics (MDRI, FRR) and population incidence patterns (recent sexual activity, earlier stage of infection);

[3] Interpretation of RITA in programmatic settings for population epidemic patterns and trends;

[4] Influence of changing context (ART coverage, prevention) on interpretation of recent infection assays; and

[5] Inference from routine health system data and case-surveillance across epidemic settings, especially in sub-Saharan Africa, where data have been less used.

5. INNOVATIONS AND NEW BIOMARKERS

The purpose of this session was to explore recent innovations in the development and use of HIV recency assays. Ernest Yufenyuym (CDC) described a multiplex assay developed by CDC for use on the MAGPIX® Luminex System. Usha Sharma (NIH) reviewed a series of projects exploring the use of novel biomarkers to develop HIV recency assays with improved specificity. Ha Youn Lee (University of Southern California) spoke about her new HIV-1 incidence and infection time estimator. And finally, to begin Day 2 of the meeting Sheila Keating (Blood Systems Research Institute) highlighted the use of HIV recency assays to monitor viral persistence in the context of HIV cure research.

MagPix Luminex System: An innovative multiplex assay

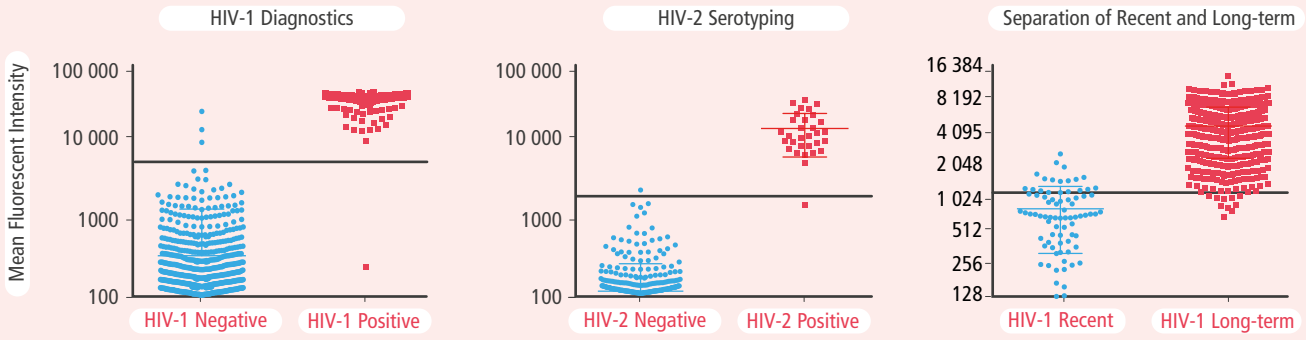
This presentation focused on CDC's assay developed for MAGPIX® Luminex System. It combines HIV diagnosis, HIV 1&2 typing, and recency classification. The basic principles are similar to an ELISA, accepting serum, plasma, and possibly DBS specimens. It has a high throughput (96 wells in ≤60 minutes). It involves HIV diagnosis using a p24-gp41 fusion protein at a relatively high (1 µg) concentration; recency classification using rIDR-M antigen with a limiting concentration of (0.04 µg), and HIV-2 typing using gp36 IDR peptide at high concentration (10 µg). It is currently being tested using a series of panels, including one large-scale panel (n=1500), where it showed high sensitivity and specificity, with fairly strong separation of recent and long-term specimens (see Figure 7).

The assay comes with a Microsoft Excel-based data management tool that supports the serial algorithm of the assay, producing diagnostic, serotyping, and recency results on each specimen. Using antibody kinetics, the developers have determined an overall MDRI of 135 days, with subtype/geographic differences ranging from 119 days in Trinidad (subtype B) to 148 days in Ethiopia (subtype C). This is a supplemental assay that would be used as an aid for diagnosis. A detailed cost-analysis has not yet been conducted, though this test is run on a standard Luminex platform, which typically costs $60,000 - $100,000.

Novel biomarkers for HIV recency assay development

Beginning in 2010, NIAID has invited research proposals for HIV incidence assays, through PA-10-212, PA-12-012 (HIV Incidence Assays with Improved Specificity) and PA-15-105, PA-15-106 (Novel Biomarkers for the Development of HIV Incidence Assays with Improved Specificity). These PAs have funded 10 R01 and 2 R21 grants since 2010.

Figure 7. Larger scale evaluation of the Multiplex Assay (n=1500)



Some important funded research includes:

[1] **R01-AI095068 (Eshleman)** - This group has been working on serologic and other algorithms that could be used for recency testing. They are currently working on accurate multi-assay algorithms, and "serosignatures" based on antibody specificity that may provide reliable biomarkers for recent HIV infection that are not impacted by viral suppression.

[2] **2R01-AI095066-06A1 (Lee)** – This research has produced the HIV-1 Incidence and Infection Time Estimator (HIITE), which was described in the next presentation. This has involved work in gene sequences and how they inform the stage of infection, therefore the ability to estimate infection timing. HIITE is the first assay to simultaneously inform HIV-1 incidence and infection time from a single blood draw, with high accuracy.

[3] **R01-AI097015 (Wu)** – This research is based on the hypotheses that changes of entropy over time vary greatly across different gene sequence segments, and certain viral genetic segments are more differentiable between acute versus chronic stages of infection. Based on the idea that classification algorithms that focusing on viral diversity of the highly informative regions can improve assay accuracy, this research has thus far demonstrated that the highly informative regions approach is an effective way to improve the predictive power of genomic-based biomarkers.

[4] **2R44-AI114365-02A1 (Mink)** – This SBIR grant to Sedia Biosciences Corporation is intended to develop a rapid HIV-1 recency assay which will use DBS and oral fluid to provide recency and prevalence data simultaneously, with reduced FRR.

In June 2018, NIAID plans to release a new series of program announcements to fund R01s and R21s to promote the identification of novel assays/algorithms which distinguish recent from chronic HIV infection, regardless of whether they have been started on ART or not, with a MDRI <365 days and <2% FRR. Assays developed should recognize all relevant HIV-1 subtypes (particularly A, B, C, and D) and be able to be used with specimen types precluding testing with RNA-based assays (e.g. oral fluid).

During discussion, the group identified that the CEPHIA Evaluation Panel could be a critical resource for evaluation of the assays developed through the new program announcements; while this will not be specified in the announcements, NIAID could request that any assays ready for validation be evaluated using CEPHIA panels. To this point, most of the successful HIV recency assays have been antibody maturation assays that have been significantly impacted by ART; this program announcement is designed to support the development of different models that will not have the same challenges.

HIITE: HIV-1 incidence and infection time estimator

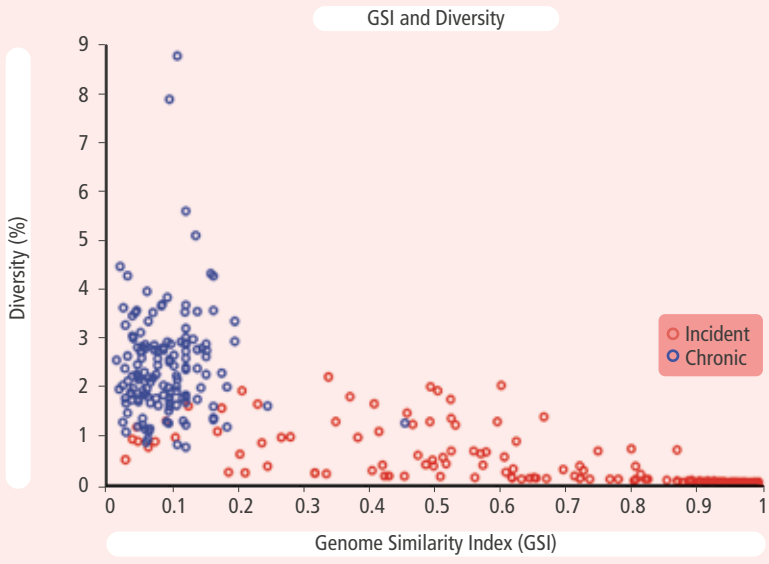
This presentation explained work meant to combine two approaches to gene sequencing (using the Genome Similarity Index and examining viral diversity) to distinguish recent from chronic infections, then estimate time since infection for those that are recent (see Figure 8).

HIV-1 Incidence and Infection Time Estimator (HIITE) is a web-based software, processing HIV-1 env gene sequences. If the Genome Similarity Index is less than a set threshold and diversity is greater than a set threshold, then a specimen is classified as chronic. If not, HIITE performs clustering to detect multiple founder incident cases and estimate single lineage diversity to classify the specimen as either recent or chronic, then moves to the next stage, estimating the time since infection for recent cases.

HIITE has been tested using 585 recent and 305 chronic specimens, using a big T of 2 years, and comparing the estimates of time since infection to Fiebig stage estimates (Fiebig, et al. 2003). With the subset of specimens tested using the full env gene, the prediction error for time since infection (compared with Fiebig stage estimates) is 13.5% (95% CI 10.8% - 16.5%), with an MDRI of 492 (404 - 582) days and FRR of 0.67% (0.0 – 2.0%) and sensitivity of 94.0% (92.0% - 95.8%).

The FRR among ART-experienced subjects was 3.2% (0.0% - 8.1%) and among the 16 virally-suppressed subjects was 12.5% (0.0% - 31.3%), suggesting that this method experiences the same ARV-related challenges the group had discussed throughout the day.

Figure 8. Relationship between Genome Similarity Index and diversity, for recent and chronic infections

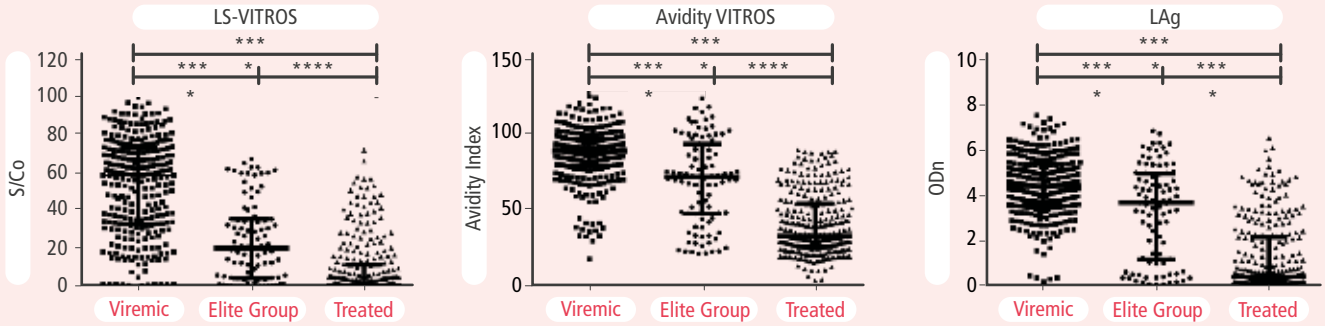


HIITE's design and validation were performed on diverse HIV-1 envelope gene sequences collected from global cohorts from Africa, America, Asia, and Europe, with specimens representing diverse subtypes, risk behaviors, viral loads, and CD4 T cell counts (Park, 2018). During discussion, the group agreed that it is important to specify a minimum input copy number, so that users of the web tool have quality output without substantial resampling. Molecular barcoding techniques would be useful to interpret HIITE's readouts on people who are virally suppressed.

Use of HIV recency assays to monitor viral persistence in the context of cure research

This presentation explained research efforts to better understand how the immune system can be used to sense HIV, and viral replication, in the context of cure research. Because of CEPHIA it has been more possible to characterize that response using available recency assays. After treatment, HIV is usually not detectable, so other immune measurements are needed to understand the size of the reservoir, or whether there's immune identification. Using the VITROS Anti-HIV 1+2 assay (Ortho-Clinical Diagnostics, USA) modified either for less sensitive (LS) or for avidity, limited seroconversion is seen following early ART; elite controllers also have a reduction in antibody production compared to regular untreated subjects (see Figure 9).

Figure 9. Differentially expressed HIV antibodies in various groups of HIV control



While these antibody kinetics pose a large problem for HIV recency assays, they may provide an opportunity to monitor whether someone has controlled their viral infection. A group at UCSF has been studying the impact of time between infection and treatment on antibody production, and found significant effect (i.e. antibodies decline rapidly after viral suppression, and are stable over time). Antibodies also correlate with reservoir measurements using both total DNA and cell-associated RNA. Longitudinal measurements of antibodies can provide a surrogate marker for systemic HIV replication over previous weeks to months. This has potential for application in the field, where antibodies may be a good alternative to viral load, as they are cheap, fast, reproducible, and provide markers of viral replication weeks to months before viral load changes. One of the limitations of this work, however, is that the VITROS platform has been used for most of the analysis, which is a very stable assay system; the LAg Avidity EIA has other technical variation and lot-to-lot variation, which may be an issue for measurement of viral suppression over time. Another challenge is that this technique will not be useful for people who never seroconvert, such as adults or babies who are very early treated.

Work in progress in this area includes:

[1] Total HIV reservoir measurement, as an alternative to ultrasensitive VL, Quantitative Viral Outgrowth Assay (QVOA), and cell-associated HIV RNA;

[2] Understanding the dynamics of HIV protein-specific antibody responses;

[3] Investigating other characteristics of antibodies for measuring the reservoir of infected cells; and

[4] Biomarkers of viral replication in cure interventions.

6. OTHER METHODS TO ESTIMATE INCIDENCE

The purpose of this session was to explore alternative methodologies for estimating HIV incidence. Angela Hernandez and Ruiguang (Rick) Song (CDC) presented about their strategy for using CD4 values on HIV case reports to estimate United States HIV incidence. John Saunders (Public Health England) spoke about similar strategies used in the United Kingdom, combining data from the GUMCAD STI Surveillance System and HARS HIV/AIDS Surveillance System. Ian Fellows (Fellows Statistics) presented strategies for using RITA data and HIV testing history to estimate HIV incidence at the sub-national level. Dimitri Prybylski (CDC) reviewed methodologies to estimate HIV incidence among key populations, using biobehavioral surveys. Lastly, Frances Cowen (Liverpool School of Tropical Medicine) presented about using RDS to estimate HIV incidence among key populations, specifically a cohort of female sex workers in Zimbabwe.

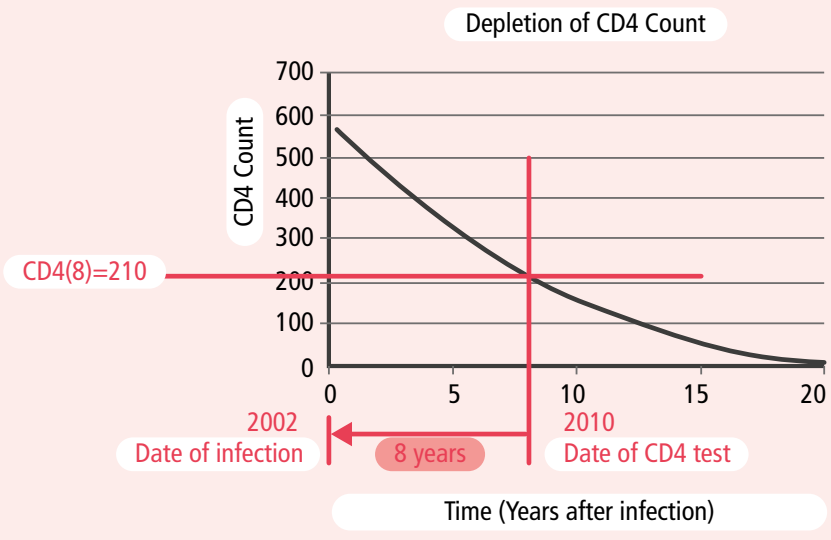
CDC HIV incidence estimation methods update

The CDC uses a combination of strategies to estimate United States HIV incidence: Bayesian-based back calculation of existing surveillance data, CD4-based back-calculation on recent (8+ years) surveillance data, and until recently, biomarker-based sample surveys (which have been discontinued due to cost-effectiveness concerns). For this presentation, the focus was on the CD4 method.

In this method, the first CD4 value after HIV diagnosis is used to estimate the distribution of delay from infection to diagnosis, under the assumption that the infected person is untreated at the time of their first CD4 test. This distribution is then used to estimate HIV incidence, and HIV incidence, combined with information on diagnoses and deaths, is used to estimate US HIV prevalence.

This strategy uses a CD4 depletion model (see Figure 10). As not all people with diagnosed HIV have a CD4 test reported in surveillance data, the number of people with CD4 test results are weighted to account for people without CD4 results. The estimated date of infection for people younger than 13 is set to the date they reached age 13. The distribution of delay (from HIV infection to diagnosis) is then estimated, and used to estimate the annual number of HIV infections (both diagnosed and undiagnosed). More parameters of the CD4 depletion model are available in a recent publication by Song, et al. (Song, et al. 2017).

Figure 10. Estimation of HIV infection date using



Advantages of this model include: 1) CD4 data is routinely collected in the US National HIV Surveillance System; 2) historical data is not required to estimate incidence, prevalence, and percent of diagnosed infections; 3) it results in single-year estimates, so trends can be assessed; 4) it allows for estimates within key populations and/or individual jurisdictions; and 5) it can incorporate information related to the duration of HIV infection. However, it relies on a fairly old CD4 depletion model that is unlikely to be updated, and requires high completeness of CD4 data (>50% within 3 months after diagnosis, and >85% overall).

It also relies on many assumptions, including:

[1] The CD4 depletion model is correct

[2] There is no treatment before the first CD4 test (may be becoming less true)

[3] All data adjustments are unbiased (e.g., multiple imputation for missing values of transmission category, the weight to account for cases without CD4 test)

[4] Diagnosis delay is stable, and its distribution can be estimated from cases diagnosed in recent years

[5] HIV infection, diagnosis, and death occur in a "closed" population (no migration)

When the trends identified by the CD4 method were compared with older methods, the trends are similar though there is some difference using absolute numbers (Hall, et al. 2017). During discussion, some working group members raised concerns about the use of CD4 counts as a basis for HIV incidence estimation, given variations in CD4 means in different geographic areas/subpopulations as well as the impact of PrEP or other treatment on CD4 depletion, likely to become a source of growing pressure for the static CD4 depletion model. There was a strong suggestion to cross-validate this model using CD4 data from CEPHIA, JHU, or other sources.

United Kingdom HIV incidence estimation methods update

This presentation focused on an effort to try to better understand the large drop in new HIV diagnoses seen in central London HIV testing clinics at the end of 2016.

The analysis combined data from two sources:

[1] GUMCAD, the STI Surveillance System that includes mandatory reporting from >600 sexual health services in England. GUMCAD only looks at STI testing services provided free by the government, and is an electronic, pseudo-anonymised patient-level dataset; and

[2] HARS, the HIV/AIDS Surveillance System that includes HIV diagnoses from all settings throughout the UK, which contains clinical information relating to HIV treatment and care; this system hopefully captures the remainder of relevant data that would be missed by GUMCAD.

Results shared from the analysis showed a significant decline in positivity among gay and bisexual men from 2013 to 2016, particularly in London, regardless of the number of prior HIV tests had. The findings are more stable in London clinics; outside of London, infrequent testers make up a larger proportion of total testers, which impacts results.

Like CDC, the UK is also using CD4 back-calculation and a Bayesian model to determine the number of new observed diagnoses of HIV compared with the number of expected infections over time. They have found that there is increased testing over time, increased frequency of testing, more rapid ART initiation, and very high VL suppression in the UK, which they believe is really helping to drive the decrease in incidence, in combination. Current analyses do not capture the effect of condom use or PrEP; PrEP particularly is probably also having a real impact on incidence, and is the next focus area of study. The analysis also has not yet integrated STI data, which may be useful for measuring the impact of PrEP (i.e. if STI rates are stable or increasing, and HIV rates are decreasing).

Use of RITA data and HIV testing history to estimate incidence

This presentation began with the motivating idea that, all other things equal, a higher infection rate in a population leads to a larger undiagnosed population, and a higher diagnosis rate leads to a smaller undiagnosed population. The transmission rate is the number of people infected per individual already infected, per time unit, and incidence is the rate of infection among those at risk. To control an epidemic, the number of individuals infected by each infected individual must be <1; and given that individuals remain infected for an estimated 33 years assuming complete ART coverage, the target transmission rate is 3% (1/33) for epidemic control.

Using a cross-sectional survey with data related to HIV status, date of last negative test, and whether ever diagnosed, it is possible to estimate the number of people undiagnosed for HIV, adjusting for misreporting of "ever diagnosed" using HIV viral load and ART biomarkers (recommended but not required). Certainty estimates can be generated using bootstraps. Basic calculations are shown below:

$$\tau = \frac{P(U\backslash H)}{E(TID)} \qquad \lambda = \frac{P(U\backslash H)P(H)}{E(TID)(1-P(H))}$$

λ = Incidence
τ = Transmission Rate
$P(U\backslash H)$ = Proportion infected who are undiagnosed
$P(H)$ = Proportion infected
$E(TID)$ = The expected time from infection to diagnosis

This method can be applied in complex survey design situations, by replacing sample means and proportions by weighted means and proportions, and weighting the likelihood used to calculate the expected time since last test among those who test, using a series of published bootstrap information.

The undiagnosed population is the first place that changes in incidence can be noted; changes in the size of the undiagnosed population either means incidence rates are changing, or diagnosis rates are changing. Therefore, the size of the undiagnosed population should be targeted for monitoring, along with testing rates. While this strategy provides a method to estimate incidence in subnational districts or key populations, doing so is difficult and relies on a number of assumptions, in the absence of vast quantities of data:

- The disease as at a steady state
  - If violated, the estimate will simply average the incidence rate over the distribution of time from infection to diagnosis
- HIV infection is independent of testing behaviour
  - If individuals in fact test in response to risk behaviour, then the estimate of expected time from infection to diagnosis will be too high
- People who are treated (have positive ART/viral load biomarkers) miss-report their undiagnosed status at the same rate as untreated people
  - If treated people are less likely to miss-report undiagnosed status, then the estimate of the proportion infected who are undiagnosed will be too high
- Those who have never tested with be diagnosed at onset of AIDS
  - If violated, the estimate of expected time from infection to diagnosis will be too large

There was considerable enthusiasm for this approach among the working group during discussion; multiple suggestions were made for validation of this approach using real data sets (e.g. the Rakai data), to test for robustness and accuracy compared with observed incidence.

Methodologies to estimate HIV incidence among key populations

HIV incidence can be estimated among key populations in a number of ways:

[1] **Directly observed HIV incidence through prospective cohort studies** of HIV-negative people followed over time and tested at regular intervals. These are often considered the gold standard as incidence is directly observed, but are costly, logistically difficult, and prone to selection bias, the Hawthorne effect, and loss to follow-up.

[2] **Probability-based sampling methods using biobehavioral surveys**, including time-location sampling, conventional cluster sampling (e.g. for prisoners or those in institutional settings), and respondent-driven sampling (RDS). In RDS, information from participants and social networks is used to infer information about the wider population; this is useful for many key populations who can't be easily sampled in census.

[3] **Laboratory testing, using RITAs**, which can be low-cost and fast, without required follow-up. However, as the working group has discussed at length over time, this requires very large sample sizes and more complex testing, including viral load determination (challenging for some field settings). Pooling of data from multiple RDS surveys among the same key population can sometimes work to meet sample size requirements and make this type of estimation possible.

[4] **Osmond's Algorithm**, which requires data on HIV status, date of HIV testing, and date of onset of risk behaviour, to calculate the number of HIV-positive people in a sample divided by person-years of risk (see Figure 11). This can be used with a single HIV prevalence survey, and is a simple, expensive method to monitor HIV incidence, but is best applied in young populations or recent initiators of HIV risk behaviours, and is less useful in generalized epidemics.

[5] **Serial RDS surveys**, where estimated incidence can be assessed longitudinally using the strategies in #2, above. One example shared was the ARISTOTLE Study in Greece, which used a RITA along with analysis of serial RDS surveys for measurement of the impact of a rapid combination intervention to address an HIV outbreak among people who inject drugs in the country.

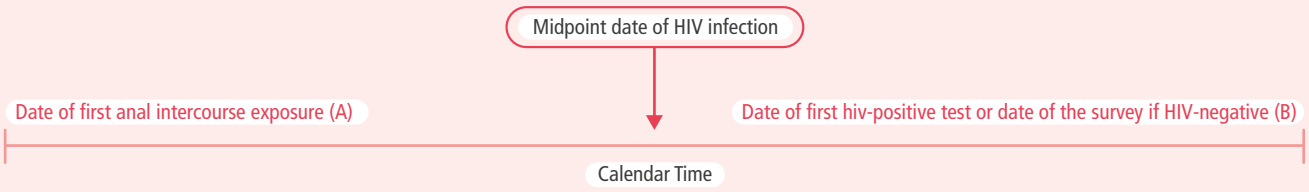
[6] **Mathematical modelling**, which can be used to estimate HIV incidence among key populations with generally easy-to-access data (such as biobehavioural surveys) in concentrated epidemics settings. However, adequate biobehavioural survey data is often lacking to dynamically model key population HIV incidence in generalized epidemics.

[7] **Viral load measures as surrogates for HIV incidence**, which is based on the idea that population viremia is a marker of forward transmission potential. This may be combined with behavioural risk measures (e.g. unprotected sex acts, number of partners) and is often a strong predictor for HIV incidence, but on its own is likely insufficient to derive a robust estimate of incidence. More research is needed in this area.

Ultimately, demonstrating consistency across multiple methods may increase confidence in estimates. An example for using RDS data and modelling to estimate HIV incidence among female sex workers in Zimbabwe was shared as the final presentation of day 2. While a prospective study is about to begin to explore the design of specific programmatic approaches for incorporating recency testing into the HIV testing algorithms at in settings frequented by sex workers, a retrospective analysis has been completed using a RITA using LAg, viral load testing, and ARV testing in combination with biobehavioural RDS data, for >9300 DBS specimens stored from a survey of >13,000 female sex workers from 2009-2013.

Preliminary results of this analysis was presented for discussion by working group members. Much of the discussion focused on the validity of the findings from the RITA, based on DBS specimens; the DBS specimens in this study were stored for about 18 months at room temperature, which JHU researchers have shown reduces their utility for recency assays compared with plasma (CDC has found that when not properly stored, DBS specimens lead to a substantial increase in incidence estimates). Others raised concerns about DBS specimens including the difficulty of controlling for elution efficiency, differences in haematocrit, whether blood is spread evenly throughout the spot, and whether the 6mm punches are consistent. Since most incidence testing is off-label, the standards for proper specimen collection, storage and processing of DBS specimens are not formally set by the assay manufacturer, and thus the end-user attempts this task. Off-label usage introduces uncertainty into incidence estimates, and therefore the group discussed the importance of approaching manufacturers to ask that they submit claims for validated uses of these specimen types with their assays.

Figure 11. Calculation strategy for Osmond's Algorithm



7. KEY MESSAGES AND DISCUSSION

The final discussion of the working group on Day 2 included a summary of what was new and notable from the meeting, along with controversial areas and/or those requiring further discussion to reach consensus. These points included:

- The release of a new, publicly-available infection dating tool, at https://tools.incidence-estimation.org/idt. Publications are forthcoming that will clarify details of the methods and opportunities available for estimation of infection dates for individuals in cohorts or clinical settings.
- Clear evidence of differences in MDRI by subtype and by sex, with longer MDRIs seen in subtype D and male specimens. These differences in MDRI by subtype and sex call for strategies to compute incidence using a weighted average MDRI based on the distribution of subtype and sex in the sample, and methods for doing this were posed in the meeting.
- While evidence does not yet exist for differences by pregnancy, this may be the result of insufficient availability of well-characterised pregnancy status on specimens for analysis. The group discussed possibilities for addressing this issue at length; one solution may be testing existing specimens with pregnancy tests, then incorporating that data. CEPHIA has a small number (n=84) of specimens from known pregnant women from CAPRISA, and Peter Hunt (UCSF) has a panel of specimens from pregnant women that might be available for sharing, though some funding would be needed to make specimens available; Oliver Laeyendecker offered to test specimens at JHU at no cost.
- It is clear that incidence estimates are sensitive to the FRR used in calculations; therefore, it is important to come to some consensus on how to properly estimate FRR in various contexts. CDC currently uses an FRR of 0.0% for all incidence estimation using the LAg Avidity EIA, including in PHIA studies worldwide; however, most estimates in any other context find a non-zero FRR for LAg Avidity EIA. FRR is particularly susceptible to issues of suppressed viremia and early treatment with ARVs, including those on PrEP. In the two most recent CEPHIA papers (Kassanjee, et al., 2016; Murphy, et al., 2016), there is a method described there of how to derive a context-specific FRR estimate. Future discussion is needed from this group on this or other methods.
- Assays manufactured by different manufacturers (i.e. the Sedia™ HIV-1 LAg-Avidity EIA and the Maxim HIV-1 Limiting Antigen Avidity assay) have different MDRIs and FRRs, and must be clearly identified whenever reporting results. Assay-appropriate MDRI and FRR estimates must be used for incidence calculations.
- There is value in including ARV testing in RITAs; however, while theoretically attractive, ARV testing is not practical in many countries. As of the time of the meeting, only one country in Africa can conduct ARV testing (South Africa), and they can test for ~20 ARVs. The group discussed the absolute priority of developing a statistical approach to interpreting RITA-based estimates, based on available data and the changing context of ARVs, assuming routine ARV testing does not become readily practical. More exploration is also needed about the impact of ARV treated but unsuppressed individuals on MDRI.
- Many pilot projects have been undertaken to explore the application of RITAs and specific assays (i.e. point of care recency assays) for case surveillance and partner notification; this has potential for larger scale-up in the near future given PEPFAR's focus on implementation of recency testing at or near to point of care. The Asanté™ HIV-1 Rapid Recency™ Assay has no calibrator; therefore, it has limited usefulness for incidence estimation, which requires a more precise assay. The huge variation seen when running the CEPHIA evaluation panels on different assays was due almost entirely to calibrators, which detect inherent variation and therefore can be used to reduce instability in estimates. While feasibility and acceptability of recency results being returned to patients has been high in pilot studies, and the Asanté™ HIV-1 Rapid Recency™ Assay is useful for providing information to individual patients at point of care, it is important to develop a series of standards and recommendations related to use and interpretation of results in the field, before information is provided to patients outside of IRB oversight in the context of pre-validation research studies. The guidance needed for physicians (who are used to integrating clinical information and providing nuanced diagnoses and prognoses) may be different from that needed for community-based HIV test counsellors, who may need more simplified strategies for interpretation and messaging.
- Promising work is underway to explore the use of recency estimates in modelling and hotspot identification; the use of these strategies to extend survey-based national estimates to the sub-national level warrants further attention.

8. NEXT STEPS

Ultimately, the working group did not make a final decision about whether to release a 2018 Technical Update, or do a broader revision of the 2011 guidance (UNAIDS/WHO, 2011). However, it is clear that further updates or guidance are needed to shape the changing field of HIV incidence estimation. The following areas must be discussed, in order to determine the best path forward for future guidance:

- **Who is the key audience?** Technical Updates tend to focus on details of recommended methods, whereas UNAIDS/WHO guidelines tend to include broad-sweeping guidance (i.e. your RITAs should include viral load) and are not very technically complicated.
- **What other information is available about experiences with rapid recency testing?** How can this information be collected, assessed, and ultimately used to improve testing programs?
- **What are the statistical methods, clinical recommendations, and laboratory standards** for which consensus exists, which can be included in a general manual for use in efforts to measure incidence and monitor impact of programs over time, in the field?
- **Is there broader representation required for the working group?** Comprehensive recommendations may require input from additional subject matter experts, yet to be defined.

There was a call from numerous working group members to hold a next meeting focused specifically on discussion, consensus-building, and decision-making related to a number of issues still unresolved at the close of this meeting. Several past meetings have focused on presentation and discussion of innovations and updates, without sufficient time to debate and ultimately resolve controversial issues; meanwhile, the field continues to move and act in the absence of updated guidance from this group.

Some issues proposed for future focused debate and consensus-building include:

[1] Methods for appropriate, practical **contextual adaptation of MDRI and FRR;**

[2] Evaluation of the **need for and role of ARV testing**, in relation to RITAs or other methods of incidence estimation;

[3] Strengthening capacity to **estimate incidence using methods beyond RITAs**; and

[4] **Maintenance and continued development of specimen** repositories and/or panel sets to support further research and testing of assays and serologically-based incidence estimation strategies.

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ANNEX 1: PLANNED MEETING PROGRAMME

Saturday, 03 March 2018

Time	Topic	Presenter
08:30 - 09:00	Breakfast	
09:00 - 09:20	Welcome remarks: Introductions, objectives and expected outcome, review agenda and review of outstanding issues from last meeting	Txema Calleja, WHO Kim Marsh, UNAIDS
	Session 1: Current methods and advances in HIV incidence assays	
09:20 - 09:40	Summary of Final CEPHIA assay validations and products	Gary Murphy, PHE
09:40 - 10:00	MDRI and FRR estimationn updates	Alex Welte, SACEMA
10:00 - 10:20	Pooled analysis of JHU and CEPHIA data for estimation of MDRI by sub-type	Eduard Grebe, SACEMA
10:20 - 10:40	Experience from the field in the use of HIV incidence assays: Rakai, Uganda	Oliver Laeyendecker, JHU
10:40 - 11:00	WHO recommendations on post-market surveillance of in vitro diagnostics	Anita Sands, WHO
11:00 - 11:30	Coffee Break	
11:30 - 11:50	Estimating recent infection testing algorithm performance characteristics among pregnant women, Zvitambo	Bharat Parekh, CDC
11:50 - 12:10	R-RITA (Revised RITA) to include ARV in the testing algorithm	Thomas Rehle
12:10 - 12:30	Comparison of HIV incidence estimates using a standard RITA and R-RITA (with ARV) using population survey data	Bharat Parekh, CDC
12:30 - 13:30	Lunch	
	Session 2: Point of care and programmatic use	
13:30 - 13:50	Preliminary findings from Malawi pilot incorporating recent infection surveillance and return of results among adolescent girls and young women	Elfriede Agyemang, CDC
13:50 - 14:10	Preliminary findings from integration of Asante in HIV testing services for case-based surveillance and partner notification activities in Central America	Sanny Northbrook, CDC
14:10 - 14:30	MeSH pilot study: assesing integration of recent infection testing algorithms into routine testing services among pregnant women, at testing facilities and among female sex workers, with return of results	Brian Rice, LSHTM
14:30 - 14:50	PEPFAR planned expansion activities of rapid HIV incidence assays among newly diagnosed in 2019	Katie Curran, CDC
14:50 - 15:10	Incorporating incidence measurements from population surveys into model-based epidemic estimates	Jeff Eaton, Imperial College London
15:10 - 15:30	Discussion	
15:30 - 16:00	Coffee Break	

(cont.)

Saturday, 03 March 2018

Time	Topic	Presenter
	Session 3: Innovations and new biomarks	
16:00 - 16:20	Innovative Multiplex Assay for Simplifying HIV Surveillance	Ernest Yufenyuy, CDC
16:20 - 16:40	Other use of HIV of incidence assays: monitoring viral persistence in the context of cure research	Sheila Keating, UCSF
16:40 - 17:00	HIITE: HIV-1 Incidence and infection time estimator	Ha Youn Lee, USC
17:00 - 17:30	Novel Biomarkers for the development of HIV incidence assays with improved specificity	Usha Sharma, NIH

Sunday, 04 March 2018

	Session 4: Other methods to estimate incidence	
08:30 - 08:50	Using HIV case reporting for estimating HIV incidence: USA	Angela Hernandez, CDC
08:50 - 09:10	Using HIV case reporting for estimating HIV incidence: UK	John Saunders, PHE
09:10 - 09:30	Approaches to estimating HIV incidence at the sub-national level using recent infection testing algorithm and HIV testing history	Ian Fellows, Fellows Statistics
09:30 - 10:00	Review of methodologies to estimate HIV incidence among key population using biobehavioral surveys	Dimitri Prybylski, CDC
10:00 - 10:30	KP population approach to estimate HIV incidence using RDS, Zimbabwe Cohort FSW	Frances Cowen, LSTM
10:30 - 11:00	Coffee Break	
11:00 - 12:00	Discussion and key messages for technical update	WHO | UNAIDS
12:00 - 12:45	Final recommendations how to improve HIV incidence measurement	WHO
12:45 - 13:00	Meeting close	

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