Welcome

Dear Attendees:

Welcome to the 2010 HIV Diagnostics Conference, co-sponsored by the Association of Public Health Laboratories and the Centers for Disease Control and Prevention. We are excited to be back in Orlando, Florida, host of the 2005 HIV Diagnostics Conference, and look forward to continuing the dialogue on HIV testing technologies and methods.

The landscape of HIV testing has changed significantly in recent years – from increased point-of-care screening, to the arrival of new testing technologies. Our meeting focuses on many of the challenges currently faced in point-of-care and laboratory HIV testing settings and highlights novel technologies and methods in research and development.

Partnerships and networking are critical to implementing improved HIV testing practices. The sessions, exhibits and posters presentations all provide opportunities to learn about, develop and strengthen our partnerships. We urge you to make the most of these opportunities to learn from each other. Please make time to visit our exhibitors to see the latest they have to offer and find out what they may have in store for us in the future.

On behalf of the planning committee, welcome to Orlando! We hope that this conference provides you with the knowledge you need to bring HIV testing into the next decade.

Mark Pandori, Ph.D.
San Francisco Department of Public Health Laboratory
Chair, 2010 HIV Diagnostics Conference Planning Committee
Conference Planning Committee

Chair: Mark Pandori, PhD
San Francisco Department of Public Health Laboratory

Berry Bennett, MPH
Florida Bureau of Laboratories

Bernard Branson, MD
Centers for Disease Control and Prevention

Elliot Cowan, PhD
Food and Drug Administration

Kevin Delaney, MPH
Centers for Disease Control and Prevention

Joanne de Vries, MPH
Massachusetts Department of Public Health

Steven Ethridge, MT(ASCP)
Centers for Disease Control and Prevention

Carol Fridlund, BS
Centers for Disease Control and Prevention

Timothy Granade, MS, MBIS, RM(NRM)
Centers for Disease Control and Prevention

Kathleen Krchnavek, MSSW
Wisconsin AIDS/HIV Program

Kenneth Landgraf, MS
Association of Public Health Laboratories

William A. Meyer III, PhD, D(ABMM), MT(ASCP)
Quest Diagnostics

Sandra Neal, MS, MT(ASCP), PMP
Centers for Disease Control and Prevention

Sheila Peel, PhD
Walter Reed Army Institute of Research

Eric Rosenberg, MD
Massachusetts General Hospital/Harvard Medical School

Barbara G. Werner, PhD
Massachusetts Department of Public Health

Kelly Wroblewski, MPH, MT(ASCP)
Association of Public Health Laboratories
Hotel Floor Plan

DoubleTree Hotel Universal Orlando

Registration – outside Citrus Crown Ballroom

General Sessions – Citrus Crown Ballroom

Exhibit Hall and posters – Citrus Crown Ballroom

Reception, breakfasts and breaks – Citrus Crown Ballroom

Lunches (Wed. & Thurs.) – Sun and Surf Rooms
Conference Program

Wednesday, March 24

Poster Viewing Times: 7:00am-5:30pm, with Q&A from 2:30-3:00pm | Exhibit Hall Hours: 7:00am-5:30pm

(6.0 contact hours for this day, 588-801-10)

At the conclusion of this day, participants will be able to:

- Discuss the role of public health laboratories in HIV testing and how that role has changed since the 2006 HIV Diagnostics Survey was conducted.
- Describe real-world implementations of rapid multi-test algorithms in point-of-care settings.
- Discuss three of the benefits and costs associated with rapid multi-test algorithms in point-of-care setting.
- Review the outcomes of an expanded HIV rapid testing program in an emergency department.
- Describe an HIV testing quality assurance program and list the key components to its success.
- Explain the purpose of the HIV Bridge Algorithm.

7:00am-8:00am  Continental Breakfast in the Exhibit Hall

8:00am-9:30am  Opening Session

Citrus Crown Ballroom  This session will include a conference introductions and an overview of past conference proceedings. There will also be report summaries from the APHL HIV Testing Practices Survey and the Model Performance Evaluation Program HIV Rapid Testing Survey.

Moderator – Mark Pandori, PhD, San Francisco Department of Public Health, HIV Diagnostics Conference Planning Committee Chair

- Evolution of HIV Diagnostics and Goals of the 2010 Conference
  Bernard Branson, MD, Centers for Disease Control and Prevention

  Barbara Werner, PhD, Massachusetts Department of Health

  Steven Ethridge MT(ASCP), Centers for Disease Control and Prevention

9:30am-10:00am  Morning Break in the Exhibit Hall
10:00am-11:30am  **Rapid Testing Algorithms at the Point-of-Care/Contact**

Citrus Crown Ballroom  
The presentations in this session describe the implementation of rapid multi-test algorithms at the point-of-contact and evaluate the performance of these algorithms in diverse settings.

Moderator – Kevin Delaney, MPH, Centers for Disease Control and Prevention

- **Lessons Learned from San Francisco’s Experience with Rapid Testing Algorithms**  
  Thomas Knoble, MSW, San Francisco Department of Public Health

- **Two-Rapid Test Strategy in Anonymous HIV Counseling and Testing (ACT) Sites in New York State 2008-2009**  
  April Richardson-Moore, RN, MPH, New York State Department of Health

- **Comparison of multiple laboratory-performed rapid HIV tests with standard confirmatory assays for assessment of multi-rapid test algorithms**  
  Linda Styer, PhD, Wadsworth Center, New York

- **Statewide Implementation of Rapid-Rapid Testing in New Jersey**  
  Eugene G. Martin, PhD, UMDNJ – Robert Wood Johnson Medical School

11:30am-1:00pm  **Lunch (provided) in the Sun and Surf Rooms – Sponsored by Abbott**

1:00pm-2:30pm  **Rapid Testing Algorithms at the Point-of-Care/Contact**

Citrus Crown Ballroom  
This session will continue the discussion of rapid testing algorithms and address other issues in POC testing including expanded testing programs, alternative uses of rapid tests, and new POC testing technologies.

Moderator – Michael Pentella, PhD, D(ABMM), University of Iowa Hygienic Laboratory

- **Use of a three rapid HIV test algorithm at point-of-care settings to facilitate same day receipt of a final HIV test result, referral to medical care, and address false-positive screening tests**  
  Jacqueline Rurangirwa, MPH, County of Los Angeles, Department of Public Health, Office of AIDS Programs and Policy

- **Examination of an alternative algorithm for HIV diagnostics using HIV-1/2 rapid tests as confirmatory assays**  
  Silvina Masciotra, MS, Centers for Disease Control and Prevention
2:30pm-3:00pm  
**Afternoon Break in the Exhibit Hall**

Poster presenters should be available by their posters to answer questions

3:00pm-4:30pm  
**Bridging Point-of-Care/Contact & Laboratory Testing**

Citrus Crown Ballroom  
The session will focus on the interface between POC and laboratory HIV testing. It will highlight innovative collaborations to improve quality assurance, discuss the implications of alternative testing algorithms for surveillance, and introduce a novel proposal to link POC and laboratory testing in a single diagnostics algorithm.

Moderator – Kathleen Krchnavek, MSSW, Wisconsin Department of Health

- **Establishing Ongoing Competency Assessment to Maintain HIV Testing Skills**  
  Teri Dowling, MA, MPH, San Francisco Department of Public Health

- **A Successful HIV Testing Quality Assurance Program; New York State Experience**  
  Mara L. San Antonio-Gaddy, RN, MSN, New York State Department of Health

- **The Potential Effect of Alternative Algorithms on HIV Surveillance**  
  Kristen Mahle, MPH, Centers for Disease Control and Prevention

- **The HIV Bridge Algorithm: Linking Point-of-Care, Laboratory and Patient Care**  
  Berry Bennett, MPH, Florida Bureau of Laboratories

4:30pm-5:30pm  
**Reception in the Exhibit Hall**

- **Expanded HIV Rapid Testing in Emergency Departments (ED) – Chicago, Illinois 2007-2008**  
  Nancy R. Glick, MD, Mt. Sinai Hospital, Chicago, IL  
  Karen Reitan, MA, Public Health Institute of Metropolitan Chicago

- **Multiplexed POC Device for HIV and Co-Infection Serodiagnosis**  
  Michael Lochhead, PhD, mBio Diagnostics, Inc.
Thursday, March 25

**Poster Viewing Times:** 7:00am-5:00pm, with Q&A from 3:00-3:30pm | **Exhibit Hall Hours:** 7:00am-5:00pm

(6.0 contact hours for this day, 588-802-10)

**At the conclusion of this day, participants will be able to:**
- Summarize some of the challenges in serologic HIV testing.
- State the importance of HIV recency testing.
- Describe three methods for detecting recent HIV infection.
- Compare fourth generation immunoassays to previous HIV testing technologies.
- Discuss the performance of some of the HIV testing technologies currently in development.
- Describe recent developments in nucleic acid testing technology.
- Discuss the potential applications of nucleic acid testing in laboratory and point-of-care settings.

7:00am-8:00am  **Continental Breakfast in the Exhibit Hall**

8:00am-9:30am  **Sерologic Testing Methods**

Citrus Crown Ballroom  The session will focus on HIV serology, from the analysis of screening immunoassay data to a comparison of confirmatory methods. Challenges like false positives, indeterminate results, and HIV-2 will also be discussed.

**Moderator – William A. Meyer III, PhD, D(ABMM), Quest Diagnostics**

- *Using all the data: Immunoassay signal-to-cutoff values provide useful information that should be considered in HIV diagnostic algorithms*
  Kevin P. Delaney, MPH, Centers for Disease Control and Prevention

- *False-positive HIV Test Results Using a Peptide-based Enzyme Immunoassay (EIA) in Pregnant Women and Others*
  Laura G. Wesolowski, PhD, Centers for Disease Control and Prevention

- *Dramatic Reduction in Indeterminate Results from HIV Antibody Testing of Blood Donors by Conversion from Western Blot to an Immunofluorescence Assay*
  Michael P. Busch, MD, PhD, Blood Systems Research Institute and UCSF

- *Use of Multispot (MS) HIV-1/HIV-2 Rapid Test to confirm HIV-1/HIV-2 Plus O Enzyme immunoassay (EIA) results shorten reporting time for HIV testing and identify cryptic HIV-2 infection*
  Robert W. Coombs, MD, PhD, FRCPC, University of Washington

9:30am-10:00am  **Morning Break in the Exhibit Hall**
10:00am-11:30am  Testing for Recent HIV Infection

Citrus Crown Ballroom  This session will include an overview of HIV incidence testing followed by presentations of novel methods and technologies for the detection of recent HIV infection.

Moderator – S. Michele Owen, PhD, Centers for Disease Control and Prevention

- Recent advances in development and application of assays/algorithms for detection of recent HIV infections and estimation of incidence
  Michael P. Busch, MD, PhD, Blood Systems Research Institute and UCSF

- Optimization and Calibration of Less Sensitive and Avidity Modified Protocols for the VITROS Immunodiagnostic Products Anti-HIV1+2 assay for Detection of Early HIV Infections and Incidence Estimation
  Sheila M. Keating, PhD, MSPH, Blood Systems Research Institute

- Antibody avidity-based assay for identifying recent HIV-1 infections based on Genetic Systems TM 1/2 plus O EIA
  Silvina Masciotra, MS, Centers for Disease Control and Prevention

- IgG3 as a Biomarker for Distinguishing Recent from Established HIV-1 Infection
  Kelly A. Curtis, PhD, Centers for Disease Control and Prevention

11:30am-1:00pm  Lunch (provided) in the Sun and Surf Rooms, sponsored by APHL Global Health Programs

- “A Global Perspective: International Laboratory Poster Highlights”

1:00pm-3:00pm  Fourth Generation Immunoassays

Citrus Crown Ballroom  In this session, clinical data as well as laboratory evaluations and assay comparisons will be presented for fourth generation antigen/antibody combination immunoassays.

Moderator – Bernard Branson, MD, Centers for Disease Control and Prevention

- Performance of the AxSym Ag/Ab HIV Combo assay, a 4th generation assay for routine HIV screening
  Christiane Claessens, MSc, INSPQ/LSPQ, Sainte-Anne-de-Bellevue, QC, Canada

- Performance Evaluation of the Bio-Rad GS HIV Combo Ag/Ab EIA
  Kathleen Shriver, PhD, Bio-Rad Laboratories

- Development* of an HIV-1/2/O Antigen-Antibody Combo Assay for Use on Ortho Clinical Diagnostics VITROS® Integrated and Immunodiagnostic Systems
  Patrick Kilmartin, B.Sc., M.Sc., Ortho Clinical Diagnostics
• Clinical and Analytical Evaluation of the ARCHITECT® HIV Ag/Ab Combo Assay
  Barbara A. Kaesdorf, MS, MT(ASCP), CCRA, Abbott Diagnostics

• Assessing the sensitivities of laboratory-based and point-of-care HIV antigen-antibody combination tests using a panel of specimens from recently and acutely infected individuals
  Mark Pandori, PhD, San Francisco Department of Public Health

3:00pm-3:30pm Afternoon Break in the Exhibit Hall
Poster presenters should be available by their posters to answer questions

3:30pm-5:00pm Applications of Nucleic Acid Testing Technologies
Citrus Crown Ballroom
This session will present current research in nucleic acid testing, from evaluations of NAAT for screening nontraditional specimen types to its use as a confirmatory test. Additionally, new technologies for POC nucleic acid testing will be introduced.

  Moderator – Sally Liska, DrPH, San Francisco Department of Public Health

• Optimization of Abbott m2000 RealTime HIV-1 Viral Load Assay on Breastmilk, Dried Blood Spots, Genital Secretions, Cerebrospinal Fluid, Urine, and Throatwash
  Julie A. E. Nelson, PhD, University of North Carolina at Chapel Hill

• Evaluating Kit-Based HIV-1 DNA PCR Protocol for Confirming Infection
  Carolyn D. Dawson, BS, Centers for Disease Control and Prevention

• New HIV-1 RNA Assay Validation: Roche COBAS TaqMan and Abbott RealTime
  James Bremer, PhD, Rush University

• Point-of-Care Molecular Diagnostics
  Marco Schito, PhD, Henry Jackson Foundation, DAIDS, NIH, Bethesda, MD
**Friday, March 26**

**Poster Viewing Times:** 7:00am-11:30am  |  **Exhibit Hall Hours:** 7:00am-11:30am

(3.0 contact hours for this day, 588-803-10)

**At the conclusion of this day, participants will be able to:**
- Describe two novel technologies for the detection of HIV infection.
- Explain how signal-to-cutoff data can be used in prevention.
- Review the findings from the 2010 HIV Diagnostics conference.
- Identify next steps for improving domestic HIV testing practice.

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7:00am-8:00am  **Continental Breakfast in the Exhibit Hall**

8:00am-9:30am  **New Approaches in HIV Testing**

Citrus Crown Ballroom

This session will highlight novel methods in prevention and recent advances in diagnostic testing technology.

**Moderator – Steven Ethridge, MT(ASCP), Centers for Disease Control and Prevention**

- **ELISA Strength of Signal in a Protocol to Prevent Mother-to-Child HIV Transmission**
  
  Michael Loeffelholz, PhD, ABMM, University of Texas Medical Branch

- **Rapid Detection of HIV-1 p24 Antigen and of Antibodies to HIV-1 and HIV-2 Using Magnetic Immunochromatography**
  
  Timothy C. Granade, MS, Centers for Disease Control and Prevention

- **Europium Nanoparticle-based Immunoassay (ENIA) for Sensitive and Early Detection of Human Immunodeficiency Virus Type 1 Capsid (p24) Antigen**
  
  Shixing Tang, MD, PhD, Food and Drug Administration

- **A novel method of creating molecular control used in the NLHRS HIV-2 Viral Load Assay and subsequent results in a NIBSC-WHO study to evaluate candidate international standards for HIV-2 RNA**
  
  John Kim, PhD, National Laboratory for HIV Reference Services, Public Health Agency of Canada

9:30am-10:00am  **Morning Break in the Exhibit Hall**

10:00am-11:30am  **Conference Wrap-Up & Next Steps**

Citrus Crown Ballroom

This session will include concluding remarks and a review conference findings.

Dr. Bernard Branson will moderate a panel discussion on next steps coming out of the 2010 HIV Diagnostics Conference.

**Moderated by Bernard Branson, MD, Centers for Disease Control and Prevention**
List of Exhibitors

**Abbott** – Booth 16  
1300 E. Touhy Ave.  
Des Plaines, IL 60614  
224.422.7000  
[www.abbott.com](http://www.abbott.com)  

In 1985, Abbott launched the world’s first HIV antibody assay and has been committed to HIV every since. From prevention and diagnosis to treatment and care, Abbott combines science and innovation to continually improve both serological and molecular methods. Visit Abbott at booth #15 to find out more.

**Avioq, Inc.** – Booth 7  
9700 Great Seneca Highway  
Rockville, MD 20850  
301.947.0202  
[www.avioq.com](http://www.avioq.com)  

The Avioq HIV-1 Mieroelisa System is an FDA approved assay for the qualitative detection of antibodies to HIV-1 in human specimens collected as serum, plasma, dried blood spots or oral fluid specimens obtained with OraSure® HIV-1 Oral Specimen Collection Device.

**Bio-Rad Laboratories** – Booth 14  
4000 Alfred Nobel Dr.  
Hercules, CA 94547  
510.724.7000  
[www.bio-rad.com](http://www.bio-rad.com)  

Bio-Rad manufactures quality assays for HIV antibody, including the HIV-1/HIV-2 PLUS O EIA assay which detects the broadest range of HIV and hepatitis (antigen and antibody). Additional products include the HIV-1 Western Blot, and Multispot HIV-1/ HIV-2, a rapid test approved for detection and differentiation of HIV-1 and HIV-2 antibodies.

**Chembio Diagnostic Systems, Inc.** – Booth 19  
3661 Horseblock Rd.  
Medford, NY 11763  
631.924.1135  
[www.chembio.com](http://www.chembio.com)  

Chembio Diagnostic Systems, Inc. develops, manufactures, licenses and markets point-of-care (POC) diagnostic tests and technology for the detection of infectious diseases. Chembio’s Dual Path Platform (DPP®) POC technology enables development of assays that provide unique features and capabilities such as multiplexing and improved control of challenging sample types such as oral fluid.

**Gen-Probe** – Booth 24  
10210 Genetic Center Dr.  
San Diego, CA 92121  
858.410.8996  
[www.gen-probe.com](http://www.gen-probe.com)  

Gen-Probe is a global leader in the development and manufacture of innovative molecular diagnostic assays for sexually transmitted diseases and microbial infectious diseases, including the APTIMA® HIV-1 RNA Qualitative Assay for acute phase and confirmatory testing, as well as testing for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC).

**Inverness Medical** – Booth 21  
2 Research Way  
Princeton, NJ 08540  
609.627.8060  
[www.invernessmedical.com](http://www.invernessmedical.com)  

Inverness offers two formats for rapid HIV 1 & 2 testing: Clearview COMPLETE and STAT-PAK.
**OraSure Technologies** – Booth 9
220 East First St.
Bethlehem, PA 18015
610.882.1820
www.orasure.com

The OraQuick ADVANCE® HIV-1/2 Test is the first and only FDA approved and CLIA waived rapid point-of-care test that can detect antibodies to both HIV-1 and HIV-2 in 20 minutes using oral fluid, finger-stick or venipuncture whole blood and plasma specimens. For more information call 800.ORASURE or visit customercare@orasure.com.

**Ortho Clinical Diagnostics** – Booth 4
1001 U.S. Highway 202
Raritan, NJ 08869
908.218.8721
www.orthoclinical.com

VITROS® Anti-HIV 1 & 2 Assay. For qualitative detection of antibodies to HIV type 1 and/or HIV type 2 in human serum and plasma. Time to first result is 48 minutes. Random-access testing.

**Roche Diagnostics Corporation** – Booths 22 & 23
9115 Hague Rd.
Indianapolis, IN 46256
317.521.6210
www.mylabonline.com

Roche Diagnostics will be highlighting their latest FDA approved HIV viral load test, clinical interpretations, and supporting laboratory automation platforms that increase productivity and quality of results.

**SeraCare Life Sciences, Inc.** – Booth 8
37 Birch St.
Milford, MA 01757
508.244.6400
www.searcare.com

SeraCare provides a total quality solution for HIV donor screening, *in vitro* diagnostic testing and research and development. Offerings include ACCURUN® independent Quality Controls for serology and nucleic acid testing, as well as a variety of BBI Panels for training, method validation, linearity assessment, genotyping and detection of seroconversion.

**Siemens Healthcare Diagnostics** – Booth 1
1717 Deerfield Rd.
Deerfield, IL 60015
847.267.5300
www.siemens.com/diagnostics

The Siemens Healthcare Sector is one of the world’s largest suppliers to the healthcare industry and a trendsetter in medical imaging, laboratory diagnostics, medical information technology and hearing aids. Siemens offers its customers products and solutions for the entire range of patient care from a single source – from prevention and early detection to diagnosis, and on to treatment and aftercare. By optimizing clinical workflows for the most common diseases, Siemens also makes healthcare faster, better and more cost-effective.

**Trinity Biotech USA** – Booth 5
400 Connell Dr., Suite 7100
Berkeley, Heights, NJ 07922
800.325.3424
www.trinitybiotech.com

Uni-Gold is the only CLIA-waived rapid HIV screening test in a third generation format providing 100% sensitivity. Uni-Gold utilizes a simple three-step procedure with results in 10 minutes.
Exhibitors
Lessons Learned from San Francisco’s Experience with Rapid Testing Algorithms

T. Knoble, T. Dowling, N. Underwood, G. Colfax
Department of Public Health, HIV Prevention Section, San Francisco, California, USA

Project: As part of a CDC-funded study, the San Francisco HIV Counseling, Testing and Linkage (CTL) program offered a rapid testing algorithm (RTA) at seven agencies that offer HIV testing in non-clinic settings. We describe lessons learned during this research project and the steps taken to implement a RTA similar to proposed POC algorithm 3 as standard practice now that the research study has ended.

Issue: Point-of-care (POC) rapid HIV testing requires confirmatory testing for all specimens with reactive test results. Until the results of confirmatory testing are available, all reactive rapid HIV tests are considered preliminary positive results. Unfortunately the confirmation process can have many delays and flaws. Those whose test is an initial false positive must wait for laboratory confirmation to be determined as uninfected, often 7 days. Those who are truly positive are informed of the preliminary nature of the rapid test result, but may hear a confusing message, e.g. “It is likely you have HIV, but we need to do further testing, please return in 7 days.” In both situations the lack of clarity is unsatisfactory to both the client and the counselor. Further complicating the problem, as many as 40% of CTL clients in San Francisco fail to return for their confirmatory test results. As a result, many clients who receive a preliminary positive result may experience a delay in being linked to HIV care or receiving partner services and many may not receive these essential components of the HIV CTL process at all.

Results: During the CDC-funded study, 7,165 persons were tested at the seven RTA sites using an algorithm similar to POC algorithm 4, with 153 (2.1%) testing positive on the screening test. Of these, 123 were confirmed positive by additional rapid tests, but 30 (20%) were negative on subsequent rapid tests performed onsite, and confirmed HIV-negative by the San Francisco Public Health Laboratory. All of these clients received the results of their rapid tests the same day, and 123 were immediately referred to HIV care and partner services. We implemented QA/QC for this three test algorithm at all seven sites, and found that we used the third test more for quality control and training rather than for testing clients. In order to minimize the impact of a performing a second test on clinic flow we found that it was best to identify one person per shift who was tasked with performing the 2nd and 3rd rapid tests, rather than training all counselors to do so. Still, for sites with a very low testing volume (some sites test less than 20 clients per month) maintaining the skill level of a technician who almost never runs the 2nd test proved too difficult to justify. After the study ended we implemented a revised algorithm using only two tests, similar to POC algorithm 3, at sites testing more than 20 clients per month and those that serve our most transient populations.

Lessons Learned: A POC rapid HIV testing algorithm is feasible in non-clinical settings and allows for immediate differentiation of clients who are likely to be truly infected from those who tested false-positive on the initial screening. We were able to develop training and quality assurance for three tests, but, since the study ended, have found it easier and more cost-effective to implement POC algorithm 3. We also found that not all sites may be able to implement or benefit from the additional work involved in adding a 2nd rapid test. However, we feel the POC testing algorithm has proved invaluable in renewing the confidence of both clients and counselors in the HIV rapid testing process, and have implemented it in additional sites that conduct large numbers of HIV tests in transient populations.

Presenter: Thomas Knoble, MSW, Coordinator for Community Interventions, San Francisco, Department of Public Health, 25 Van Ness, #500, SF CA 94102. (415) 703 7279. Thomas.Knoble@sfdph.org

Two-Rapid Test Strategy in Anonymous HIV Counseling and Testing (ACT) Sites in New York State 2008-2009

San Antonio-Gaddy, M; Richardson-Moore, A.
New York State Department of Health

Project: Based on the work of a CDC/APHL HIV Steering Committee to determine the best combination of tests for use at POC, NYSDOH implemented a two-test algorithm in April 2008 and provided training to staff in the state’s Anonymous HIV Counseling and Testing Program to offer a second rapid HIV test for clients who initially test rapid reactive.

Issue: Early diagnosis of HIV and linkage to medical services are crucial to disease management and decreasing HIV transmission. Rapid HIV testing clients receive immediate
Results: Between May 2008 and August 2009, the NYSDOH ACT Program tested 25,629 individuals. One hundred twenty-four individuals screened preliminary-positive; of these 92 clients accepted a second rapid test; 72 had a reactive result and 20 were negative on the second rapid test and WB negative. Fifteen clients declined a second rapid and confirmatory testing and one was WB negative. Of the 86 HIV positive clients identified, 70 (81.4%) returned for results, 69 (98.6%) changed their status to confidential and were referred to care. Sixty-two of 69 clients (89.9%) were confirmed to be in care through provider or self-report.

Lessons Learned: A greater percentage of clients returned to learn their confirmatory result and were confirmed to have accessed medical care after the two-test algorithm was implemented. All clients do not accept a second rapid test and client acceptance depends upon their time available, their previous HIV status and counselor messages.

Presenter: Mara San Antonio-Gaddy, RN, MSN, Bureau Director, NYSDOH, AIDS Institute, 315 Corning Tower, ESP, Albany, NY 12237. (518) 474 3671. mls07@health.state.ny.us

Comparison of multiple laboratory-performed rapid HIV tests with standard confirmatory assays for the assessment of multi-rapid test algorithms

Linda M. Styer, Robert Blum, Mara San Antonio-Gaddy, April Richardson-Moore, Monica M Parker
Wadsworth Center, New York State Department of Health, Albany NY AIDS Institute, New York State Department of Health, Albany, NY School of Public Health, University of Albany, Albany, NY

Objective: To retrospectively evaluate the concordance of laboratory-performed rapid HIV tests with standard confirmatory assays using rapid-reactive plasma samples submitted to our laboratory for confirmatory testing. This information will help determine the optimal sequence of rapid tests in multi-rapid test algorithms.

Methods: We evaluated rapid test referral specimens submitted between Jan 2008 and Oct 2009 from two sites: NYSDOH HIV Counseling and Testing Service (CTS) and Community Health Care Network (CHCN). Both sites perform a similar number of rapid screening tests per year (15,000-18,000). CHCN screens with a single rapid test (UniGold, Clearview, or OraQuick-oral or blood) whereas the CTS program uses a dual rapid test algorithm for screening (UniGold then Clearview or OraQuick-blood). All rapid reactive specimens submitted to our laboratory for confirmation receive an EIA (1X), Multispot and Western blot (WB). Specimens with an indeterminate WB receive a qualitative RNA test. For this study, plasma specimens were retrospectively tested in the lab using three waived rapid HIV tests: UniGold, Clearview Complete, and OraQuick.

Results: We tested a total of 494 rapid referral specimens, 370 from CHCN and 124 from CTS. HIV infection was confirmed by a positive WB or detectable RNA in 94% of CHCN specimens and 82% of CTS specimens. The final confirmatory test result (HIV-1 positive or negative) was concordant with results of all 3 rapid tests (reactive or non-reactive) in 99% of CHCN specimens and 93% of CTS specimens. Thirteen specimens had at least one rapid test result that was discordant with the confirmatory test result. Nine false positive results (7 UniGold, 1 OraQuick, 1 Clearview) were produced from 8 WB negative specimens, 5 false negative results (2 OraQuick, 3 Clearview) were produced from 3 WB indeterminate/RNA detected specimens and 2 false negative results (1 OraQuick, 1 Clearview) were produced from 2 WB positive specimens. A total of 7 specimens in our study had confirmatory test results (WB indeterminate/RNA detected) suggesting early HIV infection. Of these, UniGold was reactive for all 7, OraQuick for 5 and Clearview for 4.

Conclusions: In the specimens tested in this study, UniGold was the most sensitive test, detecting all probable cases of early HIV infection and producing no false negative results. However this test had the highest rate of false positive results (1.4% versus 0.2% for OraQuick and Clearview). These results indicate that UniGold should be used as the first test in a multi-rapid test algorithm, followed by a rapid test with higher specificity, such as OraQuick or Clearview.

Presenter: Linda Styer, Ph.D., Wadsworth Center, New York State DOH, Albany, NY 12208. (518) 474 2163. lstyer@wadsworth.org
Statewide Implementation of Rapid-Rapid Testing in New Jersey

Martin EG, Salaru G, Paul SM, and Cadoff EM.
UMDNJ – Robert Wood Johnson Medical School, Department of Pathology & Laboratory Medicine, Somerset, NJ.

Project: Following validation[i] of a rapid testing algorithm (RTA) to establish its ability to verify initial rapid HIV tests with a second (different) rapid HIV test, a state-wide RTA program with immediate linkage to care was begun at 3 high prevalence pilot sites in January, 2009 and rolled out to 20 lower prevalence sites in the ensuing months. The rapid-rapid program couples on-site verification of preliminary positive (PPos) result with efforts to provide immediate linkage to care.

Issue: Between 35 and 45% of clients receiving rapid HIV preliminary positive (PPos) results fail to receive results of confirmatory testing, do not enroll in subsequent treatment and are lost to follow-up.

Results: As of 11/30/09, more than 17,095 tests had been performed using a two-test rapid HIV testing algorithm (RTA). There were 149 Ppos results obtained using either Clearview StatPak (12282) or Orasure Oraquick (4706) (2466 - oral) or (2240 - fingerstick) as the initial device. A second, different device (Trinity Unigold) verified 138 results (92.6%), 11 PPos results did not verify (7.38%). Of the 149, Western blot testing confirmed 132 RTA PPos results, 6 clients refused testing (4%), 3 results are still pending and 2 results identified a false neg 2nd rapid test. Overall, 13 discordant results were obtained, 11 associated with a false pos initial rapid test and 2 associated with a false neg second rapid test. All tests identified as discordant were resolved by follow-up NAAT, EIA and western blot (identified by the mandatory western blot still being performed). In both false negative, further testing suggested operator error at the testing facility. Of the 138 Unigold verified clients, 75.4% were linked to a healthcare provider on the day of their final result. Individuals identified with HIV infection were referred into HIV medical care the same day; those with a false–positive screening test result were also resolved at the same visit, 94 (94%) whom were reactive on two different rapid tests and 6 (6%) whom were determined to have a false–positive screening test result based on non-reactive results on an additional two rapid tests. Among the 163 clients who did not go through the RTA 44 (27%) received a final result within a median of 8 days following the initial rapid test.

Lessons Learned: Rapid testing strategies are as effective in determining the correct HIV serostatus of patients with PPos rapid tests and increase the linkage to care for clients who are HIV positive.


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Use of a three rapid HIV test algorithm at point-of-care settings to facilitate same day receipt of a final HIV test result, referral to medical care, and address false-positive screening tests

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Project: The County of Los Angeles Department of Public Health, Office of AIDS Programs and Policy (OAPP) piloted a three-test, point-of-care (POC) rapid testing algorithm (RTA) similar to POC algorithm 4 (detailed in the HIV Testing Algorithms: A Status Report 2009), through a CDC-funded grant, in four diverse publicly-funded point-of-care settings in Los Angeles to evaluate the RTA’s performance and assess its impact and feasibility.

Issue: With rapid HIV testing nearly all clients receive their initial rapid HIV test results; however, many who test preliminary positive fail to return for their confirmatory test results. In 2008, among publicly-funded HIV counseling and testing sites in Los Angeles County, 48% of all testers with an initial reactive rapid test received a final confirmed result. Use of a rapid HIV RTA would eliminate the need for off-site confirmatory testing and potentially increase direct linkage to HIV care. This pilot sought to address the HIV Testing Algorithms: A Status Report 2009 “Key Data Needs” – data for sequences of three different point-of-care rapid tests performed prospectively in high and low HIV prevalence settings.

Results: The pilot was implemented in four publicly funded point-of-care settings in Los Angeles from August 2007 to March 2009. During this time frame, 10,857 rapid tests were conducted at these four sites. Of those, 263 had an initial reactive rapid test (2.42%). Of those, 100 (38%) went through the RTA. All (100%) RTA clients received a final result within the same visit, 94 (94%) whom were reactive on two different rapid tests and 6 (6%) whom were determined to have a false–positive screening test result based on non-reactive results on an additional two rapid tests. Among the 163 clients who did not go through the RTA 44 (27%) received a final result within a median of 8 days following the initial rapid test.

Lessons Learned: Using a RTA, all (100%) clients received their final result. Individuals identified with HIV infection were referred into HIV medical care the same day; those with a false-positive test result were also resolved at the same visit. Results suggest using a RTA in point-of-care settings allows more individuals identified with HIV infection to be referred into care, with fewer lost to follow-up. During the 20-month pilot period, the third rapid test in the RTA was used only 6 times; therefore, maintaining a third test at the RTA sites was not cost effective. During the same time period, California
Examination of an alternative algorithm for HIV diagnostics using HIV-1/2 rapid tests as confirmatory assays

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Objective: The current algorithm for HIV diagnosis involves screening with an EIA and confirmation with Western blot (WB) or immunofluorescence assay. The technical aspects and cost of confirmatory assays along with the improved sensitivity and specificity of screening tests led to examination of alternative diagnostic strategies. We evaluated the performance of two FDA approved HIV-1/2 rapid tests (RTs) as confirmatory assays in an alternative algorithm for HIV diagnosis that requires concordant results between the screening test and the RT.

Methods: Previously tested plasma specimens from the United States (54 HIV-negative and 56 HIV-1 WB-positive), Cameroon (5 HIV-negative and 40 HIV-1 WB-positive) and Ivory Coast (82 HIV-2 positive by Multispot (Bio-Rad)) and 26 HIV-1 seroconversion panels (230 specimens, subtype B) were tested with Clearview COMPLETE HIV-1/2 and HIV-1/2 STAT-PAK (Inverness Medical). Specimens were also evaluated with Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio-Rad), APTIMA HIV-1 RNA Qualitative Assay (Gen-Probe), ARCHITECT HIV Ag/Ab Combo assay (Abbott) and 202 have HIV-1 Western blot results available. Cumulative frequency (50%) of positive results relative to the number of days before the WB was first positive was calculated in seroconversion panels.

Results: STAT-PAK had 99.4% sensitivity (95% CI: 96.9-99.9%) and 100% specificity (95% CI: 93.9-100%), while Complete had 100% sensitivity and specificity at confirming HIV infection with previously characterized specimens from the United States, Cameroon, and Ivory Coast. The cumulative frequency analysis of seroconversion panels showed that APTIMA, ARCHITECT, Genetic Systems, COMPLETE, and STAT-PAK were positive 26, 18.5, 12, 6, and 5 days before WB respectively. Eleven percent of 106 EIA-positive seroconversion samples would have been missed by WB and RTs.

Conclusions: STAT-PAK and COMPLETE showed good sensitivity and specificity in confirming HIV infection and became positive a few days before the WB in seroconversion samples. These data indicate that the two rapid tests would perform comparably to WB for confirmation of EIA reactive samples. However, HIV-1 RNA, Ag/Ab combination, and third-generation Ab assays detected HIV-1 infected-individuals earlier after infection than RTs and WB. Further evaluation of alternative approaches for HIV diagnosis is warranted based on the findings of this study.

Presenter: Silvina Masciotra, MS, HIV Diagnostics and Incidence team, Centers for Disease Control and Prevention, 1600 Clifton Rd NE MS A-25, Atlanta, GA 30333. (404) 639 1004. svm6@cdc.gov


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Project Description: The Chicago Department of Public Health initiated an expanded HIV testing project in 4 urban EDs in October 2007. Objectives were to increase routine HIV testing, increase the number of people aware of their HIV status, identify persons with HIV infection, and facilitate linkage to care to reach African Americans living in high incidence communities.

Needs Addressed: Project implementation was facilitated in EDs with prior HIV testing experience; two EDs initiated new testing programs. HIV testing integration relied on engaging key organizational stakeholders and working closely with ED and hospital staff at all stages of project development.
Hospitals selected the HIV rapid test administered by health educators in the ED. Health educators provided pre-test information, administered the test, provided test results, and facilitated confirmatory testing, referral and linkage services.

**Results:** In the first 2 years, more than 44,000 HIV tests were performed. In year two, 32,269 tests were done, and identified 195 (0.6% positivity) confirmed HIV cases. Persons tested were 48% were female, 72% Black, and median age was 56 (range 11-101). Among HIV-infected persons where risk was known, 38% (46/120) were MSM, 38% (46/120) female, 23% (28/120) heterosexual male, and 7% (14/195) reported IDU. Implementation themes were integrating testing into ED flow and working with hospital departments. Training, TA, and continuous quality improvement were integral to successful project implementation.

**Lessons Learned:** Implementation of routine rapid HIV testing programs in ED settings is feasible, and reaches a population at risk. Programs identify a large number of HIV infected persons in emergency medical care who might have been missed if testing were not available, and create opportunities for HIV prevention education, awareness, and early disease intervention that facilitate efforts to increase the number of people who know their status.

**Presenters:** Nancy R. Glick, MD, Mt. Sinai Hospital, Chicago, IL 60608. (773) 257 5442. Nancy.Glick@sinai.org; Karen Reitan, MA, Associate Executive Director, Public Health Institute of Metropolitan Chicago, Chicago, IL 60604. (312) 556 0285. karen.reitan@phimc.org

### Multiplexed POC Device for HIV and Co-Infection Serodiagnosis

**Objective:** New HIV rapid tests that deliver antigen-specific information as part of confirmatory algorithms are potentially useful. In addition, disease management for HIV infection is significantly enhanced if one can define the subset of co-infections for which patients are at risk. A simple POC system that delivers HIV and syphilis antigen panel serology is presented here. It is comprised of single-use cartridges and a simple fluorescence reader that potentially bridges POC and laboratory testing.

**Methods:** Disposable cartridges were designed for simultaneous detection of HIV and T. pallidum antibodies in serum or plasma. The HIV assay measures Abs against p24, gp41, and gp120. The syphilis assay measures Abs against p17, p47, and TmpA. The assay uses 6 microliters of sample diluted into carrier buffer. Cartridges are read on a fluorescence imaging unit powered by a USB port. Total test time is < 30 min per sample and up to 12 samples can be run simultaneously by a single user for batch mode testing. IRB-approved and commercial clinical samples had known primary infection status determined by clinical laboratory methods. Known negative samples were used to establish empiric cutoff values. Signal/cutoff values were then calculated and these values were compared to known disease status.

**Results:** Excellent correlation between the prototype system results and known disease status was observed. For the HIV samples, 62 of 62 known positives and 122 of 124 negatives were called correctly. The two false negatives were also negative on a commercial HIV rapid test. 19 of 19 known RPR-positive samples were positive on the Trep-specific assay. At least 3 of 88 RPR-negative samples were reactive on the Trep-specific test, suggesting resolved infection.

**Conclusions:** The low cost POC system provides parallel measurement of antibodies against a panel of HIV and co-infection antigens. It has been successfully demonstrated for a HIV /syphilis combination assay.

**Presenter:** Michael J. Lochhead, Ph.D., mBio Diagnostics, Inc., 3180 Sterling Circle, Boulder, CO 80301. (303) 952 2810. Mike.Lochhead@precisionphotonics.com

### Establishing Ongoing Competency Assessment to Maintain HIV Testing Skills

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**Project:** To develop an effective method of ensuring the competency of HIV testing personnel at government-supported HIV testing sites to accurately run, read and document a variety of CLIA-waived rapid HIV tests.

**Issue:** The San Francisco Department of Public Health, HIV Prevention Section supports 18 community organizations to provide HIV counseling and testing; 75% of the 17,000 annual HIV tests provided are rapid. While persons running and reading the rapid HIV tests have to pass a required and rigorous 4-hour certification training prior to providing testing, it quickly became apparent that on-going assessment of testing competency was critical to ensure testing quality.

**Results:** The Department implemented a Competency Assessment Test (CAT) for each rapid HIV test used within San Francisco. The CAT is a checklist of critical steps that must be followed and serves as a standard against which to evaluate testing staff to maintain the quality of testing for a particular HIV rapid test. The CAT is conducted at regular intervals after initial certification and requires testing staff to be visually observed running, reading and documenting test results. Failure to pass the CAT results in a temporary
Lessons Learned: (1) A written checklist of required steps to run and read waived rapid HIV tests provides a uniform standard for assuring quality assurance. (2) Regularly scheduled CATs help identify and correct problems early. (3) MPEP is an effective QA tool removing bias from the personnel observing the CAT. (4) Regular site visits help to identify and correct issues before they become problems. (5) Running routine reports on testing data can quickly spot invalid tests and allow for remedial training to reinforce details of the test procedures required to run a waived HIV rapid test. (6) Expanding QA to allow for the use of multiple rapid HIV tests can be streamlined if standardized procedures for monitoring such as the CAT are provided. (7) Running a second rapid test when the first rapid test is reactive provides immediate QA on the first test; it helps support the result of the first rapid test and can identify a false positive test result.


A Successful HIV Testing Quality Assurance Program; New York State Experience

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Project: New York State (NYS) public health testing program has conducted rapid HIV testing Statewide since 2003. Thirty-five staff regionally out stationed conducts multiple CLIA-waived tests in community, correctional and untraditional settings. To ensure the delivery of high quality test results by non-laboratorians, a Quality Assurance Program with Wadsworth Center’s partnership has been instituted.

Issues: Although waived rapid HIV antibody tests are simple to use and can provide reliable results when the manufacturer’s directions are followed, a number of activities must be instituted to reduce mistakes that can occur in the testing process. This presentation will describe the QA program activities and quality control tools used by NYS.

Results: To comply with applicable state requirements and CLIA, NYS addressed operational issues for point-of-care settings through the development of QA practices including protocol development, staff training, knowledge assessments, proficiency testing exercises, direct observation of staff performance and other measures to ensure delivery of quality results. Outcomes in staff competency scores and negative and positive predictive values demonstrate that NYS QA program ensures accurate testing outcomes.

Lessons Learned: The ability to generate accurate POC results depends not only on the intrinsic quality of the test itself but also on extrinsic qualities such as the skills of the performer and quality of the specimen. The overall accuracy of the testing algorithm depends on the characteristics of the specific tests, the order in which the tests are performed, and the protocol for resolving discordant results. Protocols and messages for clients with preliminary reactive results are essential to the testing process. Continuous review of testing outcomes helps to address test performance issues and aids in developing future best practices.

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The Potential Effect of Alternative Algorithms on HIV Surveillance

Kristen Mahle, MPH, Centers for Disease Control and Prevention

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The HIV Bridge Algorithm: Linking Point-of-Care, Laboratory and Patient Care

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Introduction: The HIV Bridge Algorithm is intended to assist point-of-contact/care (POC) healthcare providers and clinical laboratory staff desiring a stand-alone algorithm that combines POC HIV screening strategies and laboratory supplemental testing options. The Bridge Algorithm should serve as a communication tool, informing the POC providers of possible options to resolve their rapid test(s) referrals and provides the laboratory preliminary screening results useful in determining the most appropriate supplemental testing option. The algorithm starts with a single reactive rapid test or a discordant multi-rapid test referral from POC then proceeds to laboratory support with an optional intermediate immunoassay (IA) testing step followed by four separate supplemental testing options: (I) WB or IFA, (II) Qualitative NAAT, (III) HIV-1 viral load or (IV) Dual IA. Because some of the laboratory options incorporate testing methodologies in non-traditional roles, the individual laboratory may be required to validate the assay performance for its use. It is anticipated that this algorithm may be advantageous for sites that prefer POC screening and a supplemental testing process that leads to prompt medical care referrals. For example, concordantly reactive persons with POC reactivity and laboratory Option III or IV reactivity can be referred to care in a timely manner. False-positive results may be resolved by clinical follow-up including further testing without the time and expense of an intervening supplemental test (WB, IFA, or qualitative NAAT). The Bridge Algorithm is a concept derived during the development of the 2009 APHL/CDC Status Report (www.aphl.org/hiv/statusreport) with its multiple POC and Laboratory-based algorithms. This presentation will focus on the algorithm purpose, design and data needs.

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Using all the data: Immunoassay signal-to-cutoff values provide useful information that should be considered in HIV diagnostic algorithms

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Background: Serologic testing algorithms for some diseases, such as Hepatitis C, rely on immunoassay (IA) signal-to-cutoff (S/CO) values to guide interpretation of algorithm results and identify specimens for additional testing. For HIV, evaluation of S/CO data from a variety of IAs is needed to assess their utility in testing algorithms.

Objectives: 1) To characterize the initial S/CO values of HIV serologic assays, 2) To consider S/CO values of repeated IAs and evaluate the benefit of repeating initially reactive IAs on the same test and 3) To compare S/CO results when repeating the same IA to results of a different IA.

Methods: Four public health laboratories, Florida, Kansas, Missouri and San Francisco, provided IA S/CO data for all initially reactive specimens tested between July 2008 and June 2009. All of these laboratories reported using BioRad HIV-1/2 Plus O IA (BioRad) to test their specimens. Additionally, specimens from a CDC study with results for the BioRad IA as well as the chemiluminescent Abbott Architect 4th generation IA (Architect) and the Abbott HIVAB HIV-1/HIV-2 (DNA) IA (HIVAB) were included in the analysis. Specimens were categorized into four groups based on IA and serologic confirmatory test (Western blot (WB) and/or immunoforescence assay (IFA)) results, (1) negative (IA negative), (2) false-positive (IA initially reactive but negative on repeat or IFA repeatedly reactive (RR) with negative WB, and/or IFA), (3) indeterminate (IA RR with indeterminate WB or IFA results), and (4) positive (IA RR with positive WB or IFA results). The median and range of S/CO values were calculated for initial and repeat IA results, and the area under a Receiver Operating Characteristic curve (AUC) was calculated for different S/CO threshold values.

Results: Test results were available for a total of 5286 specimens; 3029 negative, 450 false-positive, 16 indeterminate, and 1791 positive. The median and range of initial BioRad IA S/CO values for each group were: negative 0.21 (-0.07-0.99); false-positive 2.14 (1.00-16.30); indeterminate 4.60 (1.20-14.58); and positive 13.62 (1.2-21.10). A signal-to-cutoff value of 10 maximized the AUC at 0.954; 34 (8%) false-positive specimens, 4 (25%) indeterminate specimens and 1763 (98.4%) positive specimens had values...
A total of 1892 specimens had repeat S/CO data available for the BioRad IA. Of 1446 positive specimens, 1445 (99.9%) had RR results, the median initial S/CO was 14.0 and 98% of specimens had a repeat S/CO ≥ 10. Of 446 false-positive specimens with repeat S/CO data, 92 (20.6%) had RR results, the median repeat S/CO was 0.15 (negative) and 441 (99%) of specimens had a repeat S/CO < 10. The 5 false-positive specimens with initial and repeat S/CO >10 had negative IFA(4) or WB(1) but no follow-up or other IA results are available. 462 specimens from the CDC study had S/CO values for all three IAs. All 349 positive specimens were reactive on both Abbott IAs. For the Architect the median S/CO was 384.72 (range: 8.91-674.98); for the HIVAB the median S/CO was 15.87 (range: 2.74-19.30). Only 1 (0.9%) of 113 BioRad reactive, false-positive specimens had a reactive result on the Architect (BioRad=1.41, Architect=1.77, HIVAB=0.16); this specimen had negative results on repeat testing with both the BioRad and Architect IAs. No BioRad reactive, false-positive specimen was reactive on the HIVAB IA.

**Conclusions:** We found that specimens that were confirmed to be HIV-positive had very high initial S/CO values, as well as high S/CO values on repeat testing with the same or different IAs. For initially reactive specimens that were false-positive, a second different IA was more likely to have low or negative S/CO than repeat results using the same IA. S/CO values from two different IAs can be used to differentiate HIV-positive specimens from ones that are likely to be false-positive or indeterminate and to determine next steps for testing within an algorithm.

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**False-positive HIV Test Results Using a Peptide-based Enzyme Immunoassay (EIA) in Pregnant Women and Others**

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**Objective:** To examine whether false-positive HIV EIA test results occur more frequently among pregnant women than among women who are not pregnant and men ("others").

**Methods:** We retrospectively identified specimens from pregnant women and others tested using the Genetic Systems HIV-1/HIV-2 Plus O EIA from July 2007 to June 2008 at 31 labs affiliated with a large US reference laboratory. Pregnant women were identified by pregnancy-specific laboratory tests or ICD-9 codes on the date of HIV test-

**Dramatic Reduction in Indeterminate Results from HIV Antibody Testing of Blood Donors by Conversion from Western Blot to an Immunofluorescence Assay**

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**Objectives:** Information on abnormal test results on blood donations should be accurate and informative. This is particularly important for HIV, given health implications for donors and consequences for hospitals who must notify recipients of prior donations from donors with positive or indeterminate HIV test results (lookback). Indeterminate and unreadable results on HIV-1 Western blot (WB) are especially troublesome since donors and hospitals are confused by the non-specific message. To address this our testing laboratory replaced HIV-1 WB with the licensed HIV-1 immunofluorescence assay (IFA) on 02/19/07.
Methods: We compared HIV-1 confirmatory results performed using WB for one year prior to IFA implementation (2/19/06 to 2/18/07) with results performed with IFA between 2/2/07 to 2/18/09.

Results: 3,636,276 donations were tested during the study period, with 668 HIV-1 WB and 872 HIV-1 IFA confirmatory tests performed on 1,540 EIA repeat-reactive donations. 51% of the WB results were either indeterminate (33%) or unreadable (18%), compared to only 2% of IFA results reported as indeterminate and none as unreadable. All 24 IFA indeterminate samples and 118 WB unreadable samples were negative for HIV RNA by nucleic acid amplification testing (NAT). All WB or IFA negative donations also tested negative by NAT. 1 of 225 WB indeterminates was NAT positive. Donor notifications of indeterminate or unreadable results decreased from 343/year with WB to 12/year with IFA. Lookback notifications to hospitals triggered by indeterminate/unreadable results decreased from 588/year with WB to <40/year with IFA.

Conclusion: WB indeterminate and unreadable results rarely represent true infection with HIV-1 in EIA reactive donors, yet still convey a message of uncertainty when reported to the donors and hospitals. Conversion to HIV-1 IFA dramatically reduced indeterminate/unreadable results decreased from 588/year with WB to <40/year with IFA.

Presenter: Michael P. Busch, MD, PhD, Director, Blood Systems Research Institute, Vice President, Research/Scientific Affairs, Blood Systems, Professor of Laboratory Medicine, UCSF, 270 Masonic Avenue, San Francisco CA 94118. (415) 749 6615. mbusch@bloodsystems.org

Use of Multispot (MS) HIV-1/HIV-2 Rapid Test to confirm HIV-1/HIV-2 Plus O Enzyme immunoassay (EIA) results shorten reporting time for HIV testing and identify cryptic HIV-2 infection


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Objective: To find an alternative HIV testing strategy that provides timely and accurate results for clinicians.

Methods: Routine clinical serum specimens were submitted for HIV testing. Specimens that were non-reactive with the EIA were reported as “no evidence of HIV infection.” Specimens that were reactive with the EIA were immediately retested with the MS. EIA/MS reactive specimens were reported immediately as “presumptive HIV infection” with pending confirmation. All EIA reactive specimens were forwarded for confirmatory HIV-1 Western blot or other testing as required.

Results: Between July 31, 2008 and October 8, 2009 a total of 13,943 HIV serologies were performed, identifying 242 EIA reactive specimens: 203 (1.5% of total samples) confirmed by HIV-1 WB, 26 were non-reactive upon duplicate EIA retest, 7 were duplicate EIA reactive/WB negative and 6 were duplicate EIA reactive WB indeterminate. One of six available WB negative or indeterminate specimens tested was HIV-1 RNA positive. Of the 203 HIV-1 WB confirmed specimens, 201 were MS HIV-1 reactive and 2 (with WB bands gp160, p55, p31, p24 and gp160, p31, p24) were only MS HIV-2 reactive, HIV-1 RNA negative and confirmed by HIV-2 WB. The MS was 95.5% sensitive and 100% specific for detecting HIV infection. The median TAT was 1 day for the EIA/MS strategy and 3 days (interquartile range 2-4 days) for final reporting after confirmatory WB testing was completed.

Conclusions: Adjunctive MS testing allowed us to confirm reactive EIA results immediately and report “presumptive HIV infection” results pending confirmation, identify false-positive EIA results, and shorten the reporting TAT by two days. In addition, two HIV-2 infections presented with confirmed HIV-1 WB would have been missed without MS testing (1% of HIV-1 confirmed infection). These findings have important implications for clinical management and underscore the need for both HIV-1 and HIV-2 screening in clinical practice.

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Recent advances in development and application of assays/algorithms for detection of recent HIV infections and estimation of incidence

Michael P. Busch, MD, PhD

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Reliable estimates of HIV incidence are critical for characterization of HIV epidemics, evaluation of prevention programs, and design and evaluation of intervention/prevention trials. Moreover, recently infected persons contribute substantially to HIV transmission due to behavioral and biologic factors, so their detection could have important public health applications. Standard methods to estimate HIV incidence are unsatisfactory. Direct measurement of incidence through the prospective follow-up of a cohort of HIV-negative persons is expensive, unrepresentative, and not sustainable even in resource-rich settings. It is possible to estimate HIV incidence by detection of p24 Ag and/or HIV RNA before seroconversion; however, due to the very short period of antigenemia/viremia before seroconversion, this method requires very large sample sizes and is often impractical due to the need to test all seronegative samples for p24 Ag or RNA. A decade ago, Jannsen et al established the concept of identifying recently infected...
Optimization and Calibration of Less Sensitive and Avidity Modified Protocols for the VITROS Immunodiagnostic Products Anti-HIV1+2 assay for Detection of Early HIV Infections and Incidence Estimation

SM Keating, D Hanson, L Lebedeva, O Laeyendecker, L All-Napo, P Contestable, S Edwards, S Ethridge, B Branson, PJ Norris, MP Busch

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Objective: Over the past decade “recent infection testing assays/algorithms” (RITAs) have been developed to identify individuals whose antibody response against the virus is still evolving following serocconversion. Few RITA assays have been developed and none are FDA-approved for detection of early infection.

Methods: The VITROS Anti-HIV1+2 assay, widely employed outside the U.S., was recently licensed by the FDA to detect HIV-1 and HIV-2 infection. We developed a Less Sensitive (LS) (detuned) protocol for this system by pre-diluting (1:400) HIV+ samples and modifying the assay cutoff. Using 710 first-time and repeat blood donors, a signal/cut-off ratio (S/C) of 20 on the LS-VITROS has been calibrated to be equivalent to the LS-Vironostika cut-off of 1.0 formerly widely used for the identification of recent infection. The VITROS assay can also be used to test for antibody avidity, and an avidity index (AI) is calculated using the ratio of results for samples incubated in guanidine relative to PBS. Serocconversion panels (70 subjects) were tested to determine the window period (WP) of these assays. HIV positive patients on HAART (n=134) and off (n=297), and individuals with low CD4 counts (AIDS; n=140) were tested for assay specificity.

Results: The S/C of 20 by LS-VITROS has a RITA WP of 239 days (+/-75 days) and the AI of 0.6 has a WP of 180 days (+/-53 days). As with all RITA assays, these are subject to limitations such as false recent classification of samples from patients with advanced disease or on HAART. For the LS-VITROS assay, we found that 18% of HIV+ patients, 14% of AIDS patients and 29% of individuals on HAART were within the RITA WP. In the avidity version, 22% of HIV+ patients, 6% of AIDS and 40% of HAART were within the RITA WP.

Conclusions: In both the detuned and avidity modified versions of this assay, we have identified cutoffs and WP that can be used to estimate the length of time since infection and in calculations for HIV incidence.

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Antibody avidity-based assay for identifying recent HIV-1 infections based on Genetic Systems TM 1/2 plus 0 EIA

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Background: Identifying recent infections to quantify HIV incidence remains a challenge. Several laboratory methods have been developed that distinguish recent from long-term HIV infection based on the evolution of the immune response to HIV infection (antibody quantity/titer, proportion, avidity or specific subtype response). To overcome limitations of previous assays, we developed a new antibody avidity-based EIA for discriminating recent (low avidity antibodies) from long-term (high avidity antibodies) HIV infections.

Materials & Methods: The Genetic Systems TM 1/2 + 0 EIA (Bio-Rad) was modified by changing the initial sample incubation to 4oC and performing paired incubations in the presence and absence of 0.1M diethylamine (DEA). Optical density values (OD) were used to calculate the ratio of OD in the presence and absence of DEA (avidity index (AI)). Longitudinal specimens from 273 individuals with 1144 observations from multiple cohort studies, including subtypes B, B’, A, G, CRF01 and CRF02 were tested. Mean recency periods corresponding to AI values ranging from 20-80% were estimated using survival methods. Assay sensitivity and specificity were calculated from the number of specimens testing higher or lower than assay cutoff values relative to those observed earlier or later.
IgG3 as a Biomarker for Distinguishing Recent from Established HIV-1 Infection

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Objective: The use of serological assays for determining recent HIV-1 infection has proven invaluable for estimating incidence in cross-sectional cohorts. We have expanded on previous reports that demonstrated IgG3 antibody as a potential biomarker for distinguishing recent from long-standing HIV-1 infection. In this study, we developed a bead-based multiplex assay, using the Luminex format, to evaluate the feasibility of identifying recent infection based on IgG3 levels specific for HIV-1 p24, p66 and gp41.

Methods: Plasma samples of known duration of infection (949 specimens from 350 subjects) were diluted 1:50 and individually tested for reactivity against recombinant HIV-1 p24, p66, and gp41 coupled to microspheres. IgG3 reactivity was detected using a monoclonal mouse anti-IgG3 secondary antibody conjugated to PE, followed by analysis on the Bio-Plex 200 System. A normalized value was calculated by dividing the raw data output (mean fluorescence intensity, MFI) for each sample by the MFI of a calibrator.

Results: The mean reactivity period ranged from 139 days (95% CI: 120-159) at 20% AI to 408 days (95% CI: 367-448) at 80% AI. Assay sensitivity peaked at 93.2% (80% threshold) and specificity at 86.4% (40% threshold). The area under the ROC curve was 0.952 (95% CI: 0.940-0.963). All specimens with CD4 <200 cells/μl were identified as long-term. For ARV-treated samples, 54.3% were misclassified by BED compared to only 13.6% by Avidity assay (40% threshold).

Conclusions: The ROC curve analysis indicates good accuracy of this modified assay for distinguishing between recent and long term infection. Furthermore, the assay performed well where titer-based assays misclassify long-term infections as recent. Further research is needed to assess the accuracy of the assay to estimate HIV incidence for varying subtypes and prevalence.

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Performance of the AxSym Ag/Ab HIV Combo assay, a 4th generation assay for routine HIV screening

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Objective: Since 2008, the majority of HIV serology tests in Québec have been performed with a 4th generation assay: AxSYM Ag/Ab HIV Combo (Combo; Abbott Laboratories), a test licensed in Canada but not available in the US. The objective of this study was to assess the impact of the introduction of this new Ag/Ab test on the specificity of HIV serological results and on the detection of acute infections.

Methods: Test results obtained during 2008 with Combo (n=300,923) were compared to those obtained in 2007 (n=294,308) with a 3rd generation assay (3rd g; AxSYM HIV 1/2 g0). The trends in reactivity and confirmatory rates were analyzed. Cases of acute infection were examined. The patient population was similar in 2007 and 2008.

Results: The number of reactive and HIV confirmed sera were 2290 (0.78%) and 1622 (70.8%) in 2007 and 2228 (0.74%) and 1595 (71.6%) in 2008. There were 10 and 9 false-positive p24 Ag tests in 2007 and 2008 respectively. Twenty-two (22) WB negative/p24 Ag positive specimens were detected by Combo whereas 12 were detected by the 3rd g. in 2007. A detailed analysis revealed that Combo failed to detect acute infection in one patient. A significant decrease in the index value of Combo between the first and second serum obtained days apart was observed in patients with acute infection.
Conclusions: The performances of the Combo and 3rd g. were similar. However, Combo detected more patients with acute infection. Supplemental tests to detect HIV RNA or p24 Ag were needed to clarify the status of a Combo reactive/WB negative sample. There is a window period with Combo although reduced when compared to 3rd g. A second window period can occur with Combo during the transition from decreasing p24 Ag levels and increasing Ab levels. Despite the sequential nature of the data analyzed, results show that the Combo assay did not result in more false-positives in routine use and allowed for early identification of individuals with acute HIV infection.

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Performance Evaluation of the Bio-Rad GS HIV Combo Ag/Ab EIA

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Objective: to evaluate the performance of an investigational 4th generation HIV diagnostic assay designed to detect both HIV-1 p24 core antigen and HIV antibody (HIV-1 groups M and O and HIV-2) in a single microplate well.

Methods: The GS HIV Combo Ag/Ab EIA combines specific recombinant and synthetic peptide antigens representing HIV-1, HIV-2, and HIV-1 variant sequences for detection of HIV envelope antibody, as well as specific monoclonal and polyclonal antibodies for detection of HIV-1 p24 antigen. The performance parameters of the new assay were evaluated, using both “manual” microplate equipment and the Evolis™ automated microplate processor.

Results: Sensitivity of the new Ag/Ab assay was estimated to be 100% based on the results of testing 94 known HIV positive samples (54 HIV-1, 40 HIV-2) and selected dilutions of HIV-1 group O samples. In testing of 84 early HIV-1 seroconversion samples derived from 27 panels, 49/84 (58.3%) were detected by the new HIV Combo Ag/Ab EIA vs. 16/84 (19.0%) detected by a licensed “3rd generation” HIV antibody assay. Seroconversion detection was earlier with the GS HIV Combo Ag/Ab EIA for 23 of the 27 panels tested (85.2%), and improvements of 0-11 days vs. antibody testing were evident from these data. Specificity of the new Ag/Ab assay was 100.0% in testing of 1001 normal donor samples (501 sera and 500 plasma) using manual microplate equipment, with a mean signal/cutoff of 0.14 for serum and 0.15 for plasma samples. Excellent correlation with the manual results was obtained when a subset of these samples were tested on the Evolis microplate processor.

Conclusions: The investigational GS HIV Combo Ag/Ab EIA exhibited a significant improvement over current “3rd generation” antibody assays for detection of early HIV infections. The excellent performance parameters of the new 4th generation HIV Ag/Ab assay and the automated Evolis instrument are well suited for use in HIV diagnostic settings.

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Development* of an HIV-1/2/O Antigen-Antibody Combo Assay for Use on Ortho Clinical Diagnostics VITROS® Integrated and Immunodiagnostic Systems

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Objective: To develop an assay for use on VITROS Immunodiagnostic Systems that is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier detection of HIV infection.

Materials and Methods: Anti-envelope antibody detection in the VITROS HIV-1/2/O Ag-Ab assay was accomplished using recombinant transmembrane envelope proteins for HIV-1, HIV-2 and HIV-1 Group O. HIV-1 p24 antigen detection was accomplished using monoclonal antibodies (MoAb) against HIV-1 p24. Envelope recombinant proteins and p24 MoAbs were biotinylated and used in the first step of the assay to capture anti-HIV-1/2/O antibodies or HIV antigen in the sample. The micro-wells used in the assay are coated with streptavidin and thus bind the biotinylated proteins. After washing, HRP conjugates of the envelope recombinant proteins and anti-p24 MoAbs are employed in the second step. After a final wash bound HRP conjugates are detected using the chemiluminescent substrate common to all VITROS Immunodiagnostic assays.

Results: When tested on 12 commercially available seroconversion panels the HIV-1/2/O Ag-Ab assay was positive on average of 4.6 days (range of 0 to 12 days) before the VITROS Anti-HIV 1+2 antibody assay. Of 163 known HIV-1 or HIV-2 antibody positives all were reactive in the HIV-1/2/O Ag-Ab assay. Testing dilutions of two HIV p24 Ag standards (WHO and AFSAAPS) demonstrated detection of approximately 1.25 units/mL and 25 pg/mL respectively. To assess specificity, 2000 unfrozen volunteer blood donor EDTA plasmas were tested. Six (6) of these donor plasma were positive on the Ag-Ab assay, giving a specificity of 99.7%. All clades of HIV-1 p24 antigen were detected including HIV-1 Group O. In addition, HIV-2 p26 was also detected.

Conclusion: We have developed an HIV-1/2/O Ag-Ab combo assay for use on VITROS Immunodiagnostic Systems with
Clinical and Analytical Evaluation of the ARCHITECT® HIV Ag/Ab Combo Assay

Objective: To evaluate the performance of the ARCHITECT HIV Ag/Ab Combo assay on the i2000SR system in a diagnostic population.

Methods: ARCHITECT HIV Ag/Ab Combo is a chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of HIV-1 p24 antigen and antibodies to HIV type 1 (HIV-1 group M and group O) and/or type 2. Reproducibility was assessed using a negative sample and anti-HIV-1 group M, anti-HIV-2, anti-HIV-1 group O, and HIV-1 antigen samples (target 3 and/or 10 S/CO). Specificity and sensitivity were determined from 6,164 prospectively collected specimens from individuals at risk for acquiring HIV in a low prevalence setting (<1% or lower than other settings in the community), apparently healthy individuals including pregnant females and 1,267 samples that were comprised of HIV-1 antigen (n=63), anti-HIV-1 (n=1003), and anti-HIV-2 (n=201) positive samples and from internal testing of 31 commercial HIV seroconversion panels. Performance was also evaluated in individuals at increased risk for HIV in the US (n=896).

Results: For reproducibility, the within site total CVs for the positive samples ranged from 3.2 to 4.7%. Specificity in the low risk population was 99.77% (95% CI: 99.62-99.88). Sensitivity was 100% (95% CI: 94.31-100) in HIV-1 antigen samples, 100% (95% CI: 99.63-100) in anti-HIV-1 specimens and 100% (95% CI: 98.18–100) in anti-HIV-2 specimens. The median reduction in time to detection of HIV (i.e. window period) in the seroconversion panels was 7 days (range: 0-20 days) compared to the HIV antibody assay. The negative and positive percent agreement to HIV status for individuals at increased risk for HIV in the US was 99.27% (95% CI: 98.43–99.73) and 100% (95% CI: 94.87–100) respectively.

Conclusion: The ARCHITECT HIV Ag/Ab Combo assay detects HIV-1 p24 antigen and antibodies to HIV-1 (group M and group O) and HIV-2, including acute or primary HIV-1 infection and demonstrates acceptable clinical performance and precision.
Optimization of Abbott m2000 RealTime HIV-1 Viral Load Assay on Breastmilk, Dried Blood Spots, Genital Secretions, Cerebrospinal Fluid, Urine, and Throatwash

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Background: The latest technology for measuring HIV-1 RNA in plasma is real-time PCR. Abbott’s m2000 RealTime HIV-1 assay is CE-marked and FDA-approved for plasma only, and has not been optimized for off-label use with other sample types. Measurement of HIV-1 RNA levels in other body fluids is important for understanding pathogenesis and transmission of HIV, so more accurate and precise methods for measuring HIV RNA levels in other sample types are needed.

Methods: Plasma, BMK, whole blood (for DBS), semen, cervical swabs, CSF, urine, and throatwash were obtained from uninfected donors. HIV-1 virus stock was added to generate series of samples containing 0-6 log10 copies of HIV-1 per ml. Each sample type was lysed in different volumes of lysis buffer, with BMK and semen treated with proteinase K prior to extraction on the m2000sp, and the run on the m2000rt. The data from the different sample types were compared with the results from the spiked plasma samples. To evaluate the assay on patient samples, 20 samples were obtained from patients with different plasma viral loads and the samples were tested on both the Abbott assay and a comparator assay (Roche Amplicor or NucliSens).

Results: The results from the dilution series in plasma, BMK, genital secretions, CSF, urine, throatwash, and DBS had linear results compared to the nominal cp/ml. Comparison of the samples to plasma indicated that HIV-1 RNA recovery after adjusting for sample input and dilution was near 100% from semen, CSF, urine, throatwash, cervical swabs, and DBS, and approximately 50% from BMK. Patient samples that were tested on Roche Monitor assay demonstrated similar results to the Abbott assay, while patient samples that were tested on NucliSens were not as consistent with the Abbott assay.

Conclusions: The Abbott m2000 RealTime HIV-1 assay works well for measuring HIV-1 RNA levels in BMK, genital secretions, CSF, urine, throatwash, and DBS.

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Evaluation of a Kit-Based HIV-1 DNA PCR Protocol for Confirming Infection

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Background /Objective: The traditional enzyme immunoassay (EIA)/Western blot (WB) algorithm for diagnosing HIV infection has been in place for over 20 years. To evaluate alternative diagnostic algorithms, the performance of new confirmatory tests must be assessed. The purpose of this study was to evaluate a kit-based HIV-1 DNA PCR protocol for detecting HIV-infected cells as a supplemental test to confirm preliminary-positive HIV test results. The study outcomes were to determine the specificity and sensitivity of the protocol for detecting HIV infection and to evaluate whether sub-optimal cell counts and/or hemolysis were associated with discordant test results.

Methods: Overall, 1066 blood samples were obtained from HIV-infected persons 18-55 years of age who participated in the Centers for Disease Control and Prevention’s Validating Supplemental Testing to Confirm Preliminary Positive Rapid HIV Tests study and had not been taking antiretroviral therapy for at least 3 months before their blood was collected. HIV infection among study subjects was confirmed by a reactive WB following a reactive BioRad 1-2+O EIA test result. A total of 1509 samples were obtained from blood donors who were determined to be HIV-uninfected on screening with an algorithm consisting of an HIV-1 EIA and pooled plasma HIV-1 RNA PCR testing. Peripheral blood mononuclear cells (PBMC) separated from whole blood samples were initially cryopreserved and then thawed, counted, aliquoted (target = 1 x 106 cells/pellet) and refrozen at -70oC until analyzed using the Roche Amplicor HIV-1 DNA Test (version 1.5) according to the manufacturer’s instructions.

Results: An interim data analysis of 2575 samples revealed that the protocol had a sensitivity of 99.0% (1055/1066) and a specificity of 99.7% (1505/1509). Seventeen (0.7%) samples with concordant EIA/WB and HIV-1 DNA PCR results had total PBMC counts that were lower than the target count (p=.005) compared with 2 (13.3%) of 15 with discordant EIA/WB and HIV-1 DNA PCR results. Significant hemolysis was observed in 926 (44%) of 2073 samples with concordant EIA/WB and HIV-1 DNA PCR results compared with 5 (41.7%) of 12 with discordant results. Additional EIA and HIV-1 RNA testing of samples with discordant results are ongoing.

Conclusions: Preliminary findings using a kit-based HIV-1 DNA PCR protocol for confirmatory testing indicate that its sensitivity and specificity are high. Discordant EIA/WB and HIV-1 DNA PCR results may be associated with low cell counts.
counts, but they do not appear to be associated with hemo-
lysis. We are currently processing additional samples using
the kit protocol and collecting information about potential
barriers and facilitators of its use.

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New HIV-1 RNA Assay Validation: Roche
COBAS TaqMan and Abbott RealTime

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Point-of-Care Molecular Diagnostics

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Project: The development of safe and effective prevention
and therapeutic interventions to control HIV-1 infection
or disease remains an elusive tool for fighting the AIDS
pandemic. Low cost, accurate diagnostic and monitor-
ing technologies, suitable for field-use would assist this
effort. To this end, DAIDS issued a request for proposals
(NIHAI2008027) to develop a simple-to-operate, rapid HIV
point-of-care diagnostic device for use in resource-limited
settings. The device will be capable of distinguishing HIV-
infected individuals from those who have been vaccinated
with candidate constructs and subsequently developed
vaccine-induced seropositivity.

Issue: The high rate of false reactive serologic tests require
the detection of virus, typically through the use of nucleic
acid testing, which is difficult to perform in resource-limited
settings. Additional applications include early infant diagno-
sis and identifying acutely HIV-infected individuals. More-
over, some of the platforms may be particularly useful in
high incidence resource-rich settings such as dental offices,
medical clinics and emergency room facilities. Recently, the
Division of AIDS has awarded three contracts for the devel-
opment of molecular point-of-care HIV diagnostics to meet
clinical trial and end-user needs.

Results: The design specifications and requirements of the
device, as stipulated in the request for proposals will be
presented. Proposals were peer reviewed, scored and three
awards were made. A description of the three technologies
will be presented.

Lessons Learned: Although several platforms exist for
the amplification and detection of HIV, the integration of
sample-in and results-out testing using whole blood in a
cost-effective manner requires further development and op-
timization. The NIH contract mechanism allows developers
an opportunity to pursue unique design goals in a milestone
driven manner to ultimately manufacture an FDA approv-
able point-of-care diagnostic device. HJF/DAIDS contract
No. HHSN272200800014C

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ELISA Strength of Signal in a Protocol to
Prevent Mother-to-Child HIV Transmission

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Mother-to-child transmission of HIV has been successfully
prevented by testing and treatment of pregnant women.
Routine HIV testing at onset of labor at the Univ. of Texas
Medical Branch (UTMB) hospital has been through the
use of EIA tests performed in the central laboratory. Since
February 2009, we have used the Vitros HIV1/2 chemilu-
minescent immunoassay (Ortho Clinical Diagnostics), with
the results available within 6 hrs around-the-clock. False
positive HIV antibody tests are known to occur in pregnant
women; however, the confirmatory Western blot (WB) assay
requires more than 24 hr turnaround-time. The delay results
in poor decisions for prophylactic treatment of the mother
and baby and anxiety in the mother.

Objectives: We investigated the distribution of signal/cutoff
(S/C) ratio of the Vitros test results to predict the results of
WB in pregnant women in labor.

Study design: This was a retrospective study of the Vitros
HIV1/2 and HIV1 WB results of pregnant women who were
tested in labor between February and October 2009.

Results: Of 4798 specimens screened using the Vitros test,
35 (0.7%) were reactive. Eighteen (51.4%) of the Vitros reac-
tive specimens were confirmed by HIV1 WB. Among the 17
reactive specimens that failed to confirm, 15 were negative
and 2 were uninterpretable by WB. S/C ratios of Western
blot positive specimens ranged from 33.7 to 75.9. S/C
ratios of WB negative or uninterpretable specimens were all
less than 4.

Conclusions: This is the largest study to-date of the new
Vitros HIV1/2 test in pregnant women. S/C ratios of < 10
can predict negative WB results while ratios of > 30 can
predict positive WB results. S/C ratios between 10 and
30 require caution in interpretation as we do not have ade-
quate information. Our results suggest that the reporting
Rapid Detection of HIV-1 p24 Antigen and of Antibodies to HIV-1 and HIV-2 Using Magnetic Immunochromatography

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Background: Magnetic immunochromatographic tests (MICT) are constructed on lateral flow platforms and provide a quantitative measurement of bound reagents that eliminates subjective interpretation of results and can significantly improve assay sensitivity. This study applies MICT to the detection and differentiation of HIV-1 and HIV-2 antibodies, and extends the use of rapid lateral-flow assays to the detection of HIV-1 p24 antigen.

Methods: MICT uses a standard lateral flow assay platform with super-paramagnetic beads conjugated to target-specific reagents. HIV-1 p24 was detected using a rabbit polyclonal capture and a mouse monoclonal antibody/super-paramagnetic bead conjugate. HIV-1 and HIV-2 antibodies were captured using a multi-subtype recombinant antigen (HIV-1) and a multi-branched peptide (HIV-2) and were identified using Protein A conjugated to 300 nm super-paramagnetic beads. Immune complexes at the test and control lines of both assays were quantitatively measured using the Assay Development System (ADS) from Magna-Biosciences, LLC (San Diego, CA). The p24 assay performance was determined using buffer and plasma spiked with exogenous p24 or HIV-1 viral stocks, viral culture supernatants, and eleven commercial seroconversion (SC) panels. MICT HIV antibody detection was evaluated using 1) a blinded 370-member panel containing HIV-1 and HIV-2 antibody-reactive specimens and HIV antibody-negative samples from the US, Cameroon, and Cote d’Ivoire; 2) twelve HIV-1 SC panels; and 3) over 100 prospective specimens. Reference data was determined by enzyme immunoassay (EIA), Western blot (WB) and other rapid test results.

Results: MICT detection of HIV-1 p24 had a lower limit of detection of 30 pg/mL, which is similar to current commercial EIA detection (range 10-125 pg/ml). The coefficient of variation was <12% for both p24 antigen spiked into assay sample buffer and 50% plasma at 50 pg/ml concentra-

tions. P24 was also detectable in virus-spiked buffers and in culture supernatants from HIV-1 subtypes A-G and O. MICT detection of HIV-1 p24 in commercial SC panels was either equivalent to or only slightly later than that detected by more complex EIAs (2-7 days depending on the panel). The detection of HIV-1 and HIV-2 antibodies was 100% sensitive and specific when compared to EIA/WB result and was similar to HIV antibody detection in SC panels when compared to second and third generation EIAs. The MICT assay was reproducible with coefficients of variation of <12% for HIV-1 and <16.5% HIV-2 for both intra- and inter-run assays.

Conclusions: MICT detection of HIV-1 p24 antigen is quick, simple and effective and only slightly less sensitive than conventional HIV-1 p24 specific EIAs. MICT could provide a rapid, low-cost method of identifying early HIV infection requiring no subjective interpretations and providing a semi-quantitative measurement of the HIV-1 p24 present in the specimen. Further optimization of the p24 assay is underway to reduce matrix effects and to improve the lower limit of detection. The development of an antigen/antibody combination assay is in progress.

Presenter: Timothy C. Granade, MS, MBIS, RM(NRM), Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA. txg1@cdc.gov

Europium Nanoparticle-based Immunoassay (ENIA) for Sensitive and Early Detection of Human Immunodeficiency Type 1 Capsid (p24) Antigen

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Objective: To improve assay sensitivity for detection of HIV-1 p24 antigen and develop a simple assay for point-of-care use, we have evaluated the feasibility to adapt a sensitive europium nanoparticle-based immunoassay (ENIA) platform for detection of HIV-1 p24.

Methods: HIV-1 p24 is captured by monoclonal anti-p24 antibody coated on the surface of microtiter plate wells, and complexed with a polyclonal anti-p24 antibody (step 1) followed by binding to an anti-species antibody labeled with biotin molecules (step 2). In the presence of target, the antigen-antibody sandwiched complex is coupled to streptavidin (SA)-conjugated Eu3+ NPs through biotin-SA interaction (step 3). After extensive washing between steps to remove unbound or nonspecifically bound conjugates, fluorescence from the resulting complex is directly measured by a fluorometer in a time-resolved mode.
Results: ENIA exhibits an analytical target concentration range of three orders of magnitude, with a lower LOD of 0.5 pg/ml of HIV-1 p24 antigen, and is 20~30-fold more sensitive than conventional ELISA. ENIA can detect the window period cases earlier than ELISA. Two enhanced ENIAs have been developed by using biotinylated anti-SA and SA-coated europium molecules to increase signal intensity; or combining tyramide-mediated signal amplification (TSA) with ENIA. By incorporation of such enhancements, the detection sensitivity for HIV-1 p24 was increased to 0.1 pg/ml, which is the same detection limit obtained using gold nanoparticle (NP)-based biobarcode amplification (BCA) assay. Further evaluation in clinical samples is on-going. ENIA for detection of anthrax toxin has been reported.

Conclusion: Our studies demonstrate that ENIA provides a highly specific, ultrasensitive and relatively simple format for detection of HIV-1 p24, and has the potential for broad applications in clinical diagnosis and laboratory research of HIV infection.

Presenter: Shixing Tang, Staff Scientist, Food and Drug Administration, Building 29B, Room 4E16, 8800 Rockville Pike, Bethesda, MD. shixing.tang@fda.hhs.gov

A novel method of creating molecular controls used in the NLHRS HIV-2 Viral Load Assay and subsequent results in a NIBSC-WHO study to evaluate candidate international standards for HIV-2 RNA.

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Objective: A method of creating user-defined RNA targets within pseudovirions was developed in efforts to create standards and controls for molecular assays.

Methods: The HIV-1 gag and HIV-2 LTR-gag gene regions were cloned into a lentiviral vector and used to transfect 293T cells in the presence of packaging mix extracts. The resulting pseudovirions containing a single RNA of the HIV-1 and HIV-2 gene regions were purified. RNA from these particles were quantified by the Roche Cobas HIV-1 viral load assay and based on a 1:1 stoichiometric ratio, HIV-2 controls were created and used in the standardization of an in-house method using the Lightcycler. This assay was then used in a worldwide NIBSC-WHO study (30 labs, 15 countries) to evaluate 4 candidate international standards for HIV-2 RNA.

Results: The quantitation of pseudovirions by the Roche Cobas HIV-1 assay was very high approaching 10(5) to 10(6) cp/ml. Lack of cross reactivity between the HIV-1 and HIV-2 targets was confirmed by HIV-1 and HIV-2 specific PCRs. In the NIBSC-WHO study our results were virtually at or very near the group-mean for each of the 4 candidate RNAs [log10/ml]: S1, 3.10 [2.11-4.20], S2, 3.15 [1.96-4.18], S3, 3.58 [2.11-4.53] and S4, 3.58 [2.38-4.60].

Conclusion: The use of external controls and standards in molecular assays is critical especially in commercial HIV viral load assays where intra-sample variation of 0.5 log (3-fold) is considered acceptable. HIV-2 quantitation is exacerbated by the absence of a commercial assay in addition to the absence of an international standard. The ability to create pseudovirions containing HIV-1 and HIV-2 gene regions on the same RNA is advantageous because it allows different labs to standardize their HIV-2 controls to a readily available HIV-1 viral load assay. The usefulness of this method in the development of the NLHRS in-house HIV-2 viral load assay is further strengthened by the results obtained in the NIBSC-WHO study.

Presenter: John Kim, PhD, Chief, National Lab for HIV Reference Services, PL0603A, Tunney’s Pasture, Ottawa, K2A 1W1, CANADA. john_kim@phac-aspc.gc.ca
Poster Presentation Abstracts

Point-of-Care/Contact HIV Testing

Poster #1
HIV Test Preferences in an Urban Hospital Emergency Department

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Project: Anonymous surveys were distributed in an urban, level 1 trauma emergency department (ED) in the Bronx, NY to determine patient demographics and HIV test preferences. Patients were asked to complete a short demographic survey and HIV testing history in addition to answering whether they would prefer a 15-minute rapid fingerstick HIV test or a 20-minute oral swab HIV test. Means and standard deviations were calculated for continuous variables and proportions for categorical variables using SPSS software. Group comparisons were made using Chi-Square and Student’s t-tests using STATA 9.

Issue: Routine rapid HIV testing in the ED depends on patient acceptance of the offer to test. Patients may prefer one type of test over another and assessing these differences could maximize the efficacy of an ED rapid HIV testing program.

Results: Demographic characteristics of the patients surveyed (n=412) were: mean age 35.7 years ± SD 13.3; 61.5% female; 52.7% Hispanic; 39.1% black; 8.9% white. Overall, 73.9% of respondents preferred a rapid oral swab test for HIV compared to a rapid fingerstick. There were no statistically significant differences in test preferences across ethnicity (p=0.52), race (p=0.84), age over 35 (p=0.56) or age over 50 (p=0.16). A prior HIV test did not have a significant impact on test preference (p=0.08) and 81.2% of patients said they had been tested for HIV at least once. Most patients (71.1%) wanted to receive information about HIV prevention during their ED visit and there were no significant differences between demographic groups.

Lessons Learned: Patients in an urban ED are more likely to prefer a rapid oral test to a rapid fingerstick regardless of race, ethnicity or age. The majority of patients surveyed wanted to receive information about HIV prevention in the ED setting. Rapid oral ED testing with provision of informational materials and the option to use a rapid fingerstick test could maximize the number of patients tested.

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Poster #2

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Objective: To demonstrate the performance across a range of geographically diverse populations of a rapid HIV-1/2 immunoassay using a non-invasive (oral mucosal transudate) sample.

Methods: In the last five years, Calypte Biomedical has sponsored or taken part in twelve separate evaluations in 9 different countries (Thailand, China, Russia, India, Uganda, Kenya, Cameroon, South Africa and Tanzania) of its oral fluid rapid test for antibodies against HIV-1 and HIV-2, Aware OMT. Most studies used commercially available EIA tests as the gold standard, although one study relied entirely on alternative rapid tests and another used only a single western blot test. Here we present a compilation of the results of these studies.

Results: Overall sensitivity (n = 7,697) was 99.3% (95% CI = 98.8-99.6%) and overall specificity was 99.86% (95% CI = 99.2-99.8%), with individual study results ranging from 97.6% to 100% sensitivity and from 98.9% to 100% specificity. Seven of 12 studies recorded 100% sensitivity, eight recorded 100% specificity, and five recorded 100% for both parameters. The overall accuracy of the test was 99.7%, and the studies included locations endemic for subtypes A, B, C, D, CRF01_AE (“E”) and CRF02_AG.

Conclusions: The Aware™ OMT test has shown acceptable sensitivity and specificity for use as a screening test across a wide range of localities in which are endemic all of the most prevalent subtypes of the global HIV epidemic. Such geographically broad studies are increasingly important as public health efforts focus on testing algorithms that use rapid tests for initial point-of-care screening.

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**Poster #3**  
Rapid HIV Testing Programs in Urban Emergency Departments – Comparison of Results from Inner-City and Urban Sites in Baltimore, Maryland

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**Objective:** Our objective is to compare the outcomes of two emergency department (ED)-based rapid HIV testing programs one of which utilized on site ED laboratory based testing, and the other point-of-care (POC) bedside testing.

**Methods:** Two non-targeted ED based HIV testing using different operational approaches were instituted. Both were staffed by exogenous ‘facilitators’ who worked 16 hours/day, 7 days/week and obtained written consent, provided brief pre-test counseling, collected the oral fluid test, performed post-test counseling, collected confirmatory tests for reactives, and coordinated early linkage to care. Operational differences were as follows: Site A, an inner-city academic ED used an on-site ED laboratory to run HIV tests, and allowed ED medical staff to test during overnight hours; Site B, an urban teaching hospital ED used bedside testing and was run exclusively by exogenous staff. Descriptive data analysis was performed to compare the outcomes of the two programs from September 2008 to August 2009.

**Results:** Site A: 6,245 patients were offered testing by facilitators of which 3,388 (54.3%) were tested; an additional 185 patients were offered and tested ED medical staff. Site B: 9,460 patients were offered testing, of which 3,976 (42.0%) were tested (p<0.01). Patient demographics characteristics were similar except for African Americans (75% vs. 33%). Self-reported risk behaviors were also similar: any sexual risk behaviors: 31% vs. 32%; injection drug use: 6% vs. 8%; any risk of HIV: 14% vs.12%. Reactive and confirmed positive rates were higher for Site A, vs. B, 0.9% vs. 0.4% and 0.8% vs. 0.3%, respectively (p<0.01). Similar proportions of positive subjects were linked to care (58% vs. 55%).

**Conclusions:** Both rapid HIV testing programs in urban EDs with different testing approaches are acceptable by patients with varied demographics and risk profiles and have identified considerable number of individuals with unrecognized infection.

**Presenter:** Yu-Hsiang Hsieh, PhD, Assistant Professor, Johns Hopkins University School of Medicine, Baltimore, MD 21209. (410) 735 6413. yhsieh1@jhi.edu

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**Poster #4**  
HIV Rapid Testing Quality Assurance Program and Evaluation of HIV Rapid testing technologies in Ethiopia

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**Objectives:** The application of quality assurance program using EQA and assessment ensures quality test results and reports at different point-of-care (POC) services for HIV infection and implementation of the evaluated test kits nationwide.

**Methodology:** To address the problem indicated above the national program devise a methodology of evaluating HIV rapid test kits every two years, implementing the quality assurance procedures and increasing the use of these rapid test kits at different settings. External quality assessment is established to see their level of perfection in relation to each other and to the national level. Moreover, field assessment was conducted using a well designed and pretested questionnaire to see the status of the laboratories in terms of infrastructure, human resource and required supplies in a total of 568 testing sites from Dec 2008 to Jan 2009.

**Results:** In the previous years we had a very good experience in evaluating HIV rapid test kits and development of HIV rapid test kits national algorithms. The sensitivities and specificities of the evaluated test kits were in the range of 97-100% and serial testing algorithm was adopted to be the national algorithm. From a total of 228 assessed health facilities (41 hospitals and 187 health centers), 228(40%) were main laboratories and the rest 341(60%) were POC testing sites. The assessment indicates that most of the sites had proper work setup and performance of HIV testing. Two rounds of EQA panel distribution, in July 2008 and Oct 2008, showed that 197 and 315 testing points were included with response rate of 90 and 92%, respectively. There was a significant overall performance from 73.4 to 84.3% and high participation in EQA.

**Conclusion:** Establishment of strong quality assurance program at national and regional level and such assessments should be prioritized for possible corrective measures and support. In addition, evaluations of test kits are essential within a given time period that will increase confidence to use evaluated test kits at national levels.

**Presenter:** Getachew Belay Kassa, Training Manager, Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia. gechbel@gmail.com
**Poster #5**

Retesting as a Tool for Quality Assurance of HIV Rapid Test at Point-of-Care in Resource Limited Settings

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**Project:** Leadership, Management and Sustainability-Prevention, Organizational systems – AIDS Care and Treatment (LMS-Pro-ACT) is a PEPFAR funded project through the USAID implemented through Management Sciences for Health in six States of Nigeria. The project employs the WHO approach of Provider Initiated Testing and Counseling (PITC) at points-of-care using trained counselor/testers according national guidelines.

**Issue:** Rapid antibody tests for the detection of HIV remains an effective innovation in scaling up access to care and treatment in resource limited settings. The increased use of rapid HIV antibody tests outside the laboratory has necessitated the need for a retesting quality assurance program that bridges non-laboratory-based points-of-care testing and the laboratory to guarantee quality HIV test result by preventing the occurrence of problems related to testing and interpretation at the point-of-services in a provider initiated testing and counseling setting in Nigeria.

**Results:** Retesting data from eleven health facilities spread across 3 States in North-Central Nigeria with a total of 117 tests were reviewed. All sites participating in the program have trained counselor/testers using a cocktail of three test kits in a serial algorithm (Determine, Uni-gold and Stat Pak) at both point-of-care and laboratories. False positive rate was 1.7%. There was 100% concordance between positive results obtained at points-of-care and the laboratories.

**Lessons Learned:** The retesting program ensures quality of point-of-care HIV test results and is recommended for resource limited setting where manpower for routine Laboratory based HIV testing is scarce. Retesting also informs continuous quality improvement through training and retraining towards improving proficiency of non-laboratory testing staff.

**Presenter:** Ibiang Livinus, Advisor, Laboratory Systems, Management Sciences for Health, Abuja, Nigeria. ilivinus247@gmail.com

**Poster #6**

Assessment of HIV test Discordance in a Serial Testing Algorithm


**Study Objective:** Nigeria recently transitioned from parallel to serial HIV testing algorithm using rapid test kits, following a laboratory-based (phase-1) evaluation of rapid test kits using well characterized control panels. There is therefore need to generate additional data from the field on the discordant rates between the two test kits (Determine and Sat Pak) in the serial testing strategy in order to guide its utilization in the field for reliable and accurate HIV testing.

**Method:** A total of 13,231 HIV test sessions were assessed in a retrospective assessment of results of HIV testing done in different HCT program settings namely, PMTCT, Mobile HCT, and Facility integrated HCT. These tests sessions were performed by using Determine and Stat Pak test kits in a parallel strategy, and when there was discordance in the results obtained by the two test kits, a third test kit, Genie-2 was used as the tie breaker. The rate of discordance between the test results obtained using the first test, Determine and the second test Stat Pak was assessed and this was used to determine the rate of discordance between the two test kits if they were used in a serial testing strategy.

**Results:** Out of the 13,231 individuals tested, 9 test sessions (0.07%) showed discordant results and required the use of a third test (the tie breaker). This study showed a 99.93% concordance between the two test kits. The study also showed that concordance rates varied from 99.97% (8 discordant test results out of 2,573), in a PMTCT setting, 99.98% (1 discordant test result out of 6,208 tests), in a mobile HCT setting, to 100% (0 discordant result out of a total of 4,450 tests) for testing done in a facility integrated HCT site.

**Conclusions:** This assessment indicates that the rate of discordance between Determine and Stat Pak when used in a serial testing strategy is very low. The rate of discordance between the first and second test may vary from one setting to another however, and additional data is required to further evaluate the importance of these variations.

**Presenter:** McPaul Okoye, USAID/Nigeria, Abuja, Nigeria. mokoye@usaid.gov
Poster #7


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NJ Dept of Health and Senior Services, NJ Medical School and School of Public Health, Newark, NJ
CDC, Division of HIV/AIDS Prevention, Atlanta, GA, USA

Objective: As optimal point-of-care HIV testing strategies are developed and piloted, data to support algorithms are needed. An evaluation of New Jersey’s rapid HIV counseling and testing program sought to determine whether or not clients testing positive with a rapid test returned for a confirmatory laboratory test and the likelihood of getting into treatment as a result of their HIV diagnosis.

Methods: Data on all clients with a positive rapid test in New Jersey between January 1, 2005 and December 31, 2006 was collected both from the NJ Department HIV/AIDS Services counseling and testing database and eHARS. Data collected included date of rapid test and whether or not confirmatory results were received, client demographic information, date and result of laboratory and clinical tests, and HIV risk factors.

Results: Of our cohort of 655 clients diagnosed with HIV as a result of the rapid test, only 66% have a reported CD4 count or viral load test. Of these clients, 60% got into treatment within 3 months. Only 54% of the 655 clients definitely returned for their confirmatory laboratory result while 20% definitely did not receive their results; it was uncertain for 26% if they received their result. Of those clients that definitely received their confirmatory result, 80% were in treatment compared to only 53% of clients who definitely did not receive their result (OR = 3.6, 95% CI [2.3, 5.6]). Black clients and clients reporting injection drug use were less likely to receive their results.

Conclusions: When clients must return to the counseling and testing site for their confirmatory laboratory test result, at least 20% never return and are likely to not get into treatment, or to get into treatment late. A point-of-care HIV testing algorithm that did not require a second return visit from the client is likely to improve the numbers of HIV-positive persons testing positive at one of NJ’s counseling and testing sites to get into treatment at the time of diagnosis.

Presenter: Amy Piatek, UMDNJ, School of Public Health, Newark, NJ, USA. amy.piatek@gmail.com

Poster #8

Evaluating and balancing quality and costs in rapid HIV Quality Assurance

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Project: Misinterpretations of the concept of quality assurance (QA) and ignorance of the integral components of a complete QA package contribute to performance failures periodically observed in rapid HIV testing. NJHIV is a state-wide rapid HIV testing support group. NJHIV implemented policies and analyzed QA methods and usage in order to improve QA effectiveness.

Issue: Components of a successful QA program include: quality control (QC), proficiency and competency. QC is defined by manufacturers and is generally product specific. While minimum requirements are established by manufacturers, users need to analyze procedures to avoid unnecessary testing. Proficiency testing materials are available through CDC or other laboratory support organizations. With the integration of new reagent kits and testing algorithms into the public health arena, the potential for excess increases. Competency not only represents adequate theoretical knowledge of policies, procedures and testing algorithms, but also being able to perform and practice the theory. Assessment of competency requires direct observation of the personnel.

Results: Statewide usage data was analyzed for the past five years, including separately the rapid-rapid algorithm currently in use in 19 facilities in NJ. Between 2004 and 2006, mainly due to anonymous testing support, the QC usage was 35% to 70% of the kits in distribution. In accordance with a drop in anonymous testing, QC usage dropped dramatically 2007 with 21.29%, 2008 with 17.75% and 2009 only 15.68%. The rapid-rapid program initiated in 2009 amounted to 82% kits for QC; 22 kits used for each preliminary positive detected but only 10 kits for each positive verified in the rapid-rapid algorithm.

Lessons Learned: QA plans need to be modified for each setting, especially in regards to the prevalence of HIV. In low prevalence sites, QC performance at the minimum requirements, especially for a second rapid test is likely not beneficial.

Presenter: Gratian Salaru, MD, Assistant Professor Pathology, UMDNJ – Robert Wood Johnson Medical School, New Brunswick, NJ 08901. (732) 425 9060. salarugr@umdnj.edu
**Poster #9**

Performance of a Rapid HIV Test as the Primary Screening Tool at a Community Health Center in Houston, Texas

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**Objective:** Houston Department of Health and Human Services (HDHHS) has seven community health centers providing STD, TB, maternity, and family planning services to the Greater Houston area. Four of the seven health centers have a CLIA certified STAT laboratory on-site to provide point-of-care (POC) diagnostic testing for the detection of sexually transmitted diseases (STD’s). It has been well established that there is a direct correlation between success rates in patient care and result turnaround times; thereby, prompting a need to expedite delivery of patient results to the clinician. A pilot study was conducted to determine the feasibility of on-site rapid HIV testing in a clinic setting supplanting the traditional EIA methods. The Clearview® Complete HIV 1/2 rapid test was performed over a six week period from April to June 2009 to assess its performance as the primary screening procedure for HIV 1/2 antibodies in a POC setting.

**Methods:** The Clearview® Complete HIV 1/2 rapid test was performed in a STAT laboratory on patient samples submitted for HIV EIA antibody screening. The testing was performed in accordance with the manufacturer’s guidelines. Once rapid testing was completed the samples were forwarded to the central laboratory for analysis by traditional EIA procedures. Samples that tested positive by EIA for HIV antibodies were confirmed via the western blot. Performance characteristics of the Clearview® Complete HIV 1/2 rapid test, in comparison to the traditional testing algorithm performed in the central laboratory were assessed.

**Results:** Specimens from 416 clients were tested using a traditional screening and confirmation algorithm. The majority (290; 69.7 %) of the clients were male. Blacks and Hispanics were the predominant (89.2%) ethnic groups tested (Blacks: 227; 54.6%, Hispanics: 144; 34.6%). 4.3% (18) specimens tested positive at the STAT lab using the rapid HIV test. Three hundred ninety-eight (398) specimens tested negative for HIV antibodies were confirmed via the western blot. Performance characteristics of the Clearview® Complete HIV 1/2 rapid test, in comparison to the traditional testing algorithm performed in the central laboratory were assessed.

**Conclusions:** There was 100% correlation between the results obtained by the Clearview® Complete HIV 1/2 rapid test performed in the clinical setting and the traditional testing algorithm performed at the central laboratory. The utilization of rapid POC HIV testing method as a primary screening tool followed by confirmation of positive results by reference methods would enable immediate diagnosis and effective implementation of patient care in the infected HIV population seen in community health clinics in the Houston area.

**Presenter:** Jennifer Scott, Bureau of Laboratory Services, Houston Department of Health and Human Services, Houston, TX. (713) 558 3461. jennifer.scott@cityofhouston.net

**Poster #10**

Performance of the OraQuick Advance® Rapid HIV-1/2 test in a HIP HOP for HIV Awareness and Intervention Program in Houston, Texas

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**Objective:** Houston Department of Health and Human Services (HDHHS) teamed with local businesses and community organizations to conduct the third annual HIP HOP for HIV Awareness Intervention program at the NAACP Family Technology Center. The main objective of this event was to raise community awareness about sexually transmitted diseases and provide on-site testing, treatment and educational programs. This event was targeted to individuals aged 15-40 years and provided free HIV and syphilis testing services. OraQuick Advance® Rapid HIV-1/2 Antibody was piloted as a point-of-contact (POC) test during this four day event from 22nd to 25th of June, 2009.

**Methods:** During the HIP HOP event, individuals were tested for Human Immunodeficiency Virus (HIV) using the OraQuick Advance® Rapid HIV-1/2 Antibody test using either whole blood or oral fluid. All HIV rapid tests were performed on-site in accordance with the manufacturer’s guidelines and results were provided to individuals. For the individuals who tested positive with the rapid test, blood samples were sent to the central HDHHS laboratory for further testing. The traditional two-step method, of screening using the Bio-Rad HIV-1/2 Plus O EIA and confirmation of positives with the Bio-Rad Genetic Systems HIV-1 western blot kit was performed. This testing algorithm conformed to Point-of-Contact testing algorithm 1, proposed by APHL/CDC, April 2009.

**Results:** 18 (0.6%) of the 3200 people tested, were found to be preliminary positive for HIV using the rapid test. All 18 preliminary positives were confirmed to be HIV positive using the reference EIA and western blot methods. This rate is slightly higher than the rate of 0.4% population living with HIV/AIDS in the Houston area.

**Conclusions:** The testing algorithm of using an HIV rapid test at the point-of-contact with confirmation by more complex HIV EIA and western blot was effective in detecting HIV positive
The utilization of OraQuick Advance® Rapid HIV-1/2 Antibody test for the HIP HOP for HIV Awareness event was an effective interventional strategy and provided thousands of people with same-day HIV testing, counseling and follow-up.

**Presenter:** Shannon York, Bureau of Laboratory Services, Houston Department of Health and Human Services, Houston, TX. (713) 558 3461. shannon.york@cityofhouston.net

**Poster #11**

Improved Oral Fluid Specificity of the Enhanced OraQuick ADVANCE Rapid HIV 1/2 Oral Fluid Antibody Test

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**Objective:** To analyze the specificity of the enhanced version of the OraQuick ADVANCE® Rapid HIV 1/2 Antibody Test by comparing oral fluid performance between 2008 and 2009.

**Methods:** Oral fluid performance data was obtained from 11 public health jurisdictions representing over 300 individual testing sites in order to assess the statistical significance of any specificity improvement provided by the enhanced product which was approved by the FDA in December of 2008. Specificity performance in 2009 was compared with performance observed at the same testing sites in 2008. Each jurisdiction’s performance was analyzed with respect to its point estimate and 95% confidence intervals for specificity for each year. In addition, the aggregate data for 2008 and 2009 were analyzed and compared statistically.

**Results:** Overall specificity improved considerably from 99.75% in 2008 (n=236,751) to 99.92% in 2009 (n=151,981). A statistically significant improvement was also observed at 11 jurisdictions. All jurisdictions observed improved specificity in 2009 compared to 2008. These data indicate the oral fluid specificity of the rapid test has improved significantly with the release of the enhanced version of the OraQuick ADVANCE® Rapid HIV 1/2 Antibody Test.

**Conclusion:** The enhanced version of the oral fluid OraQuick® test has demonstrated improved specificity in addition to an extended (12 months) shelf life. While aggregate 2008 data and 2009 data were both within the expectations established in the package insert (99.6-99.9), the improvement in oral fluid specificity in 2009 data was highly significant. Specificity of the enhanced version of the oral fluid test compares favorably to that obtained with HIV tests that utilize blood specimens and the improved specificity should improve the positive predictive value observed in routine screening.

**Presenter:** Stephen R. Lee, PhD, Executive Administration, OraSure Technologies, Inc., Bethlehem, PA 18015. (610) 882 1820. mshine@orasure.com

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**Poster #12**

Translation isn’t just about foreign language: bridging the language gap with HIV testing

**S. Facente**

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**Project:** The availability of point-of-care HIV antibody tests has moved HIV testing out of the complex laboratory and into community-based settings. This has led to a need to bridge the gap between these lay organizations and the various entities involved in ensuring high quality laboratory testing, who are usually unfamiliar to community organizations.

**Issue:** Laboratorians at both the local level and at the state laboratory level, program administrators at the local department of public health, officials at the FDA, CDC, and CMS, manufacturers of test kits, and lay counselors and technicians at POC test sites all share the common goal of high quality HIV testing. However, people in these various groups use different terminology and are familiar with different regulations and standards of practice, which can cause communication breakdown.

**Results:** Common ground can be found between these entities by identifying a person who can act as an intermediary between them. This must be a person who can move between worlds (i.e. is comfortable in a laboratory and understands the rules of CLIA but also has direct experience providing rapid HIV testing in a non-traditional test site). POC testing sites need guidance to develop protocols that are that are accessible and can be clearly understood by lay counselors while still addressing and satisfying all local, state, and federal testing requirements. These protocols can be standardized and a template provided, which all sites can then customize to meet their needs.

**Lessons Learned:** This strategy was used successfully in the city of San Francisco and has been replicated on a smaller scale in cities throughout the country. By implementing a strategy like this one, frustration between parties will be lessened and the quality of HIV testing will increase.

**Presenter:** Shelley Facente, MPH, Facente Consulting, Richmond, CA 94804. (914) 999 1310. shelley@facente-consulting.com
Poster #13
Using photographed rapid HIV test results to pilot an EQAS

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Objectives: HIV rapid diagnostic tests (RDTs) maximise testing in various settings. An external quality assessment scheme (EQAS) using photographed results of RDTs was implemented, to assess accuracy of interpretation assessing whether photographed results of RDTs could be used in EQAS to increase operators’ proficiency, and assessing training.

Methods: Four RDTs were selected. Panels of 10 samples’ results were designed to assess if participants [non-laboratory personnel (n=19), laboratory personnel (n=22) and pathologists (n=34)] could recognise common patterns illustrated in kit inserts. The same photographed samples were provided in the second session and discussed before reporting was allowed. All reported results were compared with reference results. Differences were assessed using the Chi square test.

Results: Before training, there were significant differences in the accuracy of interpretation by the three groups of participants with all RDTs except Determine which is used extensively in the region. After training, differences in interpretation between the 3 participant groups were with SD Bioline suggesting that training standardised the quality of interpretation of results. Training increased the correct interpretation in the RDTs: SD Bioline from 44% to 82%; Determine from 85% to 98%; Advance Quality from 86% to 99.5%; Insti from 87% to 99%; p<0.001. Non-laboratory personnel made the least accurate interpretations: 74% for the subtyping RDT SD Bioline to 96% for the non-subtyping RDTs, after the training session.

Conclusions: Photographed RDT results can improve the accuracy of their interpretation, and can provide information to allow tailoring of appropriate training for specific participants. The relatively poorer accuracy of interpretation of SD Bioline highlighted need for continuing training.

Presenter: Dr. Elizabeth M. Dax, National Serology Reference Laboratory, Fitzroy, Australia. lizdax@gmail.com

Poster #14
The Significance and Value of External Quality Assessment for HIV-1 Testing

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Objective: Participation in quality assurance is a basic requirement of quality management standards. External Quality Assessment Schemes (EQAS) are designed to check the integrity of testing processes. EQAS are administered in different ways, and with different numbers of samples.

Methods: Statistical and empirical methods were used. A simple probability argument assessed the likely error rate, as a function of the number of samples tested. The basis data were accumulated in the NRL’s 2007 anti-HIV-1 serology EQAS to determine empirically the detection rate of errors dependent on the number of samples tested based on the 114 laboratories that sent complete results; 3420 results. o extrapolate to higher sample numbers, a simple model describing the true extent of testing errors within the dataset were developed.

Results: Within the selected dataset, the net error rate was 0.5 ± 0.1% per sample. Within the statistical uncertainties, the data were accurately and completely described by a simple scenario where 15.4 % of laboratories committed errors at a mean rate of 3.22% per sample. By comparison, on the basis of 30 samples, errors would be reported by 9.6 % (11/114) of laboratories i.e. the model suggests that 60 % of laboratories with errors were identified. Based on the model, 95 % of laboratories with errors would be identified after three years’ testing.

Conclusions: The quality assurance value of EQA testing depends on the number of EQA samples tested. Small numbers of non-aberrant tests do not provide strong limits on a given laboratory’s testing efficacy: at 95% confidence, it takes five successful tests before a laboratory’s testing procedure can be distinguished from a simple coin toss. The testing of large numbers of samples (> 100) are required to discover testing errors at rates typical for HIV-1 testing, as derived from the NRL 2007 EQA data. The strength of EQA testing thus lies in protecting against gross testing errors.

Presenter: Dr. Elizabeth M. Dax, National Serology Reference Laboratory, Fitzroy, Australia. lizdax@gmail.com

Poster #15
Comparison of Dried Tube and Plasma Specimens in an External Quality Assurance (EQA) Scheme and Evaluation of Quality of HIV Rapid Testing Performed by Non-Laboratory Staff in Lesotho.

APHL, Silver Spring, MD, CDC/GAP ILB; CDC Lesotho, and Lesotho Ministry of Health and Social Welfare (MOHSW)

Study Description: The National Laboratory Quality Assurance Unit (QAU), MOHSW in Lesotho with assistance from APHL and CDC/PEPFAR conducted a pilot study at 15 sites in the district of Mafeteng to compare HIV antibody test results for plasma
specimens and Dried Tube Specimens (DTS) made from the plasma specimens. The DTS is stable and can be transported without refrigeration. Use of DTS enables distribution of EQA specimens nationwide even with challenges of lengthy transport and limited refrigeration at test sites. QAU developed a comprehensive operational plan that included training, certification of testers, and quality assurance (QA) implementation at the sites. One-hundred thirty-nine testers from 15 sites participated and most tested both proficiency panels each with positive and negative specimens, one panel with plasma and one with DT specimens. Specimens were tested in accordance with the national serial testing algorithm for HIV rapid testing (Determine, Double Check and Bioline).

Results: Test results were received for 126 DTS panels and 129 plasma panels. One hundred twenty-one of 126 (96%) testers scored 100% (6/6) for DTS and 120/129 (93%) testers scored 100% for plasma specimens. For testers who tested both panels, 100% correct results were 4% higher for DTS than plasma, 113/116 (97%) vs. 108/116 (93%). Five sites had <100% pass rate, and one site accounted for half of testers with incorrect results. Sites with <100% pass rate have been followed up by the district laboratory quality officer.

Conclusion and Discussion: The performance standard for HIV Rapid Test EQA is 100%, the appropriate standard which can be achieved with non-laboratory testers if there is a strong quality assurance (QA) system. DTS is a tool that can be used in environments where maintaining cold chain requirements are difficult if not impossible. QAU using DTS panels for monitoring proficiency and external QC and a comprehensive QA system can assure quality testing services despite the challenges of widely dispersed sites. QA is critical to testing services and DTS is a key tool for supporting a comprehensive assurance program.

Presenter: Mathabo Lebina, Quality Manager, Ministry of Health and Social Welfare, Lesotho

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Serologic HIV Testing Methods

**Poster #16**

Correlation of preliminary positive HIV antibody assays performed on the ADVia Centaur CIA to WB: A one year look back

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Objective: The objective of this study was to evaluate the concordance between positive HIV antibody tests performed by chemiluminescence (CIA), a screening test to the Western Blot (WB), a confirmatory test. All reactive results, obtained by Centaur are considered preliminary and need to be confirmed by Western blot. We performed a one-year look back to ascertain the Centaur Index (CI) for these specimens.

Methods: ADVia Centaur HIV 1/O/2 Enhanced assay (in vitro diagnostic immunoassay for the qualitative determination of antibody to HIV 1/O/2) was used for screening testing of all samples. All samples that are initially reactive are repeated twice by lab protocol. If two of three patient results are reactive (CI>1), the final interpretation is based on the confirmatory Western blot assay. There are three outcome results: Positive, Negative and Indeterminate.

Results: Of the 476 initially reactive samples by Centaur, 141 (29.6%) resulted as either negative (24.6%) or indeterminate (5.0%) based on Western Blot. While the majority of these (76.6%) specimens had indices below 5, 5.7% (5 indeterminate, 3 negative) had indices over the upper limit of detection (CI>50) and 2.8% (2 indeterminate, 2 negative) had indices between 30 and 50.

Conclusions: While some of these indeterminate and negative results might be attributed to HIV-2 in this series, it is improbable to assume that all of the patients fall into that category, as many were in fact tested for HIV-2 and found to be negative. Based on the finding of this study and the previous work by this group demonstrating the lack of specificity in WB detection, we suggest revising the protocol for patients with negative confirmatory WB results to include nucleic acid testing or mandatory follow up within one month. A longitudinal study looking at patients with high CIs and negative WB by nucleic acid testing would be of particular interest in the development of appropriate testing algorithms.

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**Poster #17**

Is positive antibody detection on a chemiluminescence (CIA) HIV immunoassay in the presence of a negative western blot (WB) always a false positive?

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Montefiore Medical Center, Bronx, NY, USA

Objective: The objective of this study was to compare the specificity of CIA used as a preliminary HIV test, to WB used as a confirmatory HIV test in detecting the presence of HIV antibodies. Based on the suspicion of false negatives in confirmatory testing, it was hypothesized that the WB might have a lower level of detection than the CIA, suggesting the possibility that patients with low level antibody may be missed.

Method: Serum and plasma was acquired from 5 known HIV-positive patients, each with different starting levels of
viral load. The samples were serially diluted by a dilution factor of 10 with defibrinated and filtered human plasma, confirmed negative for HIV antibodies. These serial dilutions were tested for HIV antibodies using the CIA on ADVIA’s Centaur System (Siemens Co.) and on the HIV-1 WB (BIORAD). As standards of comparison, the positive and negative controls from CIA and WB were run by both methodologies.

**Results:** In all of these known HIV-positive patients, there were multiple serial dilutions which recorded positive results on the Centaur (above an index of 1) but had negative or indeterminate WB results. For example, serum from Patient 5 (initial viral load - 50128 copies/ml) was negative on the Centaur at a dilution of 1:100,000,000, while WB already returned an indeterminate result at 1:10 and a negative result at 1:1,000. In testing the controls, notable results were that the Centaur’s positive control (index of 3.5) was negative on WB, and that the WB control used as the lower cutoff for WB readings was above the upper-detection limit of the Centaur.

**Conclusions:** The consistent lack of sensitivity in the WB test for HIV antibodies, as evident by this limited pilot study in known HIV-positive patients compounded with the lack of sensitivity of WB controls suggests that this test is not a viable confirmatory test. Replacing the WB as confirmation with nucleic acid testing for CIA positive patients should be recommended.

**Poster #18**

The High Positive Immunoassay Confirms HIV-1 Infection and Avoids the Need of Confirmatory Tests

The Department of Internal Medicine and the Clinic of Sexually Transmitted Diseases, Regional Hospital of Colima; the Department of Internal Medicine, General Hospital 1, Colima, and the Health Research Council in Jalisco State, Mexican Institute of Social Security.

**Introduction:** The laboratory diagnosis of HIV infection starts with testing for antibodies to HIV, by immunoassays (IAs). Every positive HIV IA must be confirmed by supplemental testing. A high reactivity index (RI) of the HIV antibodies IA is associated with true positive result of confirmatory tests. We hypothesized that a high RI of the HIV IA would predict positive supplemental testing.

**Methods:** In a diagnostic test study, we included 393 positive anti-HIV subjects in the Colima State Laboratory of Public Health, Mexico, between February 2005 and April 2009. A third-generation enzymatic IA was used to detect HIV antibodies. The gold standard for HIV infection was a positive result of the Western Blot (WB) test or HIV-1 RNA ≥ 2000 copies/ml.

**Results:** By receiver-operating characteristic analysis, the RI ≥ 11.6 was associated with highest combined specificity and sensitivity for prediction of supplemental testing, and so was defined as high positive level. There was a significant difference in the proportion of true positive results by supplemental testing between subjects with low positive IA and those with high positive IA (87.4% Vs 100%, respectively, p < 0.01). The high RI of the HIV IA showed specificity for true positive result of 100% (95% CI, 97.2-100) and likelihood ratio of 72.5.

**Conclusion:** A high positive HIV IA test (RI ≥ 11.6) yields positive predictive value of 100% for HIV-1 confirmatory results in patients and allows a more efficient diagnostic algorithm to confirm HIV infection.

**Poster #19**

Evaluation of the ADVIA Centaur® HIV 1/O/2 Enhanced (EHIV) Assay

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**Introduction:** The CDC expanded HIV screening guidelines in 2006 to include routine testing of all individuals between the ages of 13-64 in all health-care settings. This widespread screening approach may be facilitated by the recent FDA approval of two automated anti-HIV 1/2 serologic diagnostic assays. The aim of this study was to evaluate one of these two assays, the ADVIA Centaur® HIV 1/O/2 Enhanced (EHIV) assay (Siemens Diagnostics, Tarrytown, NY) (ADIA). We performed a retrospective comparison study with our current manual method, Bio-Rad GS HIV-1/HIV-2 Plus O EIA (Bio-Rad Laboratories, Redmond, WA) (GS) using Western blot result as confirmation of HIV infection.

**Materials and Methods:** Ninety-six (96) patient samples that had Western blot (WB) confirmation results available were tested using both the GS and the ADIA assays according to the package inserts. The samples consisted of 28 positive, 34 negative, and 34 indeterminate WB results. Because WB patterns but not nucleic acid testing (NAT) results were available, we further stratified the indeterminate group into “indeterminate 1” and “indeterminate 2” based on WB pattern, in an attempt to distinguish between possible cross-reaction and seroconversion. “Indeterminate 1” is defined as having only non-cardinal bands. “Indeterminate 2” is defined as having at least one cardinal band present at any intensity and is the group that more likely represents acute HIV infection (AHI) or patients with advanced disease (AIDS). Based on these criteria, 16 samples were “indeterminate 1” and 18 samples were “indeterminate 2”...
Objective: Validate supplemental oral fluid testing for preliminary positive rapid HIV tests.

**Poster #20**

Validating supplemental oral fluid testing for preliminary positive rapid HIV tests

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Objective: To evaluate the sensitivity of oral fluid (OF) enzyme immunoassays (EIAs) and the OF Western blot (WB) for use following reactive whole-blood fingerstick and OF rapid HIV tests.

**Methods:** Serum and OF samples from 2201 previously diagnosed HIV-positive persons not on antiretroviral treatment were tested by OraQuick Advance Rapid HIV-1/2 Antibody Test (fingerstick (FS) and OF media), bioMérieux Vironostika HIV-1 Microelisa System (OF), Bio-Rad Genetic Systems HIV-1/HIV-2 PLUS O EIA (serum and OF), OraSure HIV-1 Western blot (OF), and the Genetic Systems HIV-1 Western blot (WB) (serum). A total of 926 samples were not tested with the bioMérieux OF EIA which is no longer available. For discordant samples, all EIAs were repeated in duplicate, and WB assays were repeated once. We calculated the sensitivity of all tests using the serum WB as the gold standard.

**Results:** All 2201 serum samples were positive by the serum EIA and serum WB. The OraQuick FS sensitivity was 99.77% and the OraQuick OF sensitivity was 99.91%. Two samples had false-negative OraQuick OF and FS results, and both of these had indeterminate OF WB results. The sensitivity was 98.68% for the BioRad OF EIA and 99.53% for the bioMérieux OF EIA. The sensitivity of the OF WB was 98.59% if 32 indeterminate specimens were included in the analysis and considered to have false-negative results and 99.95% if these specimens were eliminated from the analysis.

**Conclusion:** All OF EIAs and OF WBs analyzed from persons with positive serum WB results had at least two false-negative results. The sensitivity of the OF EIAs and the OF WB ranged from 98.59 to 99.53%. These data provide evidence that the OF EIA and WB tests may be less accurate than the serum EIA and WB following reactive rapid test results.

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**Poster #21**

Correlation on the Use of a Reactive Signal to Cutoff Threshold Value in a 3rd Generation HIV-1/2 Antibody Immunoassay to Expedite Supplemental Testing and Reduce Screening Costs

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Objective: The objective of this study was to establish a signal to cutoff (S/CO) threshold value for initially reactive blood specimens with the BioRad HIV-1/2 Plus O EIA that yields a high positive predictive value (PPV), i.e. high concordance with Western Blot reactivity. It is anticipated that the use of a S/CO threshold value for initially reactive
EIAs may reduce reporting turn-around-time (TAT) and costs associated with repeat screening.

Methods: A prospective analysis was performed on 1,002 initial reactive HIV-1/2 Plus O EIA results, comparing the initial reactive S/CO values to repeatedly reactive screening results, Western Blot results and follow up as needed. Fresh serum specimens were routinely submitted for this study. Based on Western Blot reactivity, the PPV, including all initial reactive S/CO values, was 92.5%, 867/937 (65 initially reactive EIAs did not repeat and were reported as negative). Cost savings were based on our current costs of $1.25 per EIA result ($3.75 per repeatedly reactive screening result) and reporting TAT was compared to our present third generation EIA/Western Blot algorithm.

Results: Of the 856 cases with initial screening S/CO values of greater than or equal to 11.0, the PPV was 98.9% (847/856). Of the nine discordant results, six were initially reactive EIA that did not repeat, two were repeatedly reactive/Western Blot indeterminate and one was repeatedly reactive/Western Blot negative. These 856 cases (S/CO ≥ 11.0) represent 85.4% (856/1002) of the total initially reactive EIAs. In contrast the PPV of the 146 cases with initial S/CO values of <11.0 was 13.7% (20/146).

Conclusions: In this study population, the use of a S/CO threshold of 11.0 on initially reactive BioRad HIV-1/2 Plus O EIA results as an indicator to proceed to supplemental testing, instead of intermediate repeat EIA testing, demonstrated excellent concordance with Western Blot reactivity (98.9%). The majority of initial reactive S/CO values <11.0 did not demonstrate concordant repeat screening results or Western Blot reactivity. The cost savings based on the S/CO threshold would be approximately $2 per initial reactive result. The use of the S/CO threshold could reduce TAT since the majority of the initial screens are greater than or equal to the threshold value (85%). If a traditional third generation EIA/Western Blot or IFA algorithm is used, a day set aside for intermediate repeat screening may be eliminated on the majority of initial screens. If a Dual Immunoassay method is used, there is a potential for screening and confirmation to be performed and reported on the same day.

Presenter: Berry Bennett, MPH, Retrovirology Section Chief, Florida Bureau of Laboratories, Jacksonville, FL. (904) 791 1527. berry_bennett@doh.state.fl.us

Poster #22
Performance of the Ortho Vitros HIV1/2 Assay in a Pregnant Population, Including at Delivery

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Objective: Pregnancy/multigravidity is a known cause of false positive results in tests that detect antibodies to human immunodeficiency virus (HIV). The Vitros HIV1/2 assay (Ortho Clinical Diagnostics) is a chemiluminescent immunoassay approved by the U.S. Food and Drug Administration in March 2008. Notwithstanding FDA approval, there is a paucity of data on the performance of the Vitros HIV1/2 assay in women at delivery. The objective of this study was to evaluate the performance of the Vitros HIV1/2 assay in a pregnant population receiving prenatal care, and women at delivery.

Study design: HIV1/2 immunoassay and HIV1 Western blot results between February and October 2009 were retrieved from the Univ. Texas Medical Branch Department of Pathology laboratory information system.

Results: Between February and October 2009, 13,371 prenatal specimens and 4798 peripartum specimens were screened for anti-HIV1/2 antibodies. Of these, 82 (0.6%), and 35 (0.7%), respectively, were reactive. HIV1 Western blot confirmed 23.2% of the reactive results in prenatal specimens, and 51.4% in peripartum specimens. Uninterpretable Western blot results, a category often not described in studies, were observed in 6.1% of prenatal specimens, and 5.7% of peripartum specimens. There were a total of 31 patients with initial reactive Vitros HIV results, for whom follow-up HIV testing was performed on specimens collected on different dates. Repeat specimens from 27 (87%) of these patients were also reactive by the Vitros HIV1/2 assay.

Conclusions: This may be the largest single study evaluating the performance of the Vitros HIV1/2 assay in pregnant women. The proportion of false-reactive Vitros HIV1/2 results was similar to that reported for other immunoassays used for HIV screening in prenatal populations. Follow-up HIV testing performed on a number of patients created the opportunity to evaluate the reproducibility of the Vitros HIV1/2 and HIV1 Western blot assays.

Presenter: Mike Loeffelholz, PhD, ABMM, Associate Professor, Department of Pathology, Director, Clinical Microbiology Division, University of Texas Medical Branch, Galveston, TX 77555. (409) 747 2484. mjloeffe@utmb.edu
Testing for Recent HIV Infection

Poster #23
Standardization and Monitoring of Laboratory Performance and Quality Assurance Using the Less-Sensitive HIV Incidence Assay: Seven Years of Results

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Objective: To assess the performance of the less sensitive version of the Vironostika HIV-1 MicroElisa System assay (V-LS) from 2001-2008.

Methods: The Performance Evaluation Program for HIV-1 Incidence Tests provided quality assurance (QA) services to laboratories conducting the Serological Testing Algorithm for Recent HIV Serocconversion using the V-LS assay. The performance of the V-LS assay was monitored using a standardized set of quality control (QC) and calibration materials and proficiency testing (PT) panels. Two sets of serum PT panels were produced over the 7-year period. Each panel consisted of 8 blind-coded specimens representing recent HIV infection (less than one year) and long-term infection (greater than one year). The PT panels were sent to participants each quarter. Participants returned data consisting of the screening standardized optical density (SOD) and confirmatory SOD for each panel. SOD values for calibration and QC materials were also reported. The mean, standard deviation, and coefficient of variation were calculated for all PT and QC materials. Results were analyzed for clinical misclassifications: false recent HIV-infection errors (long-term infection classified as HIV infection less than one year), false long-term infection errors (HIV infection less than one year classified as long-term infection), and differences in SOD means and variances over time for all of the QA materials.

Results: The false recent rate was 1.26% (N = 2219). The false long-term rate was 0.25% (N = 1618). No significant trends were observed for misclassification rates by year, and no significant trend in SODs was observed. However outliers for negative and high positive QC were detected.

Conclusions: Laboratories using the V-LS assay produced consistent results using standardized calibration and QC materials. The QA system continually monitored performance and provided confidence for population-based estimates of HIV incidence using the V-LS assay.

Presenter: Joanne V. Mei, PhD, Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention, Atlanta, GA. (770) 488 7945. jmei@cdc.gov

Poster #24
Development of a Bead-based, Multiplex Assay for Estimation of Recent HIV-1 Infection

Kelly A. Curtis, M. Susan Kennedy, Debra Candal, Debra Hanson, Kevin Delaney, Man Charurat, Steve McDougal and S. Michele Owen
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Background: Several laboratory-based methods have been developed for distinguishing recent from long-term infection; however, each exhibits some degree of misclassification, particularly among AIDS patients and those taking anti-retroviral therapy (ART). To improve upon limitations associated with measuring responses to a single analyte, we have developed a bead-based, multiplex assay for determination of HIV recent infection based on total antibody binding and antibody avidity.

Methods: An HIV-specific multiplex panel was created by coupling the recombinant HIV-1 proteins p31, p66, gp120, gp160, and gp41 to microspheres. Plasma samples were diluted 1:50 and tested for reactivity to the coupled microspheres using the Bio-Plex 200 System. Antibody avidity was measured following treatment with 0.1M diethylamine (DEA). 1287 specimens from 368 subjects were analyzed for sensitivity, specificity, and Receiver Operating Characteristic (ROC) curves. Individuals with low CD4 counts, AIDS or receiving ART (n= 720) were also evaluated for misclassification using an algorithm of analytes with cutoff values adjusted to a common window period of 250 days.

Results: For each analyte, HIV-specific antibody binding and avidity increased at different rates for 1-2 years post-seroconversion. Estimated area under ROC curves ranged from 0.88 to 0.96 for avidity measurements, and from 0.69 to 0.83 for total antibody measurements. AIDS or low CD4 counts had a minimal effect on misclassification. ART increased misclassification of long-term specimens as recent by 16% if treatment was initiated <365 days post-infection.

Conclusions: This bead-based, HIV-specific multiplex assay can accurately measure multiple immune responses, allowing for consideration of single or multiple analytes in estimation of HIV incidence. Individuals on ART, particularly those initiated within 1 year post-infection, may continue to be problematic for serological-based assays.

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Poster #25
Fiebig Stage and Recency: What’s the Relationship?

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Public Health officials have a vested interest in both Fiebig staging (to characterize HIV infection in its earliest days and months) and recency testing (to distinguish recent from long-standing infection). Fiebig staging is a widely used algorithm that estimates time since infection up to 2-3 months based on lab results for several HIV markers. Studies have demonstrated that early infection is disproportionately transmissible, propelling the epidemic; thus, detection of a Fiebig IV infection should prompt extra effort to counsel recently infected individuals on risk behavior, and to contact partners. Recency testing as currently used picks up at Fiebig III-VI, and allows the estimation of HIV incidence as well as prevalence in a given population, providing important epidemiological information to policy makers and funders. Fiebig I-III stages may not be captured at all in a recency testing algorithm, but should still be counted in an HIV incidence estimate. These are not competing algorithms; rather, for public health applications they complement one another. Both algorithms have limitations, including dependence on test methods that vary widely in different settings, implementation of new test methods not accounted for, and higher than desirable misdesignation rates. But both also are contributing to HIV research and to understanding the dynamics of the worldwide epidemic. This talk will focus on Fiebig staging, with definitions, examples of its utility, and some suggestions for adaptation to new test methods.

Presenter: Dr. Patricia E. Garrett, PhD, Sr. Director, Science and Technology, SeraCare Life Sciences, Inc. Milford, MA 01757. (207) 871 7145. pgarrett@seracare.com

Fourth Generation Immunoassays

Poster #26
Seroconversion Panels for the Validation of 3rd and 4th Generation HIV Assays

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Objective: To determine the stability of HIV Seroconversion panels that have been stored frozen for >10 years and assess their usefulness validating 3rd and 4th generation assays.

Methods: Seroconversion panels have been used for more than two decades to examine the sensitivity and specificity of HIV diagnostic assays. Having a complete series of bleeds from the time of infection to the production of donor HIV antibodies, allows the researcher to challenge the limits of their assay. Testing these early stage bleeds also allows for assays to be optimized for ultimate sensitivity which reduces the time between infection and detection. This work has resulted in the development and commercialization of many molecular based assays that can now identify an HIV donor within a few days of infection. However, these same molecular assays now make it almost impossible to collect a complete series of bleeds because the donor is identified and deferred from the center before they complete the entire seroconversion process. Because collections of these panels are no longer possible, we decided to revalidate our current inventory of >90 HIV panels to assess their long term stability. To accomplish this we retested panels with the original test kits that were run >10 years ago when the panels were first developed. These included but were not limited to assays developed by Abbott, GenProbe, BioRad and Ortho Diagnostic Systems.

Results: In all cases the molecular, antigen and antibody-based markers in these panels remained stable throughout their frozen storage in our Bioepository. Once the stability of these panels was verified, we then ran additional tests using contemporary assay formats available in the marketplace today. These included recently cleared point-of-care HIV rapid tests (BioRad, Trinity Diagnostics, Oraquick and ChemBio Diagnostics) and 3rd and 4th generation antigen/antibody combo assays (Abbott and Roche).

Conclusions: Seroconversion panels maintained stability for >10 years when stored frozen in our secure biorepository. In most cases, rapid tests had comparable sensitivity to current antibody based HIV assays used in the domestic marketplace. Automated platform based 3rd and 4th generation assays currently used in Europe had equal or better detection in comparison to earlier generation assays. In some cases, 4th generation antigen/antibody combo assays demonstrated the same level of sensitivity as some molecular testing formats.

Presenter: Gregory Chiklis, Ph.D., Vice President, Research and Development, ZeptoMetrix Corporation, Franklin, MA. (508) 553 5804. chiklis@zeptometrix.com

Applications of Nucleic Acid Testing Technologies

Poster #27
Evaluation of HIV RNA detection to increase ascertainment of primary HIV infection

G Murphy, S.Carne, B.Patel, C. Hill, A.Charlett, E.Mckinney, O.N. Gill, J.V. Parry and J. Tosswill
**Objective:** Evaluating RNA detection to increase ascertainment of primary HIV infection.

**Methods:** 768 residual serum specimens, from Men who have Sex with Men (MSM) attending STI clinics for syphilis serology, that had been shown to be anti-HIV negative by a ‘3rd generation’ HIV1/2 antibody assay were tested for the presence of HIV specific RNA. Pools of 48 specimens were prepared from mini-pools of 8, and tested using an in-house quantitative/qualitative real-time PCR assay for the detection of HIV-1. Two regions of the HIV-1 genome were targeted, providing enhanced detection of HIV-1 variants. Reactive pools were broken down to reveal the individual positive specimen. Each individual RNA reactive specimen was then tested with an exemplar ‘4th generation’ combination HIV antigen/antibody assay to replicate recommended UK HIV testing practices.

**Results:** Of the 16 pools of 48 specimens 3 were initially reactive for the presence of HIV RNA. On breakdown, a total of 3 individual specimens were shown to contain HIV RNA, each of which was reactive in the HIV Ag/Ab EIA.

**Conclusions:** Although presently on limited numbers, our preliminary study in a UK population at increased risk for HIV infection showed no advantage of using pooled HIV RNA over the application of ‘4th generation’ Ag/Ab assays. However, it does show a benefit of ‘4th generation’ combination and/or HIV RNA over ‘3rd generation’ antibody only assays. The benefit of a slightly shorter diagnostic window during primary infection, which would be afforded by HIV RNA over antigen detection, may be negated by the increase in turnaround times for pooled PCR. It may take several days to collect enough specimens for cost effective testing, and individual PCR testing for this purpose would be prohibitively expensive. This study is being expanded to cover another 9000 specimens, including further specimens from MSM and heterosexuals from sub-Saharan Africa, and results will be available in January 2010.

**Presenter:** Gary Murphy, PhD, Head of Seromolecular Services, Virus Reference Department, HPA Centre for Infections, London, UK. +44 (0) 208 327 6935. gary.murphy@hpa.org.uk

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**Poster #28**

Evaluation of an FDA-approved qualitative RNA detection assay for diagnosis of HIV-1 infection in perinatally exposed infants

T. J. Sullivan, T. T. Miller, B. Warren, and M. M. Parker
Wadsworth Center, New York State Department of Health, Albany, NY

**Objective:** Our objective was to evaluate an FDA-approved qualitative RNA detection assay for diagnosis of HIV-1 infection in perinatally exposed infants.

**Methods:** From 1995 to 2008, the New York State Department of Health’s Pediatric HIV Testing Service conducted diagnostic testing of perinatally exposed infants using a laboratory-developed proviral DNA PCR assay. In this retrospective evaluation, a total of 96 archived specimens collected from infants who were previously confirmed as HIV-1 positive or negative by testing of sequential specimens were tested using the APTIMA® HIV-1 RNA Qualitative Assay (Gen-Probe, San Diego, CA). Results were compared to previous DNA PCR results.

**Results:** Of 61 DNA PCR-positive specimens tested, all 61 (100%) were positive by the RNA assay. Of 35 DNA PCR-negative specimens, 28 were negative and 7 were positive by the RNA assay. All 7 RNA-positive/DNA PCR-negative specimens, including 2 from the same infant, were early specimens from infants who were later confirmed as HIV-1 positive by DNA PCR testing of follow-up specimens. For these 6 infants, the first HIV-1 RNA-positive specimen was collected an average of 27.7 days (range 18-65) earlier than the first DNA PCR-positive specimen. The 28 specimens that were negative by both the DNA PCR and the RNA assay were true negatives based on established criteria for excluding HIV-1 infection in perinatally exposed infants. The RNA assay was also conducted on a subset of specimens using 100 ul of patient plasma, rather than 500 ul as recommended by the manufacturer. All HIV-positive specimens tested using 100 ul of patient plasma were positive at this reduced specimen volume.

**Conclusions:** Our results indicate that the APTIMA® HIV-1 RNA Qualitative Assay is well suited for diagnostic testing of perinatally exposed infants. In addition, the volume of infant specimens is often limited and we have demonstrated that the assay can be used reliably with a reduced specimen volume.

**Presenter:** Timothy Sullivan, Research Scientist II, New York State Department of Health, Wadsworth Center, Albany, NY. (518) 486 9605. tjs03@health.state.ny.us

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**Poster #29**

An evaluation of pooling strategies for acute HIV-1 infection screening using nucleic acid amplification testing

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**Objective:** To evaluate a 128-member specimen pooling scheme for acute HIV infection (AHI) detection using specimens from AHI patients previously identified by nucleic acid amplification testing (NAAT) of 16-member pools.

**Methods:** AHI specimens from the CDC AHI Study that were seronegative during initial antibody screening and
were HIV RNA reactive by pooled and individual NAAT using the Aptima® HIV-1 Qualitative RNA assay were analyzed retrospectively. The CDC AHI study’s 16-member pools were created by one-stage pooling (100 ml of each of 16 plasma specimens). In this study, the RNA-reactive pools were used for second-stage pooling, whereby 200 µl from 1 RNA-reactive and 7 HIV-negative 16-member pools were combined to create 128-member pools. Each 128-member pool was tested using the Aptima assay. HIV viral load testing of individual RNA-positive specimens was conducted using the Versant® HIV-1 RNA 3.0 assay.

**Results:** All 21 individual AHI specimens evaluated had quantifiable viral loads (9 were in the range: 1,520 - 477,386 copies/ml and 12 were >500,000 copies/ml). RNA was detected in all 128-member pools except one which included a specimen with low viral load (1,827 copies/ml; 7.1 RNA copies per reaction). This pool was initially non-reactive but reactive in duplicate repeat testing. The pool which included the AHI specimen with the lowest viral load (1,520 copies/ml; 5.9 RNA copies per reaction) was RNA-reactive in 3 of 3 independent assays. The mean sample/cut-off (s/co) ratio for the low viral load specimen pool was 12.21 compared with 24.54 for the other reactive pools.

**Conclusions:** Our results suggest that most AHI cases would be detected by screening 128-specimen pools using the Aptima® HIV-1 Qualitative RNA assay. However, for specimens with viral loads less than 2,000 copies/ml, which occur in persons with very early infection, false negative results may occur. Therefore, an intermediate pool size between 16 and 128 members may offer sufficient sensitivity for AHI detection.

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**Poster #30**

**Goldsmith J*, Smith R*, Kowalski A, Blank, S, Faruki H* **National Genetics Institute, Laboratory Corporation of America, Los Angeles, CA**

**Objective:** To evaluate the use of a high-throughput HIV pooled specimen nucleic acid amplification test (p-NAAT), previously validated for human plasma sample screening, to detect Acute HIV Infection (AHI) in select New York City (NYC) Health Department Sexually Transmitted Disease (STD) clinics.

**Methods:** Using samples collected from select NYC Health Department STD clinics, a high throughput and cost-effective p-NAAT assay was used to identify AHI in individuals attending NYC STD clinics. The p-NAAT assay, optimized for a mean sensitivity of 2 to 3 copies per milliliter, uses a proprietary three-dimensional pooling algorithm which allows detection of a single infected sample in as many as 512 specimens. All specimens, collected over a 17 month period were initially screened for HIV antibodies using the OraQuick® Advance Rapid HIV-1/2 antibody test. Specimens that were HIV negative by rapid HIV-Ab testing were forwarded for HIV p-NAAT screening.

**Results:** A total of 66,663 specimens were screened using the OraQuick® Advance Rapid HIV-1/2 antibody test and 647 were found positive (0.97%, 647/66,663). The 66,016 anti-HIV non-reactive samples were then further screened by HIV p-NAAT in pools up to 512 specimens and AHI was detected in 35 specimens (0.05%, 35/66,016). HIV p-NAAT reactive samples were confirmed by individual HIV NAAT testing. By implementing AHI screening as a standard of care across these clinics, the overall rate of HIV detection increased by 5.4% to 1.02% (682/66,663).

**Conclusion:** The use of HIV p-NAAT screening can identify otherwise asymptomatic individuals in a highly infectious state. As this method is routinely used for screening millions of blood plasma donations yearly using pools of up to 512 specimens and the cost for testing is far lower than individual sample analysis (~$10 per subject), this method could be used in other STD programs to increase detection of early infections.

**Presenter:** Josh Goldsmith, Ph.D., Business Development Director, National Genetics Institute, Los Angeles, CA. (310) 689 4929. jgoldsmith@ngi.com

**Poster #31**
Sensitivity of OraQuick and Early Generation Enzyme Immunoassay (EIA) within a Pooled HIV Nucleic Acid Amplification Testing (HIV NAAT) Program

**J Stekler1,2, PD Swenson2, RW Coombs1, J Dragavon1, RW Wood1,2, MR Golden1,2**

1University of Washington, Seattle, WA
2Public Health - Seattle & King County, WA

**Objective:** To describe results of HIV testing during acute and early HIV infection.

**Methods:** In September 2003, Public Health - Seattle & King County (PHSKC) implemented pooled HIV NAAT for antibody-negative men who have sex with men (MSM). We offered rapid HIV antibody testing with OraQuick (OraSure Technologies, Inc) or a 1st or 2nd generation EIA (Viro-nostika HIV-1 Mikroeliisa System, bioMerieux; or Genetic Systems rLAV EIA, Bio-Rad Laboratories). OraQuick used
finger-stick blood samples or oral fluids, depending on the testing site. Since November 2005, when we identified one individual with early HIV infection who was OraQuick-negative but antibody-positive by the 1st generation EIA, we have screened all OraQuick-negative MSM with the 1st or 2nd generation EIA prior to pooling to reduce costs associated with NAAT and to decrease the time between testing and receipt of results. HIV RNA levels were quantified in a subset of specimens using one of three sensitive assays.

Results: From September 2003 to September 2009, 427 (2.0%) of 21451 specimens were HIV antibody-positive, and 53 (0.3%) of 21024 antibody-negative specimens were HIV NAAT-positive. One HIV-infected individual tested HIV-negative by EIA and NAAT four days after an exposure to an acutely-infected partner. The median HIV RNA level of 46 persons with acute HIV infection was 5.9 (IQR 5.3 to >6.0, range 2.9 to 7.5) log10 copies/mL. Symptoms consistent with acute HIV infection were prospectively recorded in 25 (47%) men. Between November 2005 and September 2009, we prospectively identified 29 HIV-infected MSM with discordant antibody test results (OraQuick-negative and 1st or 2nd generation EIA-reactive). Among rapid testers, OraQuick was 78% sensitive (detecting 220 of 281 HIV-infected MSM), OraQuick plus EIA was 89% sensitive (249/281), and pooled HIV NAAT had 100% sensitivity. The median HIV RNA level among the 14 OraQuick-negative/EIA-reactive testers with results available was 3.3 (IQR 2.2 to 4.6, range <1.6 to >6.0) log10 copies/mL; two had undetectable HIV RNA levels and five others had levels below the limit of detection of our current pooling strategy (2.9 log10 copies/mL). Symptoms of seroconversion were reported for only 5 (17%) OraQuick-negative/EIA-reactive testers.

Conclusions: OraQuick may be less sensitive than EIAs during early HIV infection, and individuals identified by OraQuick as antibody-negative/NAAT-positive may actually have early and not acute HIV infection. Furthermore, persons who remain asymptomatic during acute HIV infection may have lower HIV RNA levels in the first months after HIV acquisition than we would predict from results of studies of viral dynamics among cohorts of predominantly symptomatic subjects. An HIV testing strategy using OraQuick followed directly by pooled HIV NAAT would have failed to detect half of this group of OraQuick-negative/EIA-reactive men due to these low HIV RNA levels and the dilution factor associated with pooling.

Presenter: Joanne Stekler, MD, MPH, Assistant Professor, Division of Infectious Diseases, Deputy Director, Public Health - Seattle & King County HIV/STD Programs, Seattle, WA. jstekler@u.washington.edu

New Approaches in HIV Testing

Poster #32

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Background: Since the launch of nationwide free antiretroviral treatment (ART) in Ethiopia in 2005, the number of patients on ART has been on the rise. With increasing availability of ART, the potential for the emergence and transmission of drug-resistant HIV strains increases. Simple and inexpensive procedures are required to monitor the prevalence of drug resistance. Dried blood spots (DBS) are considered a convenient alternative to plasma for HIV drug resistance testing in resource limited settings.

Objective: To evaluate the efficiency of amplification and genotyping of HIV-1 subtype C from DBS and assess the similarity between pol sequences from paired plasma and DBS specimens stored at -20°C with desiccant for 40.7±5.4 months.

Design: Sixty three DBS specimens were prospectively collected from newly diagnosed, treatment-naive HIV-positive subjects in Addis Ababa in 2005. DBS were prepared by pipetting 50µl of whole blood onto 903 filter paper cards (Schleicher & Schuell, Keene, NH). Cards were dried overnight at room temperature, placed in a gas impermeable, sealable plastic bag containing a silica gel desiccant, and stored at -20°C for 40.7±5.4 months. The median log10 HIV-1 RNA (copies/ml) was 5.37 [IQR: 4.99 – 5.69]. Resistance testing was done from one spot of DBS using an in-house nested reverse transcription-PCR method that amplifies 1,023bp HIV-1 pol fragment. Resistance genotypes from plasma were obtained using both the ViroSeq HIV-1 assay and in-house nested reverse transcription-PCR method. Genotypes obtained from DBS were compared with genotypes derived from plasma.

Results: Using the in-house assay, 62(98.4%) DBS specimens were successfully genotyped; 32 of these DBS had matched genotypes derived from plasma. The mean nucleotide similarity of the pol sequence of the paired plasma and DBS was 99.64±0.33% and ranged between 98.9% and 100%. All resistance-associated mutations detected in plasma specimens were also detected in the corresponding DBS specimens.
**Conclusions:** DBS are appropriate for drug resistance surveillance among treatment naïve subjects in resource limited settings where logistic difficulties could prevent the use of plasma and serum for HIV drug resistance testing. The high efficiency of HIV drug resistance genotyping from DBS stored at -20 °C with desiccant suggested that -20 °C may be suitable for long term storage of DBS.

**Keywords:** Dried blood spot, HIV drug resistance, surveillance

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**Poster #33**
Continued Repetitive HIV Western Blot Testing Post-HIV Diagnosis and Association with Care Engagement

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**Objective:** To establish associations between a positive HIV diagnosis and continued Western Blot (WB) testing and HIV medical care engagement.

**Methods:** South Carolina (SC) HIV/AIDS Reporting System (eHARS) data for individuals diagnosed from January 1997 to December 2007 were used for analysis. All positive WB tests are reportable by law, and the first positive WB test is used as serostatus confirmation. All repeat positive WB tests done after 90 days of the first positive test are considered separate tests. Persons diagnosed with HIV from 1997 to 2005 and receiving a viral load test or CD4+ T-cell count reported at least once during each calendar year of 2006, 2007 and 2008 were defined as being In Care (IC) for the 3-year period. Other patterns of care were: Not In Care (NIC), no viral load/CD4 cell count reported across the period; and Transitioning Care (TC), viral load or CD4+ T-cell count reported during 1 or more years but not for all 3 years.

**Results:** There were 4,010/11,562 (35%) individuals with repeat positive WB tests during the study. These individuals had 7,098 repeat positive WB tests during this period. Repeat testers had a median of 1 repeat positive WB test (range 1-13); 2,197 (19%) had 1; 1,072 (9%) had 2; and 741 (6%) had 3 or more repeat tests. The median time between first positive WB and any repeat test was 884 days (range 91-4,531). From January 1997 to December 2005, there were 7,807 individuals diagnosed and alive during 2006, 2007, 2008. Of these, 3,594 (46%) were IC, 1,843 (24%) were TC and 2,307 (30%) were NIC. Repeat testers were more likely to be IC for each year under study than non-repeat testers. The odds of being IC (OR: 1.69; 95% CI: 1.51, 1.89) and in TC (OR: 2.68; 95% CI: 2.36, 3.04) for repeat testers were significantly greater than for non-repeat testers.

**Conclusion:** Receiving multiple positive HIV tests is associated with care engagement but it is an inefficient means and waste of resources to continually reconfirm serostatus.

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**Poster #34**
Evaluation of the IsoAmp® HIV detection kit Using Whole Blood Samples

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**Objective:** Early infant diagnosis (EID) of HIV infection remains a major challenge globally; dried blood spots (DBS) are transported long distances to central labs where high complexity RT PCR testing is performed using specialized instrumentation. Having a simpler molecular method might allow provincial-level labs to do testing. This change could improve turn-around-time and enable quicker treatment for infected infants. We evaluated the IsoAmp® HIV detection kit (under development, Biohelix Corp, Beverly, MA), which couples helicase-dependent isothermal amplification (HDA) with amplicon detection using a disposable, BEST™ cassette; the system only requires a heat block.

**Methods:** Patients seen at George Washington University Infectious Diseases clinic for routine HIV-1 RNA quantitative viral load (VL) and CD4 assessments were consented and enrolled. EDTA-blood was collected and extracted using a manual magnetic bead total nucleic acid (TNA) extraction kit (HandyLab, Ann Arbor, MI) and quantified using Nanodrop technology (Thermo Fisher Scientific, Pittsburgh, PA). Extracted TNA was combined with RNase free water, reaction mix, enzyme mix and mineral oil overlay. Reactions were incubated at 64 °C for 75 min, placed in lateral flow detection cassettes at room temperature and visually inspected after 20 min +/-5 min for color development at control and/or test line(s).

**Results:** To date, 19 patient samples have been tested using this kit and compared to HIV VL results. Specimens with HDA positive results had HIV VL ranging from 15,456 to 386,116 RNA copies/ml (mean; 132,888), while VL from HDA negative specimens ranged from 158 to 89,296 copies/ml (mean; 14,988). Early on we observed significant numbers of invalid HDA results, but changes in enzyme formulation resolved 6 of 8 specimens; 2 were QNS and could not be retested.

**Conclusions:** Although many more specimens (both positive and negative) need to be analyzed, we found that, with
one exception, specimens having viral loads >15,000 copies/ml were detected using the IsoAmp® HIV detection kit. We are moving towards evaluating DBS specimens with this kit to be consistent with specimens received by provincial laboratories for HIV EID testing.

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**Poster #35**

**Identification of Performance problems in the Abbott HIV-1&2 gO EIA by multiuser external quality control monitoring and real-time data analysis.**


National Laboratory for HIV Reference Services, Public Health Agency of Canada, Ottawa, Ontario.

**Objective:** To perform a root-cause analysis of defective lots of an HIV EIA kit identified by multiuser external quality control monitoring and real-time data analysis software.

**Methods:** A program was implemented in Canadian laboratories to monitor the performance of the Abbott HIV-1&2 gO EIA. A multiuser external QC reagent and the EDCNet ‘real-time’ data software analysis program were used. After entering results from the external QC reagent, results on intra- and inter-lab analysis in worldwide users of the Abbott HIV-1 gO EIA and the same external QC were generated in ‘real-time’ and displayed as a Levy-Jennings plot.

**Results:** Within six months, laboratories reported higher-than-expected calibrator rate values in the test kit. These aberrations were readily tracked by the ext QC and EDCNet ‘real-time’ data software analysis program were used. After entering results from the external QC reagent, results on intra- and inter-lab analysis in worldwide users of the Abbott HIV-1 gO EIA and the same external QC were generated in ‘real-time’ and displayed as a Levy-Jennings plot.

**Conclusion:** Laboratories should be employing third-party external controls in serological and molecular assays. Here, external QC monitoring was instrumental in identifying and confirming performance problems in the Abbott HIV-1&2 gO EIA. This program employing a common external QC reagent for multiuser use along with the EDCNet ‘real-time’ software further allowed us to examine and compare the external QC performance in labs in different parts of Canada and/or using different kit lots. Finally this example highlights the importance and benefit of implementing similar programs in a national or multilaboratory setting for laboratories performing diagnostic or clinical monitoring testing.

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**Poster #36**

**Rapid extraction and amplification of HIV-1 DNA from whole blood using a disposable microfluidics device**

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**Background:** Rapid detection of HIV nucleic acids (NA) would be useful for detection of HIV-infected infants and for routine monitoring of therapeutic effectiveness. However, placement of this technology in low-resource settings is hampered by high cost per test, complexity of the testing protocols, and the technical expertise needed to perform the testing. Microfluidic technology permits the miniaturization of the basic functions of NA extraction, amplification and detection in inexpensive, portable devices. In this study, DNA was extracted and specific HIV-1 sequences were amplified by PCR using a credit-card sized device and microfluidic principles for both NA extraction and amplification.

**Methods:** HIV-1 uninfected whole blood (WB) was spiked with various concentrations of 8E5 cells, which contain a single, integrated copy of HIV-1 DNA. Two hundred microliters of the spiked specimens ranging from 100,000 cells/ml to 100 cells/ml were extracted using the microfluidic device and the QiAMP DNA blood mini-kit. Extracts were amplified using a double-stranded primer from the long terminal repeat (LTR) region on the Stratagene MX 3000 for real-time detection of amplicons, or using equipment designed for the microfluidic cards. HIV-1 amplicons produced using the microfluidic protocols were detected by end-point fluorescence. HIV-1 infected (n=26) and uninfected (n=20) WB specimens were extracted and amplified by both methods.

**Results:** The limit of detection for the 8E5 spiked extracts from the two devices was similar: 10 copy input for Micronics; 4 copy input for Qiagen. The threshold cycle for the initial detection of HIV-1 LTR amplicons was also similar between the two methods (10 copy input: Micronics Ct = 32.35; Qiagen Ct = 31.87). All HIV-1 infected specimens extracted by both methods were detected with similar threshold cycles when amplified by the reference method. HIV-1 uninfected WB extracted by either method did not
produce any amplified material. All of the HIV-1 infected WB specimens that were extracted and amplified by the microfluidic protocols were detected, while the HIV-1 uninfected specimens did not produce any amplified material.

**Conclusions:** Microfluidic processing of WB specimens can effectively extract and amplify viral NA and could provide for point-of-care NA amplification testing in a variety of clinical venues. The level of sensitivity provides excellent qualitative detection of HIV-1 DNA in infected individuals.

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### Poster #37
### A Rapid Portable HIV Detection & Monitoring System for Low Resource Settings

**Joel W. Grover, Ph.D.** and **Katherine Luzuriaga, M.D.**

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This paper will present a summary of a proposal made to the National Institute of Health having two objectives: 1. Develop a prototype of a rapid portable instrument for detecting and measuring the amount of HIV in samples of patient blood, particularly in low resource settings. 2. Carry out a pilot study to establish the effectiveness and comparative performance of the instrument against an established reference system. The aim is to provide clinicians tools to diagnose and monitor HIV that exist in resource abundant settings but are not readily available in low resource settings. In particular, the instrument system will provide clinicians with detection methods that could be helpful in identifying acute HIV-1, early diagnosis of infection in pregnant women and infants, monitoring viral load following treatment and monitoring for the emergence of antiretroviral resistance. Viral load monitoring (VLM) of patients on antiretrovirals is a good example of the difference in treatment between resource abundant and low resource settings. While VLM is routinely done in the former because of the threat to patients and community of developing drug resistant strains, millions of recipients of antiretroviral therapy currently are not being monitored due to its cost and complexity. In this project Thermal Gradient will design and test a compact, rugged, low-cost, point-of-care diagnostic system based upon the company’s novel rapid PCR amplification device. Researchers at UMass Medical School will develop an effective HIV PCR assay and will lead the clinical studies. At the conclusion of the project the performance of the system (instrument, cartridge and assay) will be validated, first in internal tests using prepared blood samples and then in a significant pilot study.

**Presenter:** Joel W. Grover, Ph.D., Thermal Gradient Inc, Pittsford, NY, jgrover@thermalgradient.com

### Poster #38
### Detection of Human Immunodeficiency Virus Type 2 (HIV-2) Using a Real Time RT-PCR Assay with a Whole Virus Internal Control

**Linda M. Styer, Binshan Shi, and Monica M. Parker**

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**Objective:** Human immunodeficiency virus 2 (HIV-2) is closely related to the more widespread HIV-1. Each year, our lab detects several HIV-2 infections through the use of HIV-2 antibody assays. The lack of FDA-approved assays to detect HIV-2 RNA presents a barrier to diagnosing HIV-2 infection in individuals with inconclusive antibody results, and particularly in infants born to HIV-2-infected mothers. Our objective was to develop an assay to detect HIV-2 RNA. HIV-2 is often present at low titers in plasma, and thus we aimed to develop a sensitive assay that can distinguish negative samples from those that fail to amplify.

**Methods:** We developed a HIV-2 real time RT-PCR assay in which whole virus internal control (mouse hepatitis virus-MHV) is added to each sample during viral lysis and carried throughout the assay. Detection of MHV at the expected Ct range signifies proper assay function. The assay simultaneously amplifies a conserved region of the HIV-2 5’ long terminal repeat and a portion of the MHV M gene. These products are detected using two 5’ nuclease probes labeled with FAM (HIV-2) or HEX (MHV).

**Results:** We verified the presence of the two expected amplicons (1 for HIV-2 & 1 for MHV) by detecting two clearly defined bands of the expected lengths on a 15% PAGE gel. Amplicons were also confirmed by sequencing. Standard curve analysis shows good efficiency and linearity for HIV-2 & MHV (Eff=92-96% R2>0.99) when amplified separately or together, showing no interference between the two amplicons. The assay is robust, detecting multiple HIV-2 strains (subtypes A & B) and functioning with sample volumes ranging from 100ul to 1ml of plasma.

**Conclusions:** We have developed an efficient assay for HIV-2 RNA detection that will aid in the diagnosis of HIV-2 infection. This assay will be validated further to determine limit of detection, reproducibility, and accuracy. Additionally it will be adapted for HIV-2 RNA quantitation which will further increase our clinical monitoring capabilities for HIV-2 infected patients.

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**Poster #39**

Development of a simple, rapid and inexpensive method for the qualitative detection of HIV-1 RNA

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**Background:** New technologies for nucleic acid extraction, amplification, and detection offer opportunities to streamline molecular testing methods from hours to minutes while reducing the technical difficulties and instrumentation costs. In this study, a simple, instrument-free extraction protocol using magnetic beads with a silica-like surface chemistry was integrated with a rapid thermocycler and an inexpensive, battery-powered fluorometer.

**Methods:** Optimization of HIV-1 RNA detection used commercially available panels and plasma specimens spiked with known concentrations of purified virus. An internal control for RNA extraction and amplification (Qb bacteriophage, Attostar, Edina MN) was added to each specimen prior to processing. RNA was extracted using a commercial magnetic bead protocol (Invitrogen, Carlsbad, CA) modified to reduce the processing time from 45 min to 22 min. Extracts were amplified using a double-stranded primer that contained a fluorophore [Cal Red 610] and a quencher molecule [BHQ2] and the Finnzyme thermocycler (Finnzymes, Espoo, Finland). Amplicons were detected by endpoint measurements with the fluorometer (ESE Gmbh, Stokach, Finland). The optimized protocol was then evaluated using banked and freshly collected plasma from HIV-1 infected individuals in the United States (US) (n=33), banked plasma and commercial panels including individuals infected with known HIV-1 subtypes (n=17; 4 A, 3 B, 1 C, 1 D, 4 AE, 1 F, 1 G, 1 H, 1 AG), and fresh and frozen plasma from HIV-1 uninfected individuals (n=53). RNA amplification results were compared to data obtained using the Qiagen QIamp kit for RNA extraction with subsequent amplification by real-time PCR using the same primer system.

**Results:** Sensitivity for HIV-1 RNA plasma extracts was reproducible to a five HIV copy input (~ 200 HIV copies/ml). All of the US HIV-infected specimens (n=32) and the seventeen specimens of known HIV subtype were detected using the rapid method and all data were consistent with the real-time PCR results. None of the HIV negative specimens were detected by the rapid assay. Using this protocol, twelve specimens could be processed for qualitative detection of HIV-1 RNA in approximately 70 minutes.

**Conclusions:** Existing technologies can be adapted to provide a rapid, inexpensive nucleic acid diagnostic method for HIV-1 infection in resource constrained settings.

**Presenter:** Chou-Pong Pau, Ph.D., Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA.

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**Poster #40**

Innovative point-of-care HIV viral load detection in RLS

D. Laser, L. Mazzola, A. Arsham, A. Pedersen, R. Bhatia and D. Stern

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**Objective:** Wave 80 is funded by the NIAID to develop an easy-to-use, portable, rapid HIV viral load diagnostic appropriate for resource-limited settings. Nucleic acid testing of viral load is necessary to differentiate true infection from vaccine-induced serological response in vaccine trials, to detect acute HIV infection, for PMTCT infant diagnosis, and for HIV ART monitoring. Unfortunately because of cost and laboratory requirements, viral load analysis is currently unavailable in many resource-limited settings.

**Methods:** This new HIV viral load diagnostic employs direct-capture (non-PCR) nucleic acid detection for low probability contamination/misamplification; inorganic (non-protein) signal amplification for high heat/humidity tolerance; and whole-blood fingerstick sample processing within a single-use, enclosed cartridge format.

**Results:** Early results demonstrate assay miniaturization into cartridge format, enclosed on-board reagent storage, quantitation of HIV8E5-LAV RNA in spiked 100 microliter plasma samples above a detection threshold of 10,000 copies/mL, with a turnaround time of less than six hours. Initial stability testing shows minimal performance degradation after storage at 50C for 48 hours. Early prototypes of a rugged, portable, battery-powered instrument/cartridge analyzer are presented.

**Conclusions:** A new HIV diagnostic device has been designed to meet critical specifications for viral load analysis in remote and resource-limited settings. Further development is underway to 1) improve detection limit to 1000 copies/mL RNA, 2) integrate whole blood sampling and processing, and 3) reduce turnaround time to 2 hours. Full-scale validation and clinical testing begins 2011, targeting sensitivity of 95% and specificity of 99.5%.

**Presenter:** Laura T. Mazzola, PhD, Vice President, Global Health Products, Wave 80 Biosciences, San Francisco, CA. (415) 405 5228. laura.mazzola@wave80.com
**Poster #41**

**SMARTTube™ as a Test for Recent Infection**

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**Objective:** Tests for recent HIV infection have traditionally been based on antibody avidity, proportion or titre. High false recent rates (ε) or low recency durations (ω) tend to hinder incidence estimation. In vitro stimulation of HIV-antibody production by SMARTube™ technology, which declines with time after seroconversion, suggests a novel biomarker for recent infection: namely a stimulation index (SI) defined as the ratio of stimulated to unstimulated antibody levels measured by a semi-quantitative assay. We investigate the performance of a test for recent infection based on an SI threshold.

**Methods:** Using maximum likelihood analysis, we tested H0: ε = 5% vs H1: ε > 5% and H0: ω = 155 days vs H1: ω < 155 days (assuming infection events are uniformly distributed over an interval of at least two years preceding the survey). Rejecting either Null Hypothesis would imply little performance gain, relative to available recent infection tests such as those based on the BED assay. Data collected by the CDC in China were used, in populations infected for over a year for testing ε (n = 70 and n = 101 collected by the CDC in China were used, in populations infected for over a year for testing ε (n = 70 and n = 101 using Wantai and Abbott kits respectively), and in a survey of a high-incidence population (n = 57 using Wantai) for testing ω.

**Results:** At an SI threshold of 1.2, we fail to reject H0: ε = 5% with p-values of 0.51 and 0.97 for Wantai and Abbott respectively. Increasing the threshold has the advantage of decreasing ε and the disadvantage of decreasing ω. Even at a higher threshold of 1.4, conservatively assuming ε = 5%, we fail to reject H0: ω = 155 days with a p-value of at least 0.44.

**Conclusion:** A recency test based on SMARTube™ technology may attain a low false recent rate and adequate recency duration. These encouraging results support further investigation, and suggest a fundamentally new type of biomarker for constructing recent infection tests.

**Presenter:** Reshma Kassanjee, University of the Witwatersrand, School of Computational and Applied Mathematics, Johannesburg, South Africa. 278 326 7306 6. r.kassanjee@gmail.com

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**Poster #42**

**Preparation of Reference Panels for Current and Emerging HIV Variants**

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**Objective:** An unmet need among HIV researchers, regulators and assay developers is access to fully characterized virus and plasma from individuals recently infected with currently circulating HIV strains. A Working Group has been established to demonstrate feasibility of assembling plasma and virus panels from acutely infected donors in diverse geographic regions for initial coverage of frequently encountered HIV subtypes. These initial studies were aimed at securing supporting data and establishing the infrastructure for expanding the effort to include other geographic locations.

**Methods:** Samples representing large plasma volumes were donated by participating organizations for possible inclusion in a pilot panel. For initial studies, plasma samples provided by South African National Blood Service and American Red Cross were selected based on evidence of recent infection (HIV RNA+ only, or LS EIA+). Plasma was characterized by HIV RNA, p24 antigen, EIA and Western blot to assign a Fiebig stage. One ml of HIV positive plasma was used to infect PHA stimulated PBMC from uninfected blood donors. Virus sequence was attempted for plasma samples at all HIV RNA levels using single genome amplification.

**Results:** Successful virus isolation was observed for the majority of plasma samples from the U.S. and South Africa with ≥10e4 IU/ml RNA, at both early (Fiebig II) and later (Fiebig V) stage infection. The presence of early stage antibody does not interfere with virus isolation.

**Conclusions:** The results of these studies will provide criteria for selecting plasma samples for full virus characterization. Expansion of the project to other major and minor HIV strains will be initiated through a DAIDS contract. Panels consisting of plasma and virus samples and extensive characterization data will be made available through this project for use in basic research, in therapeutics and vaccine development, and in assay development, validation, and evaluation.
Characterization of Immune Responses to Capsid Protein (p24) of Human Immunodeficiency Virus Type 1: Implications for Detection


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Objective: We investigated immune responses to p24 to identify diagnostically significant immune dominant epitopes, to address cross-reactivity of anti-p24 antibodies to different subtypes, and to identify new biomarkers that distinguish acute from chronic HIV infection.

Methods: Epitopes of HIV-1 p24 were determined by using 9 peptides that span the entire p24 protein sequence. Immune responses to HIV-1 p24 were studied in 54 HIV-1 positive serum specimens from acutely infected individuals, AIDS patients and asymptomatic HIV-1 positive donors. The cross-reactivity of anti-p24 antibodies with different HIV subtypes was tested.

Results: We identified two major epitope regions located within the CypA binding loop and adjacent helices and at the end of the C-terminal domain. Three immune response patterns were observed in HIV-1 positive sera: polyclone-like, monoclonal-like, and none or very weak responses. Most (86%) plasma from acutely infected individuals reacted with multiple peptides while 60% and 30% of plasma from AIDS patient reacted with multiple or single peptides, respectively. In contrast, 46% and 43% of chronically HIV-1 infected individuals reacted with either one or none of the peptides, respectively, and only 11% reacted with multiple p24 peptides. The differences in immune response toward p24 peptides indicate a progression of immune responses from polyclone-like during acute infection to monoclonal-like or non-response to linear epitopes during chronic infection. Anti-p24 antibodies (subtype B) show broad cross-reactivity with different HIV-1 subtypes and the synergistic action of different combinations of anti-HIV antibodies improved capture and detection of divergent HIV-1 subtypes.

Conclusion: Our results provide the foundation for development and refinement of new assays for improved p24 antigen testing as future tools for rapid and accurate diagnosis as part of early intervention strategies and estimation of incidence.

Poster #44
Mother to child transmission of HIV-1 infection in UK—how soon can a diagnosis be made?

Centre for Infections, Health Protection Agency, London, UK

Objective: In order to inform the optimal choice and timing of tests for early and accurate HIV diagnosis in infants in a resource rich setting, a retrospective audit on laboratory findings on over 14,000 samples from 5,000 infants born to HIV positive mothers in UK was undertaken.

Methods: Samples had been referred to the UK Health Protection Agency and covered the birth cohort 2000-2006. The mode of delivery, timing of maternal diagnosis and maternal drug treatment histories were compared between those infants deemed to have been infected in utero with those that were infected during or after delivery. Nearly all samples were whole blood collected on EDTA, and these were tested for HIV proviral DNA. When only plasma or serum samples were available, these were tested for HIV-1 RNA. p24 antigen detection was performed on samples with sufficient volume. All samples were tested for HIV-1/2 antibodies to verify and type the mother’s HIV infection status.

Results & Conclusions: Of the 235 HIV infected infants identified, 109 had been tested at <1 year of age. HIV p24 antigen did not prove to be a sufficiently sensitive marker of infection. The proportion of intra-uterine infections was higher (82.3%) among infants whose mothers were diagnosed during pregnancy than among those diagnosed prior to pregnancy (50%). The mean gestation at delivery was lower among infants that became infected at or post delivery, suggesting that premature labour is associated with a higher risk of intrapartum transmission. Current UK guidelines recommend screening infants at risk by PCR at birth, six weeks and three months and a final antibody test at 18 months. In this audit, negative PCR results at both six weeks and three months of age indicated freedom from infection in all but four cases. Longer follow was required to detect all cases, but guidelines on timing of sample collection are not always followed.