

ARE SEROLOGICAL ASSAYS FOR RECENT INFECTION EFFECTIVE TO ESTIMATE HIV-1 INCIDENCE IN AFRICA?

C. Sakarovitch¹; F. Rouet²; G. Murphy³; A. Alioum¹; A. Minga⁴; B. Liandier⁵; T.D. Toni⁶; F. Dabis⁷; D. Costagliola⁸; R. Salamon⁷; J. Parry³; F. Barin⁵;

1-INSERM EMI 03 38, Institut de Santé Publique, Epidémiologie et Développement (ISPED), Université Victor Segalen Bordeaux 2, Bordeaux, France ; 2-CeDRes, Abidjan, Cote D'Ivoire; 3-Health Protection Agency Centre for Infections, Virus Reference Division, London, United Kingdom; 4-Projet ANRS 1220 PRIMO-CI, Programme PAC-CI, Centre Hospitalier Universitaire de Treichville, Abidjan, Cote D'Ivoire ; 5-Laboratoire de virologie and Centre National de Référence du VIH, EA3250, Tours, France ; 6-CIRBA, Abidjan, Cote D'Ivoire ; 7-INSERM Unité 593, Institut de Santé Publique, Epidémiologie et Développement (ISPED), Université Victor Segalen Bordeaux 2, Bordeaux, France; 8-INSERM U720, Université Pierre et Marie Curie, Paris, France;

Objectives: The objective is to assess the performance in West Africa of four assays to detect recent HIV-1 infections.

Methods: One commercial assay (Calypte IgG-capture BED-EIA), two modified commercial assays, the Vironostika HIV-1 EIA (Khotte et al) and the "avidity" Abbott HIV1 1/2gO assay (Suglioli et al), and an in-house assay, the IDE-V3 EIA (Barin et al), were assessed on 135 samples from HIV-1-positive regular blood donors with a known date of seroconversion from Abidjan, Côte d'Ivoire (ANRS 1220 PRIMO-CI cohort). The 135 samples included 26 from recently infected patients (≤ 180 days), 94 from non recently infected subjects (> 180 days), and 15 from patients with AIDS. The sensitivity and specificity of these methods were evaluated for individual diagnosis. The performance of an assay to estimate incidence depends also on the real incidence and prevalence in the sampled population, therefore we investigated that performances through simulations.

Results: The modified commercial assays gave the best results for sensitivity (100% for both), while the IDE-V3 technique was the most powerful for specificity (96.3%). All specimens from patients with AIDS were classified as non recent infections by three tests (Vironostika, BED and IDE-V3). The estimated HIV-1 incidence was highly overestimated with these four assays: from up to 3 times with the IDE-V3 test to 36 times with the Avidity in the case of a 30% prevalence associated with a 1% incidence in the population. Most of the false positive results were found among specimens from patients infected for more than 180 days but less than one year.

Conclusions: Our study showed that none of the four methods can currently be used to accurately estimate HIV-1 incidence in Ivory Coast. In our setting, further adaptations are required, including the use of a longer window period or a lower cut-off favouring an increased specificity.

COMPARISON OF TWO ASSAYS FOR IDENTIFYING INCIDENT INFECTION AMONG CASES OF NEWLY DIAGNOSED HIV INFECTION

Ann M. McDonald¹; Philip Cunningham²; Anthony Kelleher^{1,2}; John M. Kaldor¹;

1-National Centre in HIV Epidemiology and Clinical Research, Sydney; 2-NSW State Reference Laboratory for HIV, St Vincent's Hospital, Darlinghurst, NSW;

Objectives: To compare estimates of sensitivity and specificity of the detuned ("sensitive-less sensitive") and BED assays for diagnosing early HIV infection, using as the reference standard available information on HIV antibody testing history and clinical diagnoses of primary HIV infection among cases of diagnosed HIV-1 infection.

Methods: Consecutive cases of HIV infection among people who were voluntarily tested at St Vincent's Hospital, Sydney, as part of their clinical assessment, were tested using the detuned and BED assays. Cases with both a detuned and a BED test result were matched to cases of newly diagnosed HIV/AIDS notified to the National HIV/AIDS Registry, to retrieve information on HIV/AIDS diagnoses and prior testing history.

Results: Among 219 cases of HIV infection diagnosed in 2005, the estimate of sensitivity of the detuned and BED assays was similar among 23 cases with evidence of HIV acquisition within 30 days of assay specimen date (both 95.6%), among 18 cases with evidence of HIV acquisition 30 – 180 days (both 77.7%) and among 16 cases with evidence of HIV acquisition 180 – 365 days prior to assay specimen date (detuned 62.5%; BED 50.0%). Specificity of the detuned and BED assays among 52 cases for which HIV infection was diagnosed at least 180 days prior to assay specimen date was 82.7% (both assays) and was 72.2% and 83.3%, respectively, among 18 cases with AIDS. Among 92 cases without evidence of timing of HIV acquisition for which infection was diagnosed within 180 days of assay specimen date, 34 (37.0%) and 32 (34.8%) were diagnosed with early infection by the detuned and BED assays, respectively.

Conclusions: In a population predominantly affected by HIV-1 subtype B, the detuned and BED assays provide comparable estimates of sensitivity and specificity. They complement surveillance for newly acquired infection by providing a basis for more complete ascertainment of the recent pattern of HIV transmission.

HIV-1 INCIDENCE STUDIED BY THE IGG CAPTURE BED ENZYME IMMUNOASSAY IN IDU POPULATION OF CHINA

Yan Jiang¹; Yao Xiao¹; Jigang Feng¹; Wenyan Xu¹; Minjie Wang¹;

1-National AIDS Reference Laboratory (NARL) of China CDC, Beijing China

Objectives: The research aimed at evaluating BED EIA technique in IDU population of China, providing technology support for surveillance on new recent infection in IDU population of China.

Methods: Total 2909 HIV positive serum (plasma) of IDUs were collected from NARL (6 are seroconversion, 300 are long term infection more than 2 years) and 4 provincial HIV/AIDS confirmatory laboratories. All positive specimens were confirmed with WB. All specimens were tested with BED EIA, and HIV incidence was calculated with BED statistics software.

Results: 1. 6 seroconversion specimens (CRF-BC) are verdicted recent HIV infection with BED. 2. The false classify rate (long term infection are falsely classified as recent infection) is 12% (6/50) 5.5% (11/200) and 6% (3/50) respectively when CD4 counts is <50/ μ L, 50-400/ μ L and >400/ μ L. 3. The HIV incidence of IDU population of province A is 8.3% (95% IC is 5.46-11.0) in 2002 using BED assay, it is close to the incidence of 8.8% (95% IC is 6.3-12.0%) from HPTN cohort study. The HIV incidences of high prevalence, medium prevalence and low prevalence regions of province D in 2004 are 4.18 - 4.39%, 1.66% and 0.42-0.6% respectively.

Conclusions: 1. BED EIA is an effective, simply and convenient technique in detecting recent HIV infection and estimating incidence. 2. Some long term infection specimens are falsely classified as recent infections by BED EIA, samples used for BED assay should be selected careful. 3. The HIV incidence of IDU population of province A and D are 8.3% and; the incidence in high prevalence, medium prevalence and low prevalence regions of province D are 4.3%, 1.66% and 0.51% respectively in 2004.

PREPARING FOR BED INCIDENCE TESTING: ASSESSING THE QUALITY OF HIV SURVEILLANCE TESTING IN THAILAND

W. Chalermchan¹; A. Sriburi¹; P. Unpol¹; S. Nookhai²; V. Pobkeeree²; T. Plipat³; B. Parekh⁴; K. Fox^{2,4}; J. Tappero^{2,4}; P. Sawanpanyalert¹;

1-National Institute of Health, Department of Medical Sciences, Ministry of Public Health (MOPH), Nonthaburi, Thailand; 2-Thailand MOPH – U.S. CDC Collaboration, Nonthaburi, Thailand; 3-Bureau of Epidemiology, Department of Disease Control, MOPH, Nonthaburi, Thailand; 4-CDC, Atlanta, GA, USA;

Objectives: The recently validated BED assay allows HIV incidence estimation using cross-sectional samples; however, BED testing of HIV false-positive samples can produce false-positive BED results and incidence overestimation. To prepare for BED testing in Thailand, we assessed the quality of national HIV serosurveillance testing among pregnant women (ANC) and female sex workers (FSW).

Methods: National HIV sentinel serosurveillance is conducted in a cluster sample of 24 (of 76) provinces. For each province, consenting FSW in a random sample of sex establishments receive linked anonymous testing; ANC samples come from routine opt-out clinical testing. HIV surveillance testing is performed locally by hospital or provincial laboratories. Prior to annual surveillance rounds in 2004 and 2005, local surveillance and ANC staff were trained on specimen collection and handling techniques. During 2004, all HIV-positive and 5% of negative ANC and FSW samples were sent to Thai National Institute of Health (NIH). Samples were screened by enzyme immunoassay (EIA) and confirmed by another EIA or gel particle agglutination (GPA), depending on sample volume. During 2005, all HIV-positive ANC samples, along with all HIV-positive and HIV-negative FSW samples were sent to NIH. Samples were screened by EIA and confirmed by another EIA and GPA. Samples reactive by two (2004) or three (2005) assays were considered true positives.

Results: NIH received 768 HIV-positive and 2572 HIV-negative samples in 2004, 644 HIV-positive and 6823 HIV-negative in 2005. Virtually all samples (99.9%) were acceptable quality for testing; however, 13.0% in 2004 and 3.8% in 2005 were low volume, hemolyzed, or lipemic. Re-testing identified 0.4% false-positives in 2004, 0.5% false-positives in 2005, and no false-negatives.

Conclusions: The vast majority of locally tested HIV surveillance specimens were accurately classified. Sample quality should continue to be monitored and directed training conducted as needed.

A SIMPLE AND INEXPENSIVE PARTICLE AGGLUTINATION TEST TO DISTINGUISH RECENT AND ESTABLISHED HIV-1 INFECTION

Niel T. Constantine¹; Hong Li²; Fassil Ketema¹; **Anne M. Sill**³; Kristen M. Kreise⁴; Farley R. Cleghorn⁵;

1-Department of Pathology, University of Maryland Baltimore, Baltimore, Maryland, USA; 2-Visiting scientist at the University of Maryland from the China Scholarship Council, Yunnan CDC, Kunming, Yunnan, China; 3-Department of Pediatrics, University of Maryland Baltimore, Baltimore, Maryland, USA; 4-Department of Epidemiology and Preventive Medicine, University of Maryland Baltimore, Baltimore, Maryland, USA; 5-Center for HIV/AIDS, Futures Group, Washington, DC, USA;

Objectives: All currently used serologic assays that distinguish recent and established HIV infection for the purposes of measuring HIV incidence require a high degree of technical expertise and laboratory infrastructure which limit their applicability in resource limited countries. Further, the cost of these tests is relatively high. We sought to modify the Serodia HIV-1/HIV-2 particle agglutination assay (PA), a simple and cost-effective HIV assay that is used globally for the detection of HIV antibodies, as a sensitive/less sensitive (S/LS) test to identify recently infected individuals and to estimate HIV incidence.

Methods: The Serodia PA was procedurally modified and subjected to validation to be a S/LS assay. Following verification of HIV antibody positive status using a 1:10 dilution of sera (sensitive mode), HIV antigen-coated gelatin particles were diluted 1:68, and sera were diluted at intervals from 1:10 to 1:80,000 to calibrate the assay (less sensitive mode, PA-LS). Thirty-seven HIV clade B seroconversion panels (n=309) from Trinidad and from a commercial source (BBI) were tested at each sample dilution to determine the last positive reaction. The greatest sensitivity for correctly classifying samples from recent and established infections was determined by receiver operator curve analyses.

Results: At a 1:40,000 sample dilution and a days post seroconversion cutoff of 190 days, the PA-LS test yielded a 97% sensitivity for correctly classifying recent and established infection samples, and a 99% positive predictive value at the 1:20,000 dilution for identifying recently infected individuals when tested on clade B HIV seroconversion panels. The PA-LS offers a 30-44 fold cost savings over currently available S/LS tests.

Conclusions: A low cost, simple-to-perform PA test was modified to be a S/LS test and exhibited excellence in distinguishing recent from established HIV infection. The PA-LS test offers important advantages over current S/LS tests, particularly for use in resource-limited countries.

REGENCY ASSESSMENT IN NEWLY DIAGNOSED HIV-1 INFECTIONS BY MEANS OF A STANDARDIZED LINE IMMUNOASSAY USED FOR HIV-1/2 CONFIRMATION

Jörg Schüpbach¹; Martin Gebhardt²; Christoph Niederhauser³; Luc Perrin⁴; Philippe Bürgisser⁵; Lukas Matter⁶; Meri Gorgievski⁷; Rolf Dubs⁸; Detlev Schultze⁹; Peter Erb¹⁰; Corinne Andreutti¹¹; Jean-Claude Piffaretti¹²; B. Guentert¹³; Roger Staub²; Synove Daneel¹⁴; Pietro Vernazza¹⁴;

1-University of Zurich, Swiss National Center for Retroviruses, Zurich; 2-Swiss Federal Office of Public Health, Berne; 3-Blood Donation Service SRK Bern AG, Berne; 4-Hopital Cantonal Universitaire, Laboratoire Central de Virologie, Geneva; 5-CHUV, Service d'Immunologie et d'Allergie, Lausanne; 6-Institut Dr. Viollier AG, Basel; 7-University of Berne, Institute of Infectious Diseases, Berne; 8-University Hospital, Department of Medicine, Zurich; 9-Institute for Clinical Microbiology and Immunology, St. Gallen; 10-University of Basel, Institute for Medical Microbiology, Basel; 11-Clinique de la Source, Laboratoire, Lausanne; 12-Istituto Cantonale di Microbiologia, Bellinzona; 13-Labor Dr. Guentert, Lucerne; 14-Kantonsspital St. Gallen, Department of Medicine, St. Gallen (all Switzerland);

Objectives: Knowledge of the number of recent HIV infections in a population is important for epidemiologic surveillance. A crude estimate of this number can be obtained by "detuned" assays measuring concentration and affinity of HIV antibodies. It would be desirable if recency information could be gained with assays used anyway during confirmation of HIV infection.

Methods: We evaluated whether a Western blot-like confirmatory assay, Innogenetics' InnoLia HIV-I/II Score, could replace Calypte's HIV-1 BED Incidence EIA. The InnoLia uses standardized HIV-1 and HIV-2 antigens as targets. It distinguishes HIV-1 and HIV-2 and provides also some information as to the presence of HIV-1 group O, informations important for viral load assessment and optimal antiretroviral therapy. Antibody reaction to each antigen is quantitated by means of three internal standards.

Results: In a nationwide anonymized study we are testing all HIV infections newly diagnosed in Switzerland (proportion of non-B subtypes above 30%) during one year with both the InnoLia and BED assays and relate their results to the physician's anonymized information mandatorily forwarded to public health authorities. In an interim analysis after 5 months, both test and physician's data were available for 210 infections, of which 64 (30.5%) were recent based on physician's information (primary HIV infection diagnosis or a documented negative HIV test within the past 12 months). BED assay classified 56% of the 64 recent infections as recent and 76% of the 146 others as older. Exactly the same figures were obtained with the InnoLia using a simple algorithm (recent if product of the reaction intensities (sgp120 * gp41 * p31) < 18). Overall "correct" prediction by both tests was 70% (P < 0.0001). Overall result concordance was 73.8%.

Conclusions: Recency information similar to that obtained by the BED assay can be gained from InnoLia-based confirmatory testing. Updated results will be presented at the Symposium.

HIV-1 INCIDENCE IN BLOOD DONORS IN FRANCE BETWEEN 1992 AND 2004: USE OF AN IMMUNOASSAY TO IDENTIFY RECENT INFECTIONS

Josiane Pillonel¹; Francis Barin²; Syria Laperche³; Pascale Bernillon¹; Stéphane Le Vu¹; Benoît Liandier²; Georges Andreu⁴; Jean-Claude Desenclos¹;

1-Institut de Veille Sanitaire, Saint-Maurice; 2-Université François-Rabelais, Centre National de Référence du VIH and EA 3856, Tours; 3-Institut National de la Transfusion Sanguine, Paris; 4-Etablissement Français du sang, Paris, France.

Objectives: An immunoassay (EIA-RI) for recent HIV-1 infections (≤ 180 days) has been developed in France in 2002. We used this EIA-RI on blood donations found HIV-1 positive between 1992 and 2004 to analyze the characteristics of recently infected donors, to estimate HIV incidence in blood donors and to compare these estimates with those derived from repeat donor histories.

Methods: One third of HIV-1 positive blood donations between 1992 and 1999 and all HIV-1 positive donations since 2000 were retrospectively tested with the EIA-RI. Multivariate analysis was performed to determine the donor characteristics (donor status – first-time versus repeat –, sex, age, geographic origin, mode of transmission and HIV-1 subtypes) associated with recent infection. Incidence rates obtained with the EIA-RI [recent infections/(donations*(180/365))] were compared to classical estimates among repeat donors [Seroconversions/Person-Years].

Results: Of the 399 HIV-1-positive donors studied between 1992 and 2004, 93 (23.3%) were identified as recently infected. Factors independently associated with recent infection were repeat donor status (adjusted odds ratio (AOR), 5.1) and non-B subtypes (AOR, 2.2). The proportion of recent infections decreased when the interval between the positive and the last negative donation increased, from 76% for intervals ≤ 180 days to 5% for intervals > 36 months. Incidence decreased from 4.3 per 10^5 (95% CI: 1.9-9.4) in 1992-94 to 1.7 per 10^5 (0.8-3.4) in 2002-04 in first-time donors and from 3.2 per 10^5 (95% CI: 2.0-5.0) to 0.6 per 10^5 (0.3-1.2) in repeat donors. These rates were similar to those derived from the classical method in repeat donors.

Conclusions: This study confirms that the EIA-RI is a valid tool to estimate HIV-1 incidence. This immunoassay allowed us to estimate HIV-1 incidence in first time donors showing that incidence was slightly higher in this population than in repeat donors and that it has decreased overtime in both populations.

INCIDENCE TESTING OF HIV CASES BY CALYPTE BED ASSAY USING DRIED BLOOD SPOTS FROM A HIGH PREVALENT DISTRICT IN THE STATE OF ANDHRA PRADESH, SOUTH INDIA

V. Lakshmi¹; T. Sudha Rani¹; Bhanu Rekha¹; Lalit Dandona²;

1-Department Of Microbiology, Nizam's Institute Of Medical Sciences; 2-Center For Human Development, Administrative Staff College Of India, Hyderabad, Ap, India;

Objectives: HIV-1 incidence measures the instantaneous risk of infection for an individual in a given population and is the best measure of the current force of the epidemic, ultimately determining HIV-1 prevalence and AIDS incidence. There is an urgent need to identify the areas with recent infections in Andhra Pradesh, a State with the largest prevalence for HIV infection in South India. This would help to develop targeted interventions and implement effective control measures to slow the spread of HIV. The HIV-1 BED Incidence EIA (Calypte® HIV-1 BED Incidence EIA (IgG-Capture HIV-EIA) (Calypte Biomedical Corporation, Maryland, USA), is a second-generation assay to detect recent HIV-1 seroconversion. The test categorizes recently infected persons based on the concept that specific HIV antibody titers rise and plateau over time and that increases in antibody avidity occur as infection progresses. Recently, the HIV-1 BED Incidence EIA has been used in a number of cross-sectional populations to estimate incidence and evaluate association with various risk factors.

Methods: A stratified random cluster sampling strategy was utilized to get the population-based sample from a HIV high prevalent district of AP for this study (3.5% as per the 2005 annual sentinel survey). Dried blood spots were collected from 12,940 respondents by a house-to-house survey. The assays were performed at the Department of Microbiology, Nizam's Institute of Medical Sciences, Hyderabad and AP, INDIA.

Results: 240 dried blood spot samples that were reactive for HIV-1 Antibodies by Murex HIV Ag-Ab Combination assay (Murex Biotech Ltd., Dartford, United Kingdom), were used to measure HIV-1 incidence by the Calypte BED assay. There were 31 new / incident cases out of 240.

COMPARISON OF THREE METHODS TO DETECT RECENT HIV-1 INFECTION IN SPECIMENS COLLECTED CROSS-SECTIONALLY IN A COHORT OF FEMALE SEX WORKERS IN THE DOMINICAN REPUBLIC

S.B. Gupta¹; E. Koenig²; G. Murphy³; C. Adon²; C. Beyrer⁴; S. Khawaja¹; J. Parry³; W. Straus¹;

1-Merck Research Laboratories, West Point, PA, USA; 2-Instituto Dominicano De Estudios Virologicos, Santo Domingo, Dominican Republic; 3-Health Protection Agency Centre for Infections, London, England; 4-Johns Hopkins Bloomberg School of Public Health, Baltimore, MD;

Objectives: Interest in estimating HIV-1 incidence using specimens obtained as part of cross-sectional surveys has led to the development of new methods to detect recent HIV-1 infection through the testing of a single anti-HIV positive specimen. These assays are based on quantitative and qualitative differences in anti-HIV-1 antibodies between recent and long-standing infections. Although several different recent infection assays have been developed, to date there have been few studies that have compared their performance.

Methods: An ongoing vaccine preparedness study enrolled female sex workers in the Dominican Republic. Specimens from women found to be HIV positive at the baseline visit were tested for recent HIV-1 infection using the following three methods: 1) detuned assay 2) avidity index; and 3) BED-EIA assay. An unweighted κ statistic in pair-wise comparisons was used to estimate the correlation and 95% confidence intervals of recent HIV-1 infection detection by the three methods

Results: Nineteen out of 536 (3.5%) women were positive for HIV-1 infection. The incidence of HIV infection was 1.3% (95% confidence interval (CI): 0.2, 4.9), 0.9% (95% CI: 0.1, 4.5), 0.9% (95% CI: 0.1, 3.7) using detuned assay, avidity index and BED-EIA techniques, respectively. The overall agreement between detuned assay and avidity index methods was 94% ($\kappa=0.8$, 95% CI; 0.3, 1.0). The correlation was highest between BED-EIA and avidity index methods (100%; $\kappa =1.0$) but remained high when comparing detuned assay and BED-EIA methods (94%; $\kappa =0.8$, 95% CI; 0.3, 1.0).

Conclusions: All three methods performed similarly in detecting recent HIV-1 infection in this region dominated by clade B HIV-1 infection; however, incidence estimates were slightly higher using the detuned assay method. These assays may be of value in both clinical research and practice. The utility of individual assays for recent infection detection will depend upon operating characteristics, HIV-1 subtype limitations, and selection of appropriate assay cut-off values.

SEROLOGICAL TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION (STARHS) IDENTIFIES A HIGH AND INCREASING PROPORTION OF NEWLY DIAGNOSED INDIVIDUALS AS RECENTLY INFECTED

D. Pao¹; M. Fisher¹; G. Dean¹; G. Murphy²; J. Parry²;

1-Brighton and Sussex University Hospitals, Department of HIV and Genitourinary Medicine, Brighton, United Kingdom; 2-Health Protection Agency, Centre for Infection, London, United Kingdom;

Objectives: To investigate whether combining clinical data with application of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) reliably identifies otherwise unrecognized recent infections (RI) and observe RI trends in a UK sexually transmitted infection clinic.

Methods: STARHS, using the modified bioMerieux Vironostika assay, was applied to serum collected between 1996 and 2005 at HIV diagnosis and routine clinical and laboratory markers of RI were determined. RI were identified by conventional means, STARHS, and both combined. Trends in RI were determined using the Kruskal Wallis Test.

Results: 1526 infections were diagnosed of which 810 were new and 603 in men who have sex with men (MSM). 714/810 (88%) new infections had serum available for STARHS, which identified 88 incident infections that would otherwise have gone unrecognized (12% of all new infections, 34% of all RI). Of these, 88% reported recent high-risk sex and 50% reported seroconversion symptoms. STARHS confirmed RI in 71/74 (96%) individuals known to be infected within 6 months by conventional methods. Combining both approaches, RI increased over time from 12/47 (1996) to 48/106 (2005) [$p < 0.001$]. Amongst MSM, 47/89 (53%) RI were identified in 2005 [$p < 0.001$]. STARHS results from 23/810 (3%) new diagnoses and 56/716 (8%) previous diagnoses were deemed false incident and were associated with antiretroviral therapy, advanced disease or undetectable viral load. In 2 individuals false incident results were inexplicable.

Conclusions: Adjunctive STARHS use combined with clinical data identified a high and increasing proportion of new HIV diagnoses as recent transmissions, confirming significant ongoing transmission, particularly amongst MSM, where since 2002 a half of new diagnoses were recently acquired. Identification of additional RI by STARHS enables effective intervention that may benefit the individual and reduce onward transmission. Significant limitations may be associated with surveillance applications if relevant clinical data are not available for individual specimens.

EXAMINATION OF AVIDITY IN STARHS TESTING FOR HIV INCIDENCE

Y. Adonsu-Hoyi ¹; B. Calder-Kent ¹; L. Malloch ¹; C. Archibald ¹; P. Sandstrom ¹; J. Kim ¹;

National Laboratory for HIV Reference Services (NLHRS), Division of Surveillance and Risk Assessment, Public Health Agency of Canada, Ottawa, Ontario, Canada.

Objectives: HIV surveillance studies worldwide have frequently relied on the STARHS protocol to distinguish early from late-stage HIV-1 infections based on increasing levels of (HIV) antibody titre from the time of exposure. This method, however, along with the newly developed BED assay, does not take into account a second equally important principle in antigen-antibody interaction, namely avidity, which describes the overall strength of binding. We describe the application of the Avidity Index Protocol as both a stand-alone protocol and as a 'confirmatory' assay for the EIA currently used in the STARHS protocol.

Methods: The STARHS protocol has been well described in the literature. The Avidity Index (AI) Protocol uses potassium thiocyanate as a dissociation reagent in a commonly used EIA (BIORAD GS rLAV). The results or avidity index (AI) is then expressed as a percentage (~80%) of the ratio of the OD of the treated (KSCN) sample to that of the non-treated control. Seroconversion panels were used to validate the protocol. Samples from a national HIV surveillance program for HIV drug resistance were also used for comparison of both methods alone and the A.I. protocol as a confirmatory test.

Results: In both cases the use of the A.I. protocol distinguished approximately half (50%) of samples initially detected as early by the current STARHS protocol.

Conclusions: In light of recent criticism that STARHS protocols may severely overestimate true incidence, the implementation of the A.I. protocol as a 'confirmatory' test for samples initially identified as 'recent' by STARHS warrants further investigation. This algorithm takes into account both principles of antigen-antibody interaction and generates a more conservative estimate of incidence.

IMPROVED HIV-1 INCIDENCE ESTIMATES USING THE BED CAPTURE ENZYME IMMUNOASSAY

John Hargrove^{1,6}; Jean Humphrey^{1,2}; Kuda Mutasa¹; Bharat Parekh³; Steve McDougal³; Robert Ntozini¹; Henry Chidawanyika¹; Brian Ward⁴; Kusum Nathoo⁵; Peter Liff¹; Ekkehard Kopp⁶; and the ZVITAMBO Study Group;

1-ZVITAMBO Project, Harare, Zimbabwe; 2-Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; 3-Centers for Disease Control and Prevention, Atlanta, Georgia, USA; 4-The Research Institute of the Montreal General Hospital, Montreal, Quebec, Canada; 5-The University of Zimbabwe Faculties of Medicine and Science, Harare, Zimbabwe; 6-The DST/NRF Centre of Excellence in Epidemiological Modelling and Analysis, SACEMA, Stellenbosch, South Africa;

Objectives: HIV incidence measurement relies on lengthy follow-up studies. The BED method, which measures increasing proportions of anti-HIV IgG to total IgG following seroconversion, could be used to estimate incidence more simply, via the proportion of recent seroconverters in cross-sectional surveys. However, the method over-estimates incidence by 2-3-fold. We derive methods for correcting this problem.

Methods: Using blood from mothers who seroconverted during 12-24 mo of *post partum* follow-up in Harare, Zimbabwe we estimate the window-period taken for BED optical density (OD) to increase to a pre-set cut-off. We compare BED and follow-up incidence estimates over identical periods.

Results: The mean window for cut-off 0.8 is 190 days. Over-estimation of incidence was due to a distinct sub-group, 5.2% in the ZVITAMBO Trial, who were HIV positive at baseline but, 12-mo later, still had a BED OD < 0.8. Excluding this sub-group from the analysis reduces BED HIV incidence estimates from 7.5% to 3.5% - close to the 3.4% estimated by follow-up over the same 12-mo *post partum* period. Corrected BED incidence estimates, among 14, 110 women recruited, declined with maternal age, as did the estimates in follow-up studies. Uncorrected estimates, conversely, did not show the expected decline.

Conclusions: This study is based on only one dataset, but reported 2-3 fold over-estimates in BED incidence suggest the existence in various populations of proportions of people with abnormally long BED windows. Correction for this, presumably variable, proportion in different settings should facilitate the general use of BED to estimate HIV incidence.

THE EFFECT OF VIRAL SUPPRESSION ON CROSS SECTIONAL INCIDENCE TESTING; THE 2001 JOHNS HOPKINS HOSPITAL EMERGENCY DEPARTMENT SERO-SURVEY AS AN EXAMPLE

Oliver B. Laeyendecker^{1,2}; Charlamaine Henson²; S. Michele Owen³; Bobbi Jo Horne²; Richard E. Rothman⁴; Gabor D. Kelen⁴; Kerunne S. Ketlogetswe⁴; Judy Shahan⁴; Renu Lal³; Thomas C. Quinn^{1,2};

1-NIAID, NIH, Baltimore; 2-The Johns Hopkins University, School of Medicine, Division of Infectious Diseases, Baltimore; 3-Centers for Disease Control, Division of HIV AIDS/Prevention, Atlanta, GA; 4-The Johns Hopkins University, Department of Emergency Medicine, Baltimore

Objectives: To determine the effect viral suppression, either natural or by antiretroviral treatment (ART) on cross sectional incidence testing.

Methods: Compare the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS), and the Affinity/Avidity assay on serosurvey of a mature HIV-1 epidemic receiving anti retroviral drugs. Additionally known HIV positive individuals who control their own infection without the aid of antiviral treatment were also tested. From 6/29/01 to 8/16/01, 1549 patients entering the Johns Hopkins Hospital (JHH) Emergency Department (ED) were enrolled by interview based, identity unlinked serosurvey. HIV serology was performed by enzyme immunoassay and western blot. HIV RNA was quantified by Roche Amplicor™ v1.5. The STARHS and Affinity/Avidity assays were used to determine recent HIV infection. Levels of ART medications were tested by reverse-phase high-performance liquid chromatography. Additionally 16 samples from 8 previously characterized elite suppressors (ES) (viral load < 50 copies/ml while not on ART) were tested by STARHS.

Results: The HIV prevalence rate was 12%. Of the HIV-infected subjects 19% did not know they were infected. STARHS determined that 11/183 HIV+ individuals were recently infected while Affinity/Avidity testing confirmed 6 such individuals. All 5 discrepant samples were western blot positive, viral load undetectable. Two of five subjects had ARVs in their serum. Of the 8 ES 4 tested incident by STARHS.

Conclusions: These results suggest that inner city EDs provide an opportunity to identify previously unrecognized HIV infection. In removing the viral load negative individuals, confirmed by Affinity/Avidity testing to be chronically infected from the incidence calculation, the incidence estimate was lowered from 1.73 to 0.94 percent/year in this population. Viral suppression affects the ability of the STARHS assay to accurately determine HIV incidence.

HIV INCIDENCE ESTIMATES: SOUTH AFRICA 2005

Thomas Rehle¹; Victoria Pillay¹; Adrian Puren²; Olive Shisana¹;

1-Human Sciences Research Council, Cape Town, South Africa; 2-National Institute for Communicable Diseases, Johannesburg, South Africa;

Objectives: The 2005 South African national household survey on HIV, Behavior and Communication included HIV incidence testing which allowed for the first time a joint analysis of HIV prevalence, HIV incidence and HIV associated risk factors. 23 275 individuals aged 2 years and older participated in the survey and 15 851 respondents agreed to be tested for HIV.

Methods: The detection of recent infections was performed on confirmed HIV positive samples, using the BED capture enzyme immunoassay optimised for dried blood spot (DBS) specimens. Analysis of recent HIV infections was initially done with an OD-n cutoff value of ≤ 0.8 and repeated with an OD-n cutoff value of ≤ 0.4 , based on comparative plotting results on BED-OD-n and HIV antibody avidity index in HIV positive South African specimens. Annualized BED HIV incidence calculation applied a window period of 180 days for HIV subtype C specimens and provided weighted estimates taking into account the complex survey design. HIV incidence was also calculated from single year age cohort prevalence in the 15 to 24 year olds ($n = 4\ 120$) using smoothed prevalence data and compared with the BED incidence estimates.

Results: In the South African setting, HIV incidence estimates based on the BED assay configuration with an OD-n of 0.4 as cutoff appear to be more in agreement with other measures of incidence, such as HIV incidence calculated from single year age cohort prevalence in the 15 to 24 year olds. Lowering the cutoff to OD-n ≤ 0.4 produced BED incidence estimates that matched closely the age cohort derived incidence.

Conclusions: Laboratory-based HIV incidence measures have great practical appeal to be included in national surveys. However, the BED-assay should only be applied with the necessary setting-specific adjustments.

THE EFFECT OF CONFOUNDING FACTORS ON THE SPECIFICITY OF TESTS EMPLOYED IN THE SEROLOGICAL TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION (STARHS)

G. Murphy¹; G. Dean²; M. Fisher²; D. Pao²; D. McElborough²; H. Munro¹; O.N. Gill¹; J.V. Parry¹;

1-Health Protection Agency Centre for Infections, London, United Kingdom; 2-Brighton and Sussex University Hospitals, Brighton, United Kingdom;

Objectives: Both effective ART and AIDS defining conditions can lead to a small reduction in anti-HIV antibody titre over time. We investigated the effect of ART and AIDS on the specificity of 3 of STARHS assays.

Methods: For the study of ART, specimens were collected from two groups infected for at least one year prior to sampling. Group 1 comprised individuals receiving ART, including a specimen prior to initiation of ART and after two years of continuous treatment; Group 2 comprised patients not receiving therapy. For the study of AIDS, specimens were collected from patients with an AIDS defining condition identified through an HIV prevalence serosurvey. STARHS was performed using one commercially available assay, the 'BED' (Calypte) and two modified assays, the bioMérieux Vironostika assay (Kothe et al) and antibody avidity determined using the Abbott AxSym HIV1/2gO assay (Suligoi et al).

Results: ART was associated with a marked reduction in reactivity in the BED and Vironostika assays, but only a minimal effect on antibody avidity. For the former two assays reactivities fell consistently over the treatment period, culminating in some patients' specimens giving reactions consistent with a false classification as a recent infection. Over the same period antibody avidity showed an average decline of only 4.9%, not affecting the interpretation. Specimens from untreated controls showed a small increase in OD in the Vironostika and BED assays and no change in antibody avidity over the study period. Results on the effect of AIDS on these assays will be known shortly.

Conclusions: Knowledge of a patient's medical and treatment history is vital for accurate interpretation of assays of recent infection. Using assays in combination, such as the BED and the antibody avidity assay, may improve the reliability of identification of recent HIV infection.

PROFICIENCY TESTING PROGRAM FOR HIV-1 BED INCIDENCE ASSAY: SUMMARY OF PILOT ROUND OF RESULTS

Xin Liu¹; Bharat Parekh¹;

1-International Laboratory Branch, Global Aids Program, Centers for Disease Control and Prevention, Atlanta, GA 30333

Objectives: To monitor the quality of laboratory performance on this quantitative EIA.

Methods: We initiated a pilot proficiency testing (PT) program for the BED assay in May 2006. There were 14 participating laboratories returning 15 sets of results.

Results: 13 of the returned results (87%) were 100% in agreement with the expected results for the PT panels. Overall, the results were highly reproducible among laboratories with expected low CVs. One lab had an invalid run with control values out of acceptable range. The lab was asked to repeat the panel and returned valid results. One lab misclassified a specimen and failed the PT test. Corrective action form was sent to investigate the cause.

Conclusions: During this pilot program, issues were identified regarding data management, PT results reporting and test performance in the laboratory. Because more laboratories are interested in participating in the BED PT program as an external assessment, we are officially launching the program and the next mail-out will be the end of August.

A CALL FOR THE DEVELOPMENT OF EPIDEMIOLOGICALLY RELEVANT STI TESTING STRATEGIES FOR A POPULATION RELEVANT METHODOLOGY THAT MEASURES SEXUAL BEHAVIOUR AND HIV-1 RISK BASED ON BIOLOGICAL MARKERS

S.K. Sgaier¹; N. Negalkerke¹; A. Kalaichandran¹; L. Chen¹; P. Milson¹; P. Arora¹; P. Jha¹;

1-Centre for Global Health Research, St. Michael's Hospital, University of Toronto, Toronto, Canada;

Objectives: The heterosexual route of transmission is the driving force of the HIV epidemic worldwide. Prevention program management of the epidemic requires a sound evidence base – monitoring of not only trends in HIV/STI prevalence/incidence but also the trends in underlying sexual risk behaviours using reliable instruments other than the conventionally used and unreliable self-reported surveys. We propose the development of a novel biomarker based methodology, which can assess population sexual behaviour/HIV risk based on selective blood-based testing of recent and past sexually transmitted infections (STI) combined with careful measurements of the correlates of sexual risk.

Methods: As groundwork for methodology development, we have conducted a comprehensive literature review on the natural history, seroprevalence/incidence patterns, and transmission dynamics of *Chlamydia trachomatis* (CT), *Nisseria gonorrhoea* (NG), Syphilis, HSV-2 and HIV.

Results: CT, HSV-2 and syphilis are highly prevalent and correlate with risky sexual behavioral patterns (increased partner number and use of commercial sex work). The epidemiology of each STI varies and indicate different aspects of this risky behavior as each have their transmission dynamics. HSV-2 is one of the most prevalent STIs with levels increasing worldwide. HSV-2 seroprevalence highly correlates with lifetime number of sexual partners and HSV-2 seroincidence is a potential marker for measuring change in sexual behavior at the population level. HSV-2 has been singled out as one of the most important STI that can act as a co-factor for HIV-1 transmission. CT highly correlates with young age in females. Patients with CT and NG belong to core populations with similar but not identical characteristics where NG seems to be much more confined and focused in populations that engage in the highest risk behavior and CT more widely distributed.

Conclusions: A biomarker based methodology to stratify populations based on sexual risk behaviour and HIV risk will revolutionize the assessment of intervention programs worldwide. This requires the development of epidemiologically sane testing strategies for both incidence and prevalence of key STIs as current testing strategies are designed for point of care diagnostic purposes. In particular, the development of an epidemiological applicable blood based incidence test for HSV-2 and incidence/prevalence test for CT and NG are much needed.