Dear Colleagues,

It has been 20 years since the FDA licensed the first test for HTIV-III/LAV antibody in 1985. Remarkable developments in the field of serologic and nucleic acid testing have occurred since that time; the introduction of one-step rapid HIV tests represent the largest shift in the platform for HIV diagnostic testing in two decades. However, the central question remains the same, posed during CDC’s 1987 workshop in Atlanta: what is the role of HIV diagnostic testing in the prevention and control of HIV and AIDS?

For many years, APHL’s Human Retroviral Testing Committee convened annually to consider new developments, share data, and formulate recommendations, but despite numerous strides in screening and diagnostic technology, there has been no such conclave in recent years. It is time, therefore, for us to gather once again, share the latest information on HIV diagnostics, and consider alternatives that will best help accomplish our common goal: using the best that laboratory science has to offer to help combat and control the spread of HIV.

Thank you for your interest, and welcome to the 2005 conference, HIV Testing: New Developments and Challenges.

With warm personal regards,

Bernard M. Branson, M.D.
Associate Director for Laboratory Diagnostics
Division of HIV/AIDS Prevention
Centers for Disease Control and Prevention
ACKNOWLEDGEMENTS

On behalf of Centers for Disease Control and Prevention (CDC) we would like to express our appreciation to the following organizations for their contributions to the success of our conference.

Association of Public Health Laboratories
bioMérieux, Inc.
Calypte Biomedical Corporation
MedMira, Inc.
Orasure Technologies, Inc.
SDS, Inc.
Trinity Biotech
HIV DIAGNOSTICS: NEW DEVELOPMENTS AND CHALLENGES

Monday, February 28

7:30 – 8:30  Continental Breakfast, Poster Set-Up

8:30 – 8:40  Welcoming Remarks
Speaker: Bernie Branson, MD
Associate Director for Laboratory Diagnostics, CDC

Scientific Session 1: New HIV Enzyme Immunoassays

8:40 – 9:10  Principles and Characteristics of EIAs Recently Approved by the FDA

- Vironostika HIV-1 Plus O Microelisa System for the Detection of Antibodies to HIV-1, Including Group O
  Speaker: Chamroen Chetty, PhD
  Senior manager Immunoassay R&D, bioMérieux, Inc.
- Principles and Characteristics of the Bio-Rad Genetic Systems™ HIV-1/HIV-2 Plus O EIA
  Speaker: M. Kathleen Shriver, PhD
  R&D Manager, Redmond Operations, Bio-Rad Laboratories

9:10 – 9:30  Comparing the New EIAs with Old Standbys: Validation Data from Public Health Laboratories
Speaker: Berry Bennett, MPH
Retrovirology Section Chief, Florida Bureau of Laboratories

9:30 – 9:40  APHL Survey on HIV Assays Currently in Use
Speaker: Barbara Werner, PhD
Infectious Disease Consultant, Massachusetts State Laboratory Institute

9:40 – 10:00  Discussion
Moderator: S. Michele Owen, PhD
Chief, HIV Serology and Developmental Diagnostics Laboratory, CDC

10:00 – 10:30  Break – Poster and Exhibit Review
### Scientific Session 2: NAAT Testing for Acute HIV Infection

**Monday, February 28**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:30 – 10:45</td>
<td><strong>Results of Donor Screening with Nucleic Acid Amplification Tests (NAT) and Implications for HIV Research, Diagnosis and Surveillance</strong>&lt;br&gt;Speaker: Michael P. Busch, MD, PhD&lt;br&gt;Professor of Laboratory Medicine, UCSF&lt;br&gt;Director, Blood Systems Research Institute</td>
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<tr>
<td>10:45 – 11:00</td>
<td><strong>Pooled RNA Testing of Antibody Negative High-Risk Persons: San Francisco and Los Angeles</strong>&lt;br&gt;Speaker: Sally Liska, DrPH&lt;br&gt;Director, San Francisco Public Health Laboratory</td>
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<td>11:00 – 11:15</td>
<td><strong>Alternatives for Detecting Acute HIV: RNA, p24, and Heat-dissociated p24</strong>&lt;br&gt;Speaker: Christopher D. Pilcher, MD&lt;br&gt;Assistant Professor of Medicine, University of North Carolina at Chapel Hill</td>
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<tr>
<td>11:15 – 11:30</td>
<td><strong>HIV NAAT Testing of HIV Antibody Negative Samples</strong>&lt;br&gt;Speaker: Robert Myers, PhD&lt;br&gt;Deputy Director, Maryland Dept of Health and Mental Hygiene Laboratories</td>
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<td>11:30 – 11:40</td>
<td><strong>Screening for Primary HIV Infection: The CDC Study</strong>&lt;br&gt;Speaker: Pragna Patel, MD, MPH&lt;br&gt;Medical Epidemiologist, Diagnostic Applications Behavioral and Clinical Surveillance Branch, CDC</td>
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<tr>
<td>11:40 – 12:00</td>
<td><strong>Discussion</strong>&lt;br&gt;Moderator: Amanda Smith, MPH&lt;br&gt;Epidemiologist, Diagnostic Applications Behavioral and Clinical Surveillance Branch, CDC</td>
</tr>
<tr>
<td>12:00 – 2:00</td>
<td><strong>Lunch – Poster and Exhibit Review</strong></td>
</tr>
</tbody>
</table>
HIV DIAGNOSTICS: NEW DEVELOPMENTS AND CHALLENGES

Monday, February 28

Scientific Session 3: Rapid HIV Testing

2:00 – 3:00  Panel: Roll-out and Implementation of Rapid Testing from a Field Perspective
Speakers:
• Tony Falvo, CTL Program Coordinator, Florida Bureau of HIV/AIDS
• Marianne Porter, BS MS, Director, Division of Laboratory Improvement, Pennsylvania Department of Health
• Mara San Antonio Gaddy, RN, MSN, Director, Bureau of Direct Program Operations, New York State Department of Health
• Susanne Norris Zanto, BS, CLS (NCA), Technical Services Manager, Montana Public Health Laboratory
• Shelley Facente, MPH, HIV Rapid Testing Coordinator, San Francisco Department of Public Health AIDS Office

3:00 – 3:30  Discussion
Moderator: Bernie Branson, MD

3:30 – 4:00  Break – Poster and Exhibit Review

4:00 – 4:20  Resolution of Discordant Confirmatory Results after POC Reactive Rapid Tests
Speaker: Berry Bennett, MPH
Retrovirology Section Chief, Florida Bureau of Laboratories

4:20 – 4:40  Post Marketing Surveillance of OraQuick Rapid HIV Testing
Speaker: Laura Wesolowski, MPH
Laboratory Determinants and Diagnostics, Behavioral and Clinical Surveillance Branch, CDC

4:40 – 5:00  Evaluation and Implementation of HIV Rapid Tests: The Experience in Eleven African Countries
Speaker: Stefan Wiktor, MD, MPH, Chief, Surveillance and Infrastructure Development Branch, Global AIDS Program, CDC

5:00 – 5:30  Implications for the Public Health Laboratory from Expansion of Rapid Testing
Speaker: Arthur Kazianis, BA
HIV/Hepatitis Laboratory Supervisor, Massachusetts Department of Public Health

5:30 – 6:00  Discussion
Moderator: Bernie Branson, MD

6:00 – 7:30 Networking Reception – Poster/Exhibit Area
Tuesday, March 1

7:30 – 8:30  Continental Breakfast – Poster and Exhibit Review

Scientific Session 4: Options for Confirmatory Testing in Different Settings

8:30 – 8:45  Challenges for Blood Donor Confirmatory Testing Algorithms
Speaker:  Susan L. Stramer, PhD
Executive Scientific Officer, American Red Cross

8:45 – 9:00  Comparison of Currently Available Assays that Detect Human Immunodeficiency Virus to Address Alternative Screening/Diagnostic Algorithms for HIV
Speaker:  S. Michele Owen, PhD
Chief HIV Serology and Developmental Diagnostics Lab, CDC

9:00 – 9:15  Performance of Rapid HIV Tests Singly and in Combination
Speaker:  Kevin Delaney, MPH
Epidemiologist, Diagnostic Applications, Behavioral and Clinical Surveillance Branch, CDC

9:15 – 9:30  HIV 1/2 Combination Screening and Outcomes of Follow-Up
Speaker:  William R. Oleszko, PhD
Acting Associate Director, Public Health Laboratory, New York City Department of Health and Mental Hygiene

9:30 – 10:00 Discussion of Next Steps:
Moderator:  Sheryl Lyss, MD
Medical Officer, Diagnostic Applications, Behavioral and Clinical Surveillance Branch, CDC

10:00 – 10:30  Break – Poster and Exhibit Review
HIV DIAGNOSTICS: NEW DEVELOPMENTS AND CHALLENGES

Tuesday, March 1

Scientific Session 5: Dried Blood Spot Testing

10:30 – 10:50  Dried Blood Spots: An Ideal Specimen for HIV Serologic and Nucleic Acid Testing
Speaker: Joanne Mei, PhD
Lead Research Chemist
Newborn Screening Quality Assurance Program, CDC

10:50 – 11:10  Usefulness and Application of Dried Blood Spots in HIV and HCV Epidemiology, Diagnostics and Drug Resistance Testing
Speaker: John Kim, PhD
Chief, National HIV Reference Lab
Public Health Agency of Canada

11:10 – 11:30  Early Diagnosis of HIV-1 Infection in Infants Using Dried Blood Spots and Real Time, RT-PCR
Speaker: Chin-Yih Ou, PhD
Chief, HIV Molecular Diagnostics Lab, CDC

11:30 – 11:45  Use of DBS with BED Testing for Recent Infection
Speaker: Bharat S. Parekh, PhD
Chief, HIV Incidence Laboratory, CDC

11:45 – 12:00  Use of DBS for HIV Drug Resistance Surveillance
Speaker: Diane V. Bennett, MD, MPH, M.ED.
Coordinator, Antiretroviral Drug Resistance Surveillance, CDC

12:00 – 12:30  Discussion
Moderator: Joanne Mei, PhD
Lead Research Chemist, Newborn Screening Quality Assurance Program, CDC

12:30 – 2:00  Lunch and Networking
HIV DIAGNOSTICS: NEW DEVELOPMENTS AND CHALLENGES

Tuesday, March 1

Scientific Session 6: Concurrent Discussion Groups

2:00 – 2:30 Updates: Status of Tests for Recent Infection & CDC’s Plans for the Detuned IND
Speaker: Bernie Branson, MD
Associate Director for Laboratory Diagnostics, CDC

2:30 – 3:30 Panel: Tests for Recent Infection
Moderators: Niel Constantine and Anne M. Sill

- **Calibration, Validation and Application of the BED Assay for Recent HIV-1 Infection in Multiple Subtypes**
  Panelist: Bharat S. Parekh, PhD, Chief, HIV Incidence and Diagnosis Laboratory, CDC,

- **A Simple and Inexpensive Algorithm for Determining Recent HIV Infection: Development of a Sensitive/Less Sensitive (S/LS) Particle Agglutination Assay**
  Panelist: Neil Constantine, PhD, Professor, University of Maryland School of Medicine

- **Identification of Factors Impacting the Determination of HIV Incidence in Non-Research-Based, Clinical Populations**
  Panelist: Anne M. Sill, BA, Research Supervisor, Research Associate, Institute of Human Virology

- **Incidence Tests for the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS)**
  Panelist: Joanne Mei, PhD, Lead Research Chemist, CDC

- **Summary of STARHS Meeting in Bangkok**
  Panelist: Robert Remis, MD, MPH, FRCPC, Professor, Public Health Sciences, University of Toronto, Canada

3:30 – 4:30 IND Investigators Meeting
Tuesday, March 1

Scientific Session 6: Concurrent Discussion Groups

2:30 – 4:30  Panel: Rapid HIV Testing in International Settings
Moderator: Mark Rayfield, PhD, Global AIDS Program, CDC

- Arguments for the use HIV Rapid Tests on a National Scale in Resource Poor Settings
  Panelist: Kyle B. Bond, MS, Surveillance and Infrastructure Development Branch, GAP, NCHSTP, CDC

HIV in Botswana; Improving Lab Capacity in the Face of the AIDS Pandemic
Panelist: Patricia Clark, MPH, Interim Virology/Immunology Section Manager, Michigan Department of Community Health

- Rapid Testing: The Kenya Experience
  Panelist: Dr. Peter Tukei, Assistant Director, Kenya Medical Research Institute, Laboratory Director, CDC/KEMRI

- Use of Rapid HIV Assays in Vietnam and Cambodia: Challenges to Improving Access, Quality and Timeliness of HIV Testing
  Panelist: Ralph Timperi, MPH, Director, Massachusetts State Laboratory Institutes

- Quality Assurance for Rapid Testing
  Panelist: Elizabeth Dax, AM, MD, BS, PhD, Director, National Serology Reference Laboratory, Australia

4:30 – 5:30  Meeting Wrap-up and Discussion of Next Steps
Bernie Branson, MD
Associate Director for Laboratory Diagnostics, CDC
The Vironostika® HIV-1 Plus O Microelisa System is an enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1), including Group O, in human specimens collected as serum, plasma, or dried blood spots. The Vironostika HIV-1 Plus O Microelisa System is intended for use as an aid in diagnosis of infection with HIV-1 and not for use in screening blood donors. This assay uses three solid phase antigens; inactivated, purified HIV-1 viral lysate proteins, a purified viral envelope protein (native gp160), and a synthetic peptide with an amino acid sequence corresponding to that of the transmembrane immunodominant domain of the HIV-1 Group O (ANT 70) isolate. Antibody to HIV-1 is captured to a microwell containing antigens, reacted with anti-human immunoglobulin (goat) conjugated with horseradish peroxidase (HRP) to form an immune complex, and detected with ABTS (2,2'-azino-di-[3-ethylbenzthiazoline-6-sulfonate]) substrate. The assay design sought to improve overall sensitivity to Group O while maintaining the specificity compared to the current licensed Vironostika HIV-1 (licensed comparator = LC) and to improve ease of use with a Sample Addition Monitoring and Color Coded Reagents. In clinical evaluation, the clinical sensitivity of the Vironostika HIV-1 Plus O was demonstrated with matched serum/plasma specimens and dried blood spot specimens collected from 1,010 HIV-1 infected individuals with various CD4+ counts; all specimens were repeatedly reactive with the assay and subsequently confirmed with Western blot. For twelve (12) seroconversion panels tested and compared to the LC, the Vironostika HIV-1 Plus O detected HIV-1 antibodies earlier than the LC test in each case. Detection of HIV-1 Group M antibodies was demonstrated with subtypes A, B, B/D, C, C/E, D, E, E/A, E/C, E/F, F, G and H. The assay exhibited higher analytical sensitivity with dilutional panels from clinical specimens for both HIV-1 Group M and HIV-1 Group O specimens compared to the LC. The results of the study indicate an enhanced detection of HIV-1 antibodies with the Vironostika HIV-1 Plus O assay.
Principles and Characteristics of the Bio-Rad Genetic Systems™ HIV-1/HIV-2 PLUS O EIA

M. Kathleen Shriver, PhD
R&D Manager, Redmond Operations, Bio-Rad Laboratories

**Issues:** Manufacturers of licensed HIV test kits were challenged by FDA in 1996 to develop improved antibody screening assays containing specific antigenic sequences for detection of HIV-1 group O. This presentation describes the design and performance parameters of the Bio-Rad Genetic Systems™ HIV-1/HIV-2 PLUS O EIA, which was licensed in August, 2003 as a screening test for serum, plasma, and cadaveric serum specimens and as an aid in the diagnosis of infection with HIV-1 (Groups M and O) and/or HIV-2.

**Description:** The HIV-1/HIV-2 PLUS O EIA combines specific recombinant and synthetic peptide antigens, representing HIV-1, HIV-2, and HIV-1 variant sequences, in a direct antibody capture format. The performance parameters of the new microplate assay, including sensitivity, specificity, and reproducibility, were evaluated in studies at six sites compared to current FDA-licensed tests for detection of HIV-1/HIV-2 antibody. HIV-1 sensitivity was estimated to be 100% based on the results of testing 313 patients with AIDS, as well as 490 HIV-1 positive samples from the U.S., and 199 HIV-1 positive samples from diverse regions outside the U.S. (total N=1002). All 77/77 (100%) HIV-1 Group O specimens and 302/302 (100%) known HIV-2 samples were correctly detected. In testing of HIV-1 seroconversion panels, 34/46 (74%) demonstrated at least one bleed improvement in detection by the new assay compared to a previously licensed test, and 12/46 (26%) were equivalent. All positive samples (N=130) from four commercial panels, representing low titer samples and/or HIV subtypes of worldwide origin, were detected by the new assay (100%), vs. 95.4% and 98.5% detection using other licensed HIV-1/HIV-2 tests. In testing of 11,159 normal donor samples, specificity was 99.89% (IR=0.16%, RR=0.11%).

**Lessons Learned:** The new HIV-1/HIV-2 PLUS O EIA provides a significant improvement in sensitivity over previously licensed assays for detection of HIV antibody, while offering equivalent or better specificity. The improvement in sensitivity was demonstrated for samples containing HIV-1 Group O antibody, as well as challenging HIV-1 Group M seroconversions and low titer specimens.
Comparing the New EIAs with Old Standbys: Validation Data from Public Health Laboratories

Berry Bennett MPH, Retrovirology Section Chief, Florida Bureau of Laboratories
Sally Fordan BS, Retrovirology Supr., Florida Bureau of Laboratories

**Background:** In January 2004, the Florida Bureau of Laboratories, Retrovirology Unit, conducted a verification process of the new BioRad HIV-1/HIV-2 Plus O EIA as a possible replacement for the BioRad HIV-1/2 Synthetic Peptide EIA as the primary screening assay for blood specimens. Manufacturer’s anticipation of an eventual assay replacement was the primary reason for the evaluation.

**Issues:**
1) To conduct a comparative performance verification between the two assays to satisfy our “in-house” requirements as well as CLIA’s new performance specifications, outlined in 42 CFR 493.1253.
2) In anticipation that the new screening assay is more sensitive than supplemental assays, use of molecular testing, if possible, and client follow up on non-confirmed repeatedly reactive screens may be required. Seroconversion panels may assist in sensitivity determinations.

**Lessons Learned/Recommendations:**
Communication between the laboratory and the manufacturer is essential to rule in or out automation, reagent or operator technique issues that may be associated with sensitivity or specificity issues.
Timely client follow up, in some public health settings, proves to be difficult to determine seroconversion in the time allowed for assay verifications. The use of molecular testing, provided an appropriate specimen was submitted, proves to be an asset.
APHL Survey of HIV Assays Currently in Use

Barbara G. Werner, PhD, MA State Laboratory Institute, Boston, MA

**Issues:** In response to changes in HIV diagnostic testing, such as new EIA screening tests, increased use of rapid tests and suggestions for broader application of nucleic acid amplification tests, public health labs were surveyed to characterize current test utilization and identify planned changes.


**Lessons Learned:** Public Health Laboratories are involved with many aspects of HIV diagnostic testing. Changes in assays used will require validation studies. Some public health laboratories are assuming additional roles. Changes in testing practices are presenting new challenges of communication.

**Recommendations:** Improve communication between public health labs and APHL, CDC, clinical and blood donor laboratories, and manufacturers of HIV diagnostic assays as well as HIV/AIDS program staff and community based organizations. Repeat the survey to get updated utilization information. Improve survey to improve clarity of responses.
Results of donor screening with nucleic acid amplification tests (NAT) and implications for HIV research, diagnosis and surveillance

Michael P. Busch, MD, PhD
Professor of Laboratory Medicine, UCSF
Director, Blood Systems Research Institute

**Issues:** Highly sensitive, qualitative tests for rapid detection of nucleic acids of HIV and other blood-borne viruses (HCV, HBV and WNV) were developed and implemented as minipool (MP)-NAT assays in blood, plasma and organ donor screening settings beginning in the late 1990s. This presentation will review the design of donor NAT assays, summarize the yield of MP-NAT screening of U.S. donors, discuss the impact MP vs. single-sample NAT on window period closure and blood safety, and illustrate applications of donor NAT assays in HIV research, diagnostic and public health surveillance settings.

**Description:** NAT assays with sensitivities of <15 copies/mL have been developed by several manufacturers and licensed by FDA for donor screening. Over the first 3 years of donor NAT screening HIV-1 RNA yield was 12 confirmed-positive donors in 37 million donations screened, or 1 in 3.1 million, of which only two were detected by HIV-1 p24 antigen. For HCV, the yield was 170 RNA confirmed-positive donors in 40 million donations screened, or 1 in 230,000. WNV NAT was quickly implemented following documented transfusion cases in 2002, and resulted in detection of ~1000 viremic units in 2003 and over 500 in 2004. HBV MP-NAT implementation is now under consideration.

**Lessons Learned:** Although MP-NAT screening of donor was successfully implemented and has enhanced transfusion safety, this technology is expensive and the cost effectiveness of NAT is low (>$1,000,000 per QALY) due to very low incidence of HIV and other agents in blood donors. There is continued pressure to expand NAT to both additional established agents, as well as in response to threats from emerging viruses. Advances in technology, including automation allowing for high throughput individual sample NAT and increased multiplexing of multiple targets, are evolving rapidly.

**Recommendations:** The experience with donor NAT screening has been positive, with excellent performance of assays and yields as predicted by incidence-window period models. Preliminary applications of donor NAT assays to HIV research, diagnostic, therapeutic monitoring and vaccine trial settings have shown promise. Research is critical to establish a rational, cost-effective role for NAT screening outside the donor screening context.
Testing HIV Antibody Negative High Risk Individuals for HIV RNA

Sally Liska, DrPH
Director, San Francisco Public Health Laboratory

Background: The impact of sexually transmitted diseases is especially severe in HIV infected individuals. Therefore, to determine if detection of HIV infection could be increased by incorporating RNA testing, we pooled samples from clients seeking HIV counseling and testing services at the San Francisco STD Clinic (City Clinic) and tested for HIV RNA in a pilot program. For similar reasons, Los Angeles County Health Department was interested in determining if the detection rate of HIV infection among high risk clients could be increased by testing for RNA. Stored samples collected from STD clinics were pooled and tested for HIV RNA.

Methods: Starting in November, 2003, all clients seeking HIV screening at the San Francisco City Clinic were offered the option of additional testing for HIV RNA. After counseling and giving consent, plasma samples were screened for HIV antibody. EIA negative specimens were pooled and tested for HIV RNA using Bayer’s Versant™ bDNA. Samples from a pool testing positive were tested individually. Results of both antibody and RNA tests were given to the client and follow-up specimens were collected. In the Los Angeles study, stored serum samples collected from at STD clinics for HIV screening were unlinked, pooled and tested for HIV RNA using Roche PCR. Specimens from any positive pools were tested individually.

Results: In the San Francisco pilot, 4,126 samples collected through December, 2004 were screened for both HIV antibody and RNA; 164 (3.97%) were antibody positive and 17 (0.41%) were antibody negative/RNA positive. Los Angeles Health Department tested 33 pools, comprised of 2939 HIV antibody negative specimens collected during a four month period, and detected two (0.068%) PCR positive specimens.

Conclusions: Testing for HIV RNA can be done using pooled samples of serum or plasma and does increase the detection of HIV infection in high-risk populations compared to standard HIV screening methods.

Presenter: Sally Liska, DrPH Email: SLiska@sfdph.org
Institution: San Francisco Public Health Laboratory
Alternatives for acute HIV testing: RNA, p24, heat dissociated p24

Christopher D. Pilcher, MD
Assistant Professor of Medicine, University of North Carolina at Chapel Hill

Background: Since 2002, NC DHHS’ STAT program has supplemented traditional HIV viral lysate EIA testing with HIV NAAT for all public VCT, using specimen pooling of seronegative specimens. We have detected acute HIV infections in real time, increasing case identification by 4% with high accuracy (Sp>0.999). This has allowed efficient integration of public VCT with highly efficient prevention and surveillance activities. Recently, we have investigated the implications of using newer rapid tests and ELISAs for the potential identification of acute infections at VCT.

Methods: HIV VCT-based diagnostic performance evaluations were conducted in sites with expected high prevalence and incidence of HIV to compare new tests with NAAT/pooling for identification of HIV infection: 1) downtown Atlanta; 2) Lilongwe, Malawi; and 3) Porto Alegre, Brazil. For each study, HIV infection was defined by a positive antibody test or a positive HIV NAAT or antigen with seroconversion. Ultrasensitive NAATs (Roche 1.5 ultra, Gen-Probe TMA) and small pool sizes (1:12-1:50) were used.

Results: No false positive NAAT results were returned in any study. In Atlanta (n=2202), we assessed performance of Genetic Systems HIV-1/2+O and HIV-1 rLAV 3rd generation EIAs. Overall 70 (3.3%) subjects were HIV+. 66 EIA+ and 4 EIA-NAAT+ [3rd gen EIA sensitivity for detectable HIV, Se=94.3% (95% CI 86.2 to 97.8%)]. In Porto Alegre (n=933) Genscreen-Biorad HIV Ag/Ab 4th generation EIA followed by Abbott HIV MEIA and Biomanguinhos IF assays correctly identified all 183 HIV Ab+ and 5 Ab- infections (4th gen EIA Se=1.00, Seacute=1.00). In Lilongwe (n=1440), 587 subjects (40.8%) were HIV+. 555 were antibody+ on parallel Unigold and Determine rapid testing (Unigold Se=96.5%, Determine Se=99.0%, 2RTs Se=96.2%). 20 had RT- or RTdiscordant/NAAT+ acute HIV. For evaluation of antibody negative specimens, the performance of p24 Ag for true infection (std. p24 Se=0.75; Sp=0.995; PPV=0.75) was improved using the heat-dissociated (HD) assay (HD p24 Se=0.84; Sp=1.00; PPV=1.00). Interestingly, 7 of 21 subjects with discordant RT results (33.3%) had acute infection in this study.

Discussion: While more sensitive rapid tests and 3rd generation EIAs may reduce the window period relative to viral lysate EIA, our findings suggest that acute HIV detectable only by antigen or NAAT can still be disturbingly prevalent in high risk testing sites. p24 Ag testing (either alone in standard format, with an added heat-dissociation step or as part of a combination 4th generation assay) has acceptable sensitivity for acute HIV. Because HIV prevalence in the US is relatively low, however, the positive predictive value of p24 Ag detection for acute HIV is questionable and merits further study. Numerous formats for acute HIV detection are available, however all require that some provision be made for follow-up testing to confirm HIV status and to link patients to emergency prevention services.
Utilizing a non-commercial real-time PCR assay in a pilot study to detect HIV-1 RNA in HIV antibody negative diagnostic sera submitted to the Maryland Public Health Laboratory

Robert Myers, PhD
Deputy Director, Maryland Department of Health and Mental Hygiene

Background: The detection of HIV-1 RNA in acutely infected individuals by nucleic acid amplification testing (NAAT) can theoretically reduce widow period prior to seroconversion by several days or weeks and improve the diagnostic capabilities of HIV testing laboratories. Preliminary NAAT studies by public health laboratories have demonstrated a higher frequency of HIV-1 RNA (+)/antibody (-) samples from acutely infected individuals within their diagnostic testing populations in comparison to the frequency of NAAT (+) samples in blood donors. Additionally, rapidly identifying NAAT (+) acutely infected HIV individuals from diagnostic samples has provided epidemiologists with invaluable insights into the HIV transmission dynamics within selected networks of sexual contacts. However, the long-term utility and feasibility of routinely performing HIV-1 RNA testing of HIV antibody negative samples in the public health laboratory setting to detect acutely infected individuals remains to be determined. We have attempted to find a cost-effective approach to conduct HIV-1 NAAT that utilizes the existing molecular biology diagnostic testing infrastructure that is accessible in most public health laboratories. Methods: In this pilot study we have modified and validated a previous published real-time reversed transcribed HIV-1 gag PCR assay (RT(rt)-PCR) [J. Clin. Micro. Vol.39 (12) 4302-4308.2001] to detect HIV-1 RNA in specimens from pools of 20 HIV antibody negative blood specimens that are submitted to Maryland Department of Health Retrovirology Laboratory for routine diagnostic testing. The existing staff and expertise of the molecular diagnostics laboratory was utilized to validate, implement and perform this HIV-1 RT(rt)PCR assay. Results: During the validation process sensitivity studies established that the assay could reproducibly detect viral loads of at least 2000 RNA copies/ml of an individual HIV-1 RNA (+) sample within a pool of 20 samples. The reagent and supply costs for performing this RT(rt)-PCR are estimated to be less than $0.85/individual sample tested. As of early February 2005 over 15,000 individual HIV antibody negative serology specimens have been tested for the presence of HIV-1 RNA since the RT(rt)-PCR assay was implemented in our diagnostic testing algorithm in early October 2004. To date no HIV-1 RNA (+) samples have been found. Conclusions: The inability to detect HIV-1 RNA (+) acutely infected individuals could be due to the limited number of samples tested to date, possible non-B HIV-1 subtype insensitivities of the RT(rt)PCR assay or shifts in public health testing paradigms in Maryland that rely on testing a large proportion high-risk individuals with non-blood based technologies. The utility of continuing to perform this cost-effective HIV-1 RT(rt)-PCR assay in our laboratory remains to be determined.
Rollout and implementation of Rapid Testing from a Field Perspective

Tony Falvo
Counseling, Testing and Linkage Program Coordinator
Florida Department of Health, Bureau of HIV/AIDS, Early Intervention Section.

Issues: Ensuring compliance with state and federal regulations surrounding waived testing. Developing data collection tools and collaborating with the bureau of laboratories to ensure lab standards are met. Developing a system for processing confirmatory specimens and resolving discordant test results. Converting OraSure testing sites to a blood-based, waived testing program. Incorporation of rapid testing in a wide variety of settings, designed to target high-risk individuals and development of a new model for quality assurance. Stressing confidential versus anonymous rapid testing.

Description: The Florida Department of Health has worked with county health departments, jails, substance abuse treatment centers, CBOs, hospitals, mobile testing units and STD clinics to implement rapid testing in a wide variety of settings, while maintaining a high level of quality assurance.

Lessons Learned: Having a strong partnership with the lab, and developing a systems process for tracking rapid testing data is essential to a successful program. Continuous work is needed with field staff to ensure appropriate quality assurance practices are maintained. Education is needed for the general testing community about the difference between waived testing and the collection of specimen samples. Rapid testing sells itself as a viable means for convincing clients to test confidentially who might otherwise only test anonymously.

Recommendations: Develop a systems process for ensuring the collection of all appropriate data and design a new algorithm with the lab prior to implementation. Set standards for quality assurance, and monitor sites closely after implementation to encourage good practices.
Panel: Roll-out and implementation of Rapid Testing from a Field Perspective

Marianne Porter BS MS
Director, Division of Laboratory Improvement
Pennsylvania Department of Health

Issues: Implementation of rapid HIV testing programs faces several hurdles, including issues of maintaining a reliable testing environment, accessing training resources for testing personnel, making appropriate changes in counseling practices, and complying with federal and state laboratory licensure requirements. This presentation addresses lessons learned for implementation of a rapid testing program in publicly funded sites as well as a sampling of private initiatives.

Description: The Pennsylvania Department of Health has implemented a three-site pilot program, and successfully worked through training, staffing, and regulatory issues with the pilot sites, as well as with other, privately funded sites in the Commonwealth. There was an initial perception that the personnel requirements defined under the regulations pertaining to the Pennsylvania Clinical Laboratory Act would be a major impediment to implementation, but other factors, including clinic access, funding, and counseling have been as significant. Preliminary proficiency testing results have underscored the critical role of training and testing environment.

Recommendations: Deal directly with program implementers, to increase their confidence in the Department’s commitment to making rapid testing both available and reliable. Plan carefully, train well, and begin slowly. Determine a system for data monitoring, including elements prone to human error, such as the time, temperature, and lighting under which the test is conducted, and the use of controls to ensure test kit functionality, and provide potential testing sites with guidance on preparing procedures and setting up record keeping formats. Work closely with those individuals actively providing rapid testing services, to be sure that proposed procedures will be feasible in practice.
Panel: Roll-out and implementation of Rapid Testing from a Field Perspective

Susanne Norris Zanto BS CLS (NCA)
Technical Services Manager, Montana Public Health Laboratory

**Issues:** Implementation of rapid HIV testing programs that are acceptable to laboratory directors, feasible for health departments and/or community-based organizations (CBOs), and provide high quality client services can be challenging to implement, especially in a rural setting. This presentation addresses lessons learned for implementation of such programs, as well as recommendations for comprehensive systems of quality assurance.

**Description:** The Montana Public Health Laboratory partnered with the Montana HIV/AIDS Program to develop training and provide quality assurance oversight for the publicly funded counseling and testing sites. The Rapid Testing program began with the training of six pilot sites. After a six-month evaluation period, the program is gradually expanding to all sites, including CBOs.

**Lessons Learned:** The Public Health Laboratory, working closely with the HIV AIDS Program is key to successful implementation. This also leads to greater integrity in lab practices, especially important for social service agencies, whose work usually requires less attention to detail. Early clarity about expectations for data and program quality prevents problems with quality assurance later.

**Recommendations:** Plan carefully, train well, and begin slowly. Determine a system for data monitoring, including elements prone to human error, such as the time and temperature under which the test is conducted, or the use of controls to ensure test kit functionality. Work closely with those individuals actively providing rapid testing services, to be sure that proposed procedures will be feasible in practice.
Panel: Roll-out and implementation of Rapid Testing from a Field Perspective

Shelley Facente, MPH and Teri Dowling, MA, MPH.
San Francisco Department of Public Health, AIDS Office, HIV Prevention Section.

**Issues:** Implementation of rapid HIV testing programs that are acceptable to the laboratory community, feasible for health departments and/or community-based organizations (CBOs), and provide high quality client services can be challenging to implement. This presentation addresses lessons learned for implementation of such programs, as well as recommendations for comprehensive systems of quality assurance.

**Description:** The San Francisco Department of Public Health works with subcontracted CBOs to design, implement, and continually assess rapid HIV testing programs. This has been successful, despite initial resistance from lab personnel and agency staff. Quality assurance efforts have intensified as both the number and maturity of rapid testing programs have increased.

**Lessons Learned:** Working closely with laboratory policymakers to design and implement rapid testing protocols is key to successful implementation. This also leads to greater integrity in lab practices, especially important for social service agencies, whose work usually requires less attention to detail. Early clarity about expectations for data and program quality prevents problems with quality assurance later.

**Recommendations:** Speak directly with lab personnel, asking what would increase their confidence in the proposed rapid testing program, and then act upon those suggestions. Plan carefully, train well, and begin slowly. Determine a system for data monitoring, including elements prone to human error, such as the time and temperature under which the test is conducted, or the use of controls to ensure test kit functionality. Work closely with those individuals actively providing rapid testing services, to be sure that proposed procedures will be feasible in practice.
Resolution of Discordant Confirmatory Results after POC Reactive Rapid Tests

Berry Bennett MPH, Retrovirology Chief, Florida Bureau of Laboratories
Sally Fordan BS, Retrovirology Supr., Florida Bureau of Laboratories

Background: In August 2003, the Florida Bureau of HIV/AIDS initiated several pilot sites throughout the state to evaluate the feasibility and acceptance of POC HIV rapid testing. The Florida Bureau of Laboratories was enlisted to perform confirmatory laboratory-based testing on POC presumptive positive cases using the CDC recommended algorithm.

Issues:
1) The state program office desired an expedited laboratory confirmation and reporting process because of the transient nature and/or the temporary housing (county jails, etc) of the projected clients to be offered POC rapid testing.

2) The laboratory needed to modify its traditional testing algorithm to follow CDC’s guidelines for these select presumptive positive cases as well as revise its data entry and reporting processes.

3) The laboratory would be responsible for developing additional in-house algorithms to resolve discordant cases and provide specimens to CDC upon request.

Lessons Learned/Recommendations:
Cooperation between the state program office and the state laboratory produced a new HIV requisition form to allow easy identification of presumptive positive rapid tests upon arrival at the laboratory. This allows the laboratory to expedite data entry, testing and reporting. Clear identification of presumptive positives will hopefully avoid delayed and incomplete confirmation testing.

As discordant cases were detected, the laboratory identified the need to repeat the initial rapid tests in-house, test with other rapid assays, perform molecular testing and enlist CDC to resolve.

Any presumptive positive is a potential discordant case. It was quickly determined that the desired confirmation sample should be a serum or plasma as opposed to an oral fluid specimen to allow broader infectious disease testing, if applicable, at both the state laboratory and CDC. Unfortunately, some of the pilot sites are not able to perform phlebotomy.
Post Marketing Surveillance of Oraquick Rapid HIV Testing

Wesolowski, L., Burstein, G., Mackellar, D., Branson, B.
Diagnostic Applications, Division of HIV and AIDS Prevention, NCHSTP, Centers for Disease Control and Prevention

Background:
In January 2003, OraQuick Rapid HIV-1 Antibody Test (OraSure Technologies, Inc.) became the first HIV antibody test to receive a Clinical Laboratories Improvement Amendment (CLIA) waiver. In order to increase the use of rapid HIV tests, CDC purchased and distributed rapid test kits to over 100 state and local health departments, community based organizations, and other testing entities. Post-marketing surveillance (PMS) was conducted to monitor Oraquick rapid HIV test accuracy and utilization, evaluate discordant confirmatory test results, and characterize quality assurance methods and outcomes.

Methods:
From July 2003 to December 2003, 14 project areas participated in PMS-1 (phase 1 of PMS). Phase 2 of PMS (PMS-2) will be conducted from August 2004 to June 2005 in 18 project areas. HIV testing surveillance data was submitted to CDC for analysis during PMS-1. Additional follow-up was conducted on discordant test results which had a reactive Oraquick and either non-reactive EIA or negative or indeterminate Western blot or IFA. Historical 2002 data was requested from project areas for the same time period that PMS-1 was conducted in 2003.

Results:
There were 30,320 total HIV tests reported during PMS-1, 20,319 of which were rapid HIV tests and 10,001 were EIA. 615 (2.0%) persons were confirmed positive: 378 (61.5%) were tested initially using rapid tests and 237 (38.5%) were tested using EIA. Twenty-one discordant test results were identified. In the project areas with available historical 2002 data, (1) seropositivity rates were similar for 2003 (1.2%) as 2002 (1.1%) and (2) more positive and negative HIV rapid results were returned to clients in 2003 when compared with conventional results in 2002. Data for PMS-2 are still being collected.

Conclusions:
Rapid HIV testing was widely used in the surveillance area during PMS-1. The incidence of discordant cases was relatively rare (1/1000). A greater proportion of persons received their rapid HIV test results in 2003 than conventional test results in 2002.
Evaluation and Implementation of HIV Rapid Tests: 
The Experience in Eleven African Countries

Stefan Wiktor, MD, MPH
Chief, Surveillance and Infrastructure Development Branch, 
Global AIDS Program, CDC

Background
Access to HIV testing is a critical component of HIV prevention and care services. Because of their ease of use, HIV rapid tests are being widely used to provide HIV testing services in resource-poor countries, and in-country evaluations and quality control of point-of-service testing are important steps to assure the quality of this testing. To date there is limited information concerning the outcomes of these evaluations and of the quality of rapid test performance when used in clinical settings.

Methods
A standardized questionnaire regarding the evaluation and implementation of HIV rapid tests was administered to personnel in ministries of health and reference laboratories in 10 African countries where the U.S. Centers for Disease Control and Prevention has provided technical assistance to improve laboratory capacity. Additional information was obtained from reports, presentations, personal communications, and other documentation. The existing HIV testing algorithms in each country served as the gold standard for measuring the performance of each rapid test.

Results
Results are available from HIV rapid test evaluations conducted between 2001 and 2003 in reference laboratories in 11 countries. In all, 18 different rapid tests were evaluated, and the median sensitivity was above 99% for 12 of these tests (range 92.5% - 100%), and the median specificity was above 99% for 14 of the 18 rapid tests (range 95.3% - 100%). Subsequent evaluations at point-of-service sites in 5 countries demonstrated sensitivities and specificities of rapid tests comparable with the results of reference laboratory evaluations. To date, rapid testing has been implemented in 8 of the countries in over 600 sites including voluntary counseling and testing centers, antenatal clinics, blood donation centers, hospitals, and clinics. A median of 96,000 people were tested in each country in 2003 (range: 15,000 to 315,000 people). A serial (consecutive) testing algorithm was used in 5 countries and a parallel testing algorithm was used in 3 countries. Quality assurance systems in four countries involve retesting a sample of specimens at a reference laboratory. Concordance between on-site rapid testing and retesting at the reference laboratory ranged from 95.7% to 99.5% (median: 98.7%).

Conclusions
In-country evaluations of HIV rapid tests in settings with varying levels of technical skill and different HIV-1 subtypes yield results comparable to those obtained in more controlled evaluations. Furthermore, the excellent validity of rapid testing algorithms at point-of-service sites argues for wider implementation of HIV rapid testing.
Implications for the Public Health Laboratory from Expansion of Rapid Testing
Arthur Kazianis, Massachusetts State Laboratory Institute

**Issues:** Massachusetts Department of Public Health C&T sites began using the OraQuick Rapid HIV-1 screening test as part of a continuing mission to provide accurate and timely reporting of HIV antibody results. Such screening was initially limited to a subset of DPH funded C&T clinics participating in the Post-marketing Surveillance (PMS-1) of this product. The information presented here describes the success of HIV rapid testing in Massachusetts, practical concerns for its implementation, and the HIV laboratory’s commitment to provide continuous training, quality assurance and technical support.

**Description:** Informational and practical training sessions for C & T staff were conducted. Training modules included the performance of OraQuick, QA/QC, document requirements and laboratory safety. The QA program followed our laboratory practice by including a written test, interpreting a visual panel and performing the assay using proficiency panels. Participants were instructed to submit serum follow-up specimens to the lab and were advised to consult with the HIV lab for answers to technical concerns. Trainers also assessed environmental conditions at all proposed testing venues.

**Lessons Learned:** MA State Lab Institute received 101 serum follow-up specimens for confirmation of which 85 tested positive for HIV-1 antibody using EIA and Western blot. Six were indeterminate and 10 were negative. All negative and indeterminate samples tested non-reactive by HIV-2 EIA. The HIV lab staff also tested these follow-up samples using OraQuick and all were again reactive. Despite the training, there was lack of understanding by clinical/C&T personnel that OraQuick is a screening assay. Reactive OraQuick interpretations followed by negative “final” results, and the use of the “preliminary positive” language was interpreted as evidence of poor OraQuick performance. In fact, results were well within the 95% CI for this test.

**Conclusions:** OraQuick Rapid HIV-1 screening can be successfully implemented in traditional C & T settings. Laboratory participation is a practical way to train C & T staff. Whenever possible, attempts should be made to mimic pre- and post-analytical processes of the traditional laboratory. Trainers should be mindful to caution counselors about misuse of screening results. Laboratory staff must be available to provide continuous support.
Challenges for Blood Donor Confirmatory Testing Algorithms
Susan L. Stramer PhD
Executive Scientific Officer, American Red Cross

**Issues:** Blood donation samples that are repeat reactive by FDA licensed HIV-1/HIV-2 screening assays currently undergo a confirmatory algorithm that involves HIV-1 western blot (WB) and HIV-2 EIA/WB for those samples that do not contain HIV-1 antibody. Issues with both sensitivity and specificity exist with the current blood donor confirmatory algorithm. The major issue with this algorithm includes high rates of samples that can neither be called positive or negative according to the FDA licensed inserts; these “indeterminate” results account for 40-50% of HIV repeat reactive donations, 30-40% of which do not even have bands to viral proteins but cannot be interpreted as WB negative. Fewer than 1:2,500 donors who test HIV-1 WB negative or indeterminate is HIV infected. Poor specificity is exacerbated when an individual falsely confirms positive on the WB (current rate approximately 1:700,000). Blood donors receiving these indeterminate and false-positive messages are confused and anxious, especially when their HIV uninfected status is corroborated by RNA negativity. Western blots and comparable confirmatory assays have not evolved since 1987 in contrast to screening assays which have made significant improvements in both sensitivity and specificity. As an example of improvements in sensitivity, one newly licensed HIV-1/HIV-2 (plus O) EIA is 74% more sensitive than the WB during seroconversion. This presentation provides options to the current use of only the HIV WB in blood donor confirmatory algorithms.

**Description:** Blood centers have implemented HIV-1 RNA testing by pooled NAT in 1999. Integration of NAT with serological test results improves the quality of the donor counseling message for repeat reactive donors by increasing the sensitivity early in infection when the WB typically would not be positive. It also helps stratify WB positive donors into those most likely infected versus those who likely are not (especially when coupled with S/CO ratios of the screening assay, RNA negativity of an individual sample, and donor follow up). However, greater than 95% of HIV-1/HIV-2 repeat reactive blood donors are negative for RNA in the pooled NAT format (and of those, the vast majority will test single unit NAT negative). Therefore, simply integrating NAT into confirmatory algorithms may address the sensitivity issue but does not address the specificity issue. Since the screening EIAs have ungone vast improvements in sensitivity and specificity, those samples testing RNA negative should be tested by a second licensed HIV-1/HIV-2 EIA and only those samples testing concordantly repeat reactive require further testing by WB. Feasibility of this “dual EIA” approach is based on the concept that if two assays with comparable sensitivity are composed of differing antigens, the false-positive populations should have limited cross over; the more unique the tests, the greater the separation of false-positive populations.

**Lessons Learned:** The dependence on the use of viral lysate-based confirmatory assays in use for HIV since 1987 should be addressed. The inclusion of new versions of HIV-1/HIV-2 screening assays that are not dependent on viral lysate antigens and licensed RNA testing should be included.

**Recommendations:** Modifications in the confirmatory algorithms for HIV-1/HIV-2 EIA repeat reactive blood donors are needed. Substitutions of a second licensed screening assay (to rule out the existence of HIV antibody) and RNA (to rule out the possibility of early HIV infection) for the WB should occur. The WB then may be used only to confirm antibody reactivity in those donors whose RNA is negative but who do have reactivity by two different licensed EIAs. Such an algorithm improves sensitivity and specificity (>98% of repeat reactive blood donors will no longer be counseled as “indeterminate”).
Comparison of Commercially Available Assays that Detect Human Immunodeficiency Virus to Address Alternative Screening/Diagnostic Algorithms for HIV

S. Michele Owen, C Yang, T Spira, CY Ou, CP Pau, B Parekh, F Cowart, D. Kuehl, S Kennedy, D Rudolph, D Candal, W Luo, N Delatorre, S Masciotra, T Barnett, R Lal and JS McDougal, Laboratory Branch, NCHSTP, Centers for Disease Control and Prevention

Background/Objective: The current algorithm for diagnosis of HIV infection involves a screening EIA followed by a supplemental test, Western Blot (WB) or Immunofluorescence (IFA) for confirmation. Given the inherent problems associated with WB (cost, subjectivity, indeterminate results) and the increased number of FDA approved tests for HIV (Nucleic Acid Test (NAT), rapid test, improved EIAs), a study was undertaken to compare commercially available HIV diagnostic tools to address whether new algorithms for HIV screening/diagnosis are appropriate.

Methods: Tests used in the study included 6 EIAs, 4 Rapid tests and 2 NAT based tests as well as 2 WBs. 1002 plasma samples obtained from Boston Biomedica (BBI) that represents U.S. blood/plasma donors, and 268 non-U.S. samples were used. All or a subset of the samples were evaluated with each test and sensitivity and specificity were calculated relative to the current standard of EIA and WB. Furthermore, the testing data were used to assess potential new algorithms for HIV screening/diagnosis.

Results: The range of sensitivity observed in the study for all tests was from 92.6% to 99.4% and the specificity observed ranged from 95.8%-99.4%. When serological testing data were combined with NAT data, to address the question if NAT testing can eliminate or reduce WBs (as described in the proposed Blood Bank Screening algorithm) the number of indeterminate WB was reduced. However, samples were identified that yielded discordant results when serological and NAT based tests were compared.

Conclusions: All of the commercially available, FDA approved, assays for detection of HIV gave comparable levels of sensitivity and specificity. These data suggest that the proposed Blood Bank Screening algorithm that incorporates NAT based testing would likely decrease the number of indeterminate WB results. However, our data indicates that NAT testing can not completely replace WB as samples were identified that were serology positive and NAT negative.

Presenter: S. Michele Owen, CDC NCHSTP DHAP  smo2@cdc.gov
**Performance of Rapid HIV Tests Singly and in Combination**

Kevin Delaney, MPH  
*Epidemiologist, Diagnostic Applications, Behavioral and Clinical Surveillance Branch, CDC*

**Issue:** Rapid testing for HIV provides two distinct advantages over traditional HIV testing: the ability to provide immediate results, and the ability to take testing and a test result to people and places that were previously inaccessible. Despite the fact that all FDA approved rapid test package inserts contain language stating that: “This test is suitable for use in multi-test algorithms designed for statistical validation of rapid HIV test results. When multiple rapid HIV tests are available, this test should be used in appropriate multi-test algorithms,” rapid HIV tests are currently only being used as single screening tests which require confirmation by either Western Blot or IFA.

**Description:** In this study, persons seeking HIV testing at a high prevalence STD clinic in Los Angeles, CA were simultaneously tested with several HIV rapid tests. Additionally both serum and plasma specimens were collected and tested with a traditional EIA and Western blot. Results of individual rapid tests and combinations of rapid tests used in sequence were compared to the results of the EIA and Western blot testing.

**Lessons Learned:** Nearly all individual rapid test results (13102/13129=99.8%) agreed with the results of the serum EIA and Western blot. Sensitivity of all rapid tests was greater than 98%, with nearly all falsely negative specimens coming from clients known to be HIV positive and on treatment. Adding a second, different rapid test resulted in 100% specificity for specimens that were reactive on both rapid tests, with between 0 and 4 specimens being discordant and requiring a third “tie-breaker” rapid test. The average cost of a second rapid test was approximately half the cost of a Western blot, suggesting significant cost savings are possible. A limitation of this study is the lack of follow-up information on persons with one or more positive rapid tests whose confirmatory test was negative or indeterminate.

**Recommendations:** Rapid test only algorithms for the diagnosis of HIV infection can be as sensitive and specific as the current diagnostic algorithm. A rapid test algorithm has the additional advantage of providing same day, point of care diagnosis of HIV infection. Given the current trend towards using a rapid test to screen for HIV infection, rapid test only algorithms also could provide significant cost savings over traditional confirmatory testing (Western Blot). Further investigations which demonstrate the use of a rapid test only diagnostic algorithm at the point of care are warranted. Such investigations should include follow-up of anyone testing positive on one or more rapid tests whose confirmatory Western blot is either negative or indeterminate.
HIV-1/HIV-2 COMBINATION SCREENING AND OUTCOMES OF FOLLOW-UP

William R. Oleszko, Ph.D.
Acting Associate Director, Public Health Laboratory
New York City Department of Health and Mental Hygiene

OBJECTIVE: To demonstrate the outcome of laboratory HIV antibody testing on specimens submitted to the NYC DOHMH’s PHL after implementation of the of HIV-1/2 Plus “O” combination screening EIA.

METHOD: In April of 2004, the PHL implemented BioRad’s HIV-1/2 Plus “O” EIA screening assay for all specimens submitted for HIV antibody analysis. During the last 9 months of 2004, approx. 74,000 specimens were submitted, assayed and HIV antibody reports transmitted to submitting health care providers. The PHL’s routine HIV antibody testing algorithm requires initial replicate screening followed by supplemental testing, if required. Specimens that are repeatedly nonreactive by the EIA screening assay are reported as “Negative for Antibody to HIV-1 and HIV-2”.

Specimens that are HIV-1/HIV-2 screening EIA repeat reactive are reflexed for HIV-1 Western Blot supplemental testing. The complete testing algorithm will be presented. The demonstration of discordant replicate HIV-1/HIV-2 EIA screening results (‘splits’ or indeterminates/Ind) and discordant EIA (repeat reactive) and Western Blot (nonreactive) results (‘incompatibles’) require additional laboratory evaluation. The PHL has incorporated several supplemental assays and study protocol, including BioRad’s HIV-1 rLAV, HIV-2 EIA, Multispot HIV-1/HIV-2 Rapid EIA, V3 Peptide EIA and HIV DNA PCRs for HIV-1 and HIV-2 as a means of demonstrating whether actual or false reactivities to HIV are present in these specimens. Summaries will be presented.

RESULTS: An example of the April through December 2004 results for HIV-1/HIV-2 Plus ‘O’ “splits” and “incompatibles” are is shown in this table.

<table>
<thead>
<tr>
<th>EIA ASSAY KIT</th>
<th>HIV-1 rLAV Results</th>
<th>HIV-1 Western Blot Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reactive</td>
<td>‘Splits’ Ind.</td>
</tr>
<tr>
<td>HIV 1/2 Plus “O” (N = 73,955)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Splits’ 348 (0.47%)</td>
<td>0 0</td>
<td>348</td>
</tr>
<tr>
<td>‘Incompatibles’ 227 (0.31%)</td>
<td>0 0</td>
<td>227</td>
</tr>
</tbody>
</table>

Of the 62 of the 348 initial HIV-1/HIV-2 Plus ‘O’ EIA screening “Split” that became HIV-1 Western Blot indeterminate (each with 1 to 2 nonconsensus bands), 60 were nonreactive by HIV-2 EIA, while 2 were repeatedly reactive by HIV-2 EIA. Of the 227 initial HIV-1/HIV-2 Plus ‘O’ EIA and HIV-1 Western Blot incompatible specimens all were nonreactive by both HIV-1 rLAV and Western blot, while only 3 had HIV-2 EIA reactivity, but were negative by HIV-1/2 MultiSpot, V3 peptide.

CONCLUSION: While no HIV antibody serologic assay is 100% sensitive and 100% specific, this laboratory’s experience in its initial implementation of BioRad’s HIV-1/HIV-2 Plus ‘O’ EIA screening assay and the use of it’s HIV testing algorithm to resolve false reactive EIA screening results, ultimately resulted in a very low HIV antibody inconclusive rate.
DRIED BLOOD SPOTS: AN IDEAL SPECIMEN FOR HIV SEROLOGIC AND NUCLEIC ACID TESTING

Joanne Mei PhD

Newborn Screening Quality Assurance Program,
Centers for Disease Control and Prevention, Atlanta, GA 30341

Introduction: Dried blood spots (DBS) have been used since the early 1960s to screen newborn babies for metabolic and inherited disorders. In the late 1980s, HIV serologic technologies were adapted to the whole blood filter paper matrix for the HIV serosurveillance of childbearing women. DBS are an ideal specimen for whole blood analysis because of their ease of collection, storage, and transport. Most analytes, including antibodies and nucleic acids, are very stable on filter paper. This allows for the phenotypic and genotypic analysis of patient samples from the same specimen by using various analytical and molecular methods.

DBS Handling: The Newborn Screening Quality Assurance Program (NSQAP) conducts routine evaluations of new filter paper lots to ensure uniformity of testing. It has been providing DBS quality control and proficiency testing materials for HIV since 1989. Procedures for making, drying, storing, evaluating, and transporting DBS have been developed by NSQAP and form the basis of a clinical laboratory standard for the filter paper collection device. The quality of the DBS specimens can affect analytic results. Care should be taken during the drying process, storage, and transportation of DBS specimens to maintain specimen integrity.

DBS Testing: Testing of patient DBS should include appropriate whole blood control materials of defined reactivity. Controls are important for both serologic and molecular testing, and they can be used to assess within run and between run variability, kit lot or reagent changes, and variability between technicians or instruments.

DBS Proficiency Testing: Each quarter NSQAP distributes panels of blinded DBS specimens representing 5 individuals. We currently have 49 laboratories in 18 countries participating. The panels are tested by HIV serological screening and confirmatory methods. NSQAP gives technical assistance to laboratories that miss classify specimens.

Conclusion: DBS are an ideal specimen for HIV testing, especially in resource poor areas. DBS should be protected from heat and humidity and have excellent stability if stored appropriately. DBS controls should be part of quality assurance protocols that include blinded proficiency testing.
Usefulness and Application of Dried Blood Spots in HIV and HCV Epidemiology, Diagnostics, and Drug Resistance Testing

John Kim PhD
Chief National HIV Reference Laboratory
Public Health Agency of Canada

Issues: Whole blood collected by venipuncture has often been used as the gold-standard in sample collection for lab testing of HIV and HCV in diagnostics and surveillance studies. Several disadvantages can be associated with this method especially in resource-limited locales. This presentation describes dried blood spots (DBS) as an alternative sample sourcing method and its application and practicality in any test traditionally performed using venipuncture obtained blood.

Description: The National HIV and Retrovirology Labs (Public Health Agency of Canada, [PHAC]) have been using dried blood spots for several years in different applications. Its biggest impact has been in national surveillance programs for HIV and HCV that are conducted in conjunction with PHAC’s HIV Epidemiology & Surveillance division and its provincial partners. The use of DBS is now strongly recommended to our international partners in projects involving HIV and/or HCV testing.

Lessons Learned: DBS has been accepted as a viable and useful tool within the laboratory. Some reluctance may have been experienced because extra steps, (namely elution), not present in traditional plasma/sera testing is required. A greater challenge has been in implementing its use in surveillance studies and in convincing front line workers of its usefulness. To address this we have implemented training programs within our labs demonstrating procedures on the collection and testing of DBS.

Recommendations: Education and hands-on training, especially to front-line workers has helped significantly in gaining acceptance for DBS. Increased validation of blood collected as DBS vs peripheral blood are still required to ensure that similar performance profiles are reached especially in molecular based assays that are not licensed for use with DBS but may be used without proper validation.
EARLY DIAGNOSIS OF HIV-1 INFECTION IN INFANTS USING DRIED BLOOD SPOTS AND REAL-TIME REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION

Chin-Yih Ou1, Steven Balinandi2, Souleymane Sawadogo1, Clement Zeh1, Hua Yang1, Pius M. Tih3, Christiane Adjé-Toure3, Sam Tancho3, Leonard Kouadio Ya3, Marc Bultery5, Pauli Amornkul6, Robert Downing2 and John Nkengasong1.

Background/Objectives: Serodiagnosis of HIV-infection in infants born to seropositive mothers is problematic due to the prolonged presence of maternal antibodies. Dried blood spot (DBS) is a convenient specimen collection method because it is easy to transport and store. Our objective was to develop a simple and sensitive nucleic acid-based technology to diagnose HIV-infected infants using DBS.

Methods: A simple magnetic bead-based method is used to isolate total nucleic acid from a 6 mm DBS disc. A novel one-tube, real-time, duplex RT PCR assay is then used to detect both the HIV-1 LTR RNA and DNA. This approach was evaluated with DBS specimens from HIV-1 exposed infants in Uganda (n=128), Cameroon (n=315) and Kenya (n=410). The gold standard used to determine the infant infection status in Uganda was plasma viral load (VL) and that in Cameroon and Kenya was Amplicor DNA test on DBS. For discordant samples, we also tested them with real-time assays specific for the HIV gag and integrase sequences.

Results: We first examined the stability of HIV-1 RNA in the DBS and found that it was stable for more than 2 months if DBS was stored properly at room temperature in a humidity-free environment. We also found that when using both HIV DNA and RNA as the detection target, the detection sensitivity increased when compared with the use of DNA alone. The concordance of our assay and VL assay in the Ugandan study was 99.2%. The concordance of our assay and Amplicor DNA assay in the samples from Cameroon and Kenya was 99.7%. One sample from Uganda with negative VL and two samples from Cameroon with negative Amplicor DNA results were found to be positive by our assay. These samples were found to be positive for HIV gag and integrase sequences.

Conclusions: Because of the simplicity of specimen collection, nucleic acid extraction, detection, low cost and high sensitivity, our DBS-based HIV real-time assay will be useful for pediatric HIV diagnosis and care/treatment programs in resource-poor settings.

Presenter: Chin-Yih Ou Email: cho2@cdc.gov
Institution: Centers for Disease Control and Prevention
Status of Tests for Recent Infection & CDC’s Plans for the IND for Detuned Testing
Bernard M. Branson, M.D.

Under CDC’s IND, a participating IND labs have been using the Vironostika-LS assay for a variety of purposes; some are for incidence surveillance; some, research (and unlinked, e.g., assessing evolution of HIV antibody response in treated and untreated persons); some are investigational (e.g., results used for eligibility criteria for treatment trials like AIEDRP); and some are for surveillance unrelated to CDC’s national incidence surveillance (e.g., incidence trends in gay men in San Francisco).

The current Vironostika assay is scheduled to be withdrawn, and CDC has developed the BED HIV-1 Capture Assay for use in its assessment of national HIV incidence. CDC has also reached agreement with FDA for a “surveillance use only” label, which will not require informed consent or IRB approval. CDC is eager to protect the “surveillance use” status of the BED assay, and wants to formulate a plan to support or transition the IND labs once the current Vironostika assay is withdrawn and the IDE is terminated.

Questions were posed to the FDA about the regulatory requirements for various potential uses of the BED and other incidence assays.

This session will outline CDC’s plans for using the BED assay in estimating national incidence, and review the general principles of the FDA requirements for other uses. Examples include:

1. If research (with intention to produce generalizable knowledge) is done with either an unapproved product or off-label use of an approved product, and it involves clinical management in any way (including diagnosis, prognosis, treatment decisions, eligibility for clinical trials, counseling, notification of partners, etc.) than an IND or IDE is required.

2. For clinical lab testing, if done under CLIA as a laboratory practice (subject to CLIA-required validation) and does not violate FDA’s analyte-specific labeling, or as part of the practice of medicine for the management of individual patients, off-label use does not require an IND or IDE. The regulations do not allow this to be a subterfuge for a study; if the purpose is an organized effort to produce generalizable information, users cannot pretend it is clinical use.

3. An IND (investigational new drug application) is required for biologics (in the case of HIV tests, those approved for screening blood products). An IDE (investigational device exemption application) is required for other in vitro diagnostics. (This would apply to either the Vironostika Plus O or the BED.)
A SIMPLE AND INEXPENSIVE PARTICLE AGGLUTINATION ASSAY FOR IDENTIFYING RECENT HIV INFECTION

Niel Constantine¹, Li Hong², Kristen Kreisel¹, Anne Sill³, Fassil Ketema¹

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Background: Sensitive/less-sensitive (S/LS) serologic assays have been developed to differentiate those individuals who have recent versus established HIV infection. These assays, based on ELISA technology, may not be suitable for use in resource-limited countries where stable electricity and infrastructure are lacking, and the relatively high cost of ELISA tests may not be affordable. A simpler and more affordable method is needed to address these limitations.

Methods: The Serodia HIV-1/HIV-2 particle agglutination assay was modified to act as a sensitive/less-sensitive (PA S/LS) assay to differentiate recent from established HIV infection. Antigen-coated gelatin particles were diluted 1:68, and sera were diluted at intervals from 1:10 to 1:80,000 in specimen diluent. Sera from 75 persons classified as recently infected (< 133 days) and 77 sera from those classified as having established infection (>133 days) by the reference Vironostika (DV) S/LS using a cutoff of 1.0 were tested by the PA S/LS test at each dilution. The last dilution that produced a positive reaction (≥1+) constituted the endpoint dilution. The endpoint dilution was assessed for all samples and the dilution interval that yielded the best separation of the two populations by the PA S/LS test was selected and used when determining the concordance of the results with the DV S/LS test.

Results: At the selected serum endpoint dilution of 1:58,000, 134/152 samples were correctly classified by the PA S/LS test as compared with the DV S/LS. Three samples that had been classified as being from recently infected, and 15 samples classified as being from persons with established infection by the DV S/LS were misclassified by the PA S/LS test. Therefore, the concordance with the DV S/LS test for correctly classifying recent and established HIV infection was 88% overall, with a 96% and 81% concordance for recent and established infections, respectively.

Conclusion: A commercially available HIV gelatin particle agglutination test was modified as a S/LS test and exhibited an excellent ability to differentiate persons with recent and established HIV infection. The PA S/LS test offers unparalleled low cost, is simple to perform, and does not require any instrumentation or electrical support. Although we have shown proof of principle, the test must be challenged with a large number of well-characterized sera before being standardized for widespread use.

Presenter: Dr. Niel T. Constantine
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IDENTIFICATION OF FACTORS IMPACTING THE DETERMINATION OF HIV INCIDENCE IN NON-RESEARCH-BASED, CLINICAL POPULATIONS

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Issues: Sensitive/Less Sensitive (S/LS) assays offer the estimation of HIV incidence without the time and resources necessary to follow a cohort of at-risk seronegative individuals, and are particularly useful in resource-limited countries. For the determination of incidence using S/LS assays, cross-sectional population surveys must have a defined sampling frame within which all HIV confirmed positive cases and negative clients are documented and HIV+ specimens are available for S/LS testing. We describe our challenges and provide recommendations for making robust incidence estimates using data and specimens from non-research based, clinical and Public Health Laboratory settings.

Description: Four HVTN-participating sites in the Caribbean were initially identified as having suitable data management and specimen preservation practices to provide the necessary components for generating incidence estimates. Three sites provided clinical data and HIV+ specimens from defined screening populations (ANC, STI, VCT) while the 4th site provided data and specimens from multiple sources, which were unlinked and then reported to a public health facility for surveillance purposes. All available HIV Ab+ samples were confirmed by Western blot (WB) and “detuned” using the Vironostika S/LS EIA. Data records of samples with indeterminate or inconclusive serostatus by WB were removed from the incidence calculations.

Lessons learned: Existing clinical data and specimens derived from cross sections of clinical, screening populations and from public health laboratories may not be optimal for making robust incidence estimates. HIV+ specimens which were not originally collected for surveillance purposes often were unavailable or had insufficient volume or integrity for S/LS testing. Thus, an estimated 36%-75% of all HIV+ samples from the 4 sites did not undergo S/LS testing. The lack of having an integrated, facility-based clinical and laboratory data management infrastructure also contributed to data duplication and discrepancies. Given the necessity to define the total number of HIV+ specimens and the total samples tested within a sampling frame, we developed a correction method in which the proportion of seropositive specimens “detuned” was assessed and the equivalent and randomly selected proportion of total negative observations within the same time frame, was included in the denominator of the incidence calculation, to correct for missing specimens. Additional analyses, through modeling with known incidence estimates from previous surveys, are currently being undertaken to validate this approach.

Recommendations: The concept of a well-designed, prospective survey must be emphasized in order to estimate HIV incidence; such a survey, using the S/LS assay, requires a structured platform of data collection, data linkage, and specimen storage practices. These elements (e.g. a surveillance program and a screening database) may not be feasible to attain for existing, non-research based facilities. However, adjustments can be made to compensate for these missing elements to arrive at meaningful incidence estimates.

Presenter: Anne M. Sill
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A summary of the Satellite meeting on the STARHS HIV diagnostic assay in association with the 15th International AIDS Conference, Bangkok, Thailand, July 11, 2004

Robert S. Remis MD, MPH, FRCP, University of Toronto

In the first part of the meeting, 11 presentations were given, six on epidemiologic topics and five on laboratory aspects. A brief summary of these presentations follows:

- **HIV test-seeking behaviour may introduce significant bias in estimating incidence.** The authors developed a technique using the variable window period of the STARHS assay to quantify and remove the testing bias.

- **There is a need to establish panels of specimens from seroconverting persons to validate STARHS assays, especially for non-B clades from developing countries.** The investigators developed a protocol to identify and collect specimens and transport them to their laboratory in the US.

- **To detect a possible decrease in HIV incidence among MSM in the UK in 1995-2002, the investigators tested specimens anonymously from 16 STD clinics throughout the UK using the STARHS assay.** HIV incidence was higher in London than elsewhere but showed some decrease followed by an increase with rates in 2002 similar to those in 1995.

- **The investigators explored the challenge of measuring HIV incidence in a heterosexual population infected with mixed viral subtypes as was observed in Thailand, in particular since different subtypes may have different STARHS window period.** They propose solutions when subtyping is and is not available.

- **The authors used the STARHS assay at a clinical service in England.** Among patients studied from 1995 to 2002, they explore the possible reasons for falsely discordant results from patients with long-standing infection. They also present a case for using STARHS results for their potential clinical and public health benefits aside from their application in estimating HIV incidence.

- **The investigators presented their experience of testing recently diagnosed patients using the STARHS assay in Spain.** Epidemiologic data were available for 60 recently infected patients and showed a mixed picture with respect to gender and exposure category.

- **The investigators explore the limitations of two approaches to detecting recent HIV infection.** They suggest combining the information derived from assays using the concept of increasing antibody titres and assays using changes in antibody quality.

- **The investigators explored using oral fluid to detect recent HIV infection in a Phase I study.** They conclude that an oral fluid S/LS EIA can be developed that possesses a high concordance with S/LS serum tests though a calibrator may be needed to better standardize the method.

- **The investigators presented preliminary data on a new technique developed in Australia to detect recent infection.** The approach is based on detecting IgG isotypes that reflect duration of HIV infection.

- **The investigators sequenced 30 archived specimens from selected Caribbean islands and characterized the genetic patterns of viral strains in the region.**

- **Thai researchers presented data using the BED-CEIA assay, a competitive enzyme immunoassay that detects increasing levels of specific HIV-1 IgG.** They applied the technique to Bangkok IDUs and Thai military conscripts, obtaining estimates of HIV incidence comparable to those from independent studies.

In the second part of the meeting, a general discussion was held on several topics selected from a list. The themes that emerged may be summarized as follows:

- **There were continued concerns about test performance (sensitivity, specificity).** Results must therefore be carefully interpreted and we need to develop better methods and possibly algorithms using multiple techniques.

- **Validating newer methods is very important and should also be done in the environment (i.e. samples, equipment, reagents, personnel etc) where the test will be used.**

- **There was controversy about whether patients and clinicians should be informed of results.**

- **There is a need for more reference panels (especially non-B clades) from carefully designed protocols to validate new methods.**
HIV in Botswana: Improving Laboratory Capacity in the Face of the AIDS Pandemic

Patricia Clark, MPH
Interim Virology/Immunology Section Manager, Michigan Department of Community Health

**Issues:** In Botswana, the prevalence of HIV/AIDS among pregnant women 15-49 years old was 37.4% in 2003. Life expectancy has fallen from 72.4 years prior to the AIDS epidemic to 33.9 years in 2002. In January 2004, Botswana implemented routine HIV testing in health care settings, leading to an increase in the demand for rapid HIV tests. This presentation addresses lessons learned for the implementation of routine rapid HIV testing in non-laboratory outreach settings under field conditions.

**Description:** The BOTUSA project in collaboration with Botswana Ministry of Health (MOH) invited the Global AIDS Laboratory Project (GALP), a collaboration between APHL and CDC, to evaluate additional rapid HIV test kits for future use in surveillance and diagnosis at non-laboratory settings. A team of three U.S. laboratory scientists worked with National Blood Transfusion Service (NBTS) employees to evaluate rapid HIV test kits during July, 2004.

**Lessons Learned:** Working closely with Botswana Ministry of Health and National Blood Transfusion Service personnel to evaluate and implement rapid testing is imperative. Culturally competent training of local staff is key to the success of this project. Training of future outreach staff, quality assurance activities, and the responsibility for future rapid kit evaluations will fall to these individuals.

**Recommendations:** Routinization of HIV testing by including it in every contact with a health care provider. Expand the number of trained personnel to perform rapid HIV testing at outreach centers. Expand laboratory capacity to facilitate these advances.
Panel Discussion: Rapid HIV Testing in International Settings

Use of Rapid HIV Assays in Vietnam and Cambodia: Challenges to Improving Access, Quality and Timeliness of HIV Testing

Ralph Timperi MPH
Director, Massachusetts State Laboratory Institute

Issues: Access to HIV testing must be increased significantly in resource poor settings especially where HIV prevalence is high. Currently available rapid HIV tests offer an opportunity to provide testing in many locations including outside conventional laboratory settings. However, laboratory infrastructure that is required to assure the quality of testing is often limited in these settings. As part of the roll-out of rapid HIV testing, innovative systems must be initiated to increase access to testing while assuring testing accuracy otherwise we risk the result of adverse outcomes from inaccurate testing including effects on individuals and prevention planning.

Description: CDC/GAP has conducted technical assessments and planning visits to many GAP programs to identify actions that can strengthen laboratory infrastructure and improve the quality of laboratory testing. This presentation describes the laboratory situation in Vietnam and Cambodia and identifies strengths and weaknesses of HIV testing currently. Options for how to rapidly increase access to HIV testing and assure testing quality are considered in the context of this environment.

Lessons Learned: While rolling-out wider use of HIV rapid tests, the capacity of laboratory infrastructure must be strengthened, quality management systems must be improved and national health laboratories must play a central role in training and quality assurance for HIV rapid testing.
Quality Assurance of Rapid Testing

1Elizabeth M. Dax, 1Sandy Walker

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**Issues:** Rapid tests are becoming widely used in various types of facilities from large laboratories to community based organizations. Results of rapid HIV tests are typically qualitative. The tests may be interpreted by sophisticated densitometry or more commonly they are read subjectively and often by people not skilled in laboratory practice. Therefore, there are challenges in quality assuring performance of rapid tests not met in assuring the performance of quantitative assays.

**Description:** The NRL Australia administers quality assurance programmes for laboratories in Australia and in South-east Asia. Training programmes in laboratory practice have been carried out in South-east Asia and other areas since 1989.

**Results:** Between 2001 and 2003 6 external quality assessment exercises for HIV testing were conducted in 29 countries of South-east Asian and Western Pacific Regions. The error rates overall were 1.6%. False reactivity (3%) was higher than false negative results (0.4%). Rapid tests gave higher error rates than EIAs. Follow-up in laboratory visits and workshops show that few laboratories have written management procedures or documents for the quality assurance of their tests and testing. Few understood principles of quality assurance for qualitative tests and the use of quality controls is infrequent. Repeat and parallel testing are not carried out. Training is lacking, 2nd readings are not carried out and checking for transcription errors could eliminate many mistakes.

**Conclusion:** Rapid tests perform well in quality managed facilities where quality assurance procedures are understood and practiced but the implementation of quality management along with quality assurance is not universal among users of rapid tests.

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HIV Diagnostics: New Developments and Challenges
Poster Abstracts

1 MULTISITE EVALUATION OF BIO-RAD MULTISPOT HIV-1/HIV-2 RAPID TEST TO DETECT AND DIFFERENTIATE HIV-1 FROM HIV-2 AND AS A STAND-ALONE MULTI-TEST HIV-1 ALGORITHM BASED ON SEPARATE RECOMBINANT AND PEPTIDE ANTIGEN SPOTS.
Bentsen, Christopher

2 CLINICAL TRIAL OF THE HEMA-STRIP HIV RAPID HIV TEST USING FINGER STICK BLOOD, WHOLE BLOOD, PLASMA, AND SERUM
Constantine, Niel

3 CLINICAL TRIAL OF TWO RAPID LATERAL FLOW TESTS FOR THE DETECTION OF HIV ANTIBODIES IN FINGER STICK WHOLE BLOOD, VENOUS WHOLE BLOOD, PLASMA AND SERUM
Estandiari, Javan

4 DEVELOPMENT OF A NEW LATEX-BASED LATERAL-FLOW ASSAY FOR RAPID DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS
Kanaujia, Ganga

5 MULTI-ANTI PRINT IMMUNOASSAY (MAPIA): A NOVEL IMMUNOSCREENING TOOL FOR HIV ANTIGENS AND CONFIRMATORY ASSAY FOR HIV INFECTION
Lyashchenko, Konstantin

6 MIRACURE RAPID HIV TEST FOR DETECTION OF HIV ANTIBODIES IN WHOLE BLOOD: REPORT OF A PILOT STUDY
Ratnam, Sam

7 RAPID HIV TESTING IN DELAWARE
Gorman, Robin

8 IMPLEMENTATION OF RAPID HIV TESTING AT PUBLICLY FUNDED CTS SITES IN NEW JERSEY
Cadoff, Evan

9 Transition Year, 2004, in HIV Antibody Testing at the New York City Department Health and Mental Hygiene’s Public Health Laboratory
Punsalang, Amado

10 The impact of training and field experience on non-laboratory staff confidence and skills for conducting rapid HIV testing in point of care settings.
San Antonio-Gaddy, Mara
HIV Diagnostics: New Developments and Challenges
Poster Abstracts

11 HIV RAPID TESTING AT FEDERALLY QUALIFIED HEALTH CARE CENTERS IN NEW JERSEY
       Paul, Sindy

12 A CASE SERIES OF DISCORDANT LABORATORY RESULTS WITH RAPID HIV TESTING
       Paul, Sindy

13 An Evaluation of Proficiency Testing by Laboratories Performing HIV Antibody Immunoassays
       Howerton, Devery

14 Confirmatory testing practices among participants in the CDC's model performance evaluation program for HIV rapid testing
       Williams, Laurina

15 Evaluation of the accuracy of the OraQuick Advance rapid HIV - 1/2 test in patients with suspected HIV infection in rural India
       Pai, Nitika Pant

16 EVALUATION OF ACCEPTABILITY, FEASIBILITY AND PREFERENCE FOR THE ORAQUICK ADVANCE RAPID HIV-1/2 TEST IN A RURAL PATIENT POPULATION IN INDIA.
       Pai, Nitika Pant

17 THE COST - EFFECTIVENESS OF HIV RAPID TESTING IN THE BRAZILIAN PUBLIC HEALTH SYSTEM
       Pascom, Ana Roberta Pati

18 Rapid HIV TESTING IN NEEDLE EXCHANGE CENTRES AND PRISONS IN FINLAND
       Brummer-Korvenkontio, Henrikki

19 Rapid HIV Kit Evaluation for Non-Laboratory Settings in Botswana: A Global AIDS laboratory Project
       Clark, Patricia

20 Detection of Incident HIV Infections using Rapid HIV Antibody Detection Assays
       Candal, Debra

21 Mobile Voluntary Counseling and Testing: How Valid are HIV Test Results?
       Mutura, Catherine
22 Performance of HIV-1 Enzyme-Immunoassays on Dried Blood Spots for Quality Control of HIV Testing
   Olara, Dennis

23 EARLY DIAGNOSIS OF HIV-1 INFECTION IN INFANTS USING DRIED BLOOD SPOTS AND REAL-TIME REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION
   Ou, Chin-Yih

24 PERFORMANCE CHARACTERISTICS OF THE ROCHE AMPLICOR 1.5 ASSAY FOR QUANTITATION OF HIV-1 RNA FROM WHOLE BLOOD COLLECTED ON FILTER PAPER
   Granade, Tim

25 Modified Protocols of Commercial Enzyme Immunoassays for the Detection of Antibodies to HIV-1 and HIV-2 from Whole Blood Collected on Filter Paper
   Granade, Tim

26 DEVELOPMENT OF AN ORAL FLUID ASSAY CAPABLE OF DIFFERENTIATING RECENT FROM ESTABLISHED HIV INFECTION
   Constantine, Niel

27 INCIDENCE IMMUNOASSAY FOR DISTINGUISHING RECENT FROM ESTABLISHED HIV-1 INFECTION IN THERAPY NAÏVE POPULATIONS
   Dax, Elizabeth

28 METHOD COMPARISON OF THE HIV TESTS FOR RECENT INFECTION
   Garrett, PE

29 USE OF THE HIV-1 BED INCIDENCE EIA AND WESTERN BLOTS TO CHARACTERIZE REJECTED BLOOD DONOR SPECIMENS
   Gottfried, Toby

30 IDENTIFICATION OF RECENT HIV INFECTIONS IN SINGLE SERUM SAMPLE: COMPARISON BETWEEN THE AVIDITY INDEX METHOD AND THE SEROLOGIC TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION (STARHS)
   Martró, Elisa

31 RECENT HIV INFECTIONS IN HETEROSEXUALS: IS STARHS APPLICABLE?
   Murphy, G
HIV Diagnostics: New Developments and Challenges
Poster Abstracts

32 MEASURING ANTIBODY AVIDITY IMPROVES THE UTILITY OF STARHS IN IDENTIFYING RECENT HIV INFECTION
Murphy, G

33 ENHANCING DIAGNOSTIC DATA FOR HIV SURVEILLANCE: THE ONTARIO LABORATORY ENHANCEMENT STUDY (LES)
Remis, Robert

34 COMPARISON OF LESS SENSITIVE HIV INCIDENCE TESTS FOR THE SEROLOGICAL TESTING ALGORITHM FOR RECENT HIV SEROCONVERSION (STARHS).
Schiffer, Jarad

35 Comparison of three methods of extraction of RNA for the detection of human immunodeficiency virus viral load in human plasma
Asthana, Deshratn

36 COMPARISON OF SENSITIVITIES BETWEEN DNA AND RNA NUCLEIC ACID TESTS FOR THE DETECTION OF HIV-1 INFECTION
Luo, Wei

37 NAAT SCREENING via MULTISTAGE POOLING FOR ACUTE HIV – 1 INFECTION
McPherson, Todd

38 REAL-TIME HIV - 1 PLASMA VIRAL LOAD ASSAY
Wei, Xierong

39 REAL-TIME RT-PCR BASED ASSAY ON BLOOD CLOT SPECIMENS FOR DIAGNOSIS OF HIV-1 INFECTION IN CHILDREN, MALAWI
Yang, Chunfu

40 LOWERING THE DETECTION LIMITS OF HIV-1 VIRAL LOAD USING REAL-TIME IMMUNO-PCR FOR HIV-1 P24 ANTIGEN
Constantine, Niel

41 CD4 T-Lymphocytes Counting in a Northern Nigerian Setting: Comparison of a New Affordable Flow Cytometric and Manual Magnetic Bead Techniques
Imade, Godwin
HIV Diagnostics: New Developments and Challenges
Poster Abstracts

42  EXTENSIVE REACTIVITY OF SVCPZ, HIV-1 GROUPS N AND O V3 PEPTIDES WITH ANTI-HIV SERO-NEGATIVE PLASMA SAMPLES FROM INDIVIDUALS LIVING IN VILLAGES IN THE EQUATORIAL RAIN FOREST REGIONS IN CAMEROON WHERE A BROAD HIV-1 GENETIC DIVERSITY EXIST: IMPLICATIONS IN HIV DIAGNOSIS
Nyambi, Phillipe

43  MULTICODE RTX FOR QUANTIFYING MIXED POPULATIONS OF DRUG RESISTANT HIV
Prudent, James

44  Use of dried blood spot (DBS) or serum spot to detect recent HIV-1 Seroconversion by HIV-1 BED Incidence Assay
Phillips, Susan

45  Development of a Rapid HIV-1 Confirmatory Test
Kardos, Keith
MULTISITE EVALUATION OF BIO-RAD MULTISPOT HIV-1/HIV-2 RAPID TEST TO DETECT AND DIFFERENTIATE HIV-1 FROM HIV-2 AND AS A STAND-ALONE MULTI-TEST HIV-1 ALGORITHM BASED ON SEPARATE RECOMBINANT AND PEPTIDE ANTIGEN SPOTS.

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Background/Objective: Rapid HIV tests provide timely and accurate test results. Algorithms for HIV confirmation using combinations of rapid, simple assays have been proposed by the WHO. The utility of Multispot for differentiating HIV-1 from HIV-2 and as a stand-alone multi-test assay for HIV-1 was analyzed based on results from its separate spots with recombinant HIV-1, synthetic HIV-1, and synthetic HIV-2 antigens.

Methods: A total of 3146 fresh plasma and 2325 fresh serum samples from 3146 subjects was tested at 7 locations in the U.S.; matched serum and plasma samples from 801 known HIV-1 positive patients; 620 serum samples and 1441 plasma samples from high-risk subjects; and 905 matched serum and plasma samples from low-risk subjects. A panel of 203 worldwide HIV-1 positive samples was also tested. In addition, a total of 201 frozen serum and plasma samples from known HIV-2 positive patients was tested, as well as 500 frozen serum samples collected from West Africa. The intensity of reactivity for each Multispot HIV-1 and HIV-2 antigen spot was graded on a scale of 0 to 4, and compared to the intensity of the procedural control spot.

Results: 1903 samples (801 known HIV-1 positive serum, 801 known HIV-1 positive plasma, 70 plasma from high-risk subjects, 28 serum from high-risk subjects, and 203 worldwide HIV-1 positive samples) were positive by HIV-1 Western blot and 207 samples were positive by HIV-2 Western blot. All were detected by Multispot (100% sensitivity for both HIV-1 and HIV-2). Multispot specificity, based on blot-negative samples from both low and high-risk subjects, was 1494/1495 (99.93%) with fresh serum samples and 2272/2274 (99.91%) with fresh plasma samples. Multispot's ability to differentiate HIV-1 from HIV-2 was evaluated in 1071 samples positive by HIV-1 Western blot only (870 U.S. plasma samples and 201 worldwide samples) and 109 samples positive by HIV-2 Western blot only. Multispot correctly identified 1070/1071 (99.91%) samples as HIV-1 and 107/109 (98.16%) samples as HIV-2; the 3 remaining samples were HIV dually reactive on Multispot. Spot intensity was evaluated in fresh-paired serum/plasma samples from 871 patients known to be HIV-1 positive or HIV-1 Western blot positive. 832 patients (96%) showed strong (3+ to 4+) reactivity to both HIV-1 antigen spots; the remaining 39 HIV-1 positive patients were positive on both of the HIV-1 antigen spots, but with <3+ reactivity on one or both spots. The 4 Multispot false-positive samples from 3 subjects in the specificity study had only weak (1+) reactivity on one or both of the HIV-1 antigen spots. Strong reactivity on both Multispot HIV-1 antigens provides 100% PPV with positive HIV-1 Western blot results or known HIV-1 infection.

Conclusions: Multispot is a sensitive and specific rapid test for the detection of HIV-1 and HIV-2 antibodies, and can reliably differentiate HIV-1 infection from HIV-2. In addition, Multispot could serve as a multi-test algorithm for HIV-1 in a single device when there is strong (3+ to 4+) reactivity to both HIV-1 antigen spots.

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**CLINICAL TRIAL OF THE HEMA-STRIP HIV RAPID HIV TEST USING FINGERSTICK BLOOD, WHOLE BLOOD, PLASMA, AND SERUM**

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**Background/Objectives:** Rapid HIV tests are indispensable for providing results in a clinically-relevant time frame for occupational exposures, women in labor without known HIV status, and in public health clinics to maximize the reporting of results to subjects. Our objectives were to evaluate the performance characteristics of a novel rapid HIV test in a large number of persons from several populations, and to determine the concordance of results when using several different blood matrices.

**Methods:** Seven sites were selected to evaluate the Hema-Strip HIV rapid test (SDS). A total of 1,515 participants included 504 at low risk for HIV, 511 at high risk, and 500 persons known to be infected with HIV. Overall, the sensitivity was based on 568 true HIV positive cases and specificity was based on 947 true negative cases. The Hema-Strip HIV rapid test is a lateral flow, one-step immunoassay that incorporates a protein A colloidal gold conjugate, includes a procedural control, and provides results within 15 minutes. Results were compared with FDA-licensed EIA and Western blot assays.

**Results:** The Hema-Strip HIV rapid test produced 566 positive results and 949 negative results. This resulted in an overall sensitivity of 99.5% (95% CI: 98.5, 99.9) and an overall specificity of 99.9% (95% CI: 99.4, 100), thereby exceeding the 98% lower bounds of acceptable performance. The positive and negative predictive values among all populations were estimated at 99.8% and 99.7%, respectively. The sensitivities for the different blood matrices were between 99.5 and 99.7%, and the specificities were all 99.9%. Only one site produced sensitivity values below 99%, and all sites produced specificity values above 99.1%

**Conclusions:** The Hema-Strip HIV rapid test has exhibited excellent performance characteristics when evaluated at multiple sites and using several blood matrices. The test meets expectations for use in low risk, high risk, and HIV positive populations. The Hema-Strip HIV rapid test is simple to perform, requires minimal procedural steps, and is suitable for point of care testing applications.

**Presenter:** Dr. Niel T. Constantine  
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**Institution:** University of Maryland, Baltimore, School of Medicine
CLINICAL TRIAL OF TWO RAPID LATERAL FLOW TESTS FOR THE DETECTION OF HIV ANTIBODIES IN FINGERSTICK WHOLE BLOOD, VENOUS WHOLE BLOOD, PLASMA AND SERUM

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Background/Objective:
The objective of the clinical trial is to establish the performance characteristics of the Chembio rapid tests, HIV Stat-Pak and Sure Check HIV based on comparison to an FDA approved EIA for detection of antibodies to Human Immunodeficiency Virus. This trial assessed the performance of the assays in order to document that these products meet regulatory guidance with the respect to rapid tests for detection of antibodies to HIV. The Chembio rapid tests are quick and single-use application and are intended for use in the filed as well as professional use settings.

Methods:
Five geographically diverse clinical sites, all in the United States, enrolled a total of 2,700 participants from three risk groups: Individuals known to be positive for HIV (1,101), individuals at high risk for infection (898) and individuals at low risk for infection with HIV (701). Each participant was tested with the HIV Stat-Pak and Sure Check HIV devices at the clinical sites. Four sample matrices were tested: whole blood from fingerstick, venous whole blood collected in EDTA, serum and plasma (collected in EDTA anticoagulant). Samples from each participant were sent to a reference laboratory for confirmatory (EIA1 and Western Blot) and discrepancy resolution testing (EIA1, EIA2, WB and NAT).

Results:
HIV Stat-Pak and Sure Check HIV showed an overall sensitivity of 99.8% (1,136/1,138) and an overall specificity of 99.9% (1,551/1,553). Sensitivity is derived from the known positive and High Risk population. Specificity is derived from the Low Risk and High Risk population.

Conclusions:
Both Chembio rapid tests, the HIV Stat-Pak and the Sure Check HIV, achieve the study objectives. The performance characteristics of Chembio tests were found to be over 99%.
These results have not yet been evaluated by FDA.

Presenter: Javan Esfandiari, R&D Director E-mail: jesfandiari@chembio.com
Institution: Chembio Diagnostic Systems, Inc.
DEVELOPMENT OF A NEW LATEX-BASED LATERAL-FLOW ASSAY FOR RAPID DETECTION OF ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS

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Background/Objective: Despite of valuable efforts in the field of HIV diagnosis, early detection of infection remains a problem. Several screening and confirmatory tests are available in the market but most of them have low sensitivity when evaluated with seroconversion panels. We have developed a new rapid test based on recombinant antigens of HIV-1 and HIV-2 that can detect both IgM and IgG antibodies at earlier stages of infection.

Methods: Over 40 recombinant antigens and synthetic peptides of HIV1/2 from different sources were screened by MAPIA (multi-antigen print immunoassay; for more details, see our separate poster). Commercially available HIV1/2 serum panels were used to identify most potent antigenic reagents. A cocktail of antigens was conjugated to blue latex particles, immobilized on membrane, and test strips were made. A blue line visible on the strip within 15 minutes after sample application indicated a positive result. Test performance was evaluated with several seroconversion as well as low titer HIV-1 serum panels (Boston Biomedica, Inc.).

Results: Based on MAPIA studies, we selected recombinant gp41 and gp120 for HIV-1, and gp36 for HIV-2 antibody detection. We have also incorporated a multiepitope chimeric HIV1/2 antigen which had a superior sensitivity. The rapid test showed 100% agreement with Abbott HIV1/2 EIA and 99.5% specificity, based on testing 241 HIV-negative blood bank sera.

Conclusions: We developed a new latex-based lateral-flow test. Its sensitivity was found to be equal to that of Abbott HIV1/2 EIA and greater than the sensitivity of western blot assays when used with seroconversion panels. The rapid test is an excellent alternative to conventional HIV antibody testing methods, especially for detection of recent HIV infections in remote areas.

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Institution: Chembio Diagnostic Systems, Inc.
### Poster Abstract 5

**MULTI-ANTIGEN PRINT IMMUNOASSAY (MAPIA): A NOVEL IMMUNOSCREENING TOOL FOR HIV ANTIGENS AND CONFIRMATORY ASSAY FOR HIV INFECTION**  
Konstantin Lyashchenko, Javan Esfandiari, Ganga Kanaujia, David Greenwald, Rena Greenwald.  
Chembio Diagnostic Systems, Inc., Medford, NY.

**Background/Objective:**  
Multi-Antigen Print ImmunoAssay (MAPIA) has been recently designed to identify serodiagnostically important antigens and characterize antibody responses in infectious diseases. The method is based on immobilization of multiple antigens to nitrocellulose membranes, followed by antibody detection using standard chromogenic immunodevelopment. We utilize MAPIA routinely for screening, evaluation and comparison of different HIV antigens. It can also be used as a confirmatory assay for HIV infection.

**Methods:**  
A panel of 23 recombinant HIV proteins, synthetic peptides and polyepitope fusions from different suppliers were evaluated using HIV-1 and HIV-2 positive sera, HIV negative sera, as well as seroconversion panels. Antigens were immobilized on nitrocellulose membrane as narrow bands using a semi-automatic air-brush printing device (Linomat IV, Camag). Strips were cut perpendicular to the antigen bands, blocked, incubated with serum samples, and immunodeveloped using standard chromogenic methods. A 15x15 cm nitrocellulose membrane sheet could accommodate as many as 50 antigens and could be used to test 50 serum samples in one experiment. Results were evaluated visually and by semi-quantitative densitometry.

**Results:**  
The MAPIA data demonstrated the variability of antigen recognition patterns in the serum samples. Recombinant proteins and polyepitope fusions showed superior performance over the synthetic peptides, in particular with early positive samples of seroconversion panels. Differentiation between infections due to HIV-1 and HIV-2 was detected. The results suggested that obtained with BBI serum panels MAPIA employing carefully selected antigens could be also used as a confirmatory assay for HIV infection. In this application, MAPIA may combine HIV-1 and HIV-2 antigens in one confirmatory assay.

**Conclusions:**  
MAPIA offers an efficient and cost-effective method for screening of multiple antigens. The assay is highly reproducible, sensitive and specific. MAPIA can easily evolve into a rapid lateral-flow test format for diagnosis of HIV infection. It can also be used as a confirmatory assay for HIV infection.

**Presenter:** Konstantin Lyashchenko, Ph.D.  
**Email:** klyashchenko@chembio.com  
**Institution:** Chembio Diagnostic Systems, Inc.
MIRACARE™ RAPID HIV TEST FOR DETECTION OF HIV ANTIBODIES IN WHOLE BLOOD: REPORT OF A PILOT STUDY
Sam Ratnam
Public Health Laboratory, St. John’s, NL, Canada

Background: Rapid HIV testing can improve patient care and HIV prevention services. The MiraCare™ Rapid HIV Test is a new test developed by MedMira Laboratories based on the same principle as the FDA approved Reveal G2 Rapid HIV-1 Serum/Plasma Test (MedMira Laboratories Inc, Halifax), which detects HIV-1 antibodies in whole blood within 3 minutes. We carried out a pilot study to determine the performance of the MiraCare™ Rapid HIV Test as a point-of-care (POC) test using finger-prick blood samples.

Materials and Methods: A total of 205 patients were enrolled in the study through 3 STD/HIV clinics and included confirmed HIV positive cases being cared for at the HIV clinic. Finger-prick blood samples were tested onsite using the MiraCare™ Rapid HIV Test. Venipuncture blood samples were obtained from all subjects for standard HIV testing at the Public Health Laboratory (PHL). At the PHL, reference testing of whole blood and plasma samples was done using the MiraCare™ Rapid HIV Test in parallel with the Abbott Axsym EIA HIV screen and Western blot as the standard tests. Comparing the POC finger-prick testing results with those obtained at the PHL assessed the performance of the MiraCare™ Rapid HIV Test.

Results: Of the 205 patients, 74 were confirmed to be HIV-1 positive cases, and the remaining 131 tested negative for HIV-1/HIV-2 antibodies by the reference EIA screen. The MiraCare™ Rapid HIV Test yielded strong reactive results for 72 of the 74 confirmed positive cases and weak reactive results for the remaining 2, with both POC finger-prick blood and the PHL reference testing using whole blood and plasma. With the 131 reference EIA negative cases, the MiraCare™ Rapid HIV Test was negative in all instances with no discrepant results.

Conclusions: The MiraCare™ Rapid HIV Test showed 100% agreement with the reference method with both POC finger-prick and venipuncture whole blood samples. This data provides evidence that the MiraCare™ Rapid HIV Test can be used for POC screening using finger-prick blood samples. Also of significance is the fact that the MiraCare™ test did not have a single false positive result. This attests to its very high specificity, and bodes extremely well for its application in mass screening programs where the potential for false positive results are of a major concern. The MiraCare™ Rapid HIV Test is simple, easy to carry out, and truly rapid. Further studies are needed to provide additional data to substantiate the safety and effectiveness of this test for POC testing using finger-prick blood samples.

Presenter: Dr. Sam Ratnam
Email: Sam.Ratnam@hccsj.nl.ca
Institution: Public Health Laboratory, St. John’s, NF., Canada
Rapid HIV Testing in Delaware
Frederick P. Franze, MT (ASCP), Robin L. Gorman, MLT (ASCP), Jane P. Getchell, Dr P H

Background

Delaware began rapid HIV-1 antibody testing in March 2003 at one of the state’s 4 STD clinics. All four sites were performing the test by June of 2003. Each of these clinics conducts moderate complexity testing under a single CLIA certificate that allows multiple laboratories to engage in limited public health testing under one certificate. Prior to implementation, the HIV program office worked with the Public Health lab to develop a comprehensive Quality Assurance program for rapid HIV testing. The Technical Supervisor (TS) for the STD clinics developed appropriate logs and training materials. Clinics were trained and authorized to begin testing one at a time. Beginning Dec 04, a Community Based organization was authorized to begin testing through a contract with the HIV program office. This site is required to follow the same training and QA as the state.

Methods

Documents used for rapid testing (training, QC, etc) were designed utilizing the CDC QA guidelines. Orasure Technologies provided training to our TS, who then provided training to all public health staff involved in testing. Training was conducted in the following manner: first the lab staff was trained to perform the test and competency assessment was documented, then the HIV program office held a meeting with lab staff, clinicians and counselors to discuss protocols for testing and reporting. The first 100 patients tested using rapid tests were backed up with ELISA to validate the method. The Virology lab put together several sets of “unknowns” for Proficiency testing. All staff wishing to perform rapid testing were required to successfully interpret the results of these samples. Quality Assessment consists of the following:

- on-going QC measures as required by the manufacturer
- Proficiency testing using the CAP Anti-HIV-1 antibody – waived survey (RHIWV)
- CDC MPEP for rapid testing
- Semi-annual inspections of the 5 labs involved in testing by the TS

All Preliminary Positive rapid tests are sent to the state public health lab for confirmatory Western Blot test.

Results

For calendar year 2004, Delaware tested 6,141 patients, with 78 confirmed Preliminary Positives and 2 false positives rapid tests. All 5 testing locations successfully passed all proficiency testing surveys and no erroneous results due to technician error were reported.

Presenter: Robin L. Gorman
Email: Robin.Gorman@state.de.us
Institution: Delaware Public Health Laboratory
IMPLEMENTATION OF RAPID HIV TESTING AT PUBLICLY FUNDED CTS SITES IN NEW JERSEY

E. M. Cadoff1, S. M. Paul2, F. Jackson1, M. Wolski2, L. Nichol2, R. Williams2, K. Stralkus1, G. Salaru1, E. G. Martin1.

1RWJMS, New Brunswick, NJ, 2NJDHSS/DHAS, Trenton, NJ.

Background/Objective: Efforts by public health authorities to control the spread of HIV in the United States have been frustrated by the inability to provide HIV testing and results in a single client encounter. The New Jersey Department of Health and Senior Services, Division of HIV/AIDS Services (DHAS) funds Counseling and Testing Sites (CTS) that provide free, confidential HIV testing. The CTS sites employ state-trained HIV counselors with no laboratory background. During 2002, approximately 35% of over 70,000 clients visiting these CTS centers did not receive their results because they failed to return for a scheduled follow-up visit. Recently, the FDA approved the first CLIA waived, rapid (fingerstick) point-of-care test for HIV (OraQuick Rapid HIV-1 Antibody test, OraSure Technologies, Inc., Bethlehem, PA). With POCT testing, it is hoped that nearly 100% of clients will receive their results, appropriate counseling, and immediate referral for care and treatment, if needed. FDA approval included a contingency that mandated a Quality Assurance program be in place before testing is offered. In New Jersey, state licensure is also required. In September 2003, DHAS and UMDNJ-Robert Wood Johnson Medical School (RWJMS) started to set up a program to bring point-of-care-testing (POCT) to the CTS sites using OraQuick. Specific objectives were 1) to implement a program that included appropriate quality assurance safeguards, and 2) to bring to near 100%, the percentage of clients who receive their test results.

Methods: By expanding on the existing multi-facility POCT program at RWJMS, a statewide implementation plan was developed, consistent with the CDC's quality assurance guidelines (www.cdc.gov/hiv/rapid_testing/materials/QA-Guide.htm), state regulations, and accepted standards of laboratory medicine. The program is managed centrally by a state licensed and board certified pathologist. A centralized core staff of clinical laboratorians are responsible for: development of uniform policies and procedures, staff training and re-certification, reagent inventory control and validation, standardization and validation of equipment, review of mandated and supplemental proficiency testing, bulk management of lab supplies, and a core communication hub (www.njhiv.org). The core staff monitors compliance with key policies using an A,B,C,D,F grading scale, and works with site coordinators to improve compliance.

Results: Under this program, OraQuick HIV testing began in November, 2003, at the New Brunswick CTS. Using the “Plan, Do, Check, Act” Performance Improvement model, procedures were modified and then rolled out to 14 additional sites. Further expansion has brought the program to over 50 satellite locations, with at least 150 sites expected by the end of 2005. Compliance with quality assurance procedures has resulted in fully meeting CLIA standards. External proficiency test performance has been at 100%. Grades for compliance with other procedures not required by CLIA, such as evidence of regular review of records by site coordinators and documentation of corrective action, have shown a decrease in ‘Fs’ and an increase in ‘As’ over time. Of the first 8,000 clients tested under this program, 99.9% have received their HIV test results.

Conclusions: Based on the success of rapid testing thus far, DHAS plans to expand rapid testing to 179 publicly funded counseling and testing sites statewide.

Presenter: Evan M. Cadoff, MD
Email: cadoff@umdnj.edu
Institution: Robert Wood Johnson Medical School; New Jersey Dept of Health and Senior Services
Transition Year, 2004, in HIV Antibody Testing at the New York City Department of Health and Mental Hygiene’s Public Health Laboratory.
Amado Punsalang, Ph.D., Mona El-Fishawy and William R. Oleszko, Ph.D.
New York City Department of Health and Mental Hygiene, Public Health Laboratory

OBJECTIVE: To monitor and analyze specimens submitted to the NYC DOHMH’s PHL for HIV antibody testing, as HIV-1 EIA rapid assays are integrated into routine clinic testing, and to track rapid HIV-1 assay specificity using the latest conventional HIV antibody laboratory assays.

METHODS: As NYC’s public and private HIV testing clinics transition from conventional laboratory-based testing to rapid HIV testing systems, a code system was implemented to identify specimens submitted to the PHL as being Oraquick HIV-1 preliminary positive. The Oraquick preliminary positive specimens were retested using BioRad’s HIV-1/HIV-2 EIA and HIV-1 Western Blot assays. Specimens that were concordant as reactive by both assays were reported as HIV antibody positive, those concordant as nonreactive were reported as HIV antibody negative, and discordant test results were reported as HIV-1 antibody inconclusive. None of these submissions demonstrated HIV-2 reactivity.

RESULTS: Integration of the Oraquick HIV-1 EIA rapid assay at NYC public and privately managed HIV test sites in 2004 resulted in a net decrease of approximately 14% from that of 2003. The below table illustrates the initial 6 categories of health care centers transitioning to rapid HIV antibody testing, their 2004 submissions to the PHL for confirmatory testing and the confirmation rates for each.

<table>
<thead>
<tr>
<th>SPECIMEN SOURCE</th>
<th>PHL REPORTED HIV ANTIBODY RESULT</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>POSITIVE</td>
<td>NEGATIVE</td>
</tr>
<tr>
<td>STD-Confidential</td>
<td>120 (87%)</td>
<td>17 (12.3%)</td>
</tr>
<tr>
<td>Correctional Health</td>
<td>98 (97%)</td>
<td>2 (2.0%)</td>
</tr>
<tr>
<td>STD-Anonymous</td>
<td>84 (96.6%)</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Community Based Orgs.</td>
<td>32 (88.8%)</td>
<td>2 (5.6%)</td>
</tr>
<tr>
<td>HHC (Labor &amp; Delivery)</td>
<td>19 (90.5%)</td>
<td>2 (9.5%)</td>
</tr>
<tr>
<td>Chest Clinics</td>
<td>5 (83.3%)</td>
<td>1 (17.7%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>358 (92.0%)</td>
<td>25 (6.4%)</td>
</tr>
</tbody>
</table>

CONCLUSION: During 2004, clinics began to transition into rapid HIV EIA testing systems. The following observations were made during this initial year: (1) the total number of specimens submitted for HIV antibody screening decreased by approximately 14%; (2) an initial differential appeared in the confirmation rates for specimens submitted from confidential and anonymous public STD sites even though these clinics are located in the same facilities and are differentiated only by the clients preference for confidential verses anonymous testing; (3) Correctional Health submissions varied widely from week to week due to test kit inventory inconsistencies; and (4) high volume clinics demonstrated slightly greater confirmation rates (92.9%) than low volume clinics (88.8%).
Title: THE IMPACT OF TRAINING AND FIELD EXPERIENCE ON NON-LABORATORY STAFF CONFIDENCE AND SKILLS FOR CONDUCTING RAPID HIV TESTING IN POINT-OF-CARE SETTINGS.
San Antonio-Gaddy, M.; Richardson-Moore, A.; O’Connell, D.
New York State Department of Health, AIDS Institute

Background/Objective: Same-day rapid HIV testing in point-of-care settings requires training for non-laboratory trained HIV test counselors in test procedures, quality control, and new counseling message strategies to ensure quality testing outcomes.

Methods: The NYS Department of Health developed and conducted a 2-day training, for non-laboratorians, which consisted of instruction on counseling with rapid HIV testing and the provision of same-day results; rapid HIV test demonstration; running test controls; skill-building exercise and proficiency assessments.

Results: Staff surveys were collected from forty-seven HIV test counselors that were trained, at pre- and post-training and at 12 weeks after testing was implemented. Staff demonstrated an increase in knowledge of the rapid HIV testing process and product insert. Counselor confidence levels increased significantly from pre-training to post-training/12 week follow-up in all rapid testing skill categories. At the 12-week follow-up, 100% of counselors felt “very comfortable” interpreting test results, explaining the result to the client, and performing the finger stick. Feedback was collected from HIV test counselors on the various training tools and procedural methods used. The laminated test result card was noted as the most useful tool in training and in the field. Proficiency testing conducted at 12-weeks after the initial training demonstrated counselors tested and interpreted 100% of the samples correctly.

Conclusions: Training of HIV test counselors prior to implementation of rapid HIV testing is critical and should consist of education, skills-building exercises and competency evaluation. Provisions of a guidance manual and ongoing quality assurance through routine, periodic supervision and field observation are key elements to the continued success of the program. Counselors’ confidence improved after training and more markedly after field experience. Initial apprehension about counseling diminished quickly, and counselors were able to perform rapid tests accurately.

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Institution: New York State Department of Health
Presenter: April Richardson-Moore, E-mail: alr02@health.state.ny.us
Institution: New York State Department of Health
HIV Rapid Testing at Federally Qualified Health Care Centers in New Jersey
Sindy M. Paul, M.D., M.P.H.,1 Evan Cadoff, M.D.2, Eugene Martin, Ph.D.2, Maureen Wolski,1 Lorhetta Nichol1, Rhonda Williams1, Phil Bruccoleri1, Aye Maung Maung, Charles Taylor, Rose Marie Martin, M.P.H.1, Linda Berezny, RN1
1 – New Jersey Department of Health and Senior Services, Trenton, New Jersey
2 – Robert Wood Johnson Medical School, New Brunswick, New Jersey

Background/Objective: The New Jersey Department of Health and Senior Services, Division of HIV/AIDS Services, (NJDHSS DHAS), introduced rapid HIV testing at selected publicly funded HIV counseling and testing sites to improve the proportion of high risk persons testing for HIV and to increase the proportion of people who learn their test results. The purpose of this abstract is to highlight the successful introduction of rapid HIV testing at two federally qualified health care centers.

Methods: Staff at publicly funded HIV counseling and testing sites received counseling training, rapid HIV testing training, completed competency testing and passed proficiency testing prior to offering rapid HIV testing. All rapid HIV testing sites were licensed by NJDHSS. Data were collected using the standard Centers for Disease Control and Prevention (CDC) counseling and testing form.

Results: Henry J. Austin Health Care Center started rapid HIV testing on May 21, 2004 and the Plainfield Community Health Center started on June 1, 2004. As of September 28, 2004, 654 tests have been done. Six of the 654 (1%) patients tested positive. All 654 (100%) patients received posttest counseling and test results. One patient had a preliminary positive rapid test and a negative Western blot. The demographic results are provided below:

<table>
<thead>
<tr>
<th>GENDER</th>
<th>OVERALL</th>
<th>%</th>
<th>NEGATIVE</th>
<th>POSITIVE</th>
<th>DISCORDANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>218</td>
<td>33</td>
<td>215</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Female</td>
<td>436</td>
<td>67</td>
<td>432</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGE</th>
<th>OVERALL</th>
<th>%</th>
<th>NEGATIVE</th>
<th>POSITIVE</th>
<th>DISCORDANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 – 19</td>
<td>74</td>
<td>11</td>
<td>74</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20 - 29</td>
<td>292</td>
<td>45</td>
<td>289</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>30 - 39</td>
<td>156</td>
<td>24</td>
<td>154</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>40 - 49</td>
<td>87</td>
<td>13</td>
<td>86</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>45</td>
<td>7</td>
<td>44</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RACE</th>
<th>OVERALL</th>
<th>%</th>
<th>NEGATIVE</th>
<th>POSITIVE</th>
<th>DISCORDANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>107</td>
<td>16</td>
<td>107</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Black</td>
<td>317</td>
<td>48</td>
<td>314</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>199</td>
<td>30</td>
<td>196</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>AS/PI</td>
<td>2</td>
<td>&lt;1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>19</td>
<td>3</td>
<td>18</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusions: Rapid HIV testing provides a way to integrate HIV counseling and testing into routine care. All the patients learned their test results compared with a statewide average of 65% of patients when non-rapid HIV testing is done. Advantages of rapid HIV testing in a clinical setting include, same day results, immediate entrance into treatment, and more patients knowing their HIV status.

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Institution: New Jersey Department of Health & Senior Services
Division of HIV/AIDS Services
A Case Series of Discordant Laboratory Results with Rapid HIV Testing

Sindy M. Paul, M.D., M.P.H.,1 Evan Cadoff, M.D.2 Eugene Martin, Ph.D.2, Maureen Wolski,1 Lorhetta Nichol1, Rhonda Williams1, Phil Bruccoleri1, Aye Maung Maung1, Rose Marie Martin, M.P.H.1, Linda Berezny, R.N.1
1 – New Jersey Department of Health and Senior Services, Trenton, New Jersey
2 –UMDNJ - Robert Wood Johnson Medical School, New Brunswick, New Jersey

Background/Objective: The New Jersey Department of Health and Senior Services, Division of HIV/AIDS Services, (NJDHSS DHAS), introduced rapid HIV testing at selected publicly funded HIV counseling and testing sites to improve the proportion of high risk persons testing for HIV and to increase the proportion of people who learn their test results. The purpose of this abstract is to describe a case series of patients with discordant results.

Methods: Staff at publicly funded HIV counseling and testing sites received counseling training, rapid HIV testing training, completed competency testing and passed proficiency testing prior to offering rapid HIV testing. All rapid HIV testing sites were licensed by NJDHSS. All rapid HIV testing were completed using OraQuick, (Orasure Technologies, Inc. Bethlehem, PA), with confirmatory testing via Western blot at the NJDHSS laboratory. The protocol for discordant results included a repeat HIV by OraQuick 4-6 weeks after the initial positive OraQuick result, submission of the original HIV confirmatory specimen to the CDC for independent confirmation of the original negative Western blot, collection of additional serum for hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), HIV by standard enzyme immunoassay, Epstein-Barr virus (EBV), and Rheumatoid factor (RF); collection of additional plasma for ultra-sensitive, quantitative RNA determination of HIV. Demographic data were collected using the standard Centers for Disease Control and Prevention (CDC) counseling and testing form.

Results: Rapid HIV testing started at one publicly funded counseling and testing site in New Jersey on November 1, 2003. Through December 9, 2004, 40 sites were conducting rapid HIV testing with 9,181 tests completed. Four (0.04%) of these patients met the definition of a discordant case with a preliminary positive rapid HIV test and a negative Western blot. Two of these patients were pregnant, both immigrants from Central America. One of these patients refused follow-up testing citing a stable, monogamous relationship with a spouse who tested negative. Another patient declined follow-up testing after confirming his negative EIA status with an ID specialist; one patient agreed to return for follow-up testing, but failed to return; two patients returned for follow-up testing. Both tested HIV negative by traditional enzyme immunoassay and both were repeating OraQuick HIV positive upon re-examination 4-6 weeks later. The one patient completing the discordant protocol was repeatedly positive by OraQuick and negative by enzyme immunoassay and ultrasensitive RNA analysis. This patient was hepatitis A virus polyclonal antibody positive, but had no indications of acute hepatitis A, B or C infection. Rheumatoid factor was within the reference range. There was evidence of a distant EBV infection with IgG antibodies to EB nuclear antigen, but no detectable IgM antibodies to viral capsid antigen, or IgG antibodies to early D antigen.

Conclusions: Rapid HIV testing is a reliable, reproducible screening test for use in publicly funded HIV counseling and testing sites. The observed false positive rate of 0.04% in sites run with a rigorous QA program is acceptable to continue to offer rapid HIV testing. Analysis of discordant results to date suggests that non-specific interferences may play a significant role in the rare instances in which OraQuick results are not confirmed by Western Blot testing.

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Institution: New Jersey Department of Health & Senior Services
Division of HIV/AIDS Services
An Evaluation of Proficiency Testing by Laboratories Performing HIV Antibody Immunoassays
D.A. Howerton, Ph.D.*, Suzette A. Park, M.S. Centers for Disease Control and Prevention (CDC), Atlanta, GA 30341-3717

BACKGROUND: Under the Clinical Laboratory Improvement Amendment (CLIA) regulations, laboratories performing non-waived HIV antibody testing are required to participate in a proficiency testing (PT) program. Currently, ten PT programs for HIV antibody are CLIA-approved and five of them are active. Some of the PT programs have recently expanded to include rapid testing, even though some of the rapid tests are CLIA-waived. We were interested in reviewing the data available from PT programs to determine 1) the proportion of laboratories using the various non-waived test kits, 2) whether any performance problems were discernable, and 3) how the laboratories performed that reported results using rapid test methods.

METHODS: Two data sources were used for this evaluation. One source was the PT participant summary reports for surveys conducted during 2004 by the CLIA-approved PT providers. The second source was the Centers for Medicare & Medicaid Services (CMS) database that contains the CLIA laboratory demographics and the HIV antibody PT performance data reported by the PT providers to CMS. We extracted performance data from these two sources and calculated the rates of unacceptable results.

RESULTS: In 2004, a total of 2356 laboratories were enrolled in PT for HIV antibody according to CMS statistics. Of these, 82% were hospital (1529) and independent (395) laboratories. An analysis of CMS data available over the 10 year period 1994-2004 indicated that the rates of PT event failure ranged from 1% to 6% per year (failure is defined as a score of less than 80% correct results for a survey event). An evaluation of PT summary reports from the individual providers for surveys conducted during 2004, showed that for EIA testing 1) the most common test system used was the Abbott HIV 1/2 recombinant method, 2) in the College of American Pathologists’ surveys (the program with the largest enrollment), laboratories performing both the HIV-1 and HIV 1/2 EIA test methods demonstrated error rates of less than 1%, and 3) performance using rapid tests showed a relatively high rate of invalid results (up to 22% in some surveys) with false positive and false negative rates ranging from 0 to 10%, varying by survey provider. These results with rapid testing are most likely due to the composition of the challenge samples, some of which were diluted serum (matrix effects).

CONCLUSIONS: A review of the 2004 PT program summary reports and performance data submitted to CMS from 1994-2004 reveals changes in overall performance over time which may be reflective of the introduction of new technology, such as rapid testing, and the use of manipulated (e.g., diluted) samples that may not behave the same way in all test kits. As immunoassay technology changes, PT providers need to be aware that challenge samples acceptable for one type of method may not be acceptable for others.

Presenter: Devery Howerton  Email: DHowerton@cdc.gov
Institution: CDC
CONFIRMATORY TESTING PRACTICES AMONG PARTICIPANTS IN THE CDC’S MODEL PERFORMANCE EVALUATION PROGRAM FOR HIV RAPID TESTING

Laurina Williams1*, Leigh Vaughan1, David Cross1, Courtney Rodi2
1Centers for Disease Control and Prevention, Atlanta, GA; 2Constella Group, Inc., Atlanta, GA

BACKGROUND: Current CDC initiatives for HIV/AIDS prevention are aimed at reducing barriers to early diagnosis of HIV infection and increasing access to health services by encouraging the use of HIV rapid tests. Recommendations for confirming reactive HIV rapid test results have been published and we sought to learn if they are being followed. (http://www.phppo.cdc.gov/mpep)

METHODS: Univariate and bivariate analyses were performed on survey responses from a convenience sample comprised of all testing sites that participated in the CDC’s Model Performance Evaluation Program (MPEP) for HIV rapid testing in August 2004. Participants in the event tested a set of six challenge samples, and also answered questions about their laboratory testing practices.

RESULTS: Of the 384 participants, U.S. testing sites predominated (327; 85.2%). Of those most were hospital testing sites (227/327; 69%); 42 (18%) self-identified as “independent” or “other”; and 12 sites (3%) as community based organizations, sexually transmitted disease clinics, or drug treatment centers. 338 unique responses regarding confirmatory testing practices were reported. Testing sites using more than one type of rapid test could report more than once. A variety of confirmatory testing practices were observed. 3 testing sites reported that no confirmatory testing was required. 207 (61%) respondents indicated only referring specimens to another facility for confirmation. Of the 126 respondents indicating doing onsite confirmation testing, 58 (46%) of them appeared to have followed published recommendations. 19% (24/126) indicated using only EIA testing; 22 of those were hospital laboratories. 3% (4/126) used EIA in combination with a second rapid test (same kit). 14% (18/126) of respondents indicated that they used a second rapid test with no other type of confirmatory testing. Overall, 21% (71/338) of total respondents indicated confirmatory testing practices that did not include either WB, IFA, or referring tests out.

CONCLUSIONS: U.S. testing sites reported using a variety of confirmatory testing practices, some of which are not in compliance with current recommendations that either WB or IFA testing be used to confirm a preliminary positive HIV rapid test result. Follow up is needed to confirm these findings and to improve adherence to published recommendations. (www.cdc.gov/hiv/rapid_testing/materials/QA-Guide.htm; www.cdc.gov/mmwr/preview/mmwrhtml/mm5310a7.htm)

Presenter: Laurina O. Williams, Ph.D., MPH Email: low1@cdc.gov
Institution: CDC
**Poster Abstract 15**

**Evaluation of the accuracy of the OraQuick Advance® rapid HIV -1/2 test in patients with suspected HIV infection in rural India**

Nitika Pant Pai, Rajnish Joshi, Archana Wankhade, Sandeep Dogra, Madhukar Pai, DK Mendiratta, Pratibha Narang, SP Kalantri, Jacqueline P Tulsky, Arthur L Reingold

University of California, Berkeley & San Francisco; Mahatma Gandhi Institute of Medical Sciences, Sevagram, India

**Background:**
The new OraQuick Advance® rapid HIV 1/2 test has greatly expanded testing options for HIV by allowing oral mucosal fluid testing. We evaluated this test in patients with suspected HIV infection at a rural hospital in India. Our objective was to determine the diagnostic accuracy of the new OraQuick test using both oral mucosal transudate and finger prick specimens, and to evaluate them against the reference standard of repeat ELISA and Western Blot. In this preliminary report, we present data from the first 150 participants.

**Methods:**
In a cross-sectional study, we recruited patients with suspected HIV infection at the Mahatma Gandhi Institute of Medical Sciences, Sevagram, India. After pretest counseling, participants were administered two rapid tests (OraQuick on finger prick and oral fluid) in parallel, and blood was drawn for ELISA and Western Blot concurrently. The rapid test results were interpreted blinded to the reference standard results. Confirmatory testing with Western Blot was done for rapid test preliminary positives and ELISA positives. Rapid test and ELISA negatives were not tested further.

**Results:**
The median age of the participants was 33 years (range 18 - 88 years). A majority of patients (68% men) were from lower socioeconomic strata. Of the 150 participants, 38 (25%) were confirmed to have HIV infection using the reference standard. The oral OraQuick test had 100% (38 of 38) sensitivity and 100% (112 of 112) specificity. The finger prick OraQuick test had a sensitivity of 100% (38 of 38) and specificity of 99.1% (111 of 112). The agreement between the oral and finger prick OraQuick results was 99.3 (kappa=0.98 (95% CI 0.95, 1.0)).

**Conclusion:**
In our study, both finger prick and oral OraQuick tests had high accuracy. The oral test was found to be simple, convenient and easy to administer in our rural Indian hospital setting. We intend to evaluate these tests in a larger sample of participants, and also examine issues such as patient preference, feasibility and cultural acceptability.

**Presenter:** Nitika Pant Pai, MD, MPH  **Email:** nitika@berkeley.edu  **Institution:** Division of Epidemiology, University of California, Berkeley, CA.
Evaluation of acceptability, feasibility and patient preference for the OraQuick Advance® Rapid HIV-1/2 test in a rural patient population in India.

Nitika Pant Pai, Rajnish Joshi, Archana Wankhade, Sandeep Dogra, Madhukar Pai, Deepak K Mendiratta, Pratibha Narang, SP Kalantri, Jacqueline P Tulsky, Arthur L Reingold

University of California at Berkeley and San Francisco, Mahatma Gandhi Institute of Medical Sciences, Sevagram, India

Background:
The OraQuick Advance® HIV1/2 test, using oral mucosal transudate samples, has not been adequately evaluated in rural India. We conducted a cross sectional study in patients at high risk for HIV infection, attending a rural hospital in Central India. Our objective was to determine the acceptability, feasibility and preference of the study participants for HIV tests (rapid oral, rapid finger prick, ELISA and Western Blot). In this preliminary report, we present the results of the first 150 participants.

Methods:
Participants were administered two rapid tests in parallel, and blood was drawn for ELISA and Western Blot concurrently. Face to face interviews using questionnaires were conducted to assess acceptability, pretest and posttest experience, test preferences, and feasibility.

Results:
All the participants accepted testing after pretest counseling. A majority (78% of 150) favored oral tests to blood-based tests (finger prick and venepuncture). We questioned participants on discomfort experienced with various tests. 4% of the participants reported discomfort (itching, pain) with oral rapid test; 42% of the participants reported pain, bleeding, and fear of blood with finger prick rapid tests; 24% of the participants reported fear of blood draw, pain on repeat punctures, bleeding, swelling and pressure with use of tourniquet with venepunctures. A majority (66%) of the participants preferred the oral rapid test over blood-based tests. For re-testing, 78% of the participants preferred oral rapid tests. With respect to the timing of test results, a majority (66%) desired results in two weeks, and 17% desired results in one hour.

Conclusions:
From this preliminary data, it appears that oral fluid testing is culturally acceptable and is the preferred option in the rural Indian population we studied. Similar studies are needed in different regions of India to determine cultural acceptability, patient preference and feasibility for various HIV testing alternatives.

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Institution: University of California, Berkeley
THE COST-EFFECTIVENESS OF HIV RAPID TESTING IN THE BRAZILIAN PUBLIC HEALTH SYSTEM
Ana Roberta Pati Pascom*, John M Blandford*, William Brady+, Suzanne Westman+, Aristides Barbosa Junior*
Brazilian National AIDS Program; *Centers for Disease Control and Prevention, Global AIDS Program

Background/Objective: HIV rapid testing has been shown to be effective. Recently, an evaluation was undertaken to establish a rapid-test algorithm and to assess the field performance of this algorithm in Brazil. In choosing optimal public health interventions, the effectiveness of interventions should be considered together with the commitment of resources required for each of the alternatives. This study evaluated the cost and cost-effectiveness of standard and rapid-test algorithms to assess the relative costs and benefits of these algorithms.

Methods: We evaluated the cost of rapid-test and standard algorithms for clients tested at a large counseling-and-testing center (CTA) in Curitiba, together with the effectiveness of each of the algorithms. We constructed a model to estimate the expected effectiveness and associated costs of two HIV testing algorithms: 1) the standard algorithm, as determined by federal law [Portaria nº 59/GM/MS, 28 January 2003, http://www.aids.gov.br/final/diagnostico/portaria.htm]; and, 2) the rapid-test algorithm, based on findings of Phase I study of rapid-test performance [citation]. The measure of effectiveness—the health outcome of interest—was defined as the number of CTA clients who received accurate results of their HIV serological status. We considered the direct medical costs of HIV testing and diagnosis borne by the public health system, as well as direct non-medical costs borne by the client. Two perspectives were taken for the analysis: 1) a societal perspective, which considered the costs to the public health system and those to the CTA client; and, 2) a public health system perspective, which considered only those costs to the public health system. The cost-effectiveness study, as a component of the HIV rapid-test evaluation (Reg. CONEP 4024), received approval of the Brazilian National Commission of Research Ethics (Carta Nº 875 CONEP/CNS/MS).

Results: Our model demonstrated that both the standard and rapid-test algorithms provided near-perfect accuracy in diagnosing the HIV serostatus of CTA clients. The principal determinant of effectiveness was the likelihood that a client would receive her or his results; the rapid-test algorithm had the advantage of providing testing and diagnosis during one visit to the CTA. In contrast, the standard algorithm had an advantage on the cost side of the analysis, resulting from lower test kit and labor costs for the initial screening test. Partly offsetting the cost advantage of the standard algorithm, the rapid test offered lower time and transportation costs for the client. In the base-case analysis from a societal perspective, the standard algorithm cost R$24.36 (US$8.41) per client receiving accurate test results, compared to R$29.11 (US$10.06) for the rapid-test. This result implied that the incremental cost of the additional effectiveness achieved by the rapid-test was R$94.13 (US$32.51) per client receiving accurate test results. Influential variables in determining expected cost and cost-effectiveness of the algorithms were client return rates, HIV prevalence, and the cost of rapid-test kits for screening.

Conclusion: The data suggest that, as rapid testing is implemented, it may be advisable to give priority to contexts where return rates are low or HIV prevalence is high, or both. If a lowered cost for screening tests under the rapid-test algorithm could be negotiated, the relative cost-effectiveness of this algorithm could improve significantly. Because this study was conducted in a CTA that is not reflective of the overall reality in Brazil, it would be valuable to evaluate the cost-effectiveness of HIV rapid testing in other contexts. As rapid testing is being implemented, an opportunity exists to conduct evaluations of both performance and cost-effectiveness in contexts different from that presented in the study CTA or in programs serving high-risk populations. Assessing the cost-effectiveness of testing algorithms in alternative settings could assist decision-makers in forming national HIV testing policy.
RAPID HIV-TESTING IN NEEDLE EXCHANGE CENTRES AND PRISONS IN FINLAND

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Background/Objective: In an effort to affect injecting drug use (IDU) associated HIV epidemic a voluntary rapid HIV-testing was implemented to IDU's visiting needle exchange centers and prison inmates.

Methods: All personnel working with rapid tests are trained with short lectures and laboratory work at the Finnish National Public Health Institute (NPHI) before starting the testing to ensure the quality of testing and counseling. NPHI organizes also twice a year a quality assessment rounds for HIV serology for prisons and needle exchange centers and confirms by Western-blotting all positive rapid HIV-test (Determine HIV-1/2, Abbott) results from separately drawn venous blood.

Results: During 1999 – 2003 more than 7800 samples were tested with the Determine HIV-1/2 (spesificity 99,9 %, positive predictive value 90%). The HIV-antibody prevalence declined from 6,7 % to 0,4 % in IDU’s visiting needle exchange centers and from 2,4 % to 0,3 % in prison inmates tested with rapid HIV-test. A short training period in NPHI was found to be important before testing was initiated. Acceptance of rapid tests among health care personnel in prisons and needle exchange centers was good. Inmates and IDU’s prefer rapid testing to conventional testing.

Conclusions: Rapid HIV-testing was well received in prisons and needle exchange centers. Pre- and post-test counseling was seen as an important part of the procedure. A reference laboratory giving training for testing, and maintaining quality control program is an importat part of a testing system using rapid HIV-tests.

Presenter: Brummer-Korvenkontio, H Email: henrikki.brummer@ktl.fi
Institution: National Public Health Institute, Helsinki, Finland
Rapid HIV Kit Evaluation for Non-Laboratory Settings in Botswana; A Global AIDS Laboratory Project

Theresa Bokete¹, Phetogot Phoi¹, Wilson Kalake², Catherine Pule², Yvette Benjamin³, Patricia Somse⁴, Patricia A. Clark⁴, Jane Getchell⁵, Frances Pouch Downes⁴


Background: In Botswana the prevalence of HIV/AIDS among pregnant women 15-49 years old was 37.4% in 2003. Since April 2000, Volunteer Counseling and Test centers have tested >120,000 first-time testers using an algorithm of parallel rapid tests (with a tie-breaker third rapid test if needed). In January 2004, Botswana implemented routine HIV testing in health care settings, leading to an increase in the demand for rapid HIV tests.

Project: The BOTUSA project in collaboration with Botswana Ministry of Health (MOH) invited the Global AIDS Laboratory Project (GALP), a collaboration between APHL and CDC, to evaluate additional rapid HIV test kits for future use in surveillance and diagnosis at non-laboratory settings. A team of three U.S. laboratory scientists worked with National Blood Transfusion Service (NBTS) employees to evaluate rapid HIV test kits during July, 2004.

Methods: NBTS employees obtained test kits to be evaluated and developed criteria for kit evaluation prior to the arrival of the U.S. team. Three HIV kits meeting the criteria were tested against 448 serum samples (195 repeatedly positive by EIA and 253 negative by EIA) provided by the NBTS. Sensitivity/specificity values were calculated against two different gold standards: 1) compared to conventional EIA results and 2) compared to an agreement of at least three assays (i.e. 2 rapid tests and the EIA, or all three rapid tests, even if EIA differed). Inconclusive or indeterminate results for a particular test were excluded.

Results: All three rapid kits had sensitivities >/= 95.3% and specificities >/= 98.0% compared to conventional EIA results. Using the alternative gold standard increased sensitivities to >/= 96.9% and specificities to 100%. The EIA produced 8 discordant results (3 false positive, 5 false negative) and the rapid test kits produced 6, 3 and zero discordant results, respectively.

Conclusion: Use of an alternative gold standard may be more appropriate than comparison to conventional EIA in evaluating the performance of rapid HIV tests in a high prevalence population. Two rapid HIV kits were reported as suitable for addition to the list of kits approved for use in Botswana clinics.

Presenter: Patricia A. Clark
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Institution: Michigan Department of Community Health
DETECTION OF INCIDENT HIV INFECTIONS USING RAPID HIV ANTIBODY DETECTION ASSAYS.
Stephen D. Soroka, Timothy C. Granade, Debra Candal, and Bharat Parekh
HIV Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA 30333
Objective: To detect incident HIV infections using modified commercially available simple/rapid HIV antibody detection assays (RTs).
Methods: Three RTs, Determine (Abbott Laboratories), OraQuick (OraSure Technologies, Inc.), and Sero-Strip (Chem-Bio, Inc.) were modified to detect HIV incident infection using different dilution protocols. The appropriate dilution for assessing seroincidence was established by making serial dilutions of known HIV positive specimens and determining the appropriate dilution that would still react with these positive specimens, but not with known low-titer specimens for each RT. The modified protocols were then evaluated using a panel of HIV-1 antibody reactive samples (n=157), 11 seroconversion panels (HIV antibody positive n = 42), and 4 HIV antibody low-titer panels (n = 70). RT data were compared with the data derived by the less-sensitive method of Janssen et. al. (JAMA, 1998, vol. 280(1): 42-48) using the Abbott 3A11-LS HIV EIA.
Results: The ability to identify HIV incident infections using the modified RTs correlated well with the 3A11-LS reference method (>95%). Samples misclassified by the RT versus 3A11-LS were different for each RT, and were generally close to the cutoff of the reference method. Correlation of subjective RT scoring versus the 3A11-LS results showed a concordance of results for all three RT.
Conclusions: Modifications to HIV RTs may be useful for population-based studies for determining HIV seroincidence. Further evaluation on field samples is needed.

Presenter: Debra Candal
Institution: Centers for Disease Control and Prevention
MOBILE VOLUNTARY COUNSELING AND TESTING: HOW VALID ARE HIV TEST RESULTS?

*Catherine Mutura*; **Linus Odawo**; *Edwin Ochieng*; **Peter Tukei**; *June Odoyo*; *Elizabeth Marum*; **Manasse Amollo**; *Judy Chege*; **Kevin DeCock**; **Juma Ali**; *Iman Moody*

*CDC - Kenya
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***CDC/Kemri – Kisumu
****KEMRI

**Background/Objective:**

Kenya’s HIV/AIDS burden is still enormous. Some communities are not able to access stationary Voluntary Counseling and testing sites. Mobile Voluntary Counseling and Testing (MVCT) services are occasionally offered. Across section of blood samples collected during various MVCT were analyzed for validity in CDC Lab in Nairobi, and results are hereby presented.

**Methods:**

Blood collected from every 10th client counseled and tested in MVCT was collected on S&S#903 filter papers. The samples were dried, packaged in zip-lock bags with desiccants and transported to the CDC Lab in Nairobi. Quality Dried Blood Spots (DBS) samples were selected, punched and eluted using standard methods. DBS validated EIA test kits (Enzynost HIV 1&2 and vironostika uniform II plus O) were used. Lab HIV results were compared with MVCT results of Determine HIV 1&2 and Unigold HIV 1&2.

**Results**

Five hundred and seventy three (573) DBS samples (356 from Kenya Demographic Health Survey MVCT and 217 from routine MVCT, (a representative of 5,730 samples using 1:10 sampling protocol) were analyzed. There were 13 samples (13/573 – 2.3%) with discrepant results between lab and MVCT. Ten out of thirteen (10/13) samples had HIV positive results in VCT but Negative in the lab while three out of thirteen (3/13) had positive Lab results but Negative in VCT.

**Conclusions:**

Though the overall discrepancy between Lab and MVCT is low (2.3%), MVCT seem to report more false positives than false negatives at the rate of 1.7 and 0.5% respectively. Quality training of testing staff, proper storage of test kits, proper handling of filter papers, and good record keeping, all do contribute to the validity of HIV results.

**Presenter:** Catherine Mutura  
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Performance of HIV-1 Enzyme-Immunoassays on Dried Blood Spots for Quality Control of HIV Testing

Dennis Olara¹, Harriet Mutesi¹, Benon Biryahwaho² and Robert Downing¹

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Background: With the rapid expansion in access to ART under the PEPFAR initiative, there is an urgent need to make HIV serologic testing universally available. A pilot project has been initiated in Bushenyi District that offers home-based VCT to every household. HIV testing is conducted in the home on finger-stick blood using a sequential rapid testing algorithm. For quality assurance, dried blood spots are collected on SS 903 paper, dried and stored in ziplock bags with desiccant and shipped to Entebbe for testing by EIA. Our objective was to re-visit our DBS EIA testing algorithm (Vironostika HIV Uni-form II plus O and GS rLAV in parallel) to determine which EIAs and which testing algorithm to adopt to optimize performance at minimal cost.

Methods: Three EIAs were evaluated, Vironostika HIV Uni-form II plus O (VU), GS rLAV (GR) and Murex HIV-1.2.O (M) on DBS from known HIV-seropositive and seronegative persons. Serum elution and testing were as recommended with some in-house modifications. Mean OD values for HIV-negative samples were used to calculate cut-off values and both raw OD values and standardized OD values (SOD) were analyzed. Consensus results for sequential and parallel algorithms were determined retrospectively for all possible combinations and compared to the known serostatus.

Results: Mean OD values for HIV-positive samples were VU; 2.926 (1.861-3.5), GS: 1.764 (1.175-2.548) and M; 2.797 (1.328-3.5) and for HIV-negative samples they were VU; 0.062 (0.047-0.084), GS: 0.082 (0.003-0.558) and M; 0.166 (0.050-0.473). SOD values (by integer of 0.5) for HIV-positive samples ranged from 4.0->10.0 for VU; from 2.0-4.5 for GS and from 1.5-4.5 for M; for HIV-negative samples, the corresponding values were 0-0 for VU, from 0-1.0 for GS and from 0-1.0 for M.

Conclusions: VU performed best; SOD<0.5 for all HIV-negative samples and SOD>4.5 for all positive samples). This EIA correctly identified all positive and negative samples and could be used as a screening assay in a sequential algorithm, either of the other EIAs serving as a confirmatory assay; VU is, unfortunately the most expensive of the three assays.

Presenter: Dennis Olara            Email: dco6@cdcuganda.org
Institution: CDC-Uganda
EARLY DIAGNOSIS OF HIV-1 INFECTION IN INFANTS USING DRIED BLOOD SPOTS AND REAL-TIME REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION
Chin-Yih Ou1, Steven Balinandi2, Souleymane Sawadogo1, Clement Zeh1, Hua Yang1, Pius M. Tih4, Christiane Adje-Toure3, Sam Tancho3, Leonard Kouadio Ya3, Marc Bulterys5, Pauli Amornkul6, Robert Downing2 and John Nkengasong1.

Background/Objectives: Serodiagnosis of HIV-infection in infants born to seropositive mothers is problematic due to the prolonged presence of maternal antibodies. Dried blood spot (DBS) is a convenient specimen collection method because it is easy to transport and store. Our objective was to develop a simple and sensitive nucleic acid-based technology to diagnose HIV-infected infants using DBS.

Methods: A simple magnetic bead-based method is used to isolate total nucleic acid from a 6 mm DBS disc. A novel one-tube, real-time, duplex RT PCR assay is then used to detect both the HIV-1 LTR RNA and DNA. This approach was evaluated with DBS specimens from HIV-1 exposed infants in Uganda (n=128), Cameroon (n=315) and Kenya (n=410). The gold standard used to determine the infant infection status in Uganda was plasma viral load (VL) and that in Cameroon and Kenya was Amplicor DNA test on DBS. For discordant samples, we also tested them with real-time assays specific for the HIV gag and integrase sequences.

Results: We first examined the stability of HIV-1 RNA in the DBS and found that it was stable for more than 2 months if DBS was stored properly at room temperature in a humidity-free environment. We also found that when using both HIV DNA and RNA as the detection target, the detection sensitivity increased when compared with the use of DNA alone. The concordance of our assay and VL assay in the Ugandan study was 99.2%. The concordance of our assay and Amplicor DNA assay in the samples from Cameroon and Kenya was 99.7%. One sample from Uganda with negative VL and two samples from Cameroon with negative Amplicor DNA results were found to be positive by our assay. These samples were found to be positive for HIV gag and integrase sequences.

Conclusions: Because of the simplicity of specimen collection, nucleic acid extraction, detection, low cost and high sensitivity, our DBS-based HIV real-time assay will be useful for pediatric HIV diagnosis and care/treatment programs in resource-poor settings.

Presenter: Chin-Yih Ou
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Institution: Centers for Disease Control and Prevention
PERFORMANCE CHARACTERISTICS OF THE ROCHE AMPLICOR 1.5 ASSAY FOR QUANTITATION OF HIV-1 RNA FROM WHOLE BLOOD COLLECTED ON FILTER PAPER

TC Granade, SK Phillips, DH Candal, JN Nkengasong, M Kalov, and BS Parekh. Centers for Disease Control and Prevention, Atlanta, GA  30333

Objective: To quantitate the levels of HIV-1 viral RNA found in dried blood spots (DBS) using the Roche Amplicor 1.5 assay and to compare these results to those determined in matched plasma specimens from adult and pediatric patient populations.

Methods: Nucleic acid extraction from DBS was performed using a simple modification of the Roche protocol (60°C for 30 minutes in lysis buffer). Amplification was optimal at 25 cycles and resulted in a lower detection limit of 80 HIV-1 RNA copies/ DBS (1600 copies/ml) while maintaining the dynamic range of the assay.

Results: Of 150 serologically HIV antibody positive adults, 87 (58%) were concordantly reactive by plasma and DBS viral load, 37 (24%) were undetectable in both sample types, 3 (2%) were positive in plasma and negative in DBS and 24 (16%) were positive in DBS and negative in plasma. The amplified material from the DBS reactive/plasma negative specimens was determined to be HIV-1 DNA that was extracted from the intact cells on the DBS. Matched DBS and plasma specimens from infants from Cote d’Ivoire (N= 138) were completely concordant (44 reactive, 94 negative). Viral loads from both sample sets were slightly lower than in plasma due to the lower sample volumes of DBS specimens.

Conclusion: HIV-1 viral load determinations from DBS using the Roche Amplicor assay offer an alternative to standard plasma viral load testing in settings where plasma collection, storage and transport is difficult.

Presenter: Tim Granade

Institution: Centers for Disease Control and Prevention
**Poster Abstract 25**

**MODIFIED PROTOCOLS OF COMMERCIAL ENZYME IMMUNOASSAYS FOR THE DETECTION OF ANTIBODIES TO HIV-1 AND HIV-2 FROM WHOLE BLOOD COLLECTED ON FILTER PAPER.**

TC Granade, DH Candal, KB Bond, M Downer, J Mei, R Chiengsong, and BS Parekh. Centers for Disease Control and Prevention, Atlanta, GA 30333.

**Objective:** HIV antibody detection assays for use with dried blood spots (DBS) have been modified and approved for DBS use in the United States, however, kits used in international setting with serum or plasma specimens have not been applied to DBS testing. The goal of this study was to develop and to assess modified protocols of international HIV antibody testing products for use with DBS.

**Methods:** Six HIV enzyme immunoassays (EIA) for the detection of antibodies to HIV-1/HIV-2 were evaluated using a panel of matched DBS and plasma. The tests evaluated were: Uniform II plus O, Detect, Select HIV-1/2, Genscreen antigen/antibody, GACELISA, and Murex 1.2.O. DBS included known HIV-1 (N=70) and HIV-2 (N=27) specimens, 4 HIV-1 seroconversion panels N=(54), and HIV non-reactive specimens (N=56). An additional 156 specimens from east Africa (81 reactive, 75 non-reactive) representing different HIV subtypes were also included. All DBS were eluted in a standardized buffer and appropriate dilutions for each assay were determined empirically. Results from the DBS EIAs were compared to HIV EIA and Western blot results of the matched plasma.

**Results:** Detection of HIV antibody positive DBS were >= 98.75% concordant with the matched plasma results for all tests. Identification of DBS antibody negative specimens correlated with the plasma test results (>98%) for all tests except Genscreen and Murex which had more false positive DBS results. Detection of seroconversion varied slightly among the tests, however the detection of the first positive specimen in each panel did not vary by more than one week.

**Conclusion:** Current HIV EIAs used in international settings may be easily modified for use with DBS. HIV test results of DBS are comparable to EIA and Western blot testing of matched plasma specimens.

**Presenter:** Tim Granade

**Institution:** Centers for Disease Control and Prevention
DEVELOPMENT OF AN ORAL FLUID ASSAY CAPABLE OF DIFFERENTIATING RECENT FROM ESTABLISHED HIV INFECTION.

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1 University of Maryland, Baltimore, School of Medicine
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Background/Objective: Identifying persons who have been recently infected with HIV is important for epidemiologic investigations, including the estimation of incidence and for the enrollment of acutely infected individuals into appropriate intervention programs. Currently, sensitive/less-sensitive assays (S/LS) are available to classify recently infected persons (3-6 months post-infection), but they require the collection of blood that often limits their application. Simpler methods of specimen collection are needed to increase compliance and offer an alternative to invasive procedures that increase the risk of occupational exposure to HIV. We sought to develop, calibrate, and validate a S/LS assay that uses easy-to-collect oral fluid.

Methods: Serum/oral fluid pairs were collected from 246 HIV-infected adolescents newly enrolled in HIV care programs of the Adolescent Trials Network from 15 sites in the United States and Puerto Rico. The serum was collected by venipuncture, whereas the oral fluid was collected by the OraSure device that targets mucosal transudate containing IgG antibody. Reference testing was performed on all serum specimens by the Vironostika S/LS test using an optical density (OD) cutoff of 1.0 to distinguish recent (<170 days) from established infections (>170 days). We chose the first 20 recent and 20 established samples, as classified by the serum Vironostika S/LS, and tested the complementary oral fluid specimens at various dilutions to calibrate a modified Vironostika Oral Fluid assay as an S/LS test. An optimal dilution of 1:50 and an OD cutoff of 0.238 were selected to yield a concordance of the oral fluid S/LS assay with the serum Vironostika S/LS assay of 100% for recent samples and 85% for established samples. Using these parameters, the concordance of the serum S/LS and the oral fluid S/LS assay was then determined using the remaining 206 serum/oral fluid pairs. Of the 206 specimens, 61 were classified as being from recently infected individuals and 145 from those with established HIV infection according to the serum S/LS assay.

Results: There was a correlative trend of the OD readings of both assays when testing the 206 serum/oral fluid pairs (r=.658, p<.001). The concordance rate between the serum and oral fluid based S/LS assays using the parameters selected during the calibration phase was lower than anticipated (57% for recents and 92% for established). However, when reassessing the results from the entire specimen pool (n=246 serum/oral fluid pairs), the best agreement between the oral fluid and the serum S/LS results remained at an oral fluid dilution of 1:50, but with a new cutoff of 0.280. This adjustment to the OD cutoff of the oral fluid S/LS assay gave the highest kappa value (κ=.703), and a concordance rate of 85% for the recent samples and 87% for the established samples as compared with the serum S/LS test.

Conclusions: The HIV oral fluid S/LS test has exhibited good concordance with the routinely used serum S/LS test. This S/LS test strategy using oral fluids offers a simple and safe collection method that is suitable for use in a variety of epidemiologic investigations.

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INCIDENCE IMMUNOASSAY FOR DISTINGUISHING RECENT FROM ESTABLISHED HIV-1 INFECTION IN THERAPY NAÏVE POPULATIONS

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2Center for Immunology, St. Vincent’s Hospital, Sydney, Australia.
3National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA., USA.

Objective: To identify a specific marker of recent human immunodeficiency virus (HIV-1) infection.

Design: The humoral immune response in individuals recently infected with HIV-1 has been followed by analysing the antibody isotype, specific response generated to HIV-1 antigens in sequential samples collected during and following seroconversion.

Methods: Antibody isotype specific HIV-1 Western blots were analysed to identify interactions indicative of recent HIV-1 infection. These responses were further quantified using an antibody isotype specific ELISA based on recombinant HIV-1 antigens.

Results: During maturation of the immune response to HIV-1 infection there is a rapid and enduring IgG1 isotype response to all the major proteins transcribed by the env, gag and pol genes. Data obtained from panels of specimens collected longitudinally from individuals infected with HIV-1, has indicated that isotype-specific responses to different HIV-1 antigens appear at different time points following infection and often only appear transiently. We have observed an early transient peak of IgG3 reactivity to p24 over an interval of approximately 1 to 4 months following HIV-1 infection. The presence of IgG3 reactivity to p24 permits established infection to be distinguished from recently infected individuals during this time period.

Conclusion: An assay for anti-p24 IgG3 reactivity provides an estimate of the incidence of HIV infection that may be applicable for epidemiological surveys as well as monitoring new infections during vaccine trials and managing treatment programmes.

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METHOD COMPARISON OF HIV TESTS FOR RECENT INFECTION
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Background: Accurate laboratory assessment of incidence vs. prevalence or early vs. long-standing
HIV infection is valuable for epidemiological studies and for diagnostic assessment of individuals. We
have completed a study of samples from a large group of deferred plasma donors with unknown dates
of HIV infection using several candidate HIV incidence test methods to categorize samples as
‘consensus incident’, ‘consensus prevalent’ or ‘lacking consensus’ by a variety of methods.

Materials and Methods: Samples tested were from a repository of well-characterized plasma units
from deferred donors who were confirmed HIV positive. HIV incidence test methods included less-
sensitive, avidity index methods, and methods using novel approaches. The PRB601 panel was
assembled by aliquoting samples from units that tested incident or prevalent by at least 11 of the 12 test
methods used, and by preparing a data sheet with test results for HIV markers and for nine incidence
test methods (four less-sensitive, two avidity-index, and three other approaches).

Results: Of 78 samples tested by 12 methods, 42% were prevalent or long-standing infection by all
methods. Only 6% were incident or recent infections by all methods, but if one candidate method was
not considered, 18% were consensus incident. There was a lack of consensus (more than one method in
disagreement with others) in 29% of this sample set, some of which may be due to variations in the
definition of recent. Eight consensus prevalent and seven consensus incident samples were assembled
into a panel for evaluating incidence tests.

Conclusions: A clear understanding of HIV incidence (the rate of new infections) is important for
formulating health care policy and efforts to fight the epidemic. Although long seroconversion series
(that would constitute a gold standard) do not currently exist, useful information can be derived from
samples with unknown seroconversion dates if consensus results are found from multiple incidence test
methods based on different principles. PRB601 is a commercially available panel of consensus incident
and consensus prevalent samples.
Use of the HIV-1 BED Incidence EIA and Western Blots to Characterize Rejected Blood Donor Specimens

**Piacentini, S.C., Gottfried, T.D., George, J.R. (all of Calypte Biomedical Corporation)**

**Background/Objective:** The Calypte® HIV-1 BED Incidence EIA, originally developed by the CDC as the BED-CEIA, measures the ratio of anti-HIV specific antibodies to total antibodies using an anti-IgG capture system. Numerous studies have confirmed that specific to non-specific IgG ratios below a cutoff are correlated to HIV-1 infections of 6 months or less. A set of confirmed HIV-1 positive plasma specimens collected from rejected blood donors was evaluated using the Incidence EIA. Correlations between the Incidence EIA results and Western Blot profiles were investigated.

**Methods:** Fifty plasma specimens from blood donors rejected due to a reactive result in an HIV screening test were subjected to confirmatory testing using a serum Western Blot (WB) (Calypte, Rockville, MD). WB Indeterminate specimens were resolved using PCR Branch DNA. All specimens confirmed to be HIV-1 positive were tested in the Calypte HIV-1 BED Incidence EIA, a quantitative test where specimens are evaluated against a Calibrator to generate an ODn value. An ODn of $\leq 0.8$ is considered a recent (<6 months) seroconversion.

**Results:** Fourteen of the 50 specimens were HIV-1 negative by WB and branch DNA PCR analysis when required. Of the remaining 36 confirmed HIV-positive specimens, 20 were determined to be “recent” infections by the Incidence EIA. Associated with the recent HIV-1 infections were WB profiles that generally demonstrated weaker bands compared to specimens identified as “long term”. Additionally, the BED Incidence test result (ODn) suggested a quantitative attribute. Specifically, ODn values of less than 0.100 (n=7) consistently correlated to WB profiles with weak or absent envelope (gp41, gp120, and gp160) reactivity, whereas ODn values greater than 0.100 (n=13) generally had stronger envelope bands, although weaker than the envelope bands observed in long term infections. One specimen identified as a recent infection (ODn = 0.051) had the attributes of an early seroconvertor (only a faint gp160 band and 166 copies of HIV-1 RNA per ml).

**Conclusions:** The proportion of recent infections in the group of confirmed HIV-1 positive rejected blood donors was 55% (20/36) which is higher than the HIV-1 incidence in the general US population. This is not surprising considering that regular donation intervals would result in infection occurring since the last accepted donation. The association of weak WB banding patterns with recent HIV infections is not a new finding. However, the data here further supports the validity of the BED Incidence assay to identify early HIV-1 infections. Numerous publications have previously demonstrated the value of the Incidence EIA as a powerful research and epidemiological tool to estimate the number of new infections within a population. This data suggests that the quantitative ODn value generated by the Incidence EIA may provide additional information including a potential prediction of western blot profile and an estimate of seroconversion progress.

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Identification of recent HIV infections in single serum sample: Comparison between the avidity index method and the serologic testing algorithm for recent HIV seroconversion (STARHS)

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Background/Objective: An increasing interest on estimating the incidence of HIV-infection using cross-sectional specimens has led to the development of several laboratory methods. Serological techniques that can be performed using commercially available HIV tests while including modifications in their protocol or in sample preparation are of particular interest. The aim of the present study was to compare an avidity index method using the AxSYM platform (Abbott Diagnostics) to the serological testing algorithm for recent HIV seroconversion (STARHS), to assess their ability to detect persons with recent or long-standing HIV infections.

Methods: A total of 160 serum specimens from documented HIV-positive subjects were collected at several clinical laboratories in Spain and at an infectious disease clinic in Italy. Those with available clinical and laboratory information where classified as recent infections (n=13), long-standing infections (n=57), and AIDS (n=24). Overall agreement was calculated through a kappa statistic (κ).

Results: Among the 160 specimens κ was 0.67 (overall, fairly good agreement, p<0.001). When analyzed by group, both techniques correctly identified 12/13 recent HIV infections. However, STARHS misclassified more individuals that had a long-standing infection or AIDS as being recently infected (6/57 and 3/24 by STARHS vs. 1/57 and 2/24 by the avidity assay). The avidity index method has several advantages over the STARHS, including ease of performance and automation, no need for internal calibrators and specific controls, no need for specialized training or official approval by regulatory authorities, and a wide availability of the test (a third-generation, HIV-1/2 microparticle EIA).

Conclusions: The avidity index technique is easy to perform, inexpensive, automated, exportable, time-saving, and does not need sophisticated laboratory requirements or personnel training. Our results show that both techniques perform similarly in identifying recent infections, although STARHS misclassified more individuals that had a long-standing infection or AIDS as being recently HIV infected. The identification of recent infections based on the combination of both methods needs further investigation.

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Recent HIV infections in heterosexuals: Is STARHS applicable?

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Background: The use of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) in determining HIV incidence in an HIV-1 subtype B infected population has been well documented. However, its use in populations infected with non-B subtypes is less well studied. We describe a method that allows trends in recently acquired infections to be determined using the STARHS assay in a population largely infected with non-B subtypes, where individual infecting subtypes are known.

Methods: Between 1997 and 2000, a total of 839 specimens from heterosexuals attending 15 STI clinics were identified as anti-HIV positive as part of the Unlinked Anonymous Prevalence Monitoring Programme of England, Wales & Northern Ireland. Of these, 797 were available for STARHS testing and, for 551 of them, an HIV subtype was assigned. Regardless of subtype a STARHS standardised optical density cut-off of 0.75 was used to determine whether the infection was recently acquired.

Results: The proportion of infections that were recent fell significantly between 1997 and 2000 for those infected with HIV subtype B but it remained stable in those infected with a non-B subtype. Among those infections subtyped as circulating recombinant form an increase in recent infections was observed.

Conclusions: These results indicate continuing transmission of HIV in heterosexuals. The decline in the proportion of subtype B infections that were recent, and the relative stability of recent infections among non-B subtypes, correlate well with the changing prevalences of HIV subtypes among heterosexuals in the UK. Although an annual incidence cannot be calculated the measurement of proportions of recent infection among those infected with particular non-B subtypes provides a means of monitoring trends in transmission intensity among heterosexuals.
Measuring antibody avidity improves the utility of STARHS in identifying recent HIV infection

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Background: The Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) is increasingly used to differentiate recent from long-standing HIV infection. However, factors that reduce antibody titre such as combination antiretroviral therapy (ART) and advanced HIV disease, as well as HIV subtype, may reduce the specificity of immunoassays that identify recent HIV infection. We investigated whether a testing strategy could be developed using two assays that utilise different biological principles to improve the accuracy of the identification of recent HIV infection. One is based on antibody titres and the other antibody avidity both of which increase following infection.

Methods: Specimens were collected from two groups of patients that had been infected for at least one year prior to sampling. Group 1 comprised individuals receiving ART, and included a specimen collected immediately before initiation of ART and at six-monthly intervals for two years; Group 2 comprised patients not receiving therapy. STARHS was performed using the bioMérieux Vironostika assay and antibody avidity determined using the Abbott AxSym HIV1/2gO assay applying previously described methods.

Results: ART had a marked effect on STARHS assay reactivity, but only a slight effect on antibody avidity. STARHS standardised optical densities (SOD) fell by 50% over a two-year treatment period whereas antibody avidity declined by only 4.9%. Specimens from untreated controls showed a small increase in SOD and no change in antibody avidity over the study period.

Conclusions: Antibody avidity can help identify specimens from patients receiving ART that are falsely classified by STARHS as a recent infection. However, when used alone, the short window period associated with low antibody avidity limits its ability accurately to differentiate recent from long-standing infection. Using both techniques together improves the reliability of identification of recent HIV infection.
ENHANCING DIAGNOSTIC DATA FOR HIV SURVEILLANCE: THE ONTARIO LABORATORY ENHANCEMENT STUDY (LES)

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Background: HIV diagnostic test results provide important insights into trends in HIV infection. However, missing risk data may limit interpretation. The detuned assay identifies persons who recently seroconverted and allows the calculation of HIV incidence.

Methods: For HIV-positive and a 1:200 sample of negative test results, we sent a questionnaire on HIV-related risks and previous HIV testing. We tested positive specimens by the detuned assay using the Abbott 3A11 HIV-1 and Vironostika EIA assays modified to detect recent HIV infection. HIV incidence was calculated for each exposure category including data from the completed questionnaires. We developed an analytical approach to adjust crude HIV incidence for testing bias i.e. patients seeking an HIV test “prematurely” due to the symptoms of primary HIV infection and unusual high risk exposures.

Results: From 10/1999 to 12/2002, 3,106 patients were HIV-positive for the first time; 79% of questionnaires were returned within 10 months. Risk data was provided on only 46% of laboratory requisitions. The proportions of two exposure categories from the LES were different than that based on the laboratory requisition: HIV-endemic countries 17% (versus 7% from the requisition) and MSM 46% (versus 55%). HIV incidence (per 100 person-years) over the three-year study period was: MSM 2.2, MSM-IDU 2.4, IDU 0.23 and persons with an HIV-infected or at-risk sexual partner of the opposite sex 0.10. Incidence was considerably higher among Toronto MSM than elsewhere (2.9 versus 1.9) and among Ottawa IDUs than elsewhere (0.71 versus 0.19). Over time, HIV incidence in IDUs decreased while there was a modest increase in HIV incidence among MSM in the latest six-month period. Adjusted HIV incidence was 20 to 40% lower than crude HIV incidence for MSM in Toronto.

Conclusions: The LES provided important additional data on risk and HIV test history and yielded estimates of exposure category-specific HIV incidence over time. Though the observed incidence was likely an overestimate, the trends likely reflect the actual situation. Decreasing HIV incidence in IDUs is reassuring but the possible increase in HIV incidence among MSM will require close monitoring. Adjusting HIV incidence for testing bias provides more robust estimates of true HIV incidence, especially among MSM.

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Comparison of Less Sensitive HIV Incidence Tests for the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS).

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**Background:** To estimate incidence from cross-sectional population surveys, the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) uses the results of a standard and a Less-Sensitive (LS) EIA to classify HIV infections as recent or long standing. A modified version of the Vironostika HIV-1 MicroElisa System (V-LS) is currently used as the LS-EIA for STARHS. This assay will soon be replaced by the Vironostika HIV-1 Plus O MicroElisa System (VplusO-LS). The VplusO-LS assay has also been adapted for use in STARHS. To investigate the comparability of VplusO-LS and V-LS in STARHS, longitudinal specimens from the HIVNET Infected Participant Cohort (IPC) were tested using both kits.

**Methods:** The IPC specimen set (1009 specimens from 105 recent seroconverters with known dates of last negative and first positive HIV test) was tested in triplicate on both assays. Correlation was assessed by fitting a linear regression of the square root standardized optical densities (SOD’s) from the Plus O-LS on the square root SOD’s from the V-LS. Assay performance on low positive (LPC) and high positive (HPC) control materials was also assessed. A subset of 324 specimens from 59 patients with 3 or more specimens collected before starting antiretroviral therapy (Mean length of follow up 504 Days) was used to calculate mean window periods (number of days between detection of HIV antibody with a standard EIA and a threshold SOD of 1.0 with the LS-EIA) and 95% confidence intervals for both assays.

**Results:** V-LS classified 459 samples as incident (SOD<1), and VplusO classified 472 as incident (90.2% concordance). The two tests showed good overall correlation, with an $R^2$ of 0.848, and a slope of 0.996. The VplusO assay had a slightly better coefficient of variation (CV) on the control materials (18.9% on LPC and 19% on HPC vs. 18.4% on LPC and 26.7% on HPC for V-LS). With both assays, specimen SOD’s diminished after patients started antiretroviral therapy. The mean window period for V-LS was 218 days (95% confidence interval (CI) 193-250 days). The mean window period for the VplusO-LS was 203 days (95% CI 185-243 days).

**Conclusion:** The VplusO-LS gave comparable results to the V-LS for the classification of recent HIV infection in terms of overall specimen response (SOD) and overlap of window periods. Further validation is needed to determine if the two assays provide comparable estimates of HIV incidence when applied to specimens from cross-sectional surveys.

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COMPARISON OF THREE METHODS OF EXTRACTION OF RNA FOR THE DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS VIRAL LOAD IN HUMAN PLASMA
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Background/Objective:
The quantitation of viral load in HIV positive patients is a very important assay as part of patient care. In our laboratory, we encountered a number of difficulties with the automated NucliSens Extractor. As new technology (NucliSens miniMAG) has become available in the nucleic Acid purification system, we investigated the possibilities of implementing this technology.

Methods:
NucliSens manual isolation of total nucleic acid from biological specimen makes use of the selective adsorption of nucleic acids to silica particles under specific experimental conditions. The silica is washed to remove any contaminants after which desorption of the nucleic acids can take place. NucliSens Extractor automates the isolation process for the nucleic acid. Adsorbing to Silica, filtering, washing, and eluting into a closed vial for further processing and concentrate the nucleic acids. NucliSens miniMAG extracts DNA and RNA from a variety of specimen types using bioMerieux’s proprietary Boom Technology with magnetic silica.

Results:
Method comparison analyses were done using student t-test. We analyzed 37 patients with the automated Extractor and miniMAG extraction the correlation value was 0.98.

Further, we were interested to see if the correlation between miniMag and extractor will hold its value in patients with low CD4 absolute numbers. We analyzed 22 patients with CD4 of less than 500 cells/uL and 13 patients with CD4 of greater than 500 cells/uL, the correlation seen was of 0.98 and 0.99 respectively. In addition, a small number of patients (n=8) were analyzed with the Manual extraction and automated Extractor with a correlation of 0.97.

Conclusions:
Based on preliminary analysis at the time of abstract submission we find that the NucliSens miniMAG for Nucleic Acid Purification is as good or better than the automated NucliSens Extractor for RNA extraction of Biological specimen. In addition, the miniMAG device itself is space saving, cost effective and efficient.

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Comparison of Sensitivities between DNA and RNA Nucleic Acid Tests for the Detection of HIV-1 Infection
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Background/Objective: The current diagnostic testing algorithm for human immunodeficiency virus type 1 (HIV-1) infection generally requires the initial screening of plasma/serum samples by an enzyme-link immunoassay (EIA) followed by a confirmatory Western blot (WB) test. This testing algorithm does not detect patients at the early stage of infection. It has been shown that nucleic acid tests (NAT) can identify infected but seronegative persons in this window period. In this study, we compared the sensitivity of a proviral DNA test with that of a viral RNA test for the detection of HIV-1 infection.

Methods: Specimens from 200 WB-confirmed HIV-1 seropositive and 100 seronegative US subjects were examined for the presence of HIV-1 viral RNA and cellular proviral DNA. To detect viral RNA, we used 200 µl of plasma. To detect proviral DNA, we used 200 µl of whole blood. A reverse transcription/polymerase chain reaction (RT-PCR) assay was used to detect viral RNA, and a PCR assay was used to detect proviral DNA. Both assays used a 5'-nuclease-based real-time amplification system containing the same primers and fluorescent probe derived from a conserved LTR region of the HIV-1 genome.

Results: Both the RNA and DNA assays were 100% specific since all the plasma and whole blood specimens from the 100 seronegative subjects were found to be negative. Among the 200 plasma specimens (200 µl) from the seropositive subjects, 186 (93%) had detectable HIV-1 LTR RNA. Twelve of the 14 subjects with initial viral RNA-negative result had enough plasma (500 µl) for reexamination. Eight of these 12 were found positive. Thus the sensitivity of RNA assay increased to 97% (194/200) if 500 µl of plasma was used. On the other hand, all 200 whole blood specimens from the seropositive subjects tested positive (100%) for proviral DNA.

Conclusions: Our results showed that, in this particular US population, whole blood proviral DNA is a better target than plasma viral RNA for the detection of HIV-1 infection. Our proviral DNA assay, which is highly sensitive and specific, may be useful as an alternative test for the detection and/or confirmation of HIV-1 infection.

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NAAT SCREENING via MULTISTAGE POOLING for ACUTE HIV-1 INFECTION

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Background/Objective:
In 2001, we conducted a pilot study exploring the feasibility of multistage pooling of approximately 8300 HIV-1 antibody negative sera for evidence of acute HIV infection and successfully demonstrated the ability to detect HIV RNA in those sera. (Pilcher et.al. 2002 JAMA) These experiences enabled the NC State Laboratory of Public Health to subsequently conduct prospective screening for acute HIV-1 infection in 228,016 specimens received over a 24-month period beginning November 1, 2002.

Methods:
Patient sera were tested for antibody via the Vironostika HIV-1 Viral Lysate Microelisa System (bioMerieux). Seronegative aliquots were subsequently processed in a multistage pooling algorithm. Specimen pooling was automated during the first year of study using the Beckman Biomek FX Robot (Beckman/Coulter) and a Hamilton AT Plus (Hamilton Company) during the second year. Pools of 90 to 96 sera (termed “B” pools) were initially tested by a commercial NAAT assay for HIV-1 qualitative viral RNA. NAAT testing during the first year of study was performed with the NucliSens HIV-1 Qualitative Assay (bioMerieux) and the Procleix HIV-1 Assay (Gen-Probe Inc.) during the second year of study.

Reactive “B” pools were deconstructed to their component smaller “A” pools, and then the reactive “A” pool was deconstructed to identify reactive individual sera. Results were classified as either acute infection or false positive only after patient notification, clinical follow-up, and repeat testing which yielded seroconversion by both the bioMerieux EIA and BioRad/Genetic Systems western blot assay.

Results:
Of 228,016 sera screened for HIV-1 antibody, 224,108 had not previously tested positive and hence were at risk for acute HIV infection. The NAAT assays detected an additional 40 patients with acute HIV infection. Approximately 4% of the HIV-1 infected patients were EIA antibody negative and would have been undetected without the use of nucleic acid amplified testing. Additionally, there were three false positive NAAT tests for an overall positive predictive value of 93%.

Conclusions:
Multistage pooling can efficiently diagnose acute infection with good positive predictive value in low prevalence populations. This research shows that it is feasible for laboratories with high testing volume such as commercial and state public health laboratories to perform widespread screening for acute HIV-1 infection.

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Institution: NC State Laboratory of Public Health; Raleigh, North Carolina
Real-Time HIV-1 Plasma Viral Load assay
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Background/Objective: Plasma viral load is used routinely to predict disease progression and to monitor antiretroviral therapy (ARV). With increasing access to ARV therapy and the reduction of drug costs, the demand for a sensitive viral load assay is increasing. The objective of this study is to develop a simple, cost-effective, subtype-independent viral load assay for resource-limited settings.

Methods: To increase the throughput of plasma (200ul) RNA isolation, we examined the efficiency of RNA isolation using a low-cost magnetic bead method (Cortex) and compared it with the RNA isolated using the viral RNA kit from Qiagen. A real-time, one-step and duplex RT PCR assay was designed to detect plasma HIV-1 RNA. A DNA clone was constructed and transcribed into RNA in vitro. This RNA (EC) contained fluorinated deoxynucleotides and was resistant to RNaseA. It was added to the plasma and co-purified with HIV RNA and served as an external control to monitor the efficiency of RNA extraction. It was also used as an internal calibrator for real-time viral load calculation. One-step RT PCR reagent from Qiagen was used.

Results: We found that the recovery and quality of plasma RNA isolated by the Cortex magnetic beads was the same as those by the Qiagen method. We compared several primer pairs (derived from LTR, gag, pol and integrase) for amplification and found that an LTR pair yielded the best amplification efficiency. When tested with eight different HIV-1 subtypes, the optimal amplification temperature of this LTR pair was found to be 52°C. The dynamic detection range of the assay is 475 to 1 million copies of HIV-1 per ml of plasma. The coefficient variations (% CV) of run-to-run variability of the process, including RNA extraction and viral load assay in the designated dynamic range were between 21 to 58% (8 runs). The cost of this assay is approximately 8 US dollars.

Conclusion: A simple, one-step, real-time duplex RT PCR assay is developed to measure plasma HIV-1 viral load. This assay may be useful in antiretroviral monitoring in resource-limited countries.

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REAL-TIME RT-PCR BASED ASSAY ON BLOOD CLOT SPECIMENS FOR DIAGNOSIS OF HIV-1 INFECTION IN CHILDREN, MALAWI

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Background: Mother to child HIV-1 transmission is the most common route of HIV-1 infection in children. HIV antibody testing is unreliable for children born to HIV-infected mothers under 18 months of age due to maternal antibodies. Therefore, in children younger than 18 months of age, HIV-1 infection is diagnosed by detecting HIV-1 viral sequences. We have developed a duplex, one-tube, one-step real-time RT-PCR-based assay for HIV-1 diagnosis. In this study, we evaluated this assay for its ability to detect HIV infection in blood clot specimens in children born to HIV-1-infected mothers under the age of 18 months in Malawi.

Methods: We collected 1ml of blood by fingerstick at 6, 9, 12, 18-24, and 30-36 months of age during a study of measles vaccination of HIV-infected and -uninfected children outside of Blantyre, Malawi. We performed HIV rapid antibody testing on blood collected ≥ 18 months of age from children born to HIV-infected mothers. Antibody positivity ≥ 18 months was taken as the indicator of HIV-1 infection in children. Total nucleic acids were extracted from blood clot specimens following digestion with streptokinase, and real-time RT-PCR was performed using the primers in the HIV-1 LTR region. We tested blood clots from 34 children with positive HIV antibody results and 24 of 146 children with negative HIV antibody tests.

Results: To evaluate the sensitivity and specificity of the assay, we first tested 34 children who were consistently HIV antibody positive ≥ 18 months of age and all of them had positive RT-PCR results from samples taken on at least two time points. We then tested 24 HIV-1 antibody negative children. Of the 24 children, 23 were HIV-1 negative by RT-PCR while one had positive HIV-1 RT-PCR at 6, 12, 18 and 24 months and undetectable antibody at 18 and 24 months by two rapid HIV tests. HIV infection was confirmed in this child by amplification of the HIV-1 gag region and sequencing.

Conclusion: The one tube and one step Real-time RT-PCR used here was both sensitive and specific in detecting true HIV-1 infection from blood clot specimens in children born to HIV-1-infected mothers under the age of 18 months.

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LOWERING THE DETECTION LIMITS OF HIV-1 VIRAL LOAD USING REAL-TIME IMMUNO-PCR FOR HIV-1 P24 ANTIGEN

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Background / Objectives: Currently, nucleic acid tests can detect infection by HIV as soon as 12 days after infection, and are capable of identifying as low as 50 copies RNA/mL. The use of these tests has been instrumental in protecting the blood supply and for monitoring response to antiretroviral therapy. More sensitive methods that could detect lower levels of viremia could provide a safer blood supply and assist clinicians in better assessing virologic responses to therapy. Our objective was to develop a real-time immuno-PCR (IPCR) method that combines the specificity of serologic detection with the exquisite sensitivity of molecular techniques for the detection of HIV p24 antigen (Ag) in serum, and show: 1) that IPCR is capable of detecting extremely low levels of HIV viremia using patient samples with known viral RNA copy numbers; and 2) that HIV-1 p24 Ag can be detected by IPCR in patient samples containing <50 RNA copies/mL.

Methods: The IPCR assay consisted of (1) an ELISA for p24 Ag detection using an immobilized mouse monoclonal capture antibody and a biotinylated secondary human antibody, followed by streptavidin-HRP, and (2) the addition of 10 pg/ml biotinylated DNA (500 bp) followed by real-time PCR. Fluorescence was detected during PCR amplification by hydrolysis of hybridization probes labeled with the 5'-reporter dye: 6-carboxyfluorescein, and a 3'-Black Hole Quencher molecule. To determine the detection limit of IPCR for HIV-1 p24 Ag, we generated a standard curve by IPCR using dilutions of HIV-1 p24 Ag from HIV-1 infected cell culture supernatants quantified by ELISA. These dilutions included log fold differences from 10⁰ to 10⁸ HIV-1 p24 molecules. To assess performance of the IPCR, 14-37 replicates from HIV-1 antibody positive patients with known HIV-1 RNA viral loads (determined by the Amplicor HIV-1 Monitor Test) were diluted within groups of three logs (between 1.68 - 6514 viral RNA copies), and then analyzed by real-time IPCR. In addition to these samples, 52 samples from HIV-1 infected persons (antibody positive) who had viral loads <50 RNA copies/mL were tested by real-time IPCR.

Results: When derived from the IPCR standard curve, a dose response was observed by IPCR for samples with known viral loads diluted at 1.68 - 43.7, 60.7 - 607, and 5179 – 6514 copies/mL. In addition, IPCR detected 42 % of patient samples which could not be detected by RT-PCR (i.e., <50 RNA copies/mL). The limit of detection for the IPCR was equivalent to 20 viral RNA copies/mL and 0.66 viral RNA copies per reaction; this corresponded to 40 attograms/reaction of p24 Ag and approximately 1000 HIV-1 p24 Ag molecules.

Conclusions: IPCR for HIV p24 antigen detection was shown to be more analytically sensitive for the detection of viremia than approved nucleic acid tests. It has the potential to confirm the diagnosis of HIV-1 at an earlier time than current methods, and should be valuable to monitor the response to anti-retroviral treatment when RNA copy number is below 50.

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CD4 T-Lymphocytes Counting in a Northern Nigerian Setting: Comparison of a New Affordable Flow Cytometric and Manual Magnetic Bead Techniques

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Human Immunodeficiency virus (HIV/AIDS) burden in sub-Saharan Africa is heavy and there is an urgent need for efficient and affordable CD4 T-lymphocyte estimation in HIV positive patients. Manual methods which are commonly used for CD4 T-lymphocyte counts in developing countries are labour intensive and can only be used to test 10-15 samples per day. Because of high demand for CD4 T-lymphocyte counts, we therefore compared a new and simplified, automated flow cytometric (Cyflow) CD4 T-lymphocytes counting technique (Partec, Munster, Germany) with the manual Dynabead method to determine which technique is more feasible and cost-effective for use in our centre.

Blood samples were collected from 40 adults including healthy individuals, pregnant women and HIV positive subjects to get a wide range of CD4 T-lymphocytes counts (i.e. Low <400 cells/µl, Medium 400-700 cells/µl, and High >700 cells/µl). Assessment of reproducibility of CD4 T-lymphocyte counts was also performed.

There was no statistically significant differences between the CD4 T-lymphocytes counts obtained with Dynabead and Cyflow techniques (475 ± 270 cells/µl; range 40-888 cell/µl and 508 ± 283 cell/µl; range 49-1244 cell/µl, p = 0.057, r = 0.93). The reproducibility test produced a CV% of 7.8% and 1.9% respectively for Dynabead and Cyflow.

The two techniques produced similar results but the reproducibility was higher with Cyflow. The new and simplified, automated Cyflow technique is more cost-effective (i.e. $3.00-$5.00 per test) than the Dynabead ($12-$22 per test) especially in busy centres since as many as 200 samples can be measured per day.

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EXTENSIVE REACTIVITY OF SIVCPZ, HIV-1 GROUPS N AND O V3 PEPTIDES WITH ANTI-HIV SERONEGATIVE PLASMA SAMPLES FROM INDIVIDUALS LIVING IN VILLAGES IN THE EQUATORIAL RAIN FOREST REGIONS IN CAMEROON WHERE A BROAD HIV-1 GENETIC DIVERSITY EXIST: IMPLICATIONS IN HIV DIAGNOSIS.

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Background/Objective: HIV-1 seroprevalence studies conducted between the year 2000 and 2001 on specimens collected from individuals living in rural villages in the equatorial rain forest regions of Cameroon revealed a low HIV prevalence rate (5.8%). HIV-1 group M subtype analysis on HIV-1 seropositive plasma samples revealed the presence of seven HIV-1 group M subtypes, several circulating recombinant forms, and new intersubtype recombinant infections. These studies suggested that there are still unidentified viral variants circulating in these regions. Such infections need to be identified and the viral proteins incorporated into new HIV diagnostic tests. Therefore, plasma samples obtained from subjects living in villages in the equatorial rain forest regions that were HIV-1 seronegative in commercial HIV-1 assays were tested with V3 peptides derived from SIVcpz variants, groups N and O.

Methods: Plasma samples from 4156 persons aged 15-70 years old and living in 53 rural villages in the equatorial rain forest regions in Cameroon were tested for antibodies to HIV-½ by the Abbott Determine HIV-½ rapid assay (Abbott, Wiesbaden, Germany). Reactive samples were further tested for antibodies to HIV-1 by an HIV-1 ELISA using the HIV-1 rLAV kit (Genetic Systems Corporation, Redmond, WA) as described by the manufacturer. Plasma samples that were negative in both assays were tested for reactivity in ELISA with V3 peptides derived from SIVcpz (-gab, -ant, -us), HIV-1 group N YBF30, group O MVP5180, and group O CA9.

Results: Of the 4156 plasma samples tested for antibodies to HIV-1/2, 243 (5.8%) were reactive in both the HIV-½ Abbott Determine and the HIV-1 rLAV. Only samples that were negative in both assays were tested in the V3 peptide ELISA. For this, 1465 HIV negative plasma samples were randomly selected and tested for reactivity with V3 peptides of which 212 (14.5%) were reactive with one or more V3 peptides. The most extensive reactivity was observed with SIVcpz-gab V3 peptides (11%), followed by reactivity with SIVcpz-ant (8.8%), SIVcpz-us (5.8%), HIV-1 group N YBF30 (6.2%), group O MVP5180 (5.0%), and group O CA9 (4.6%). While the majority of the samples reactive with more than one V3 peptide exhibited OD values between >0.3-0.6, 18 samples reacted with all six peptides with OD values >1.0.

Conclusions: This study revealing extensive reactivity of anti-HIV-1 negative plasma samples with V3 peptides from SIVcpz, HIV-1 groups N and O suggest that yet unidentified HIV variants circulate in these regions of Cameroon. Identifying such new variants is crucial for an improved diagnosis to prevent further spread of HIV in these regions and other regions of the world.

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MultiCode RTx for Quantifying Mixed Populations of Drug Resistant HIV
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Background: Human immunodeficiency virus type 1 (HIV-1) can select for drug-resistant mutations in the reverse transcriptase (RT) gene when infected individuals are exposed to certain combined modalities. Some of these emerging mutations are more common than others and have become markers for antiretroviral resistance.

Methods: For the early detection of these markers, a novel MultiCode RTx one-step PCR testing system to rapidly and simultaneously characterize mixtures of HIV-1 targets was designed.

Results: For cDNA, nucleotide polymorphisms for codon M184V (ATG to GTG) and K65R (AAA to AGA) could be differentiated and quantified even when the population mixture varied as much as 1 to 10,000. Mixed population standard curves using 1-100% of mutant or wild-type generated over four logs of total viral particle input produced consistent results.

Conclusions: MultiCode RTx could be applied to other drug selected mutations in the viral genome or for other applications where single base changes in DNA or RNA occur at frequencies reaching 0.01% to 1% respectively. Supported by NIH grant AI-058888

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Use of dried blood spot (DBS) or serum spot to detect recent HIV-1 seroconversion by HIV-1 BED Incidence Assay

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**Background/Objective:** Development of a protocol to permit use of DBS or serum spot on filter paper to detect recent infection can have major implications for surveillance of incidence in many settings, where specimen collection, processing, storage and transport can be major obstacles. We have developed a protocol for the use of DBS and serum spot to detect incident infections by HIV-1 BED Incidence assay.

**Methods:** A total of 205 unlinked matched DBS and serum specimens were used with optimized protocol. DBS or serum spots were prepared by spotting 100 µl of blood or 50 µl serum onto SS#903 filter paper and drying overnight. For both DBS and serum spot, antibodies were eluted overnight from a 6mm punch using 200 µl of specimen diluent in a 96-microwell blank plate. Next day, 50 µl of eluted material was transferred to the BED assay plate containing 50 µl of diluent. Subsequent procedure was similar to serum specimens. Calibrator and controls were prepared as serum spots on filter paper and used to normalize OD values and monitor the runs. Stability studies of serum spots were conducted at various temperatures.

**Results:** The BED assay performed on sera identified 35 (17%) of 205 serum specimens as recent seroconverters (<153 days) while 170 were long-term infections. When DBS and serum spots were used, 34 and 32 specimens were classified as recently infected, respectively. Correlation coefficients (R^2) comparing raw OD or OD-n of serum and matched DBS or serum spot specimens were >0.95. Agreement between matched serum and serum spot or DBS in classifying recent and long-term infections was 95% to 98%. Ongoing stability studies of serum spots indicate that spots are stable at 25 C for at least 4 months.

**Conclusions:** The DBS or serum spots on filter paper can be successfully used for detecting recent seroconversion using the HIV-1 BED Incidence Assay. The expanded use of the BED assay with DBS or serum spot can have significant impact on incidence testing in a variety of settings.
Development of a Rapid HIV-1 Confirmatory Test

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**Background:** In the United States, only the Western blot and Immunofluorescent Antibody Assay (IFA) are approved by the FDA for confirmatory HIV testing. Both of these tests are labor intensive, expensive, require skilled readers and slow to provide results. The World Health Organization has recommended algorithms for confirmation using combinations of simple/rapid tests with different HIV antigens.

**Methods:** We developed a rapid lateral flow, immunochromatographic assay with synthetic peptides and native protein antigens representing *env* (gp41, gp120, and gp160), *gag* (p24), and *pol* (p51/66) applied on the same nitrocellulose strip. The sample is applied to a disposable cassette which then flows onto the test strip hydrates and mixes with a reporter conjugate, to which IgG antibodies in the specimen bind. Antibodies specific to a particular HIV antigen band bind and immobilize the colorimetric reporter at the site of that band on the strip. At completion (~30 min.), the device can be either read visually or by using a portable optical reader, which detects the intensities of the test bands.

**Results:** Preliminary results using colloidal gold as the reporter with 4 positive and 2 negative serum specimens demonstrated detectable banding patterns comparable to results of the Western blot. Visible bands remained detectable with 1:1600 dilutions of HIV-positive serum, suggesting sensitivity is sufficient to detect the lower level of antibodies present in oral fluid.

**Conclusion:** A confirmatory lateral flow assay for HIV is feasible, and can be optimized for specimens with low antibody concentrations, such as oral fluid.

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