DEVELOPMENT OF ASSAYS TO ESTIMATE HIV INCIDENCE

MEETING PROCEEDINGS

CHAPEL HILL, NORTH CAROLINA

MAY 13 – 14, 2009

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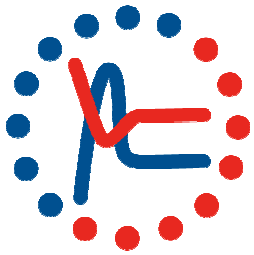

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Acronyms

AIDS	Acquired Immune Deficiency Syndrome
AIEDRP	Acute HIV Infection and Early Diseases Research Program
AI-EIA	Two-well Avidity Index Enzyme Immuno Assay
ALIVE	A Longitudinal Study of HIV-1 Infection in Intravenous Drug Users: John Hopkins Study
ANRS	Agence Nationale de Recherches sur le Sida
ARS	Acute Retroviral Syndrome
ART	Antiretroviral Therapy
B cell	A lymphocyte (a white blood cell): matures in bone marrow and produce antibodies
BED	Enzyme Immunoassay using peptides from HIV-1 subtypes B, E, and D
BSRI	Blood Systems Research Institute
CASCADE	Concerted Action on Seroconversion to AIDS and Death in Europe
CAPRISA	Centre for the AIDS Programme of Research in South Africa
CDC	U.S. Centers for Disease Control and Prevention
CHAVI	Center for HIV/AIDS Vaccine Immunology
CV	Coefficient of Variation
DHS	Demographic and Health Survey
EIA	Enzyme Immuno Assay
ELISA	Enzyme-linked Immunosorbent Assay
Env gp140	Envelope glycoprotein 140
Gag (p55,p24)	Retroviral matrix proteins
Gp41	Glycoprotein 41 (HIV)
Gp120	Glycoprotein 120 (HIV)
FDA	Food and Drug Administration
FHI	Family Health International
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIVNET	HIV Network for Prevention Trials
HPTN	HIV Prevention Trial Network
IAS	International AIDS Society
ICSS	Incidence Core Sample Set
IDE (-V3)	Immunodominant Epitope
IDU	Injection Drug User
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Inno-LIA	Line Probe Immunoassay
KAIS	Kenya AIDS Indicator Survey
LOD	Limit of Detection
LS	Less Sensitive
LSI	Lymphocyte Stimulation Index
MACS	Multi-center AIDS Cohort Study
MOH	Ministry of Health
MPER peptide	Membrane Proximal Epitope Region Peptide HIV
MTN	Microbicides Trials Network
NAT	Nucleic Acid Test
NCHECR	National Centre in HIV Epidemiology and Clinical Research
NHANES	National Health and Nutrition Examination Survey
NIAID	National Institute for Allergy and Infectious Diseases
NIH	U.S. National Institutes of Health

NK	Natural Killer cells
OGAC	Office of the U.S. Global AIDS Coordinator
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PEPFAR	President's Emergency Plan for AIDS Relief
PRD	Product Requirement Document
PRIMO	French Prospective Cohort HIV Study
RITA	Recent Infection Testing Algorithm
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
SACEMA	South African Centre for Epidemiological Modeling and Analysis
SC	Seroconversion
STARHS	Serologic Testing Algorithm for Recent HIV Seroconversion
UNAIDS	Joint United Nations Programme for HIV/AIDS
UNSW	University of New South Wales
USAID	United States Agency for International Development
V3 peptide	V3 region of HIV-1 gp120
WIHS	Women's Interagency HIV Study
WHO	World Health Organization

EXECUTIVE SUMMARY

Family Health International (FHI) convened a meeting on the Development of Assays to Estimate HIV Incidence on May 13-14, 2009 at the Carolina Inn in Chapel Hill, North Carolina. The purpose of this meeting was to assess how new and existing technologies and research could be applied to advance the development of assays to estimate HIV incidence. Nearly fifty leading experts including immunologists, epidemiologists, HIV transmission experts, assay developers, virologists, industry representatives, and potential users of assays participated in the meeting. The meeting was primarily supported by the Bill & Melinda Gates Foundation and in conjunction with and the support of the World Health Organization (WHO), the HIV Prevention Trials Network (HPTN), and the Center for HIV/AIDS Vaccine Immunology (CHAVI).

There is a global need for inexpensive, easy-to-use human immunodeficiency virus (HIV) assays that can be used to reliably estimate HIV incidence at the population level using cross-sectional methodologies. Current assays are limited by the number of samples from persons with long-standing infection that are misclassified as representing recent infection as well as HIV subtype-dependent and other factors that result in over-estimation of HIV incidence rates. None of the current assays used to estimate HIV incidence are recommended for use at the individual level, although such an assay could have both clinical management and public health applications (partner services and characterization of recently transmitted viruses for genotype and drug resistance profiles). Consensus is needed within the field to help standardize terminology, develop and refine HIV incidence assays, and provide guidelines for the use of such assays.

Reliable estimates of HIV incidence in a population are critical for epidemiologic characterization, the evaluation of HIV prevention programs, and in the design and evaluation of HIV intervention trials. Incidence data can also be used to monitor transmission patterns and better target HIV prevention efforts. Moreover, the identification of recently infected persons during a defined, brief period following HIV infection and/or seroconversion, which contributes substantially to HIV transmission due to behavioral and biologic factors, could have important applications at the public health level.

Standard methods to estimate HIV incidence are unsatisfactory. The indirect approach, based on the measurement of prevalence in repeat cross-sectional surveys, is logistically challenging, takes years to conduct, and is difficult to standardize over time. Direct measurement of incidence through the prospective follow-up of a cohort of HIV-negative persons is expensive, unrepresentative, and not sustainable even in resource-rich settings. Furthermore, the enrollment of persons into a cohort study often leads to behavior changes that result in a lower observed rate of HIV incidence than in the broader population of interest. It is possible to estimate HIV incidence from surveys via the detection of the HIV p24 antigen and/or HIV RNA before seroconversion on HIV antibody assays. However, due to the very short period of time individuals are antigenemic/viremic before seroconversion, this method requires very large sample sizes and is often impractical due to the need to test all seronegative samples for p24 Ag or RNA).

As an alternative means of estimating HIV incidence in a population from cross-sectional surveys, researchers have developed laboratory assays based on the principle that the HIV antibody response matures over time and that people with recently acquired infection can be identified during a defined post-seroconversion “window period.” These tests could then be used to determine the number of recently infected persons among the HIV-seropositives identified in

a representative sample of a population or from a surveillance system and the resulting number and window period duration used to estimate HIV incidence.

A decade ago, scientists at the U.S. Centers for Disease Control and Prevention (CDC) and collaborators reported on the use of such an assay in a method known as the Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS). This algorithm employed a sensitive commercial assay (Abbott HIV 3A11) and a customized, less-sensitive (LS or "detuned") version of this assay; the specimen from a recently infected individual is reactive on the sensitive assay, but non-reactive on the LS assay. A similar approach was used with the BioMérieux Vironostika HIV-1 assay. It was subsequently found that these assays (both based on HIV-1 subtype B, most common in the United States and Europe) had significantly different post-seroconversion window periods for non-B subtypes, which predominate in other regions of the world. Consequently, without accounting for subtype distributions among the HIV-seropositive subjects in the sample, use of these assays would yield inaccurate estimates of HIV incidence. An additional practical problem was that both of the commercial assays that formed the basis for the algorithm were removed from the market by their manufacturers. Other immune-response-maturation approaches to identifying recent HIV-1 infection have been based on i) the proportion of HIV-specific antibodies that appear against parts of gp41 from HIV-1 subtypes B, E, and D among all IgG as measured by a capture enzyme immunoassay (the "BED" assay"); ii) quantification of the avidity of anti-HIV antibodies using a modified third generation anti-HIV assay; iii) measurement of the antibody response to a gp41 immunodominant epitope (IDE) and various gp120-V3 loop peptides, known as IDE-V3 assay; iv) measurement of isotype IgG3 anti-HIV which is present early in the immune response; and v) quantification of anti-HIV antibodies on a line immunoassay (Inno-LIA HIV adaptation).

However, these STARHS approaches have been challenged by various obstacles. These obstacles include: i) variability of the immune response among HIV-1 infected individuals and the impact of antiretroviral therapy and late stage AIDS immunosuppression leading to a lack of sensitivity and specificity (especially long-term specificity) in identifying persons with recent infection; ii) variation in the window period for different HIV-1 subtypes or populations; iii) difficulty in the standardization of post-seroconversion window-periods, assay calibration and quality control measures; iv) the unpredictable availability of the commercial products used as the backbone for some HIV incidence assays; v) complexity and high cost of some of the assays; and vi) perceived lack of a commercial market for HIV incidence assays. Due to these factors, the use of HIV incidence assays has been the subject of some debate and controversy and guidance on assay use is evolving.

Interested scientists from several countries have held *ad hoc* meetings over the past decade to discuss the development and validation of these assays. To advance this effort, the World Health Organization (WHO) has convened a Technical Working Group on HIV Incidence Assays, which held meetings in January and August 2008 and April 2009. This group is made up of epidemiologists, laboratory specialists, and public health officials. The group has worked to standardize terminology in the areas of assay calibration and validation, conduct a comprehensive literature review of studies that have reported on the assessment of the assays, develop a standardized protocol for assay validation, and define the specimen sample sets required for assay calibration and validation. The WHO Technical Working Group on HIV Incidence Assays has also developed a framework for advancing assay development, evaluation, validation, and comparison. Despite a recognized and urgent need for reliable assays for the estimation of HIV incidence (preferably endorsed by WHO and approved for use by relevant regulatory agencies), their absence represents a significant scientific and public health gap. Thus, there is a need to optimize and determine the appropriate use of current

assays, alone or in combination, and drive the identification and validation of novel, improved HIV incidence assays by engaging a broad range of stakeholders in a collaborative, focused partnership.

There is evolving guidance on the use of the only commercialized HIV incidence assay, the BED assay. The Joint United Nations Programme on HIV/AIDS (UNAIDS) reference group statement on the use of the BED assay for HIV incidence estimation (2005) recommended that the BED assay not be used for routine HIV surveillance applications. This statement was made after a review of BED-based HIV incidence estimates indicated that such estimates were substantially higher than those obtained with other methods. The Office of the U.S. Global AIDS Coordinator (OGAC) statement (2006) suggested that the BED assay could be used with appropriate adjustments, in conjunction with expert consultation in sentinel or population-based surveillance and evaluation of HIV prevention interventions. More recent guidance has recommended when using the BED assay to be careful in considering the populations and uses by: determining use of antiretroviral therapy, determining CD4 counts, employing expert consultation on sample sizes and using adjustments for misclassification. The WHO Technical Working Group on HIV Incidence Assays, in conjunction with CDC scientists, is developing a methodologic guidance document (2009) for standardizing the validation of existing and future HIV incidence assays.

The Chapel Hill meeting provided a forum for scientists from around the world to discuss various ways to improve the use of current HIV incidence assays, the need to develop new assays, and the best ways to determine the global demand for such assays. A series of presentations were delivered from representatives of FHI, CHAVI, the National Centre in HIV Epidemiology and Clinical Research (NCHECR), the South African Centre for Epidemiological Modeling and Analysis (SACEMA), the National Institute for Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH), CDC, the University of Francois-Rabelais, Tours (France), and the Blood Systems Research Institute (BSRI). Collectively, these presentations provided detailed information about: 1) the current state of HIV incidence estimation, 2) a market assessment for HIV incidence assays to understand terminology, perceptions, uses and global demand, 3) the merits and limitations of current and potential new biomarkers, 4) optimal specifications and requirements for HIV incidence assays, 5) the critical path to progress an HIV incidence assay through development; and 6) a definition of the specimens and infrastructure needed for HIV incidence assay development and validation. In addition, each presenter was invited to comment on the presence of gaps in knowledge or improved HIV incidence estimation efforts as they pertained to their subject area. These comments were used as a springboard for discussions intended to identify what efforts are needed to address these gaps in order to improve the availability of HIV incidence assays and accuracy of HIV incidence estimation.

The meeting was designed to be interactive and to stimulate conversation and exchange across disciplines. A number of panel discussions, breakout sessions and thought exercises were conducted to address the challenges facing the fields of HIV incidence estimation and assay development. Session participants were encouraged to pose solutions and identify the next steps forward.

Conclusions and Next Steps

1. Current State of HIV Incidence Estimation

Standardize terms and methods: There is a clear need to establish standard terminology and a standard set of methods to be used for HIV incidence assays; the WHO Working Group will address this activity.

Develop guidance document on use of assays (including BED): UNSW/NCHECR scientists will take the lead, in conjunction with the WHO Technical Working Group, on drafting a guidance document on the use of the assays for HIV incidence estimation as part of the FHI/Gates sub-grant program.

Sustained funding: An underlying theme for this meeting was the need for sustained funding for HIV incidence assay development (due to low market incentives).

2. Market Assessment for HIV Incidence Assays and Demand Estimation

Complete the market assessment: FHI will continue refining the global demand estimation for HIV incidence based on input received at the meeting.

3. Identification of Novel Biomarkers in Development of Assays to Identify Recent HIV Infection and to Estimate HIV Incidence

Use a two-prong approach to identify biomarkers: Use a dual strategy involving 1) a biomarker discovery effort in the long-term combined with 2) a parallel approach on how to optimize the use of currently existing HIV incidence assays.

Use a combination of biomarkers: Scientists recommended using a combination of markers (and multiplex assays) and giving different weights on parameters contingent on the population and HIV subtypes.

Exploit biomarker diversity: The need to exploit antibody/biomarker diversity was discussed. The group agreed that to identify a single biomarker assay will be challenging. Using multi-subtype recombinant antigen assays or manipulating ratios of biomarkers depending on the population may be promising approaches.

Evaluate biomarkers from chronically infected individuals: Biomarkers in chronically infected individuals will need to be better understood to move the field forward.

4. Epidemiology and Incidence Study Design

Develop a protocol for assessing assays: There is a pressing need for an internationally agreed framework for validating and comparing HIV incidence assays, such as a standardized assay or algorithm validation protocol. The WHO Technical Working Group will continue to develop a guidance document on this topic.

Foster consensus around statistical issues: There is a need to develop a consensus on statistical parameters for estimating HIV incidence. Statistical parameters to be identified include the determination of the window period and the development and application of adjustment factors to address long-term specificity. The WHO Technical Working Group Meeting held in April 2009 focused on resolving these statistical issues. A meeting report is forthcoming and will be posted on the WHO website.

5. Current Issues in Technical Assay Development – Input from Industry

Explore the options and opportunities to develop a public-private partnership: It was recommended to explore the concept of a public-private partnership to move the field of HIV incidence assay development forward.

Perform risk/reward and cost/benefit analyses: For HIV incidence assay development and commercialization it is important to evaluate likely costs/risks and projected rewards/benefits.

Develop assays for different uses or applications: From a commercial standpoint, it is important to consider developing different HIV incidence assays based on the different uses or applications to expand commercial market opportunities.

Generate commercial interest and application: HIV incidence assays must have a significant commercial application to generate market interest. Small companies, compared to large companies, may be more interested in developing products for the niche market for HIV incidence estimation. Large companies may support modifications or adaptation of their existing commercial HIV antibody assays for expanded use in identification of recently infected persons and incidence estimation.

Provide existing specimen panels to industry for assay development: Companies will need to have access to specimen sample sets to validate HIV incidence assays. Making this resource available to industry may promote an interest in HIV incidence assay development.

6. Pathway for HIV Incidence Assay Validation

Refine the assay development pathway: There is a need for a clear pathway for assay calibration and validation. Assay development should follow a similar process as that of drug discovery, with sequential phases. The WHO Technical Working Group will continue to refine their document on this topic.

7. Infrastructure and Specimens for Assay Validation

Establish a virtual database of sample sets: There is a need to catalogue studies and specimens for HIV incidence assay calibration and validation. The UNSW/NCHECR is currently developing a virtual database of sample sets as part of the FHI/Gates sub-grant program.

Establish a central specimen repository for systematic assay assessment and validation: There was a consensus expressed to establish a central HIV specimen repository. A key next step would be to develop the infrastructure for the HIV specimen repository, as described by Mike Busch in Section 8.2 of this report: Assay Development and Validation Toolbox. The central HIV specimen repository would contain samples obtained prospectively combined with archived samples.

Establish guidelines pertaining to the use of core specimen sample sets: Criteria must be established on how to access HIV sample sets.

8. Assay Specifications and Performance Requirement

Refine assay specifications based on assay uses and applications: Minimum and optimal specifications and requirements must be established for HIV incidence assays based on assay uses and applications. Specificity was considered one of the most important requirements.

PRESENTATION SUMMARIES

1. SESSION I: CURRENT ISSUES IN HIV INCIDENCE ESTIMATION

1.1 Overview of the Key Issues in HIV Incidence Estimation

Timothy Mastro, MD, Vice President of Health and Development Sciences, FHI, presented an overview of the key issues in HIV incidence estimation and outlined the meeting objectives and goals. Key elements of this presentation are included in the Executive Summary.

The accurate measurement of HIV incidence is crucial for the fight against HIV. Without an accurate measure of incidence, scientists cannot identify whether the number of new HIV infections is increasing or decreasing, or whether new HIV-prevention strategies are working. Traditional methods, including cross-sectional surveys and cohort studies, are slow, expensive, logistically challenging, and difficult to sustain.

Scientists have been trying to develop better assays based on the principle that a person's immune response to HIV evolves during the early stages of an infection. In theory, a person with a recently acquired infection could be identified by an immature antibody response in his or her blood. In practice, however, current tests frequently misclassify a person with a long-standing HIV infection as being recently infected—resulting in inaccurate HIV-incidence estimates.

The need for an improved HIV incidence assay is only part of the problem. Scientists need better statistical methods to design and interpret HIV incidence surveys in various global populations. Furthermore, a recent FHI survey of scientists, program leaders, donors, clinicians, and other stakeholders found that:

- Terms that describe "HIV incidence," a "recent infection," and "assays" are used inconsistently
- The most widely used technique—the BED assay—is well known, but many experts were not aware of alternative methods to estimate HIV incidence. (BED refers to the B, E, and D subtypes of HIV-1)
- The usefulness of the BED assay is disputed because of misclassifications that give rise to inaccurate estimates
- There is a lack of consensus on the demand for HIV incidence assays

Meeting objectives

- Determine the merits and limitations of current biomarkers and candidate biomarkers (Biomarkers are the antibodies and other biological molecules that are assayed to determine a person's HIV status.)
- Establish the optimal specifications and requirements for each HIV incidence assay
- Define the materials—human specimens, laboratory supplies and infrastructure—needed to validate a new assay
- Develop standardized terms for key concepts
- Clarify the public health needs and the clinical needs for the assays
- Identify the next steps for the ongoing market assessment and estimate of demand for HIV incidence assays

Key questions during this presentation included:

- Is a multi-test algorithm the best approach?
- Are there additional biomarkers that better detect recent HIV infection and measure HIV incidence?

1.2 Market Assessment: Overview and Preliminary Findings

Megan Averill, Research Associate, FHI, presented an overview and preliminary findings of a market assessment to examine perspectives on and the global demand for assays to estimate HIV incidence.

In late 2008, FHI launched an examination of global demand for assays to estimate HIV incidence and detect acute HIV infection. To complement its global public health expertise, FHI recruited *bio*Strategies Group — a biotechnology market research firm with experience in HIV-related products. This joint endeavor seeks to characterize current and future needs across widely varied users and applications, including multilateral bodies conducting and evaluating HIV prevention programs, governments, sponsors of epidemiological surveys, researchers, institutions assessing potential clinical trial sites, and clinicians providing HIV diagnosis and treatment.

The following activities are underway to assess current use of and future demand for assays to estimate HIV incidence and detect acute infection: in-depth consultations with expert advisors; identification of key stakeholder groups and target informants; qualitative interviews with informants representing varied stakeholders and geographic areas; and construction of models to estimate demand for assays accounting for attributes and applications.

This presentation summarized the preliminary findings of the market assessment which was based on qualitative interviews completed through May 1, 2009. A comprehensive report of findings will be issued after data collection and analysis are complete.

Methods

In-depth interviews with 52 key informants on HIV incidence estimation were conducted in March and April 2009. Each interview lasted approximately one hour. Interviewees were identified to ensure geographic diversity and input from major stakeholders who develop and use assays to estimate HIV incidence and detect acute HIV infection. These included biomedical and behavioral HIV prevention researchers; HIV prevention program leaders; epidemiologists and HIV surveillance experts; basic scientists; large donors underwriting HIV prevention, treatment, and surveillance activities; multilateral coordinating bodies; biotechnology and pharmaceutical firms; blood banks; and clinicians. A structured interview guide included questions on terminology related to recent HIV infection; awareness and current use of specific assays to estimate HIV incidence and/or detect acute infection; the types of work for which such assays are used; advantages and disadvantages of various assays; necessary and desired attributes in improved assays; potential increases or decreases in future assay use; and factors influencing these projected levels of use.

Findings

Terminology: Terms around HIV incidence and recent infection are used inconsistently. Conceptions of acute and recent infection were conflated, as were the terms used to describe assays to estimate HIV incidence and detect acute infections. Many interviewees categorized infections as "recent" based on specific assays used to detect them. Some defined "recent" infection as that occurring 6 to 12 months post-transmission. Fewer considered infection 1-2 months post-transmission as "recent." Interviewees acknowledged and were concerned about variability in labeling. One interviewee reported avoiding the term "recent infection" all together due to the confusion in terminology used. Many, but not all, agreed that an assay specifically used in estimating HIV incidence should be referred to as an "incidence assay." Others recommended using "recent infection assay," "early infection assay," "acute infection assay,"

and “early seroconversion test.” An interviewee urged that assays used to estimate HIV incidence should be named such that users clearly understand their purpose.

Awareness and Use: BED is the most widely known and commonly used assay in estimating HIV incidence. While all interviewees had heard of the BED assay, not all were aware of alternative methods to estimate HIV incidence, such as avidity tests and less-sensitive (“detuned”) assays. The assays most commonly used in estimating HIV incidence were the BED assay followed by multi-assay algorithms involving the BED and avidity tests. Other interviewees used avidity tests alone or detuned assays to estimate HIV Incidence. Several interviewees reported using other methods to estimate HIV incidence, some components of which are not commercially available. Due to inaccuracies in estimates produced, some interviewees objected to using assays to estimate HIV incidence altogether. Assays are primarily being used to estimate incidence in epidemiological surveillance, with a focus on understanding transmission dynamics and identifying populations with high HIV incidence. Secondary uses are for evaluating HIV prevention studies and identifying appropriate clinical trial populations and sites.

BED Assay: While awareness of the BED assay is widespread, its acceptance and use are intensely disputed. Interviewees reported diametrically opposed views on the BED assay, and a range of opinions in between. One BED assay supporter said, “The [BED] idea is brilliant;” while another reported, “I don’t want to use [BED assays] at all. I think they’re awful.” The primary advantages of the BED assay cited were convenience and cost. Users commented positively on its high throughput and ease of use in terms of training and sample types required. Low estimated cost per sample and commercial availability were also appreciated. Current users, former users, and nonusers alike strongly criticized the BED assay’s tendency towards overestimation of HIV incidence. Other concerns included inconsistency in cross-reactivity across HIV subtypes, complicated calculation requirements, and irreproducibility of results. These drawbacks spurred some interviewees to avoid relying on assay-derived cross-sectional HIV incidence data entirely. Others suggested using avidity assays in combination with the BED assay to improve accuracy, but noted the lack of field experience in doing so.

Desired Attributes: Sensitivity, specificity, and reproducibility of results supersede cost as priorities for improved HIV incidence assays. Epidemiologists, researchers, and laboratory scientists uniformly voiced desire for assays with specificity and sensitivity superior to the BED assay. Potential users reported willingness to accept incremental increases in cost for: 1) higher specificity, 2) greater sensitivity, and 3) improved reliability of results. Other important assay attributes included sample type required, consistent availability, ease of interpreting results, and high throughput. Interviewees also desired clear guidance on which assays to use for specific applications and populations.

2. SESSION II: BREAKOUT SESSION 1: THOUGHT EXERCISE

Participants in this session were engaged in a thought exercise that was designed to expand their thinking beyond what the HIV incidence field looks like today and to envision how it could – and should – be advanced. This exercise built upon the overview of the challenges facing the field as provided in Session 1: Current Issues in HIV Incidence Estimation.

Participants were divided into four multidisciplinary groups. The exercise was limited to one hour to stimulate a rapid brainstorming environment aimed at identifying key challenges in the field. Once the challenges in HIV incidence estimation were identified by the groups, the top five

challenges were prioritized. The groups then engaged in brainstorming of both incremental and revolutionary concepts to solve these challenges.

To start the thought exercise the group was presented with the following questions:

> ***What are the current challenges facing the field of HIV incidence estimation today?***
>
> ***What would we envision if we could solve our primary challenges?***
>
> ***Would we propose "novel or revolutionary concepts" rather than "incremental changes?"***

The output of the breakout session is summarized below. See appendix III for the thought exercise handout.

2.1 Discussion of Group Reports The session was moderated by Dr. David Serwadda, Professor, Makerere School of Public Health, Kampala, Uganda. The subgroups reconvened to present their group reports. The key issues identified during the group reports are summarized below.

Terminology A consensus on the terminology used to define HIV infection is urgently needed. Standardized terminology should focus on establishing a distinction between the estimation of HIV incidence in populations and finding acutely infected individuals in a population. A key solution identified for this challenge was to establish a working group to define and publish a consensus article clarifying terminology. Progress has already been made on this issue as a subgroup of the WHO Technical Working group met in April 2009 and developed some guidelines for terminology that will be available in the meeting report.

HIV Infection Window Periods Defining the term "window period" for recent HIV infection is a critical issue. The majority of participants agreed there is a need to establish a nomenclature to distinguish the pre-seroconversion window period (which is short – about a month) and the post-seroconversion window period used for STARHS incidence assays (which should be long – at least 6 months). There was also interest expressed in an assay that could identify individuals at high risk for HIV transmission to allow for HIV prevention interventions. Such an assay may be able to identify an individual in both the pre-serconversion window period and for a short time following seroconversion during which high-titer viremia and infectivity is present.

A 'revolutionary' idea presented was to identify or develop an assay for both epidemiological use and for the classification of individual cases. This raised the question of whether the assay format would be developed as a single (multiplexed) assay or two separate assays? A majority of the participants felt it would be difficult to design an assay that would identify and discriminate both the pre- and post-seroconversion window periods. However, there was a clear consensus for a need both to estimate HIV incidence in populations and to find acutely HIV infected individuals in clinical settings. Despite this need, the market for HIV incidence assays is perceived to be small. This creates a compounding factor, in which an assay must be usable for at least five years – otherwise manufacturing may not be a viable option. To circumvent the issue of marketability, establishing private-public partnerships was suggested as a means to navigate the gap in the development and marketing of HIV incidence assays.

Assay Specificity and Sensitivity Participants uniformly voiced that the priority need is to develop assays/algorithms to identify individuals with 'recent' HIV infections in a population and to distinguish between early and long-standing HIV infection as a dominant issue. Assays and testing algorithms to detect acute pre-seroconverstion viremia using RNA amplification assays or fourth generation Ag/Ab assays are already commercially available. Additionally, there is significant investment from commercial and government sources to further improve these assays, including development of rapid tests for detection of acute infection. There is a consensus that the methods used to find 'recently infected' individuals and to estimate HIV incidence in a population are not accurate, as reflected by the high percentage of false recent infections currently being reported.

The majority of participants indicated it is important to know which population sub-groups and demographics are being infected with HIV. The relationship between the duration of the window period and assay specificity and sensitivity was discussed. Several questions were posed on how to address this issue: Is there a need to determine the false recent rate for all populations, and hope that future assays will not be as adversely affected as the BED? Is a multi-test algorithm a possible solution?

New Biomarkers and Assay Evaluation There is a pressing need to identify new biomarkers for HIV incidence assays and to determine the utility of existing assays. Unfortunately, there is no true consensus of what is "good enough" for international use. There is an urgent need to define a pathway for assay development; this development should be a similar process as that used in drug discovery with appropriate field research to help validate the approach. A perfect test may not be available or feasible, but effort should be directed at developing a test that helps move the field forward.

Sustained funding An underlying theme was the need for more sustained funding to move the field forward.

In conclusion, this preliminary discussion was successful in highlighting the key challenges facing the field and generating ideas for further exploration.

See appendix III for the thought exercise handout and for the full list of challenges and concepts generated from each team.

3. SESSION III: USE OF MARKERS IN THE DEVELOPMENT OF ASSAYS TO IDENTIFY RECENT INFECTION AND INCIDENCE ESTIMATIONS

3.1 Acute HIV Infection

Myron S. Cohen, MD, Director of the University of North Carolina Institute for Global Health presented an overview of the findings on acute HIV infection from CHAVI.

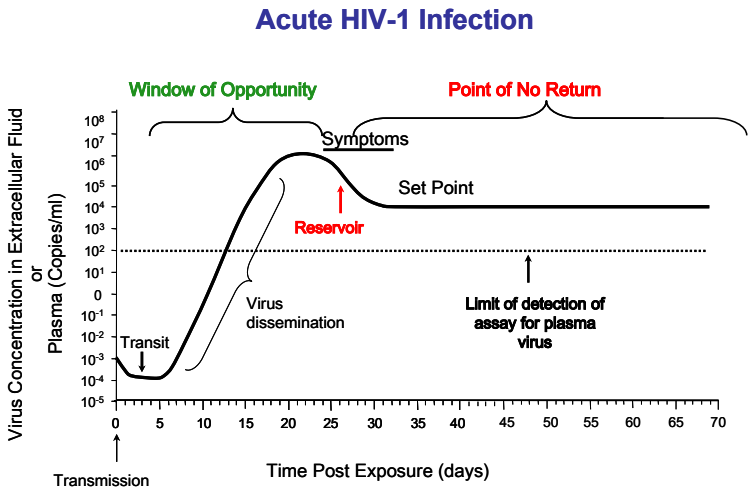
One key research focus of CHAVI is to understand the biology encompassing the early stages of HIV infection (primarily sexual transmission) and the corresponding immune response. The identification of individuals early in the acute infection phase is challenging. Several methodologies have been used by different groups in which participants have been identified by symptoms or via case findings from index patients, but these methods are typically very expensive, or result in late detection of the acute infection window. In addition, cohort studies

can detect seroconverters but they may also miss very early key events unless the individuals are bled very frequently.

To circumvent these limitations, CHAVI and others are utilizing a cross-sectional screening-RNA/p24Ag method, which can be applied prospectively or to stored samples. Retrospective studies are currently being performed using blood plasma donors through the Blood Systems Research Institute in collaboration with ZeptoMetrix. HIV-seronegative samples are concurrently screened and subjects with HIV RNA represent acute infection. No cells have been collected for use in these studies and there is a lack of data pertaining to the acquisition routes.

The CHAVI-001 study is an ongoing prospective study with the goal of identifying individuals who are viremic-prior to antibody development. The study identifies Fiebig stage 1 and 2 individuals by collecting specimens frequently and monitoring by detecting RNA (and subsequent antibody development. Subjects are followed for a period of one - two years. Samples were obtained using the North Carolina (NC) Public Health System in which samples from every person tested are pooled and all HIV-positive infections identified. To summarize the study more than 700,000 people have been screened (subtype B) to identify about 170 subjects with acute infection. Additionally, 8000 subjects have been screened in Africa (subtype C) to detect another 100 subjects. In total, CHAVI has collected more than 300,000 specimens from patients at all stages of HIV infection. Subjects followed for more than two years represent a unique resource for development of assays to estimate HIV incidence. The major problems with this approach are: high cost to manage patients and partners; lack of infrastructure to transfer study participants for other programs after the completion of the two year study; and treatment might eventually be required for acutely infected individuals altering the course of the disease.

Study of the subjects with acute infection has been revealing. Eighty percent of subjects who contracted HIV by sexual transmission are infected with single HIV virion/single quasispecies. The remaining twenty percent are infected with multiple HIV quasispecies. These data were validated in subtype B, subtype C and SIVmac251 mucosal transmission (Keele et al., PNAS 105: 7522-7, 2008).



After transmission, there is a window in which detection of the virus is difficult to observe due to current detection limits. The CHAVI- ZeptoMetrix samples have shown that as HIV-negative samples turn positive the viral load increases rapidly during a "cytokine storm" unique to the acute phase of infection (see figure). Increase in viral load ultimately leads to the destruction of immune system cells resulting in apoptosis. At the peak of viremia, the individual is thought to be most contagious; as antibody and other immune responses develop the viral load decreases to "set point"; individuals with a high set point have a poorer rate of survival.

A latent pool of HIV is established in the early viremia stage prior to set point. Curing HIV is likely impossible without eliminating the latent pool. The latent pool may be smaller in some

people that are treated early with highly active antiretroviral therapy (HAART). Currently, the primary reasons for treating acute infections are to reduce viral load potential, protect CD4 cells, protect immune response potential, reduce the latent pool, and prevent secondary HIV transmission.

In summary, understanding the early stages of HIV infection may facilitate the development of more sensitive diagnostics and may provide additional treatment options.

3.2 The Host Response to HIV Infection

Georgia D. Tomaras, PhD, Assistant Professor, Surgery, Immunology, Molecular Genetics and Microbiology, Duke University Medical Center presented an overview of the host response to HIV infection. The main components of the presentation are:

1. Multiplex detection of antibody responses
2. Ontogeny of the antibody response in acute infection
3. Understanding the B-cell response and potential biomarkers

Multiplex Detection of Antibody Responses A customized, robust high-throughput Luminex Multiplex Assay was developed for the detection of plasma and mucosal HIV specific antibodies. The principle of the assay is Luminex technology in which HIV-1 proteins are coupled to beads, which are impregnated with dye. Once the antigen is bound by its corresponding antibody in either plasma or mucosa a fluorescent signal is emitted and detected. Currently, HIV-1 antigen specificity and antibody isotypes measured by the multiplex assay are shown in the table below.

Antibody Types	Antigen (Antibody Specificities)
IgM	Env gp140 (Clade A, B, C, Consensus)
IgG, IgG1, IgG2, IgG3, IgG4	Env gp120 (Consensus, 89.6)
IgA, IgA1, IgA2	gp41
	Gag (P55, p24)
	Tat
Controls	Nef
Total Antibody Subclass levels	RT
Rheumatoid Factor	P31
IgG depletion for IgA/IgM detection	MPER peptide
	Immunodominant epitope
	V3 peptide

The Multiplex Assay has the advantage of being highly sensitive and robust while at the same time reducing the sample volume required for analysis. The customized Multiplex Assay permits high-throughput detection of > 10 antigens simultaneously. To allow for small sample volumes, the assay uses tetramers that have the peptides already in them; allowing the same coupling chemistry to be used as in the beads. The customized Multiplex Assay, combined with IgG removal, is highly sensitive at a limit of detection (LOD) of 0.5 ng/ml for specific IgA detection in seminal plasma and CVL. The HIV-1 specific envelope proteins used for detection (consensus gp140 oligomers) are at least as effective as autologous envelope oligomers at detecting HIV-specific IgG, IgA, and IgM responses.

Ontogeny of the Antibody Response in Acute Infection The nature of the earliest antibodies IgM, IgG (IgG1-4), IgA after virus transmission were studied using specimen panels from acute infection cohorts (Plasma donors, Trinidad, and CHAVI-001). Statisticians at SCHARP aligned all the specimen panels to the same start point (T0), in which all samples plasma viral load was 100 copies/ml. Using these aligned plasma donor cohorts, the timing and kinetics of the initial anti-HIV-1 IgM, IgG, and IgA antibodies in plasma and mucosal secretions were identified:

- There is a timed appearance of antibody responses to different HIV-1 epitopes during acute infection, with increasing antibody avidity over time.
- Plasma anti-gp41 IgM, Rheumatoid factor and anti-Gag IgG3 decline during acute infection.
- Systemic and mucosal anti-gp41 IgA selectively decline during acute HIV, but recover in chronic infection.

Alterations in B-cell Phenotype during Acute Infection The immediate effect of transmitted/founder HIV-1 on the B-cell arm of the immune system was studied using CHAVI 001/012 protocols. The study groups included seven uninfected healthy subjects, six subjects with acute HIV-1 infection off ARV and eight subjects with acute HIV-1 infection on ARV. The mean duration on ARV was 31 days with a range of 14-63 days. The study revealed the occurrence of early HIV-1-induced T- and B-cell germinal center damage and a loss in Peyer's patch mucosal generative microenvironments. Additionally, HIV-1-induced polyclonal B-cell activation and terminal differentiation of naïve and memory B-cells to plasma blasts/cells were identified.

In summary, these data obtained from examining the host response to HIV infection have identified potential biological markers that potentially could be used to develop an assay to estimate HIV incidence.

Potential Biological Markers for Assays to Estimate HIV Incidence

- **Kinetics**
  gp41 IgM decline
  gp41 Initial IgA decline
  IgG3 Gag decline in acute
  Rheumatoid Factor
- **Specificity Appearance**
  Absence/presence of gp120, p55Gag, CD4bs, MPER antibodies and specific epitopes
- **Antibody Quality**
  gp41avidity, gp120
- **Cellular Markers**
  B cell: Naïve versus Memory populations etc.
  Total NK increases and immunoregulatory NK decreases

3.3 Panel Discussion: What are the Prospects for New Biomarkers?

Moderator: John Parry, PhD, Deputy Director of the Virus Reference Department, HPA Centre for Infections

Panelists:
- Myron Cohen, MD, Director, University of North Carolina Institute for Global Health
- Georgia Tomaras, PhD, Assistant Professor, Surgery, Immunology, Molecular Genetics and Microbiology, Duke University Medical Center
- Neil Constantine, PhD, Professor, University of Maryland School of Medicine

- Francis Barin, PhD, Professor of Microbiology, Head of the French National Reference Center for HIV, University Francois-Rabelais, France
- Bharat Parekh, PhD, Team Leader, Serology/Incidence, Global AIDS Program, CDC

The purpose of this panel discussion was to identify the prospects for the use of biomarkers in the development of assays to identify recent HIV infection and incidence estimations. To guide the panel discussion a series of key questions were presented.

Questions:

1) Do you think we have any new biomarkers that will work for HIV incidence assays?
2) How would these biomarkers be used for assay development?
3) What technical challenges would these new assays present?
4) Should these new potential assays be used along or in combination with existing assays?

It was apparent from the panel discussion that identifying novel biomarkers to estimate HIV incidence in populations may prove to be more difficult than identifying biomarkers for acutely infected individuals. To facilitate the identification of new biomarkers, the panel discussed the development of specimen panels from recently and chronically HIV infected individuals as a key next step. These sample sets should be made available to assay developers and researchers; however, several challenges around the collection of these specimen panels were identified. First, a large number of samples would be needed to aid in the identification of new biomarkers for recent HIV infections. Second, HIV elite suppressors and long-term non-progressors specimen panels would be needed to determine the false recent rate of HIV infection. Ultimately, the generation of this sample repository could prove expensive to establish and maintain due to the quantity of samples and the follow-up time required in attaining the appropriate sample sets.

The identification of a single biomarker for recent infection will be a challenge. The panel recommended using multiple approaches to find new biomarkers – including, but not limited to, the evaluation of antibody isotypes, antibody titers, cellular biomarkers, and neutralizing antibodies. This would include manipulating multiple biomarkers or ratios of biomarkers, and determining how much variability there is in a population. To streamline these efforts, biomarker combinations should be identified systematically with the aid of biostatisticians.

There was an overall consensus that it will be technically challenging to find new biomarkers to estimate HIV incidence in populations. The probability of success could be increased by the availability of well-defined HIV sample sets and a multi-pronged approach to identify and evaluate new or multiple analytes for HIV incidence assays.

4. SESSION IV: CURRENT ISSUES IN TECHNICAL ASSAY DEVELOPMENT

4.1 The Accuracy of Serological Assays for Detecting Recently Acquired HIV Infection and Estimating Population Incidence: A Systematic Review

John Kaldor, PhD, Head of Public Health Programs and Professor of Epidemiology at the National Centre in HIV Epidemiology and Clinical Research, Sydney, Australia, presented a systematic review of the accuracy of serological tests for recently acquired HIV infection that are being widely used for measuring population patterns of HIV incidence. This work was conducted on behalf of the WHO Technical Working Group on HIV Incidence Assays to inform

the development of a protocol for systematically assessing performance characteristics of HIV incidence assays using a standardized approach.

A central objective of HIV programs is to reduce the extent of transmission in populations. Incident HIV infection rates are a key indicator of the rate of HIV transmission and help determine both the need for programs and their effectiveness, but can only be measured directly by repeat testing of longitudinally monitored populations. Standard terms are applied to monitor populations. Sensitivity is the ability to identify recent infections. Specificity is the ability to identify infections that are not recent. False recents are individuals with longstanding HIV infections who are identified as recent by STARHS assays, often due to late stage disease (AIDS) or being on ART. These individuals could potentially lead to an overestimate of HIV incidence.

In 2005, UNAIDS released a statement that recommended the BED incidence assays not be used for any purpose. The studies described in the statement were based on validation studies in which the accuracy of the HIV incidence assay had been investigated by comparing estimates of incidence that were derived from 'gold standard' estimates obtained by other means. These investigations have been conducted in a variety of settings, using a range of different gold standard estimates. There has not been an agreed approach to validation, making the comparison of findings across assays problematic.

To inform the development of a more systematic approach, Kaldor's group performed a literature review of studies that reported on the validation of HIV incidence assays. The electronic bibliographic database of PubMed was searched to the end of December 2007. Reference lists of selected studies were also checked for other potentially relevant studies. Conference presentations were included if the corresponding full report was not available. If the required review information was not available in the conference presentation, authors were contacted for unpublished data. The primary literature search was conducted using the terms 'HIV' and 'Incidence', combined with 'immunoassay' and 'surveillance'. Variations of the terms were used. Standard definitions applied to the study are listed below:

- **Measurement of performance characteristics** - the process of collecting serum specimens from people with HIV infection of known duration, applying the assay to these specimens, and calculating the sensitivity of the assay to detect infections of short duration as *recent*, and the specificity of the assay to detect infections of longer duration as *not recent*

- **Validation of assay-derived incidence estimates** - the process of collecting serum specimens from the members of a population in which a 'reference' estimate of HIV incidence was available, applying the assay for recently acquired infection to these specimens, deriving an estimate of population HIV incidence from the results and comparing it to the reference estimate.

- **Sample set** - a distinct group of samples used in a study of performance characteristics or validation

A total of 150 reports were identified, 33 assessed performance characteristics (of 13 difference assays on 74 sample sets), and 22 conducted validation (four different assays on 34 sample sets). The specimens derived from diverse sources. In most cases, the assay estimates were compared to direct HIV incidence measurements from the source cohorts. Across 13 different assays, sensitivity to detect recent infections ranged from 42 to 100%. Specificity for detecting

established infections ranged between 49.5 and 100% and was higher for long-term (greater than one year) infections. Focusing on four different assays, comparisons were made between assay-derived population HIV incidence estimates and a 'reference' HIV incidence estimate. Serological assays have reasonable sensitivity to detect recent HIV infection, but are vulnerable to misclassifying established infections as recent, potentially leading to biases in incidence estimates. The assays have not been validated in a sufficiently wide diversity of populations to confirm their routine use for public health purposes.

Most reports included information about multiple sample sets. A sample set is defined as a group of individuals/specimens assessed. Recent and established results were identified before and after the 'window period' of the assay. Other combinations included recent, established and AIDS (19), AIDS cases only (19), recent infections only (4) i.e. wide variety in timing of specimens. Of the 67 including established infections only 19 included people infected with HIV for one year or more – 'longstanding'. Individuals were excluded from analysis based on findings using a comparison to a 'gold standard' assay rather than clinical information to determine if truly a recent infection or not.

Identifying a subset of records to investigate can change specificity. This appears to be higher for longstanding infections but drops with AIDS. It was observed that increasing time on HAART seems to relate to decreasing specificity. Very small number of samples – 4 result sets actually had the same people tested on four different assays. Assays appear to be accurate enough to use at population level (but not individual level) however narrow range of specimens used. There is a clear need to confirm that specificity does decrease with AIDS and duration of antiretroviral treatment. If confirmed, there is a need to consider this when assessing assays.

In summary, there is an urgent need for an internationally agreed framework for evaluating and comparing these tests. A standardized protocol for assessing HIV incidence assays would improve the quality of assay assessment and help inform the use of assays. In addition, there is an urgent need for established, well-described HIV specimen collections for systematic assay validation.

4.2 Mathematics of Assay Use and Limitations – The Window Period and Long-Term Specificity

Alex Welte, PhD, Research Fellow of the South African Centre for Epidemiological Modeling and Analysis (SACEMA) presented statistical modeling concepts, study power, and requirements for HIV incidence estimation from recent infection testing algorithms (RITA). This work represents the method agreed upon for the analysis of incidence biomarker data by the WHO Technical Working Group during the HIV Incidence Assays Meeting held April 22-24, 2009 in Geneva. Ultimately, this work is to inform the development of a guidance document on statistical modeling and analysis for HIV incidence estimation as a component in the critical path for assay development.

The Model Beginning with a plain transition model, in which all subjects progress through a state of 'recent infection', a simple incidence was estimated by the number of recently infected subjects divided by the multiplication of the number of uninfected subjects and the mean window period. In the more realistic case, in which some subjects either fail to progress to the assay defined state of 'non-recent', or revert to the assay defined state 'recent' after having initially progressed to 'non-recent', a definition of 'true' 'recent infection' can be obtained by considering the response of the subpopulation in which the simple model applies. This leads to a notion of 'false recent', and the assay based test is seen to have a 'false recent rate', which

can be estimated from suitably representative sets of specimens from 'non-recently' infected individuals. By estimating the number of 'true recent' from the number of 'test recent' infections, the same simple estimator can be used, with the numerator being the estimate of the number of 'true recent infections.

Statistical power calculations can be used to detect an HIV incidence trend given a known false recent rate. By using the coefficient of variation, the degree of specificity and/or window period uncertainty has a substantial impact on the confidence level of the HIV incidence estimate. Study power and incidence precision erode rapidly with reduced specificity.

Specificity and the window period are the defining assay performance characteristics for the purposes of HIV incidence estimation via RITA. The window period must be known for absolute HIV incidence estimates, but not for the comparison of two different HIV incidence estimates. For RITA, ≥98% specificity could be a reasonable target to provide good performance of an assay in a cross-sectional survey, but that the specificity must be known for all applications of incidence estimation. Importantly, RITA might still be used when specificity is imperfect. However, the study statistical power declines rapidly in this case, and methods for dealing with imperfect specificity should be evaluated with respect to calculating this loss of power, not essentially as an invitation to use significantly non-specific assays in the belief that the relevant statistical methodology will counteract this problem.

The WHO Technical Working Group on HIV Incidence Assays held a subgroup meeting on this topic in April 2009. The proceedings of that meeting may be found on the WHO HIV Incidence website: (http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay). The statistical issues surrounding HIV incidence assay specificity and sensitivity in this unique setting will affect assay development. These factors, in addition to standardization of terminology, will play a central role in development of the methodology to validate new assays. Progress has been made on identifying these keys issues, but additional follow-up is required to evaluate the approach and ultimately reduce it to common practice.

See appendix IV for RITA Definitions and Concepts, Handout.

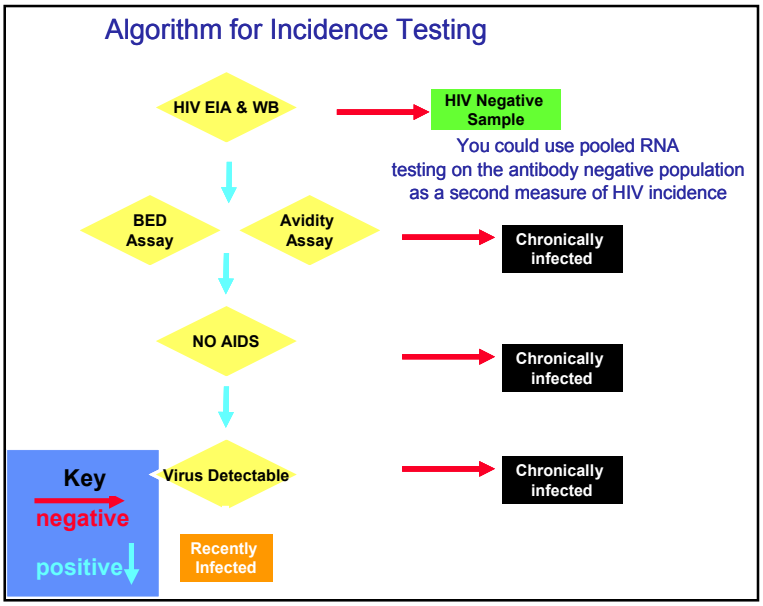
4.3 Algorithm for Incidence Testing

Oliver Laeyendecker, M.S., MBA, Senior Research Assistant, NIAID, NIH, presented an overview of the HIV Prevention Trials Network (HPTN) algorithm used to increase the specificity of HIV incidence testing.

The HIV Antibody Testing Algorithm Confirms Recently Infected Individuals

The algorithm for HIV incidence testing uses the BED assay in tandem with the Avidity Index to eliminate people with chronic HIV infection from the HIV incidence estimate. A cutoff of <1.0 was used for the BED assay and a cutoff of <90% was used for the Avidity Index. Restrictions were incorporated for individuals on ART and with low CD4 counts. The CD4 counts were used as a proxy for individuals with advanced HIV disease or AIDS. Data show that applying this testing algorithm to several FHI and HPTN data sets, the percent of individuals with long-standing HIV infection misclassified as having recent infections was reduced to 0.6%. In addition, the misclassification rate of chronic-infections/AIDS cases was consistently lower when both BED and Avidity assays were used in combination. Application of this algorithm to specimens from the Rakai 2002 cohort yielded an incidence estimate (1.8% per year) that was very close to the 1.64% per year generated for the cohort during follow-up (see figure).

The HIV Antibody Testing Algorithm Improves Incidence Estimates The BED and Avidity assays are not adversely affected by sample handling (freeze/thaw issues) or by pregnancy. However, the assays have been shown to be highly susceptible to viral suppression resulting in low antibody levels. Such samples are falsely misclassified as recent and over time; the number of HIV-positive virally suppressed individuals has been shown to increase within a population. Additionally, the development of AIDS as well as the use ART both impact the BED and Avidity assays; resulting in a significant number of "false recent" samples. Ultimately, these samples will result in over-estimations of incidence.



In summary, a multi-test algorithm can dramatically increase specificity for cross-sectional HIV incidence testing and may be able to address the problem of over-estimation of HIV incidence identified with current HIV incidence assays.

4.4 Panel Discussion: How Far Can We Go with Existing Assays?

Moderator: David Stanton, MN, MPH, Chief, Division of Technical Leadership and Research U.S. Agency for International Development (USAID)

Panelists:

- John Kaldor, PhD, Head of Public Health Programs and Professor of Epidemiology, The National Centre in HIV Epidemiology and Clinical Research, Australia
- Alex Welte, PhD, Visiting Research Fellow, South African Centre for Epidemiological Modeling and Analysis (SACEMA), South Africa
- Oliver Laeyendecker, MBA, Senior Research Assistant, NIAID, NIH
- Tim Hallett, PhD, Department of Infectious Disease Epidemiology, Imperial College London
- Bernard Branson, MD, Associate Director, Laboratory Diagnostics, DHAP, CDC

The purpose of this panel discussion was to identify how far we can go with existing assays to estimate HIV incidence. To moderate the panel discussion a series of key questions were presented.

Proposed questions:

1) Do you think antibody-based assays will remain our main tool for incidence estimation?
2) How accurate do they need to be, that is – what is "good enough"?
3) How should current assays be used?
4) What can we do with existing assays to increase confidence in their incidence estimations?

'How good is good enough' for assay specificity depends on the applications of the different assays used to estimate HIV incidence, the prevalence in populations, and the study size. Currently, with a study size of 20,000 and a 5% HIV incidence rate, the currently available assays would probably suffice. However, with a study size of 1,000 and a 1-2% HIV incidence rate, no currently available assay would suffice. Some studies have observed that HIV incidence can change overtime within a population. Thus, one must be careful not to assume that the incidence rate of HIV is stable in a population.

Accurate or absolute levels of HIV incidence may be difficult to ascertain. Trends in HIV incidence may provide a better means to monitor the HIV epidemic within a population. Several factors that were discussed to effect the measurement of HIV incidence include: (1) Co-infections could affect the serological profile of an individual; (2) Viral suppression; (3) Incidence measures can vary over time as the epidemic evolves; and (4) It is important to distinguish between individuals who have been antiretrovirally suppressed vs. those who are naturally suppressed.

Available data may be used to verify the accuracy of HIV incidence estimates. HIV incidence estimates are context specific and dependent. Using the BED assay as a specific example, the assay can aid in estimating HIV incidence on a national level; however, it is more difficult to obtain information on specific subpopulations being infected (age, demographics, etc). Some candidates for an epidemiologic or modeling approach to estimate HIV incidence might include occupational cohorts as well as the youngest age strata in antenatal surveillance, among which there is little to no mortality. It was discussed that seroconverter cohorts are important tools to identify how many people never attain the RITA progressor status because they have naturally low HIV viral loads and low anti-HIV antibody levels.

In summary, the participants indicated that there were four main issues to consider when using current, or developing new assays to estimate HIV incidence:

1. Assays need to be tailored to the study size or end users- population or individual
2. Multiple subtypes within a population need to be considered
3. AZT interventions may affect the accuracy of incidence estimates
4. Other epidemiological data should to be utilized to assess the accuracy of the HIV incidence estimates

5. SESSION V: CURRENT ISSUES IN TECHNICAL ASSAY DEVELOPMENT

5.1: Market Assessment: Projecting Global Demand

Timothy Mastro MD, FHI, presented an overview of efforts to estimate global demand for HIV incidence assays. As part of the market assessment, FHI is engaged with *bio*Strategies Group in estimating this demand. FHI is in the early phase of this activity and expects to have a report of the demand estimation for HIV incidence assays later this year. Design of the demand estimation is informed by feedback gathered from qualitative interviews with key stakeholders, including epidemiologists, researchers, funding organizations, and multilateral agencies interested in using assays to estimate HIV incidence.

Main components of demand include:

1. Public health surveillance, including large cross-sectional surveys, surveys of sentinel populations, case-based surveillance, and blood donation screening

2. Research and special studies, including screening and assessment of populations for appropriate clinical and vaccine trial sites
3. Other clinical uses, such as new case identification

Demand for assays to estimate HIV incidence is *not* consumer-driven. Rather, major public health funding organizations, researchers, multilateral bodies, and governments will drive and procure assays to estimate HIV incidence. Demand for use of HIV incidence assays in public health surveillance will be influenced by variations in surveillance methodologies across institutions; the frequency with which various surveys are conducted; support from major funding organizations (e.g. President's Emergency Fund for AIDS Relief (PEPFAR)), multilateral organizations (WHO, UNAIDS), and national governments for or against specific assays; the need for such studies (i.e. high enough incidence in a given population); and existence of infrastructure to conduct such studies. The latter already exists in some countries, such as Kenya for the Kenya AIDS indicator Survey (KAIS), and South Africa for screening of pregnant women. Some countries employ HIV incidence assays in ongoing case-based surveillance programs. These include the United States, Canada, France, and China. In some instances case-based surveillance is used to track specific population subsets rather than in national surveillance.

Special studies will also influence demand for HIV incidence assays. Assays that are well validated and affordable could be in high demand to use in identifying appropriate research sites, and as primary or secondary markers in assessing the effectiveness of various interventions. Although currently available assays, such as BED, are not recommended for clinical use, demand could be substantial for an accurate and affordable clinical tool to identify individuals with recently acquired HIV infection.

Design and utility of the global demand estimation was explored further during Breakout 3 (Session VIII: Laying the Foundation for New Assay Development, 8.3: Group 3 – Global Demand Estimation).

5.2 New Assays in Development

5.2.1 From Concept to Commercialization: The BED Capture EIA

Bharat Parekh, PhD, Team Leader, Serology/Incidence Team Global AIDS Program, Centers for Disease Control and Prevention (CDC) presented an overview of the path followed to commercialize the BED assay.

The BED assay was developed by Dr. Parekh's Laboratory at the CDC and reported in 2002. Several steps were taken to ensure quality control of the assay such as defining calibrator and controls, bulk purchasing of key reagents (e.g. goat-anti-human IgG, Streptavidin-peroxidase, etc.) as well as bulk purchase of plasma specimens and storage. The in-house development of the BED assay kit included the preparation of detailed kit insert and stability studies of reagents/kit. Ultimately, the laboratory was producing 50 kits (100 plates) per batch/2-3 months. Given the resources available in the laboratory, this effort was not sustainable; transfer to private industry was needed to make the assay widely available. In April 2004, the BED Assay was licensed to Calypte and InBios (see time-line on left). Calypte, worked very closely with the CDC during the technology transfer process. Parekh's laboratory continues to perform systematic quality control on kit lots that Calypte manufactures. In 2005, the Food and Drug Administration (FDA) permitted sales of the BED assay with the following label: "For Surveillance Use Only, Not for Diagnostic Use." Currently sales are restricted to CDC-approved

March 2004	BED Assay Presented at the Technology Transfer Conference at the CDC
April 2004	The BED Assay Licensed: • Calypte (Oregon) • InBios (Seattle, Washington)
April 2005	Interim lot was manufactured from Calypte for evaluation
Oct 2005	1st BED Assay kit lot passed QC in lab for commercial sale
	Developed systematic QC procedure for new lots
	CDC, Calypte and FDA discussions on the new incidence test
	CDC publications and data shared with the FDA
	FDA permitted sale of the BED assay with the following label: *"For Surveillance Use Only Not for diagnostic use"*
	Sale restricted to CDC approved laboratories in the U.S.
	No restriction for sale outside the U.S.
	Many countries begin to use the BED assay for incidence surveillance
2006	UNAIDS statement re-overestimation of incidence
	TWG/OGAC recommendations re-overestimation and use of the BED assay with appropriate adjustment, based on 2 studies
2006-Present	4 more companies licensed the BED assay •Trinity Biotech (Ireland) •Spaan Diagnostics (India) •Kinghawk Pharma (China) •Sedia Biosciences (U.S.)
	~30+ publications > application of the BED assay
	CDC QC checked 6 lots

laboratories in the U.S. There is no restriction on sales outside of the U.S. In 2006, concern regarding over-estimation of HIV incidence was voiced by UNAIDS.

Several key issues were identified during the process of developing the BED Assay. First, the transfer of the assay from the research laboratory and additional time and resources required to transfer the assay to a commercial entity can be very demanding and cumbersome process. This process requires solid, meticulous, and convincing science as well as a major commitment of time, money, and personnel. Second, when developing the BED incidence assay only specimens from AIDS patients were used for false recent classification. Finally, and most importantly, there does not appear to be a major market for large companies to justify investment in assays to estimate HIV incidence.

5.2.2 Development of New HIV Incidence Assays Dr. Parekh presented an overview of several new assays that his laboratory is developing. These assays include: (1) Two-well Avidity Index EIA (AI-EIA); (2) One-well Limiting Ag Avidity EIA (Lag-Avidity EIA); (3) gp41-LS EIA; and (4) Rapid Incidence-Prevalence (I-P) Assay. All of these assays utilize a newly developed multi-subtype recombinant protein, gp41-IDR (rIDR-M), which equivalently detects antibodies from divergent subtypes. Advantages of these assays include likely similar performance in different subtypes, availability of enough quantity of rIDR-M antigen and the multiple assay formats using different principles. One-well Lag-avidity EIA is a new concept that works equally well when compared to two-well AI-EIA. Both assays use pH 3.0 buffer as a dissociation reagent. The Rapid I-P Assay was designed using high and low or limiting concentration of antigen and can measure prevalence and incidence using a single rapid testing device. This assay is simple and easy to use, sample storage and transport to the central laboratory is not necessary. The assay is an inexpensive surveillance tool; making it ideal for resource limited countries.

Several next steps in characterization of these assays were proposed to improve their accuracy to estimate HIV incidence. First, an accurate determination of the cutoffs and window periods must be measured using sero-conversion panels. Next, the rate of false recent classification

must be estimated in individuals with known long-term infections (AIDS, tuberculosis/malaria and other co-infections, ARV, elevated IgG). Third, these assays must be validated in different HIV subtype populations. Ultimately, these assays will need to be either evaluated as stand-alone tools or used in conjunction with an algorithm to increase the predictive value of detecting recent HIV infection.

In summary, these promising new approaches, such as AI-EIA, Lag-Avidity Assay and Rapid I-P Test, are being developed to detect recent HIV infection independent of HIV subtype. The combined use of two different methods based on two different principles, e.g. antibody levels and antibody avidity, should significantly improve the predictive value and the accuracy of HIV incidence estimates.

5.2.3 EIA-RI: An In-House Assay for the Identification of Recent HIV-1 Infection: Rational, Results and Limitations

Francis Barin, PhD, National Reference Center for HIV, Inserm U966, Université F Rabelais, and CHU Bretonneau Tours, France presented an overview of the HIV detection assay that has been used in France since 2003.

Rational for the IDE-V3 Detuned Assay In January-March, 2003, the reporting of HIV infection became mandatory in France. The goal was to provide indicators allowing a better adaptation of preventive measures. To estimate recent infection, Dr. Barin developed an in-house assay called the IDE-V3. The IDE-V3 assay was selected because it could give a negative or weakly positive signal with early samples (< 6 months post-exposure) and a strong signal with late samples (≥ 6 months post-exposure). The assay uses viral antigens gp41 IDE region and env V3, recognized by antibodies that are present in any HIV-1 positive sample independent of the genotype.

The IDE-V3 assay is an indirect ELISA in which each antigen is coated on the solid phase. The assay was developed and validated on panels of informative sera (ANRS cohorts) consisting of: 1) 224 sequential sera from 113 seroconverters, 2) 604 sera from patients at chronic stage (without AIDS), 3) 143 sera from AIDS patients. Using these panels, there was not a precise determination of the window period. However, the time information was first dichotomized into a binary variable in which two groups of infected individuals were defined as those with infection <180 days old and those with infections >180 days old. A logistic regression model was used to determine the biomarkers threshold to best detect recent status (the best sensitivity with the best specificity).

Time since infection	Definition	EIA recent/ total	Sensitivity Specificity (95% CI)
< 6 m	HIV Ab -	1,097 / 1,429	76.8 (74.5-78.9)
	HIV Ab - and (PHI or SC)	823 / 927	88.8 (86.5-90.7)
> 6 m	HIV Ab +	257 / 1,280	79.9 (77.6-82.1)
	AIDS	204 / 1,104	81.5 (79.1-83.7)

Results of the French National Virological Surveillance Using IDE-V3 The reporting schema for virological surveillance is based on time since infection as either < 6 months post-exposure or ≥ 6 months post-exposure(see table). An individual was reported as being HIV positive for less than six months if they met the following conditions: (1) A previous HIV antibody negative test less than 6 months prior to the diagnosis was reported or (2) At least two indicators were reported: a previous negative test within 6 months combined with a symptomatic primary infection an/or an incomplete western blot indicative of a recent seroconversion. An individual was reported as being HIV positive for greater

than six 6 months if they met the following conditions: (1) A previous HIV antibody positive test more than 6 months prior to the diagnosis was reported or (2) reported at AIDS stage. The virological surveillance data for European patients (2003-2007) are summarized in the table above. Additional studies were performed comparing Europe and Sub-Saharan Africa specimen panels. The data indicate the IDE-V3 assay is less sensitive in African patients versus European; reasons for this difference in specificity are currently being explored.

Limitations of IDE-V3 Several limitations of the IDE-V3 assay include transferability, long-term availability, and low sensitivity. First, transferability may prove problematic due to the dynamic range of the internal controls (CV 20% from expected value). Second, the long-term availability of reagents is an issue; for instance, production of the conjugate (anti-human IgG, polyclonal) was stopped. The polyclonal antibody has been replaced by a monoclonal antibody, which has required new calibration of the assay. Finally, the assay had a lower sensitivity than expected, suggesting that the window period is probably less than 6 months. The conjugate replacement and the necessity to define a more precise window period has justified a new calibration of the EIA-RI with a large informative panel including hundreds of sequential sera from seroconverters of the ANRS Primo cohort (2-6 sera/pts) including: B versus non-B and European versus SS-African participants. This study will aid in defining the window period that will be used for incidence estimates.

5.3 Industry Panel Discussion: Insights and Lessons Learned

Moderator: Stuart Shapiro, MD, PhD, Medical/Program Officer, DAIDS, NIAID, NIH

Panelists:

- Cham Chetty, PhD, CEO, Avioq, Inc.
- Paul Contestable, PhD, Principal Scientist, Infectious Disease/Transfusion Medicine Assay Research and Development, Ortho Clinical Diagnostics
- John Hackett, PhD, Research Fellow, Volwiler Society, Abbott laboratories
- Rikkert Maertens, PhD, International Marketing Manager, Innogenetics, Belgium
- Ron Mink, PhD, President and CSO, Sedia Biosciences Corporation

The purpose of this industry panel discussion was to gain insight into HIV incidence assay development and commercialization. To moderate the panel discussion a series of key questions were presented.

Questions:

1. What characteristics make an assay more likely for development?
2. What are the incremental steps to progress this assay through development?
3. What gaps exist in the current in-house and/or commercialized assays for HIV incidence estimation?

The discussion resulted in a very open and frank dialogue between researchers and industry representatives on the key factors required for industry to invest in the development and commercialization of an assay. The main two factors discussed were: 1) commercialization requirements and 2) the necessity of a product requirement document.

A risk/reward analysis must be performed before designing a product. There are always commercial risks (e.g. change in market value) and regulatory risks involved in assay development. In general, a sufficient market share must exist for companies to become interested in developing assays. HIV incidence assays are currently considered niche products for epidemiologic surveillance purpose. Companies must weigh the costs versus benefits before engaging in developing these niche products. From a commercial standpoint, an assay with multiple applications may be more commercially viable. A research test with a limited market may have lower market value but may also require a different level of regulatory approval (e.g. FDA). The panel discussed that there is not a defined cut-off for the market size, but rather factors such as cost of development and goods that need to be balanced with pricing to ensure an adequate profit margin. The total sales requirement and profit margin may vary based upon the size of the company. If a product has a clear application and balanced risk to reward ratio, then a small market size may be acceptable.

Development of an assay product profile is a necessity. Prior to initiating a project, the minimum requirements for the assay need to be defined. This provides the assay developers a target and standards to benchmark progress on assay development. Ultimately, the product profile provides the criteria making decisions on whether to continue to progress development of the product. The panelists emphasized that assay development is driven by the requirements outlined in the product profile. For example, if the specificity requirement is equal to or greater than 99.0%, a project could be stopped if the specificity comes in at 98.5% or lower. Thus, the design requirement of specificity equal to or greater than 99% will not be reached by the development team. In all circumstances, one must know the assay target. It is imperative that developers have a target that they can understand and to define how it is being used in specific terms. Panelists indicated that throughout the course of the meeting several different product requirements for the HIV incidence assay have been discussed. However, many of these requirements are associated with high risk and effort; limiting company interest and investment. The panel encouraged the field to move away from mathematical formulations and think more in terms of the technical requirements for the assay.

In summary, it was suggested that for the field to move forward it is critical to prioritize needs and develop a product profile for HIV incidence assays. In addition, it was stressed that a public-private partnership should be explored in order to move assay development forward.

6. SESSION VI: THOUGHT EXERCISE

Participants in this session were engaged in a thought exercise that was designed to expand their thinking beyond what the critical screening pathway for estimating HIV incidence looks like today and to envision how it could – and should – be constructed. This exercise built upon the overview of the challenges facing the field as provided in Session 1: Current Issues in HIV Incidence Estimation.

Participants were divided into three multidisciplinary groups. The exercise was limited to one hour to stimulate a rapid brainstorming environment. To begin the thought exercise the group was presented with the following challenge:

> ***Assume a major, global HIV funding organization has announced that it wants to obtain national HIV incidence estimations in multiple countries. The funding organization will award a grant to the best idea on how to do this that is feasible, accurate, timely and cost efficient.***

To guide the group in answering this challenge the group was presented with the following questions:

Questions:

1. **What is your methodology? How would you gather information?**
   Example: large, representative national serosurveys using an incidence assay

2. **What is your assay algorithm – will you use?**
   i. Combinations of currently used/available assays
   ii. Combinations of currently used and newly developed assays
   iii. Newly developed assays-only
   iv. Novel assay alone or in combination

3. **What is your pathway and are there defined decision points?**

4. **Based upon the algorithm selection, how will this affect your timeline?**

The output of the breakout session is summarized below. See appendix III for the thought exercise handout.

6.1 Reports from Small Groups

Each group presented a different critical screening pathway for estimating HIV incidence. Each critical pathway scenario is outlined below.

Critical Pathway Scenario 1

The methodology proposed to estimate national HIV incidence was to modify existing household survey methods. The national household survey would include national surveillance via individual case identification combined with special studies to track high-risk individuals. In this example, three countries selected include Vietnam (with special focus on injection drug users (IDUs), Tanzania, and Ukraine (with special focus on IDUs).

The algorithm would include the BED assay and a second new (non-Ab) biomarker. The selected algorithm would perform consistently across different HIV subtypes and account for the Lymphocyte Stimulation Index (LSI) and ARV users. The accuracy of the algorithm in specific populations and regions would need to be determined as well as the validation of the algorithm in specific HIV sub-types.

Outline of the critical screening pathway for estimating HIV incidence:

1) Obtain Ministry of Health (MOH) and government support
2) Conduct pilot studies to accumulate a breadth of specimens to establish panels, validate assay across range of represented subtypes, and co-infections (malaria, tuberculosis), and possibly compare assay performance among HIV-negative individuals
3) Obtain additional information on each case, including clinical symptoms (for exclusion of long-standing infection, ARV users), CD4 count and other clinical markers
4) Obtain complete panels for each country

The minimum timeline to complete the project would be two years with a total budget of less than $5 million.

Major assumptions of this model would include:

- Obtained MOH and governmental support
- Established all necessary seroconversion panels for each country
- Used specialized sampling appropriate to epidemiological nuances of each country (i.e. tracking IDUs in Vietnam and Ukraine)
- Conducted studies in-country and utilized core laboratories (or set up new ones as needed)
- Laboratories would be highly trained and testing performance will be validated
- Identified CD4 counts for recent HIV-positive individuals and ARV
- Conducted using a single survey (cross-sectional survey with no case follow-up)

Critical Pathway Scenario 2

The methodology to estimate national HIV incidence was to use a nationally representative household-based survey using a sample size of approximately 20,000+ individuals (based on prevalence and power). The survey would capture demographic data. Specific questions would be added on HIV testing history, ARV use, and demographic characteristics as part of the survey. Venous blood samples would be collected.

The selected algorithm would include an HIV Ag/Ab (4th generation antigen-antibody screen), Ab test of HIV (3rd generation), and CD4 count. A tube of venous blood sample (~4 mL) sent to a laboratory and the following testing algorithm would be performed:

Outline of the critical screening pathway for estimating HIV incidence

1) Screen for HIV Ag/Ab (4th generation antigen-antibody screen)
    - Blood processed for Ag/Ab screen
        i. If positive, test with newly developed 4th generation assay in the wings (Test 1- HIV Ag/Ab);
        ii. If recent, test with Test 2- 3rd generation HIV Ab test
2) Based on results from Ab test of HIV (3rd generation), test with optimal incidence assay with specificity > 98% and window period ~ 6 months
3) Then use an algorithm to sort out the false recent individuals or use point of care with CD4 count on-site

Critical Pathway Scenario 3

The methodology to estimate national HIV incidence was a cross-sectional survey combined with modeling of available data. Countries with different stages of the epidemic and HIV sub-types would be selected. The survey would use data available from an AIS, Demographic Health Surveillance (DHS) surveys, NHANES, and antenatal clinic surveys. The survey would capture information such as test history, history of ARV use, demographical data and risk behaviors. The sample size would be derived based on expected incidence in the population.

Outline of the critical screening pathway for estimating HIV incidence

1) Rapid HIV testing followed with existing assays and HPTN algorithm
2) Collection of serum + CD4 count (plasma) + dry blood spots
3) Test kits would include: 1) BED done with parallel with modified avidity (AxSYM *in vitro*), 2) CD4 on confirmed HIV-positive individuals within 1 week and viral load of incidents (BED + avidity) to eliminate natural viral suppressors and false recents, 3) possible adjustments thereafter

4) Result comparison between HPTN algorithm, epidemiological modeling and BED correction factor

The projected timeline would be 2 years:

- 1 year to prepare sites
- 6 months for fieldwork and testing
- 6 months for analysis and report writing.

6.2 Discussion of Group Reports

Moderator: Mercy Kamupira, MBChB, MPH, Clinical Safety Physician, International Partnership for Microbicides, South Africa

The thought exercise was very informative. In general, there was a lack of consensus among the groups on the next steps for the critical screening pathway. However, the similarities between the different groups included the use of the DHS survey and the use of the HPTN-like algorithmic approach. The group concluded that testing and validating HIV incidence assays for cross-sectional surveys presents technical challenges.

In conclusion, the current challenge facing the field is to identify how to utilize existing knowledge to establish a way forward- both technically and methodologically. There is interest in both optimally using existing assays and developing new, improved assays for estimation of HIV incidence.

7. SESSION VII: CURRENT ISSUES IN ASSAY VALIDATION

7.1 Specimen Collection: Desired Sample Sets

Michael Busch, MD, PhD, Vice President of Research and Scientific Affairs, Blood Systems Research Institute (BSRI) and Professor, University of California, San Francisco, presented an overview on specimen collections and desired specimen sample panel sets.

Specimen Characteristics:

A critical component of the assay development and evaluation process is the availability of sample sets to validate assays designed to discriminate recent from long-standing infections and to estimate HIV incidence. There are two broad categories of sample sets: 1) samples from recently infected/seroconverting persons used to determine the sensitivity of incidence assays/algorithms for detection of recent infections and to derive window period estimates (calibration), and 2) samples from persons with long-standing infections used to estimate rates of false-recent misclassifications. Both types of panels are needed to balance the objectives of developing incidence assays/algorithms that have as long a recent infection window period as feasible, while maintaining a low rate of “false-recent” misclassifications.

The desired characteristics of serologic panels for window period calibration have been identified. These characteristics include: 1) Each panel should consist of at least 50 subjects with incident HIV infections and optimally include seroconvertors infected with multiple major HIV subtypes and unique characteristics such as rarer genotypes/crfs; 2) Both previously archived and prospectively collected panels should have an adequate number of serial specimens per subject. When considering the utility of archival panels it is ideal to have 3+

serial specimens in the year following HIV-infection (well-documented seroconversion). Although a number of sample archives exist that include serial samples from large numbers of seroconvertors, these samples are valuable to the primary investigators and generally have a limited numbers of aliquots (<10) and limited volumes of serum/plasma per aliquot (<1mL) are available for contributing to incidence assay panels. Consequently, specific collections (prospective panels) should be obtained for incidence assay development and window period calibration; prospective panels can be build from patients recruited to provide specimens according to an established schedule following HIV-infection (i.e. once per month for 0-6 months, every two months 7-12 months, every 6 months in years 2 and 3); 3) Adequate specimen volume must be obtained – a minimum of 2 ml for archived samples and 10 ml or more for prospective panels. 4) Specimens must be stored at -20°C or colder with minimal freeze-and-thaw cycles; 5) The HIV-1 subtype of the specimen should be known or reasonably imputed; and 6) The panel must exclude specimens from persons who received antiretroviral therapy (ART) after the diagnosis of acute HIV infection.

Long-standing infection panels can be used for assay development and HIV incidence estimation. For purposes of establishing the specificity of incidence assays/algorithms and estimating rates of false-recent misclassifications (used in incidence projection calculations), samples from persons who are known to have been infected for > 1-2 years are required. These can be obtained from existing natural history cohorts or from other large study populations. It is important to collect specimens from populations of long-standing infected persons who are known to have low antibody titers such as elite controllers, long-term non-progressors, patients with advanced stage AIDS, and patients on anti-retroviral therapy. A general goal should be to build long-standing infection panels that include >500 specimens including at least 50 specimens from each of the problem categories listed above.

Other useful data for specimen panels include patient information such as geographic source, exposure category (likely mode of HIV-acquisition), data on timing of infection/seroconversion (based on: detection of RNA prior to antibodies; acute retroviral syndrome (ARS); prior test; discrete exposure), sex, age, viral load (RNA above threshold of ECs or ART-suppression), CD4 count (above AIDS defining threshold) and co-morbidities (malaria; hepatitis; pregnancy). Additional data required to establish or impute HIV-1 subtype include: genotype (or serotype) each specimen, obtain specimens from populations in which single subtype predominates, or predict on basis of exposure category (other predictors) and known distribution of genotypes from same population and time period.

Specimen Sources:

Panels for assessing sensitivity for detection of early HIV infection and determination of window periods for incidence calculations

There are several existing specimen panels for characterization (calibration) of HIV incidence assay/ algorithm 'window periods'. These specimen panels include: 1) Plasma donor seroconversion panels which include frequent serial (2-3 per week) specimens from source plasma donors who evolved from HIV-negative, to RNA+/Antibody- to Antibody+ (these panels are currently all subtype B as source plasma donor programs exist primarily in the United States); 2) Interval seroconverters, consisting of convenience samples from persons who had an HIV-negative specimen at defined intervals (< 12 months) prior to first HIV-positive specimens (e.g., seroconverting repeat blood donors and serially tested clients from public health laboratories); 3) High-risk cohort seroconverters, such as serial specimens from subjects enrolled in natural history, prevention or vaccine trials and documented to acquire HIV during follow-up (such as MACS, WIHS, ALIVE, HIVNET, HPTN, CAPRISA, and Rakai); and 4)

Specimens from acute HIV infection studies involving active screening of individuals for acute HIV infection targeting high HIV high-incidence populations and using NAT to identify, enroll and follow acute HIV infection cases (such as Options, AIEDRP, CHAVI, AMPLIAR, CASCADE, PRIMO).

- Note: Specimens from participants in high risk and acute HIV cohorts with long term follow-up (>1-2 years) should be well represented in seroconversion panels since there can be significant delay to seroconversion by incidence assays in a minority of individuals.

Panels for characterization of specificity and derivation of false-recent rates of HIV incidence assays/algorithms

Numerous research and population surveillance studies have access to known long-term infected subjects. Archived specimens exist from seropositive subjects who enrolled in natural history, prevention, or vaccine trials (e.g., MACS, CAPRISA, HIVNET; JHU ER). The relevant specimens should be from subjects documented to have had HIV antibodies >1-3 years. There are also archives of samples from the following categories of subjects: Elite Controllers; HAART suppressed patients; AIDS patients (low CD4); and HIV-infected patients with co-morbidities such as malaria, TB, hepatitis, etc. Since the characteristics of HIV antibodies and other parameters are generally stable in long-standing infections, serial specimens in the freezers can be accessed per subject and/or large volume collections can be obtained prospectively from qualified and consenting subjects. One approach to obtain larger volumes of plasma is to recover more of the plasma from tubes designated for PBMC preparation; by either removing plasma prior to gradient separation or removing and saving all of the plasma above the PBMC layer (this simple approach could also be used to obtain more plasma from prospectively followed seroconversion cohorts discussed above).

Potential expanded use of HIV-infected blood donors for incidence panels

HIV-infected blood donors are a “convenience sample” likely to represent the larger populations of infected individuals from around the world. There are >80 million donations per year globally, with blood collection programs in every country. A total of >15,000 newly diagnosed HIV-infected donors are detected per year, with plasma components (>200 mL) that are typically discarded (due to positive test results) able to be made available for research use. HIV RNA (NAT) or 4th generation antigen/antibody screening is now performed in most countries, resulting in detection of early HIV infection stages. It is estimated that >250 pre-antibody window period donations (units) are currently interdicted per year; these donations can be accessed and the donors enrolled into serial collection protocols for building incidence assay panels with large volumes and diverse subtype representation. In addition > 2,500 seroconverting repeat donor units are collected per year; for these donors the time since the prior seronegative donation is known, which allows for derivation of crude estimates for incidence assay window periods based on known HIV incidence rates in the corresponding donor populations. Detection of HIV-infected blood donors thus allows access to large volume plasma components for test development, evaluation, and Quality Control. These are in addition to source plasma collections, which in the United States total 13 million collections annually. These source plasma donor collections are obtained by aphaeresis at ~400 centers; ~750,000 donors give 17 donations per year with 500 – 880 mls of plasma per collection. This effort identifies >100 incident HIV infections detected each year by NAT or antibody serioconversion, which have been used to develop plasma donor serconversion panels referenced above..

In summary, many archived specimens exist; however low volumes are available and access is guarded. There are many untapped opportunities for collection of suitable samples from a range

of patients and infected blood and plasma donor populations that would prove useful for incidence assay development, window period calibration, and false incidence rate assessment. There may also be an opportunity to acquire relatively large volumes of plasma that are currently discarded from samples that are processed for PBMC isolation from participants in existing cohort studies with known dates of infection and/or risk factors.

7.2 Cataloging of Specimens: Virtual Database of Specimens

Joanne Micallef, PhD, Research Fellow, National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, presented an overview of the development of a virtual database of specimens from seroconverters and acute HIV infection specimen panels. The development of a virtual database is part of a subgrant from FHI funded by the Bill and Melinda Gates Foundation.

The three steps in the development of the database were discussed. These steps included: 1) to identify cohort studies and clinical trials with potentially relevant specimens (i.e. seroconverter and acute cohorts); 2) contact the study investigators; and 3) compile data into a database.

Clinical trial registries and Medline were searched for articles published between 2000 and the end of March 2009. These searches were performed using terms: (HIV) AND (incidence OR early infection OR acute OR recent infection OR recent seroconversion OR recently infected OR newly acquired OR primary infection). The status of studies identified may be: complete studies with archived specimens, ongoing studies with archived and prospective specimens and planned studies with prospective specimens. Two study types were of interest. First, for seronegative cohort studies, HIV seronegative subjects are followed at regular time intervals and seroconversion documented. The standard time interval between follow up of subjects is ideally <=12 months. Second, for acute cohorts, individuals are confirmed as p24 antigen- or HIV RNA-positive, antibody negative or are identified as symptomatic with documented seroconversion.

Several variables were extracted from the study. Variables being extracted from the studies include author/principal investigators, countries/cities, period of recruitment, number of participants, risk groups, mean age, percent male, race, and HIV subtype distributions. For seronegative cohorts variables also include testing interval, demographics at baseline and follow-up of seroconvertors. When evaluating acute cohorts, the definition of acute HIV and length of follow-up are extracted. All data is being compiled into an Access database. To date, 5,268 articles and more than 250 clinical trials have been identified. Data from 43 seroconvertor cohorts and 13 acute cohorts has been extracted thus far. For the 43 seroconvertor cohort studies, the mean number of seroconvertors per study is 136. The subtype and follow-up for these studies was not well reported. Most of the studies were performed in Africa (n=25), followed by North America and South America, each with 11 studies, Asia with 6 studies, Europe with 4 studies and the Caribbean, Asia and Australia/New Zealand each with 1 study reported.

Following the literature review, the next step will be to contact study investigators with the following objectives: to obtain details of studies that are not available from the published literature, to determine the availability of specimens, and to determine whether investigators are willing to contribute specimens to a specimen bank (and conditions) and compile the details of relevant specimens. Options for contacting investigators include introductory letter followed by an online or paper (email) survey or phone call. Investigators would be asked if /where specimens are stored, types of specimens collected, time points, volume, if study

enrollment/follow-up is ongoing, willingness to collect extra plasma and if other studies that can contribute specimens are known.

Once the database has been completed, investigators will be able to search for studies that have relevant specimens (archived and prospective) and to identify studies in which investigators have an interest in sharing specimens in a collaborative effort. In addition, investigators will be able to identify specimen information including type and volume of specimen available, location of specimens and general study information. Ultimately, this may facilitate the process of identifying specimens for a central repository of specimens relevant for the evaluation of incidence assays and will enable assay developers to have access to specimens which will aid assay development.

7.3 Panel Discussion: What is Needed for the Toolbox?

Moderator: Sheila Peel, MSPH, PhD, Assistant Department Chief, Laboratory Director HIV Diagnostics and Reference Laboratory, Walter Reed Army Institute of Research.

Panelists:

- Mike Busch, MD, PhD, Director Blood Systems Research Institute, and Vice President. Research and Scientific Affairs, Blood Systems Institute
- Joanne Micallef, PhD, Epidemiologist, National Centre in HIV Epidemiology and Clinical Research, Australia
- John Parry, PhD, Deputy Director, HPA Centre for Infections
- Sue Eshleman, MD, PhD, Professor Johns Hopkins University School of Medicine
- Andrea Kim PhD, MPH, Epidemiologist, Global AIDS Program, CDC

The purpose of this panel discussion was to explore and define the infrastructure, resources and specimens required to develop, calibrate, and evaluate HIV incidence assays. To guide the panel discussion a series of key questions were presented.

Questions:

1) Do we know what samples we need to go forward?
2) What are the main limitations to assemble the required specimens?
3) Do we know what infrastructure is required?
4) How would we go about setting up the infrastructure to assemble the required samples?

Specimen sample sets are needed to develop and move an assay forward. The WHO "Methodologic Guidance Document for the Validation of Existing and Future HIV Incidence Assays" provides a draft critical pathway (page 17, v. January 12, 2009) describing the essential panels and iterative steps required for assay validation. First, the assay must be developed and characterized in-house by the test developer. Secondly, the developer can initiate the assay qualification phase by making a request for the blinded incidence core sample set (ICSS) to a core committee who will review the assay's intended use and in-house performance. Upon approval, a Central Repository will send the ICSS which will establish the assay's ability to distinguish between HIV-negative, recent infection, and long-term infection on blinded specimens. Screening of the core sets in new assays will enable cross-assay comparisons of assay performance with standardized samples. Assays that pass this phase will progress to the

third stage of the validation, where the assay will be transferred to a central laboratory and then evaluated in that laboratory using archived sero-converter panels and known long-term HIV infection panels of appropriate sample sizes to assess assay performance and quantify the assay's false-recent rate among long-term infections greater than 1 year. For the first two steps of the critical pathway, general sample sets will be used but for the third stage more specialized sample sets derived from specific populations such as pedigreed seroconvertors or false recent cases and from various regions and subtypes of appropriate sample sizes will be required. The specific characteristics of the sample sets were discussed by Dr. Michael Busch (Session VII: Current Issues in Assay Validation: "Specimen Collections: Desired Sample Sets"). This topic was further explored during Breakout 3 (Session VIII: Laying the Foundation for New Assay Development, Group 2 – Assay Development and Validation Tool Box).

Currently, there are several limitations to assemble the required specimens. The key issues identified to acquire archival samples are the lack of adequate record keeping that describes the sample characteristics and ownership/authorship issues. An option discussed was the development of a protocol to follow cohorts prospectively with additional follow-up for the sole purpose of building panels collecting relevant specimens for incidence assay development. A combined approach of a physical repository developed specifically for incidence assay development linked to virtual archived sample sets may lead to a more comprehensive and standardized sample inventory. As a first step, a clearinghouse for accessing these specimens should be established. A series of key challenges currently facing the establishment of a specimen repository are outlined below. These challenges highlight the need to piggyback collection efforts onto existing studies.

1) Networks and organizations have complex clearance systems (e.g. 6 months for HPTN)
2) Institutional review board regulations (such as blood volumes collected)
3) In-country challenges for sending/receiving data and samples
4) Freezer/storage costs
5) Shipping costs
6) Commercial uses of samples
7) Blood collection must be embedded in informed consent process
8) MOH restrictions in-country
9) Lack of information about how archived samples were collected and stored (volume, type, etc.)

There is a clear need to develop an infrastructure to support the acquisition of specimens and processing them into aliquotted samples and the establishment of a sample repository for incidence assay development and quality control. Once established, the process for making sample sets available to assay developers via a central repository or virtual sample sets needs to be defined. The framework for the type of oversight that would be needed to collect the necessary sample sets was discussed and included WHO, academic institutions, or a center/institute such as the Blood Systems Research Institute. There was a strong consensus that sample volumes in the specimen repository should be large, however, large blood draws present several challenges including IRB approval, investment in training so that people can understand why samples are needed, and the intellectual and scientific investment of sites in specimen collection.

In general, there was a strong consensus that the establishment of a central repository of specimen samples and the supporting infrastructure to manage the collection is an essential component for assay development and validation. A combined approach of a physical

repository linked to virtual archived sample sets would support the development of the critical pathway for assay assessment and validation.

8. SESSION VIII: LAYING THE FOUNDATION FOR NEW ASSAY DEVELOPMENT

8.1 Group 1 – Assay Specifications

The objective of this breakout session was to develop draft assay specifications for an HIV incidence assay. A product requirement document (PRD) describes the product that will be developed, i.e. the product profile. The purpose of the PRD is to articulate the purpose of the product, features, and technical requirement. To initiate discussion on the development of a PRD for incidence assays, several questions were posed to the group including:

Questions:

1. ***What features should be included in the PRD?***
2. ***What criteria should be applied to each feature in the PRD?***
3. ***How well does the performance of existing assays match the draft PRD requirements?***

The charge to the group was to determine what features and assay specifications would be required for an assay that was designed for HIV incidence estimation from cross-sectional samples for use at the population level. The group was divided into three subgroups to independently discuss and populate the draft assay specifications document. A lively discussion followed as the groups worked together to complete the draft PRD. The output from the subgroups were compiled and the summarized in the table shown below.

The draft table represents a starting point for the PRD. Additional discussion and refinement will be needed to ensure that key issues are addressed and a consensus achieved.

PRODUCT REQUIREMENT DOCUMENT (PRD)-DRAFT		
Feature	**Desired**	**Minimal**
Operational Characteristics		
Specimen type	DBS, plasma, serum, urine, oral	Serum plasma
Sample Volume	10 micro liters	0.5 mL
Time-to-result	< 2 hours	Not defined
Turn-around-time	1 day	3 months
Specimens per run	1000 +	1
Instrumentation	No	Yes
Specialized equipment required	No	Yes
Performance Characteristics		
Sensitivity	100%	90%
Specificity	100%	98%
HIV Subtype coverage	All	Well characterized
Assay specificity affected by ARV	No	Predictable & correctable
Assay specificity affected by AIDS (%)	No	Predictable & correctable
Performance Characteristics		
Assay Format	96-well plate, high through-put	Anything
Kit Stability	6 years	3 months
Reconstituted Reagents Stability	2 months	24 hours
Calibrators	No	Yes
Controls	Yes	Yes
Automation	No	Yes
Technical skill level required	Low	PhD
Result Interpretation		
Data/Result capturing & documentation	Electronic	Visual
Confirmatory algorithm	No	Anything
Maximum number of steps in algorithm	Not defined	4-5 on a subset (not all samples)
Mean Recent HIV Incident Period	6-12 months	3 months-SD 2 months
[Recent] Inclusive Window period range SD 2 weeks	18 month inclusive	2 months
Commercial Aspects		
Cost per specimen	$1.00 USD	$20.00 USD

8.2 Group 2 – Assay Development and Validation Tool Box

Participants specializing in specimen collection strategies and assay development were engaged in a thought exercise that was designed to determine the infrastructure for development of an assay assessment pathway for new HIV incidence assays. This exercise built upon information provided in Session VII: Current Issues in Assay Validation and the WHO "Methodologic Guidance Document for the Validation of Existing and Future HIV Incidence Assays." The critical pathway section of the WHO guidance document describes the essential panels and iterative steps required for assay validation defining three essential elements:

- Specimen panels: existing and new specimens
- Laboratory: central and field laboratories
- Infrastructure/scientific guidance: committees, decision points, timeline, etc

The exercise was limited to ninety minutes to stimulate a rapid brainstorming environment. To commence the thought exercise the group was presented with the following challenge:

> ***Assume a major funding organization wants to release a RFP for development and validation of new HIV incidence assays. Describe the infrastructure, facilities and reagents required to identify and progress new assay candidates?***

To guide the group in answering this challenge the group was presented with the following questions:

1. ***What are the concrete next steps to develop and sustain a specimen repository?***
2. ***What laboratory support is required to progress a new assay through validation?***
3. ***What infrastructure is required to coordinate this activity?***

The output of the breakout session is summarized below. See appendix III for the thought exercise handout.

Outline of the proposed concrete next steps to develop and sustain a specimen repository

- Establishment of qualification and calibration panels
- Designation of a central specimen repository
- Adequate blood volumes collected and a system to replenish specimens as needed
- Establishment of a central repository of specimens with a network for laboratory collaboration and goodwill for specimen sharing for different HIV sub-types
- Standard operating procedures for collecting, processing, aliquoting, cataloguing and labeling specimens following international good laboratory practice guidelines
- Statistical support
- Technical assistance to sites and quality assurance/quality control mechanisms
- Involvement of 10 or more experienced clinical research sites with intellectual interest in the development of HIV incidence assays
- Establishment of validation protocol
- Commercial collaborations (with production of prototype assays, production/supply of kits, development of incidence assay protocols, etc)

Group Report:

Establish the Core Sample Repository and Central Lab within the Core Facility
A Core Facility will be established to house the Core Sample Repository and to serve as the Central Laboratory. This Core Facility may be multi-centered, with Repository and Central Laboratory sites located in two or more locations such as in the United States and Europe. A Core Facility Oversight Committee would be formed to coordinate development of Core Facility sites, policies, and procedures, establishment of the relevant incidence specimen panels and the distribution and use of specimens for incidence assay development and ongoing quality control. This committee could be under the WHO umbrella.

While the remainder of this document discusses the role of single Core Facility, it may be logistically important for both logistical and political reasons that the specimens listed here will be housed at two to four regional laboratories, with the effort and overall specimen repository supervised by the Core Facility.

Core Sample Repository activities will include:

Inventory of existing and available sample sets and creation of a virtual repository: The project team will conduct a comprehensive review and assessment of available relevant specimens for possible inclusion in desired specimen panels. Samples will include mostly specimens from well-characterized patients with HIV infection, but some HIV negative patients may be included as well as controls. The initial assessment will address ownership of and access to the specimens and allowable uses based on appropriate consent procedures. A database representing this virtual repository will be created containing information on the physical integrity and location of these specimens, as well as subject details such as exposure category, approximate time of exposure, sex, gender, age, HIV-1 subtype and antiretroviral history. The specimens for the virtual specimen sets will be maintained at satellite locations.

The virtual repository will be established as an organized electronic inventory of relevant specimens available from committed collaborators for use in this project, but will not be transported to and maintained at the central repository supported by this initiative. Example of specimens in the virtual repository will include: acute infection (RNA+/Ab-) samples, seroconversion panels or pedigreed sets of specimens from recently infected individuals with estimated times since seroconversion and defined genotypes, and panels of specimens from individuals with long-standing infections that have been documented to yield false-incident results on relevant assays (e.g. from ART-treated subjects, persons with advanced immunodeficiency, and so-called “elite controllers”).

Acquisition and inventory of actual specimens: The Core Facility will oversee the acquisition and aliquoting of actual samples for the repositories based at the Central Laboratory (ies) as well as the creation of an electronic specimen repository database. Information to be housed in this database will include the same information included in the virtual repository data table, as well as information on the number, condition, and disposition of each aliquot once it is logged and stored.

As actual specimen sets identified in the cataloging process become available, the Core Facility will arrange for all necessary ethical and regulatory approvals for shipments to enable specimen transport and transfer from participating sites to the Core Facility. Samples will be collected to build a diverse subtype repository, including both early and late infection and possible false incident specimens. The collection and storage of samples will be done according to

established criteria to maintain sample quality. Information regarding the samples will be uploaded into the Core Specimen Database.

The core repository will develop specific panels composed of core sample sets, including samples from individuals in both early and late infection, to assist in development and monitor performance of new assays by academic and commercial labs, at the central laboratory. Selected panels will be sent to field sites at a later stage to monitor performance of the assays on site.

Types of specimens to be included in sample repositories: The actual and virtual repositories will include plasma or serum samples from individuals in the following categories:

1. Very early acute infections, spanning the period from ramp-up viremia to early seroconversion time points (including representation of subtype diversity and major geographic areas),
2. Individuals with known dates of infection with serial samples available over 1 to 3 years of follow-up (including representation of subtype diversity and major geographic areas),
3. Individuals under anti-retroviral therapy of 1 year or more duration; individuals with high CD4 and low viral loads (elite controllers); and individuals with advanced infection/AIDS (for each category, specimens must include representation of subtype diversity and major geographic areas).

The following have been preliminarily identified as sources of samples for both the actual and virtual specimen repositories:

1. Plasma donor seroconversion panels (SeraCare)
    - HIV-1 subtype B only
2. Blood donors with known inter-donation intervals (American Red Cross, SANBS; NIAID/NHLBI Panels initiatives)
    - These are available from wide range of geographic locations and at large volumes; but only 1-2 specimens are typically available from each donor
3. Public Health Laboratories with last-negative and first-positive plasma archived
    - Also small numbers per patient and typically very small volumes
4. High risk cohorts (MACS, CAPRISA, Rakai, WIHS, Vaxgen, HIVNET, IAVI, HVTN, other)
5. Acute HIV cohorts (CHAVI, CAPRISA, AIEDRP, Options, CASCADE, PRIMO, other)
    - Stored specimens 'precious' and also low-volume
        - High volumes may be best obtained through prospective collection of plasma that is discarded under existing protocols for preparation of PBMC in the conduct of many prospective cohort studies.
6. Elite controller cohorts (SCOPE, Harvard cohorts)
7. Other clinical cohorts with access to AIDS patients
8. ART treatment studies
9. Vaccine trials

Central Laboratory Activities:
In addition to facilitating the development and maintenance of the Core Repositories, the Central Laboratory(ies) will: 1) develop detailed study protocols and laboratory operational manuals and procedures in collaboration with the Core Scientific Committee and assay

development companies and satellite laboratory sites; 2) develop report templates and maintain report databases; 3) validate assays at the Central laboratory(ies) using panels compiled and held in the repository; these will include seroconversion (sensitivity) panels, false incident (specificity) panels and QA/QC panels to be used to assess whether or not assays can be used in an incidence testing capacity; 4) test and maintain sets of samples that will replenish repository stocks; 5) analyze results from assay development panels sent to companies or other assay developers, and from quality control panels sent to the field sites; and 6) participate in analysis of data and development of manuscripts.

Refine the Critical Assay Assessment Pathway and Assess New/Existing Assays

Critical Assay Assessment Pathway refined and decision points/criteria. This phase will refine the Critical Assay Assessment Pathway for identification and assessment of new and/or existing assays, using the already existing WHO critical pathway as a starting point. The progression of the assay along the critical path will be data and milestones driven with built-in go/no-go decision points. The design of the pathway will allow for standardized evaluation of both new and existing assays via the use of Central Core sample sets and the central laboratory.

The Core Scientific Committee will provide guidance on the refinement of the assessment pathway (pass/fail criteria) throughout the process. Assays will be evaluated and inserted into the critical path at key decision/insertion points that have criteria associated with each step. This will allow for subsequent progression of the assay on the pathway and access to core reagents and facilities as required. The main outcome of this phase will be the refinement of the critical pathway and the development of criteria required for progression of assay along the assessment pathway.

Qualification of new assay and inter-assay comparison of existing assays for progression to field-testing In this phase, the Core Scientific Committee will review data and select assays to be transferred to and evaluated in the central laboratory. Assays will be selected based on the following categories: 1) new assay(s) and 2) three or four existing assays requiring further evaluation/development. Using the assay specifications provided by the investigator, the Core Scientific Committee will determine the stage at which the assay should enter the critical assessment pathway (per above description). Assays will then proceed through the established critical pathway. Existing (prototype) incidence assays will be compared side-by-side with an identical sample panel in the central laboratory, allowing a standardized comparison in a controlled environment. For new incidence assays, the critical pathway will serve as the qualifying step before progressing to field-testing.

The critical pathway should involve three distinct steps outlined below.

The Primary Phase The primary assay development phase will consist of work performed by the commercial or academic development lab, in-house using sample panels labeled with known HIV infection status to allow for early development of incidence assays. This phase will involve a partnership between the Incidence Assay Core Facility and academia and industry to accelerate development of viable prototype incidence assays or modified protocols and cutoffs for incidence applications of existing HIV assays.

The Qualification Phase The qualification phase would involve the distribution of blinded and coded panels for qualification of prototype assay in the commercial or academic laboratory that developed the assay. The panels would include a supply of ~900 unlinked and blinded samples

including: 1) samples from documented seroconvertors with time points representative of the times spanning weeks to 2 years post infection (N =100), 2) ART treated and at different lengths of time on ART (N =200), 3) confirmed AIDS cases (N =200); and 4) chronically infected and long-term infected (> 2 years) panels with major subtypes (N =500).

The Calibration Phase The calibration phase would involve the fine-tuning of the incidence assay for use in incidence projections including calibration of window periods relevant to potential assay cutoffs and assessment of epsilons. Panels would include: 1) Seroconversion panels (N=100) representing all major HIV-1 subtypes and different stages of recent infection, coming from 1) plasma donors, 2) repeat blood donors from HIV incidence cohorts in Brazil, South Africa and United States, and 3) acute cohorts. The purpose of these panels would be to determine whether it is possible to distinguish between long-term and recent infection and to better characterize false recent rates and define window periods.

In addition to the qualification and calibration panels, there would be a separate set of false-incidence or specificity panels. These panels should include elite controller samples (100 ml from 50 elite controllers) and similar numbers of samples with large volumes of plasma from AIDS patients. Samples representing a cross-section of persons with HIV infection at all stages should also be represented in this group.

Estimated cost
The infrastructure for the specimen repository defined above can be established with $0.5 million per year for two years. Another $1 million per year would be needed to acquire, store, log, and distribute the desired specimen sample sets.

Discussion:
The industry representative in this session pointed out that it is important to have the panels already available for industry to become interested. This is one of the highest barriers in this field.

8.3 Group 3 – Global Demand Estimation

Timothy Mastro (FHI), Megan Averill (FHI), and Brad Theien (*bio*Strategies Group) led a discussion on estimating global demand for HIV incidence assays. This session was intended to inform the ongoing FHI-led demand estimation activity. Participants considered the main sources of demand, and how best to estimate their magnitude. These sources include:

1. Population-based surveys in countries with generalized HIV epidemics: Demographic and Health Surveys (DHS) and other large surveys with HIV testing can be used to estimate the number of HIV-positive samples to be tested by an HIV incidence assay.
2. Case-based surveillance: Several countries monitor HIV through ongoing case-based surveillance; these include the United States, France, Canada, and China. CDC and relevant Ministries of Health can provide estimates of the number of HIV positive samples collected annually.
3. Sentinel surveillance: Sentinel populations are monitored across the globe through a wide variety of periodic surveys. These populations include pregnant women visiting antenatal clinics, injection drug users (IDUs), men who have sex with men, and sex workers. This component of demand may be difficult to quantify, given the large number and types of sentinel studies for HIV. WHO, UNAIDS and US Bureau of the Census reports are a good source of information on these surveys.

4. Research and other purposes: incidence assays are also used to identify appropriate populations for clinical, intervention and preventions trials. Organizations conducting these trials can be contacted directly for information on the number of HIV positive samples collected. These include FHI's Site Identification and Development program, the Microbicides Trials Network, and the HIV Prevention Trials Network. Although currently not recommended for this purpose, some assays are also used in clinical settings to determine the recency of individual cases.

Several other factors must be considered to determine the potential market for HIV incidence assays. Currently, HIV incidence assays are used as part of the STARHS methodology, where only HIV-positive specimens are tested. It is possible that a future incidence assay would be used as the initial HIV test yielding both information on HIV-seropositivity as well as recency. Such a practice would increase demand for the assay by many fold. Also, users commonly report running samples in duplicate or triplicate in order to validate findings.

FHI and *bio*Strategies Group will call on participants in the demand estimation discussion to obtain further input on appropriate assumptions and data sources. FHI expects to complete the HIV incidence assay demand estimation later in 2009.

CONCLUSIONS AND NEXT STEPS

1. Current State of HIV Incidence Estimation

Standardize terms and methods: There is a clear need to establish standard terminology and a standard set of methods to be used for HIV incidence assays; the WHO Working Group will address this activity. This terminology should be contingent on assay use and application. There should be clear terminology to distinguish between the pre- and post-seroconversion window periods.

Develop guidance document on use of current and future assays: UNSW/NCHECR scientists will take the lead, in conjunction with the WHO Technical Working Group, on drafting a guidance document on the use of the assays for HIV incidence estimation as part of the FHI/Gates sub-grant program. A need was expressed to generate guidelines on how to develop an HIV incidence assay, but this would require more technical guidance from industry. The issue was raised as to whether the BED assay should become part of a multi-test algorithm. An eventual goal would be to develop a clear package insert for the assay.

Sustained funding: An underlying theme for this meeting was the need for sustained funding for HIV incidence assay development (due to low market incentives).

2. Market Assessment for HIV Incidence Assays and Demand Estimation

Complete the market assessment: FHI will continue refining the global demand estimation for HIV incidence based on input received at the meeting.

3. Identification of Novel Biomarkers in Development of Assays to Identify Recent HIV Infection and to Estimate Incidence

Use a two-prong approach to identify biomarkers: Use a dual strategy involving 1) a biomarker discovery effort in the long-term combined with 2) a parallel approach on how to optimize the use of currently existing HIV incidence assays.

Use a combination of biomarkers: Scientists recommended using a combination of markers (and multiplex assays) and giving different weights on parameters contingent on the population and HIV-1 subtypes.

Exploit biomarker diversity: The need to exploit antibody/biomarker diversity was discussed. The group agreed that to identify a single biomarker assay will be challenging. Using multi-subtype recombinant antigen assays or manipulating ratios of biomarkers depending on the HIV population may be promising approaches.

Evaluate biomarkers from chronically infected individuals: Biomarkers in chronically HIV infected individuals will need to be better understood to move the field forward.

4. Epidemiology and Incidence Study Design

Develop a protocol for assessing assays: There is a pressing need for an internationally agreed framework for validating and comparing HIV incidence assays, such as a standardized assay or algorithm validation protocol. The WHO Technical Working Group on HIV Incidence Assays will continue to develop a guidance document on this topic.

Foster consensus around statistical issues: There is a need to develop a consensus on statistical parameters for estimating HIV incidence. Statistical parameters to be identified include the determination of the window period and the development and application of adjustment factors to address long-term specificity. The WHO Technical Working Group Meeting held in April 2009 focused on resolving these statistical issues. A meeting report is forthcoming and will be posted on the WHO website.

5. Current Issues in Technical Assay Development – Input from Industry

Explore the options and opportunities to develop a public-private partnership: It was recommended to explore the concept of a public-private partnership to move the field of HIV incidence assay development forward.

Perform risk/reward and cost/benefit analyses: For HIV incidence assay development and commercialization it is important in to evaluate likely costs/risks and projected rewards/benefits.

Develop assays for different uses or applications: From a commercial standpoint, it is important to consider developing different HIV incidence assays based on the different uses or applications to expand commercial market opportunities.

Generate commercial interest and application: HIV incidence assays must have a significant commercial application to generate market interest. Small companies, compared to large companies, may be more interested in developing products for the niche market for HIV incidence estimation. Large companies may support modifications or adaptation of their existing commercial HIV antibody assays for expanded use in identification of recently infected persons and incidence estimation.

Provide existing specimen panels to industry for assay development: Companies will need to have access to specimen sample sets to validate HIV incidence assays. Making this resource available to industry may promote an interest in HIV incidence assay development.

6. Pathway for HIV Incidence Assay Validation

Refine the assay development pathway: There is a need for a pathway for HIV incidence assay calibration and validation. Assay development should follow a similar process as that of drug discovery, with sequential phases. The WHO Technical Working Group will continue to refine their document on this topic.

7. Infrastructure and Specimens for Assay Validation

Establish a virtual database of sample sets: There is a need to catalogue studies and specimens for assay calibration and validation. The UNSW/NCHECR is currently developing a virtual database of sample sets as part of the FHI/Gates sub-grant program.

Establish a central specimen repository for systematic assay assessment and assemble the toolkit for HIV incidence assay validation: There was a consensus to establish a central HIV specimen repository. A key next step would be to develop the infrastructure for the HIV specimen repository, as described by Mike Busch section 8.2 of this report: Assay Development and Validation Toolbox. The central HIV specimen repository would contain samples obtained prospectively combined with archived samples.

Establish guidelines around use of core specimen sample sets: Criteria must be established on how to access HIV sample sets.

8. Assay Specifications and Performance Requirement

Refine assay specifications based on assay uses and applications: Minimum and optimal specifications and requirements must be established for HIV incidence assays based on assay uses and applications. Specificity was considered one of the most important requirements.

Appendices

Appendix I: Meeting Agenda

WEDNESDAY MAY 13, 2009			
Time	**Duration (min)**	**Topic**	**Speaker**
7:00 – 8:00 am		Registration and Continental Breakfast	
Session 1: Current State of HIV Incidence Estimation			
8:00 – 8:45 am	45	Welcome and Introductions Overview of the Key Issues in HIV Incidence Estimation	Tim Mastro
8:45 – 9:15 am	30	Market Assessment: Overview and Preliminary Findings	Megan Averill
9:15 – 10:15 am	60	Breakout Session 1: Thought Exercise (4 small groups of 10-15 people each)	Moderator
10:15 – 10:30 am	15	Break	
Session 2: Group Reports			
10:30– 10:50 am	20	Reports from the 4 small groups (5 minutes each)	Rapporteurs
10:50 – 11:30 am	40	Discussion of Group Reports	Moderator
11:30 – 12:30 pm	60	Lunch Break	
Session 3: Use of Markers in the Development of Assays to Identify Recent Infection and Incidence Estimations			
12:30 – 12:45 pm	15	The HIV Transmission Event	Mike Cohen
12:45 – 1:00 pm	15	The Host Response to HIV Infection	Georgia Tomaras
1:00 – 1:45 pm	45	Panel Discussion: What are the prospects for new biomarkers?	Moderator
1:45 – 1:55 pm	10	Break	
Session 4: Epidemiology and Incidence Study Design			
1:50– 2:10 pm	15	How Accurate Are Assays for Recent HIV Infection? A Literature Review	John Kaldor
2:10 – 2:30 pm	20	Mathematics of Assay Use and Limitations– The Window Period and Long-term Specificity	Alex Welte
2:30 – 2:45 pm	15	Algorithms for Incidence Testing	Oliver Laeyendecker
2:45 – 3:15 pm	30	Panel Discussion: How far can we go with existing assays?	Moderator
3:15 – 3:30 pm	15	Break	
Session 5: Current Issues in Technical Assay Development			
3:30 – 4:00 pm	30	Market Assessment: Projecting Global Demand	FHI
4:00– 4:30 pm	30	New Assays in Development	Bharat Parekh Francis Barin
4:30 – 5:00 pm	30	Industry Panel Discussion: Insights and Lessons Learned	Industry/ Representatives Moderator
5:00 – 5:05 pm	5	Recap	Moderator
6:00 – 7:00 pm		Reception	
7:00 – 9:00 pm		Dinner	

THURSDAY MAY 14, 2009			
Time	**Duration (min)**	**Topic**	**Speaker**
7:15 – 8:00 am		Continental Breakfast	
8:00 – 8:15 am	15	Opening Remarks	Tim Mastro
Session 6: Thought Exercise			
8:15 – 9:15 am	60	Breakout Session II: Thought Exercise (3 small groups of 16 to 20 people each)	Moderator
9:15 – 9:30 am	15	Reports from the 3 small groups (5 minutes/each)	Rapporteurs
9:30 – 10:05 am	35	Discussion of Group Reports	Moderator
10:05 – 10:25 am	20	Coffee Break	
Session 7: Current Issues in Assay Validation			
10:25 – 10:45 am	20	Specimen Collections: Desired Sample Sets	Mike Busch
10:45 – 11:00 am	15	Cataloging of Specimens: Virtual Database of Specimens	Joanne Micallef
11:00 – 12:00am	60	Panel Discussion: What is needed for the tool box?	Moderator
12:00– 1:00 pm	60	Lunch	
Session 8: Laying the Foundation for New Assay Development			
1:00 – 2:30 pm	90	Breakout Session III (3 small groups of 16 to 20 people each) • Group 1: Assay Specifications • Group 2: Assay Development and Validation Tool box • Group 3: Global Demand Estimation Model	Moderator
2:30 – 3:00 pm	30	Reports from the 3 Small Groups (10 minutes/each)	Rapporteurs
3:00 – 3:20 pm	20	Break	
Session 9: Summary of Meeting			
3: 20 – 4:10 pm	50	Moderated Discussion	Moderator
4:10 – 4:30 pm	20	Recap	Tim Mastro/Gates
Adjourn Conference			

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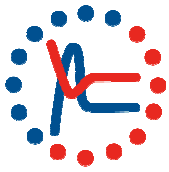


BREAKOUT SESSION 1: CHALLENGES

Sessions #1 and 2: Thought Exercise
Date: May 13, 2009 – 9:15 -10:15 am

Objectives

1. Update list of challenges facing the field
2. Rank challenges
3. Develop concepts to address challenges

Deliverables

- List up to 12 challenges facing the field – "terminology" must be one of them.
- Rank the challenges—what are the top 5? "Terminology" should be one of them.
- Capture 1-3 new concepts to address each of the top 5 challenges.

1. ***What are the key challenges facing the field?***
   - Group brainstorms and facilitator captures up to 12 challenges onto flipcharts
   - Each person votes on top 3 challenges using dot system in "Priority" column.
   - Collectively, the group picks top 5 challenges to address.

2. ***How do we address these top 5 challenges?***
   - Capture ideas to address top 5 challenges in the "Concepts" columns using post-it notes.
   - Use pink for revolutionary ideas.
   - Use yellow for incremental changes.

3. ***Summary***
   - Prepare summary flipchart using top 5 challenges. List best 3 concept ideas to address these challenges in "A", "B", and "C" columns.
   - An FHI staff will transcribe the information onto a PowerPoint slide for the group report.

Breakout Session #1: Group Discussion

Team 1 (Red): Summary of Top Challenges and Key Concepts

#	Top 5 CHALLENGES	BEST CONCEPTS			
		A	B	C	D
1	Terminology	Syphilis model – ban the use of acronyms (revolutionary)	Assays for recent infection are different from assays for acute infection (incremental)	WHO Working Group on terminology (incremental)	
2	Specificity	Biomarkers of late infection (incremental)	Alternative cut-offs (window period) (incremental)	Use of algorithm (viral load, CD4 count) (incremental)	Basic science of assay process (modeling of) (revolutionary)
3	Biomarkers	Look for host biomarkers (genomics) of HIV-1 infection to get around subtype variability (revolutionary)	If possible, assay with a long (large) dynamic range (incremental)	Hair – other types of samples (revolutionary)	Chemokines neutralizers – Ab isotype (IgG3, IgM, IgA), characterize immune response (incremental)
4	Reference panels	Representative specimen sets (incremental)	CAP-like panels (incremental)	Enriched for false recent (incremental)	Subtype diversity (incremental)
5	Broad applicability	Development of multiplex units that combine diverse biomarkers (incremental)	Modeling of multiple markers with specific weights for final yes/no determination (incremental)	Combining algorithm into one assay format (incremental)	
Additional Challenges Identified					
6	Biological variability within population				
7	Commercial viability				
8	Longevity of technology				
9	Improved statistical methodology				
10	Ease of use				
11	Consensus on discourse: window periods				
12	Applicability for individual (clinical research)				

Team 2 (Blue): Summary of Top Challenges and Key Concepts

#	CHALLENGES	BEST CONCEPTS		
		A	B	C
1	Terminology	WHO conference to address/disseminate decisions (incremental)	Recent infection not equal to recent seroconversion (incremental)	Consensus proposed by WHO (incremental)
2	Long-term specificity of the assay/false recent rate	Develop new assay/refractory to sub-type/in vitro stimulation of Ab is different in early seroconversion vs. AIDS (revolutionary)	Use of algorithm (incremental)	
3	Window period	Subtype independent assay, well-characterized (incremental)	Non-antibody dependent assay (revolutionary)	Consensus on methodology (incremental)
4	Gold standard	Standardized validation approach (incremental)	Detect and measure early infection (incremental)	Data quality and completeness and methodology validity (Incremental)
5	Statistical formulas	Consensus on methodology (incremental)	Meta-analysis of available data sets (incremental)	Account for all geographic and demographic factors (incremental) Data quality and completeness and methodology validity (incremental)
Additional Challenges Identified				
6	Sensitivity			
7	Market size (competition/funding organizations)			
8	Sample type			
9	Calibration sets, validation sample set, samples to evaluate assays			
10	Assay robustness			
11	Sample size			
12	Technological transfer (IP)			
13	Defining 100% infected			

Team 3 (Yellow): Summary of Top Challenges and Key Concepts

#	CHALLENGES	BEST CONCEPTS		
		A	B	C
1	Design assays for specific applications	New assays for reliable sensitive epitope (revolutionary)	Intensified validation of avidity (incremental)	
2	Assay performance (as a whole)	New assay for reliable specific epitope/new biomarker assay (revolutionary)	Establish gold standard for validation of assays/algorithms (revolutionary)	Determine false recent rate for different populations (incremental) New algorithm (incremental) Improve sensitivity/specificity (incremental) Clear definition of assay requirements (incremental) Examine performance of existing assays/algorithms on ART (incremental) Measure false recent rate in more settings and same setting over time to assess variability in calibration parameters (incremental) Standardize the use of two assays to improve accuracy (e.g. BED and avidity) (incremental)
3	Resources/specimen panels for assay validation	Global specimen repository (revolutionary) This issue should be supported by NIH as a new project taking population with high incidence as target population (incremental).	Small consortium of independent global laboratories to act as a repository. Partner with vaccine, microbicide and PrEP sites that have seroconversion panels (incremental) (revolutionary)	Facilitate data sharing (revolutionary)
4	Minimum sample size	Establish and disseminate minimum standard (incremental)	Modeling for small size determination and consensus for specific applications (incremental)	Prepare and get consensus on clear guidelines for appropriate application of incidence test (revolutionary)
5	Terminology	Expert panel to define terminology and publish (incremental)	Eliminate use of incidence assays for individual characterization (revolutionary)	
Additional Challenges Identified				
6	False recent rate			
7	Sub-type specificity			
8	Individual heterogeneous response			
9	Testing bias			
10	Test and treat			
12	Clinical utility			
13	Technical ease of assay			

Team 4 (Green): Summary of Top Challenges and Key Concepts

#	CHALLENGES	BEST CONCEPTS		
		A	B	C
1	Accuracy of test performance	Competitive process for best assay – funding required (incremental)	Get the right test materials like the neutralizer, antibody, panels (incremental)	Promote new approaches – funding required (incremental)
2	Consensus on the assay development pathway and dissemination	Multi-disciplinary team work on timeline to develop clear pathway/milestones for new assay development (incremental)	International specimen bank for assay development and validation (incremental)	Industry/FDA collaboration on a standard and flexible pathway (revolutionary)
3	Lack of cross-disciplinary approach	Encourage multi-disciplinary approach (incremental)	Give a prize to immunologists for coming up with a great new approach (revolutionary)	Funding initiatives that require formation of multi-disciplinary teams (immunologists, assay specialists, epidemiologists, modeling, clinical trial specialists, etc). (revolutionary)
4	Diversity in host response	Active HIV infection use genomics/proteomic to identify new target – a new approach (revolutionary)	More basic research in immunology mapping response to gp41. Define optimal epitope and genetic conservation (incremental)	Encourage research in diverse populations (incremental)
5	Terminology	Working group on terminology (incremental)	Define terms and always include definition of terms in documents and articles that detail recent or acute infection. Never assume knowledge (incremental)	Define terminology with consensus from major stakeholders (incremental)
Additional Challenges Identified				
6	Calibration of window			
7	Sample size			
8	Materials for assay development			
9	Skepticism			
10	Interpretation of results			
11	HIV diversity			
12	Varying needs/applications across stakeholders			
13	Lack of perceived market			

BREAKOUT SESSION 2: CRITICAL SCREENING PATHWAY

Session #6: Thought Exercise

Date: May 14, 8:15-9:15 am

Charge:

Assume a major, global HIV funding organization has announced that it wants to obtain national HIV incidence estimations in multiple countries. The funding organization will award a grant to the best idea on how to do this that is feasible, accurate, timely and cost efficient.

1. How would you do it?

- **Define your methodology. How would you gather information?**
  Example: large, representative national serosurveys using an incidence assay

- **Define your assay algorithm – will you use:**
  - Combinations of currently used/available assays
  - Combinations of currently used and newly developed assays
  - Newly developed assays-only
  - Novel assay alone or in combination

- **Define your pathway and decision points.**

2. Based upon the algorithm selection, how will this impact your timeline?

- Define your timeline and decision points

3. Summarize results

Terminology

- *Current assays: field test assays that are available for use. May include incidence assays (example: BED) or other types of assays (example: CD4)*
- *Newly developed assays: assays are available, but may not have been fully validated in the field.*
- *Novel assay: assay needs to be developed*

BREAKOUT SESSION 3: GROUP 1

Session #8: Assay Specifications
Date: May 14, 1:00-4:00 pm

OBJECTIVE: Develop assay specifications for an HIV incidence assay.

Charge:

> ***If designing a new assay for incidence estimation from cross-sectional samples for use at the population level, what features and assay specifications would be required?***

BACKGROUND

A product requirement document (PRD) describes the product that will be developed, i.e. the product profile. The purpose of the PRD is to articulate the purpose of the product, features, and technical requirements.

1. ***What features should be included in the PRD?***
2. ***What criteria should be applied to each feature in the PRD?***
3. ***How well does the performance of existing assays match the draft PRD requirements?***

SUMMARY

Update draft PRD and capture limitations of existing assays.

BREAKOUT SESSION 3.2: GROUP 2

Session #8: Assay Specifications
Date: May 14, 1:00-4:00 pm

OBJECTIVE

Determine the infrastructure for development of an assay assessment pathway for new HIV incidence assays.

Charge:

Assume a major funding organization wants to release a request for proposal for development and validation of new HIV incidence assays. Describe the infrastructure, facilities and reagents required to identify and progress new assay candidates?

BACKGROUND

Based upon session VII, and the WHO working group HIV Incidence Assay validation protocol (provided in your binder), the critical path for assay assessment involves these 3 elements:

- Specimen panels: existing and new specimens
- Laboratory: central and field laboratories
- Infrastructure/scientific guidance: committees, decision points, timeline, etc

4. ***What are the concrete next steps to develop and sustain a specimen repository?***

5. ***What laboratory support is required to progress a new assay through validation?***

6. ***What infrastructure is required to coordinate this activity?***

SUMMARY

Discussions of group reports and finalize report-out.

Appendix IV: Recent Infection Testing Algorithm (RITA) notes

Recent Infection Testing Algorithms
Key Definitions and Concepts

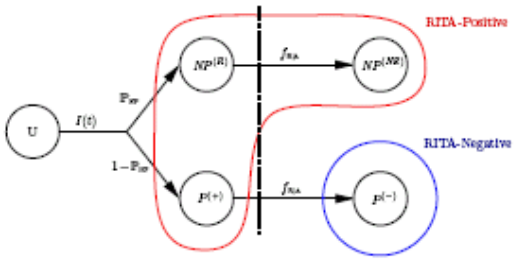
Simple Case:

- Seek a transient state of 'Recent Infection' lasting about half a year
- Use any number of incidence-assays, thresholds, clinical data, rules etc. to define a RITA-positive state
- IF everyone leaves the RITA-positive state, can equate recent with RITA-positive

- Mean time spent in the state of recency = mean window period = ω
- Simple incidence estimator:

$$I_{\text{est}} = \frac{N_{\text{R}}}{N_{\text{U}}\omega} = \frac{\text{Number of Recently Infecteds}}{\text{Number of Uninfecteds} \times \text{Mean window period}}$$

Handling RITA non-progressors:



- Declare (unobservable) transition in the RITA-non-progressors
- Use the same distribution of waiting times!
- Now a transient recency applies to everyone, but is not observable in everyone!
- Distinguish recent/non-recent from RITA-positive/RITA-negative
- RITA-sensitivity is perfect (recency guarantees RITA-positivity)
- Need to calibrate RITA-specificity (ρ_R) (Non-progressing proportion $\mathbb{P}_{\text{NP}} = 1 - \rho_R$)
- Same estimator, if survival is the same for RITA-progressors and RITA-non-progressors, but now

$$N_{\text{R}} = \text{Number RITA-positive} - \phi \times \text{Number RITA-negative}$$

where

$$\phi = \frac{1-\rho_R}{\rho_R} = \text{odds of RITA non-progression}$$

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