1985 1st Generation HIV-1 IA
1987 HIV-1 Western Blot
1989 p24 Antigen Assay
1990 HIV-2 IA
   HIV-1 IA for Dried Blood Spots
1991 2nd Generation HIV-1/2 IA
1992 3rd Generation HIV-1/2 IA
   HIV-1 IFA
   CLIA-Moderate Complexity
   HIV-1 Rapid IA
1994 Collection Device and HIV-1 IA for Oral Fluid
1996 HIV-1 IA for Urine
   HIV-1 Home Specimen Collection Diagnostic System
   Quantitative HIV-1 Viral Load Assay
2002 Rapid HIV-1 IA for Whole Blood, Plasma
2003 CLIA-Waived Rapid IA for Whole Blood
   IAs to Include Group O Detection
2004 CLIA-Waived Rapid HIV-1/2 IA for Oral Fluid
   Rapid IA Differentiates HIV-1 from HIV-2
2006 Random Access Microparticle Chemiluminometric IA
   Qualitative HIV-1 Nucleic Acid Test for Diagnostic Use
2008 FDA’s CLIA Waiver Requirements Revised
2010 4th Generation HIV-1/2 Antigen Antibody Combination IA
2012 CLIA-Waived Rapid IA Provides Immediate Test Results
   Over-the-counter HIV-1/2 Rapid IA for Oral Fluid

Sponsored by the Centers for Disease Control and Prevention, Association of Public Health Laboratories and the National Minority AIDS Council.
Identification of Early HIV Infections Using the Fourth Generation Abbott Architect HIV Ag/Ab Combo Chemiluminescent Microparticle Immunoassay (CIA) in San Diego County

Anna Liza Manlutac and P. McVay, San Diego County Public Health Laboratory

Objective: San Diego County Public Health Laboratory (SDCPHL) serves a relatively large population of at risk individuals for early HIV infection as San Diego County ranks 22nd in the United States for prevalence of cases of AIDS in Metropolitan Statistical Areas. Therefore in March 2011, San Diego County began to use the newly FDA approved Abbott Architect HIV Ag/Ab Combo Chemiluminescent Microparticle Immunoassay (CIA). This fourth generation test, by detecting the p-24 HIV-1 viral antigen as well as antibodies to HIV-1 and HIV-2, offers the advantage of decreasing the window period to improve early detection of HIV infection. It also has a rapid turnaround time since it is a random access analyzer and has saved staff time. This study describes our first 18 months of experience including numbers of positives detected and confirmatory results.

Methods: 
1. Specimens tested were all serum specimens from multiple submitters, with the majority of specimens being collected at the San Diego Public Health clinics.
2. All initially reactive specimens on the Abbott Architect CIA were repeated in duplicate. Specimens were called positive on screening if reactive on at least 2 out of 3 tests.
3. Specimens were confirmed first by HIV-1 Immunofluorescent Antibody Assay (IFA). Two readers must agree that specimen is positive before turning out a positive result.
4. CIA positive, HIV-1 IFA negative/inconclusive specimens are sent for further confirmation to two laboratories:
   a. A commercial lab performs the qualitative GenProbe Transcription-mediated amplification (TMA) assay for HIV-1.
   b. The State of California Public Health Laboratory Viral and Rickettsial Disease Laboratory (VRDL) for HIV-1 IFA, WB (Western Blot) and HIV-2 EIA, IFA and WB for discordant CIA/IFA results.
5. Biorad Multispot HIV-1/HIV-2 Rapid Test was performed on CIA reactive, HIV-1 IFA negative specimens at SDCPHL during our evaluation of that method.

Results: A total of 12,865 specimens were tested in 18 months. 207 (1.6%) were repeatedly reactive on CIA. 166 of 207 confirmed by HIV-1 IFA. 41 IFA negative specimens were further tested: 14 were positive for HIV-1 RNA by TMA, 12 were IFA reactive at the State of California Public Health Laboratory 22 were WB reactive at the State of California Public Health Laboratory. 0 were HIV-2 antibody positive. 6 IFA negative specimens were also tested by Multispot. 5 were HIV-1 positive.

Conclusions: The fourth-generation Abbott Architect HIV Ag/Ab Combo assay identified 14 patients over an 18 month period who would most-likely have been missed if we had still been using a third generation assay. The instrument is easy to use, labor saving and gives a fast turnaround time. Multispot can quickly confirm results and once it is approved for use in California (i.e. published as an approved confirmation method by the CDC in the MMWR), we could have screening and confirmation completed in a few hours.
Performance Comparison of the 4th Generation Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA on the EVOLIS Automated System Versus Abbott Architect HIV Ag/Ab Combo, Ortho Anti HIV-1/HIV-2 EIA on Vitros ECi and Siemens HIV-1/O/2 Enhanced on Advia Centaur

Elizabeth Mitchell, G. Stewart, O. Bajzik, M. Ferret and M. K. Shriver, Bio-Rad Laboratories

Background: The GS HIV Combo Ag/Ab EIA is an enzyme immunoassay based on the principle of the sandwich technique for the qualitative detection of HIV p24 antigen and detection of envelope antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. The solid phase is coated with:

- Monoclonal antibodies against HIV p24 antigen
- Purified HIV antigens: HIV-1 gp160 recombinant protein, a synthetic peptide mimicking totally artificial HIV-1 group O-specific epitope (i.e. encoded by no existing virus); and a peptide mimicking the immunodominant epitope of the HIV-2 envelope protein.

The conjugates are based upon the use of:
- Biotinylated polyclonal antibodies to HIV p24 Ag (Conjugate 1)
- Peroxidase-conjugated streptavidin and HIV antigens (gp41 and gp36 peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins, and the same synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase) (Conjugate 2). (Figure 1).

During the assay procedure, Conjugate 1 (biotinylated polyclonal antibody to HIV p24 Ag) is added to the microplate wells, followed by the addition of samples to be assayed, as well as controls, and a calibrator. If present, HIVp24 antigen binds to the monoclonal antibody on the solid phase and binds to the Conjugate 1. HIV-1 and/or HIV-2 antibodies, if present, bind to the antigens immobilized on the solid phase. The addition of Conjugate 1 and sample is validated through a color change from yellow-green to blue. After incubation, excess sample is removed by a wash step. Next, Conjugate 2 is added. Peroxidase-labeled streptavidin reacts with biotinylated Ab-Ag-Ab complexes; peroxidase-labeled HIV-1 and HIV-2 antigens bind to the IgG, IgM or IgA antibodies captured on the solid phase. After incubation, unbound Conjugate 2 is removed by washing. Working TMB Solution is added to the plate and allowed to incubate. A blue or blue-green color develops in proportion to the amount of HIV antibody and/or antigen present in the sample. Color development is stopped by the addition of acid, which changes the blue-green color to yellow. The optical absorbances of specimens, controls, and the calibrator are determined spectrophotometrically at a wavelength of 450 nm with a 615-630 nm reference.

The GS HIV Combo Ag/Ab EIA is intended as an aid in the diagnosis of HIV-1 and/or HIV-2 infection, including acute or primary HIV-1 infection. The assay may also be used as an aid in the diagnosis of HIV-1 and/or HIV-2 infection in pediatric subjects (i.e., children as young as 2 years of age). The GS HIV Combo Ag/Ab EIA is intended for manual use and for use with the Bio-Rad EVOLIS™ and Elite™ Automated Microplate Systems.
For this study, the GS HIV Combo Ag/Ab EIA evaluation was performed on the EVOLIS Automated Microplate System. The EVOLIS is a fully automated microplate analyzer workstation that performs all functions necessary for complete sample processing of microplate assays. Functions include: positive sample barcode scanning, sample pre-dilutions, sample and reagent dispensing, plate incubations, plate wash cycles, photometric measurement of completed assay plates, results evaluation and generation of results reports. The EVOLIS™ System provides this advantage: the operator loads samples and consumables onto each EVOLIS™ System, then walks away to work on other tasks while specimens are processed.

**Objective:** The objective of this study was to compare the performance of Bio-Rad Laboratories 4th generation GS HIV Combo Ag/Ab EIA on the EVOLIS Microplate System to the Abbott 4th generation ARCHITECT HIV Ag/Ab Combo assay and two 3rd generation HIV antibody only tests (Ortho Diagnostics Anti HIV-1/HIV-2 EIA and Siemens HIV 1/0/2 Enhanced EIA).

**Methods:** Testing on the Abbott ARCHITECT HIV Ag/Ab Combo assay and Siemens HIV 1/2/0 Enhanced EIA on the Advia Centaur was performed at the Muir Labs. Testing on Ortho Diagnostics Anti-HIV 1+2 EIA on Vitros ECi was performed at the Contra Costa County Public Health Lab and testing on GS HIV Combo Ag/Ab EIA was performed at Bio-Rad Laboratories.

Analytical sensitivity of the 4th generation assays for HIV-1 p24 antigen was determined using the AFSSAPS (France) standard and the WHO HIV-p24 antigen International standard 90/636. Both standards were prepared by serial dilution into normal serum according to the standard’s package insert instructions. Each serial dilution point was tested in triplicate (x3) for each standard on the GS HIV Combo Ag/Ab EIA and on the Abbott ARCHITECT HIV Ag/Ab Combo assay. Sensitivity was also evaluated by testing serially collected samples from individuals with HIV infection from commercially obtained seroconversion panels HIV9077, HIV9079, and PRB95, N = 55, with 39 specimens containing detectable HIV1-RNA.

Specificity was evaluated by testing 1000 purchased prescreened negative donor samples (EIA and pooled HIV-1 RNA), as well as two western blot/rapid test false reactive panels from Zeptometrix (HIV9027 and HIV9078, N = 14). Each sample was tested once, and initially reactive samples were retested in duplicate.

**Results: Analytical HIV-1 p24 Sensitivity**

The results for the analytical sensitivity of the GS HIV Combo Ag/Ab EIA and the ARCHITECT HIV Ag/Ab Combo assay for HIV-1 p24 antigen were verified with antigen standards derived from the AFSSAPS (France) (Chart 1) and the WHO HIV-1 p24 antigen standard 90/636 (Chart 2). The GS HIV Combo Ag/Ab EIA demonstrated an antigen sensitivity of 12.69 pg/mL (range: 11.099-14.286 pg/mL at 95% CI) on the AFSSAPS standard and an antigen sensitivity of 0.41 IU/mL (range: 0.126 to 0.675 IU/mL at 95% CI) on the WHO standard (Table 1). In comparison, the ARCHITECT HIV Ag/Ab Combo assay demonstrated an antigen sensitivity of 20.10 pg/mL (range: 19.439 to 20.767 pg/mL at 95% CI) on the AFSSAPS standard, and 1.18 IU/mL (range: 1.082-1.287 IU/mL at 95% CI) on the WHO standard.

**Results: Performance in Early RNA positive Seroconversion Samples**

Sensitivity was evaluated by testing 55 seroconversion specimens. Of these seroconversion samples, 70.90% (39/55) were HIV-1 RNA positive. The GS HIV Combo Ag/Ab EIA and ARCHITECT
HIV Ag/Ab Combo assay were equivalent, exhibiting a sensitivity of 94.87% (37/39) on the 39 HIV-1 RNA positive samples tested (Table 3). In the seroconversion panel PRB951, both 4th generation Ag/Ab assays detected HIV-1 reactivity 11 days before the 3rd generation EIAs; and in the seroconversion panels HIV9077 and HIV9079, the 4th generation assays detected HIV-1 reactivity, 7 days and 8 days respectively, ahead of the 3rd generation assays. The 3rd generation assays, Ortho Anti HIV-1/HIV-2 EIA and Siemens HIV 1/0/2 Enhanced assay, both detected 76.9% (30/39) of the HIV-1 RNA positive seroconversion samples (Table 3 and Chart 3).

Results: Specificity Performance in Low Risk Blood Donors
The specificity of the GS HIV Combo Ag/Ab EIA in the normal donor population was 100% (1000/1000), with a 95% confidence interval of 99.6 -100% (Table 2). In comparison, ARCHITECT HIV Ag/Ab Combo assay had a specificity of 99.7% (995/998) with a 95% confidence interval of 99.1%-99.9 %. The initial reactive rate for the ARCHITECT HIV Ag/Ab Combo assay (2.5%) was higher than expected from the package insert claims, perhaps due to interference from previously frozen samples. The 3rd generation Siemens HIV1/2/O Enhanced EIA had a specificity of 100% (1000/1000, 95% confidence interval of 99.6%-100%), and Ortho Anti-HIV 1+2 EIA had a specificity of 99.70% (991/994, 95% confidence interval of 99.1%-99.9%).

Results: Specificity Performance with HIV False Reactive Panels (Zeptometrix: HIV9027 and HIV9078) Specificity was further evaluated by testing two specificity panels from Zeptometrix that are characterized as HIV false positive. Zeptometrix HIV9027 (N=9) is a western blot false reactive panel, and Zeptometrix HIV9078 (N=5) is a rapid test false reactive panel. The ARCHITECT HIV Ag/Ab Combo assay was reactive with five members of the western blot false reactive panel HIV9027 (Table 4). The other three assays evaluated had no reactivity with any members of the false reactive panels tested.

Conclusion: The Bio-Rad Laboratories 4th generation GS HIV Combo Ag/Ab EIA demonstrated superior analytical sensitivity and specificity when compared to ARCHITECT HIV Ag/Ab Combo assay. Additionally, the GS HIV Combo Ag/Ab EIA demonstrated improved sensitivity and equivalent or better specificity compared to 3rd generation antibody only assays. Overall, both 4th generation assays demonstrated excellent performance in the reduction of the serological window period, detecting HIV-1 infection 7 to 11 days earlier than the third generation HIV-1/HIV-2 EIAs tested.
Table 1
Summary of Analytical HIV-1 p24 sensitivity

<table>
<thead>
<tr>
<th></th>
<th>AFSSAPS HIV-1 p24 antigen Standard Required: &lt;50 pg/mL</th>
<th>WHO HIV-1 p24 antigen International Standard 90/636 Required: &lt;2 IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS HIV Combo Ag/Ab EIA</td>
<td>Abbott Architect HIV Ag/Ab Combo</td>
<td>Abbott Architect HIV Ag/Ab Combo</td>
</tr>
<tr>
<td>12.69 pg/mL Range (11.099 - 14.286)</td>
<td>20.10 pg/mL Range (19.439 to 20.767)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Performance in Low Risk Blood Donors

<table>
<thead>
<tr>
<th>ASSAY</th>
<th>Number Tested</th>
<th>Initial Reactive</th>
<th>Repeat Reactive</th>
<th>Non Reactive</th>
<th>Specificity</th>
<th>95% CI (Wilson)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad GS HIV Combo Ag/Ab EIA on EVOLIS™</td>
<td>1000</td>
<td>0/1000 0 %</td>
<td>0/1000 0 %</td>
<td>1000/1000 100 %</td>
<td>1000/1000 100 %</td>
<td>99.6 % to 100 %</td>
</tr>
<tr>
<td>Siemens HIV 1/2/0 Enhanced EIA on ADVIA Centaur®</td>
<td>1000</td>
<td>1/1000 0.1 %</td>
<td>0/1000 0 %</td>
<td>1000/1000 100 %</td>
<td>1000/1000 100 %</td>
<td>99.6 % to 100 %</td>
</tr>
<tr>
<td>Abbott HIV Ag/Ab Combo on ARCHITECT</td>
<td>998</td>
<td>4/994 0.4 %</td>
<td>3/994 0.3 %</td>
<td>991/994 99.7 %</td>
<td>991/994 99.7 %</td>
<td>99.1 % to 99.9 %</td>
</tr>
<tr>
<td>Ortho Diagnostic, Anti HIV-1/2 EIA on VITROS® ECI</td>
<td>904</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The initial reactive rate was higher than in the assay package insert and may perhaps be attributed to interference from frozen samples.
### Table 3
**Performance in Early RNA Positive Seroconversion Samples**

<table>
<thead>
<tr>
<th>PANEL ID</th>
<th>Total Panel Members Tested</th>
<th>HIV-1 RNA Positive Samples</th>
<th>Bio Rad GS HIV Combo Ag/Ab EIA on EVOLIS</th>
<th>Abbott HIV Ag/Ab Combo on ARCHITECT</th>
<th>Siemens HIV 1/2 O Enhanced EIA Centaur</th>
<th>Ortho Diagnostic, Anti HIV-1/HIV-2 EIA on VITROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRB551</td>
<td>6</td>
<td>4 (67%)</td>
<td>4 (67%)</td>
<td>1 (17%)</td>
<td>1 (17%)</td>
<td></td>
</tr>
<tr>
<td>HIV9077</td>
<td>24</td>
<td>17 (71%)</td>
<td>16 (67%)</td>
<td>14 (58%)</td>
<td>14 (58%)</td>
<td></td>
</tr>
<tr>
<td>HIV9079</td>
<td>25</td>
<td>18 (72%)</td>
<td>17 (68%)</td>
<td>15 (60%)</td>
<td>15 (60%)</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>55</td>
<td>39/39 (100 %)</td>
<td>37/39 (94.87%)</td>
<td>30/39 (96.92%)</td>
<td>30/39 (96.92%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4
**Performance in HIV False Reactive Panels (Zeptometrix: HIV9027 and HIV9078 Signal to Cutoff (S/CO) Values ≥ 1.00 is reactive)**

<table>
<thead>
<tr>
<th>Spec ID #</th>
<th>Dates</th>
<th>Gene Probe (C of A)</th>
<th>Abbott HIV-1 p24 Ag (C of A)</th>
<th>Couter HIV-1 p24 Ag, (C of A)</th>
<th>GS HIV-1/HIV-2 PLUS O (C of A)</th>
<th>GS Combo Ag/Ab EIA EVOLIS</th>
<th>Abbott HIV Ag/Ab Combo ARCHITECT</th>
<th>Siemens HIV 1/2 O Enhanced EIA CENTAUR</th>
<th>Ortho Anti-HIV 1+2 EIA VITROS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV9027-01</td>
<td>Dec 2, 1997</td>
<td>Negative</td>
<td>0.41</td>
<td>0.381</td>
<td>0.084</td>
<td>0.276</td>
<td>0.100</td>
<td>&lt;0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>HIV9027-02</td>
<td>Dec 6, 1997</td>
<td>Negative</td>
<td>0.39</td>
<td>0.381</td>
<td>0.104</td>
<td>0.342</td>
<td>0.290</td>
<td>&lt;0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>HIV9027-03</td>
<td>Dec 9, 1997</td>
<td>Negative</td>
<td>0.37</td>
<td>0.391</td>
<td>0.091</td>
<td>0.299</td>
<td>0.140</td>
<td>&lt;0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>HIV9027-04</td>
<td>Dec 12, 1997</td>
<td>Negative</td>
<td>0.46</td>
<td>0.381</td>
<td>0.096</td>
<td>0.318</td>
<td>0.120</td>
<td>&lt;0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>HIV9027-05</td>
<td>Dec 16, 1997</td>
<td>Negative</td>
<td>0.4</td>
<td>0.381</td>
<td>0.104</td>
<td>0.342</td>
<td>2.360</td>
<td>&lt;0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>HIV9027-06</td>
<td>Dec 19, 1997</td>
<td>Negative</td>
<td>0.49</td>
<td>0.476</td>
<td>0.094</td>
<td>0.309</td>
<td>2.360</td>
<td>&lt;0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>HIV9027-07</td>
<td>Dec 23, 1997</td>
<td>Negative</td>
<td>0.44</td>
<td>0.762</td>
<td>0.064</td>
<td>0.276</td>
<td>1.720</td>
<td>&lt;0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>HIV9027-08</td>
<td>Dec 26, 1997</td>
<td>Negative</td>
<td>0.43</td>
<td>0.671</td>
<td>0.086</td>
<td>0.283</td>
<td>1.610</td>
<td>&lt;0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>HIV9027-09</td>
<td>Dec 30, 1997</td>
<td>Negative</td>
<td>0.4</td>
<td>0.476</td>
<td>0.078</td>
<td>0.267</td>
<td>1.190</td>
<td>&lt;0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>HIV9078-01</td>
<td>May 22, 2000</td>
<td>Negative</td>
<td>0.8</td>
<td>0.31</td>
<td>0.115</td>
<td>0.378</td>
<td>0.180</td>
<td>&lt;0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>HIV9078-02</td>
<td>May 24, 2000</td>
<td>Negative</td>
<td>0.3</td>
<td>0.02</td>
<td>0.105</td>
<td>0.345</td>
<td>0.100</td>
<td>&lt;0.05</td>
<td>0.15</td>
</tr>
<tr>
<td>HIV9078-03</td>
<td>July 9, 2000</td>
<td>Negative</td>
<td>0.3</td>
<td>0.26</td>
<td>0.106</td>
<td>0.349</td>
<td>0.080</td>
<td>&lt;0.05</td>
<td>0.14</td>
</tr>
<tr>
<td>HIV9078-04</td>
<td>July 17, 2000</td>
<td>Negative</td>
<td>0.3</td>
<td>0.28</td>
<td>0.09</td>
<td>0.296</td>
<td>0.080</td>
<td>&lt;0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>HIV9078-05</td>
<td>July 30, 2000</td>
<td>Negative</td>
<td>0.6</td>
<td>0.3</td>
<td>0.101</td>
<td>0.332</td>
<td>0.100</td>
<td>&lt;0.05</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Abstracts

Chart 1: AFSSAPS HIV-1 p24 antigen Standard Analytical
Sensitivity Required: <50 pg/mL

Chart 2: WHO HIV-1 p24 antigen International Standard
Analytical Sensitivity Required: <2 IU/mL

Chart 3: Closing of the Serological Window
(DAYS from Initial Bleed to WB Positive)
Objective: Fourth generation HIV antigen/antibody (Ag/Ab) combination assays represent a significant improvement in the assays used for diagnosing HIV infection. Combination assays detect both acute and chronic infections based on their ability to detect HIV-1 p24 Ag and antibodies to HIV-1 group M, group O, and HIV-2. During the acute phase of HIV infection, early detection is dependent on the Ag sensitivity of the assay. Here we directly compare the Ag sensitivity of the ARCHITECT HIV Ag/Ab Combo (Combo) assay to RNA viral load using virus isolates. The use of HIV-1 isolates allows the correlation to be done in the total absence of an antibody response to infection and thus mimics the early stage of acute infection. In addition, the impact of genetic variation on Ag sensitivity can be assessed by evaluating a variety of HIV-1 non-subtype B strains. Previous studies have shown that HIV genetic variation can adversely affect the performance of some fourth generation HIV assays. The detection of non-B strains is becoming increasingly important. In the U.S., the prevalence of non-B strains has slowly increased over the last 10-15 years to approximately 3-5% of newly diagnosed infections.

Methods: A total of 35 unique HIV-1 group M isolates consisting of subtypes A (n=2), B (n=7), C (n=6), D (n=4), F (n=3), G (n=1), CRF01_AE (n=8), CRF02_AG (n=2), and unique recombinant forms (n=2) were used in the evaluation. Cell-free supernatants from cultured HIV-1 isolates were diluted in negative human plasma to four different levels. Each dilution was tested in the Combo assay to determine a signal to cutoff (S/CO) value and in the RealTime® HIV-1 assay to quantify RNA viral load. For each isolate dilution series, S/CO values and RNA copies/mL were plotted to determine the RNA concentration corresponding to a S/CO value equal to 1.0; a Combo S/CO value greater than or equal to1.0 is considered reactive. An overall correlation of RNA copies/mL and S/CO was also determined using the entire data set.

Results: The median RNA copies/mL at a Combo assay S/CO value of 1.0 was 57,900 (4.76 log10) for the individual virus isolates (range 26,440 to 102,400; 4.42-5.01 log10). When all the isolates were plotted as a single data set, the value was 58,500 RNA copies/mL (4.77 log10). There were no apparent differences in the correlation of RNA and S/CO by subtype/CRF.

Conclusions: Based on analysis of virus isolates, the ARCHITECT HIV Combo assay begins to detect p24 Ag once RNA viral loads have reached approximately 58,000 copies/mL. The correlation of viral load and Ag sensitivity was consistent across genetically diverse HIV-1 group M strains. These results are in agreement with data showing the Combo assay detects acute HIV infection in human subjects when viral load values are above 30,000 RNA copies/mL.
4th Generation HIV Screening in Massachusetts: A Partnership Between Laboratory and Program


Objective: In January 2012, the Massachusetts Department of Public Health (MDPH) Office of HIV/AIDS (OHA) and Hinton State Laboratory Institute (HSLI) set a goal to transition from 3rd to 4th Generation HIV screening for serum specimens collected at funded HIV prevention and screening programs throughout the state by June 2012. To meet this goal, HSLI and OHA needed to proactively address laboratory system components such as test validation, algorithm and results language development, and IT systems configuration. Programmatic system components such as timely specimen submission, messaging for clients, and training/technical assistance for HIV testing providers also needed to be addressed.

Methods: A project management team consisting of HSLI, OHA, and external training partners met regularly from January – June 2012 to develop and implement the state’s transition from 3rd to 4th Generation HIV screening. This team was responsible for launching a new HIV testing algorithm, developing official language regarding new processes and test results, establishing a standard shipping mechanism to ensure receipt of specimens from all state-funded testing providers within 48 hours of collection, validating the new screening test and algorithm, developing a system to respond to potentially identified acute HIV cases including novel deployment of state Disease Intervention Specialists (DIS), and developing materials to instruct and support OHA staff and funded providers. This team was also responsible for conducting in-person and web-based trainings as well as creating, conducting, scoring, and analyzing written post-training knowledge assessments. Pilot program and senior management feedback informed policies and protocols.

Results: During this six month period, MDPH successfully met the challenges of transitioning from 3rd to 4th Generation HIV screening. New laboratory processes were validated, test results language was updated, a new shipment process was institutionalized, and the time between specimen collection and results delivery was cut in half. In total, 274 testing providers representing 46 agencies attended one of 11 day-long trainings, and 84 providers from other arenas of the prevention and care system participated in two abbreviated webinars. A knowledge assessment was utilized to determine if OHA and funded testing staff understood the new policies, technologies, and systems. Outcomes of the assessment were used to direct further technical assistance.

Between June 21st and November 15th 2012, the HSLI tested 4,850 serum specimens using the new algorithm. Of those:

- Eighty-five tested positive for HIV-1.
- No samples tested positive for HIV-2.
- Three samples tested non-reactive for both HIV-1 and HIV-2 and continued to supplemental NAAT.

Eighty-eight samples tested reactive on the antibody/antigen test and continued to supplemental Multispot testing. Of those:
Of those:
- Two samples tested non-reactive.
- One sample tested positive for HIV-1 indicating acute infection.

**Conclusions:** Successful initiation of 4th Generation HIV screening technology across an entire service system is predicated upon a team approach with both laboratory and program representation. Specific attention must be paid to the following components: laboratory process, time-sensitive shipping protocols, consistent and accessible training, implementation monitoring, and responsive technical assistance.
Evaluation of HIV Point of Care Testing Results Compared to Lab-Based Third and Fourth Generation Assays in a Public Health Setting

Ali Lanza-Case and K. Benge, Unified State Laboratories: Public Health, Salt Lake City, UT

**Background:** Standard laboratory-based HIV testing by public health systems is often performed in designated laboratories and requires that a serum or plasma sample be sent to a public health laboratory. If the initial laboratory testing is reactive, then the sample is retested in duplicate and confirmatory testing is performed. Often patients must wait up to several days for results to become available. Rapid point-of-care (POC) HIV tests are excellent options in situations where patient follow-up may be difficult, allowing for clinicians or health workers to provide patient test results in a matter of minutes, however, confirmatory HIV testing at an approved HIV testing laboratory is still required for all reactive POC results. With the advent of 4th generation laboratory-based HIV screening capabilities, which are able to detect both HIV p24 antigen and HIV antibodies simultaneously, POC rapid tests may not be the preferred screening method in many situations, especially in cases with recent exposure risk.

**Objective:** The objective of this study was to evaluate both 3rd and 4th generation lab-based screening and confirmatory testing results against POC reactive samples in an effort to elucidate costs and benefits of each assays performance characteristics in a low prevalence state.

**Methods:** Between July 2010 and May 2012, Unified State Laboratories: Public Health received 3,860 serum/blood specimens for HIV testing. Of the specimens received, 31 samples flagged as POC reactive by the submitting agencies were evaluated on the Biorad HIV 1-2-O 3rd generation assay and subjected to Western Blot testing on the Biorad HIV-1 Western Blot assay according to standard testing algorithms. Sample aliquots were stored at -20 and retrospectively evaluated on two separate 4th generation screening assays including BioRad 4th generation HIV Ag/Ab and Abbott Architect HIV Ag/Ab combo as well as the BioRad Multispot platform (part of new CDC recommended 4th generation testing algorithm for public health laboratories).

**Results:** Of the 31 samples flagged as POC reactive, two were reported as HIV negative by the laboratory. One sample had reactive result interpretations on all three screening assays (BioRad 3rd gen., BioRadand 4th gen., and Abbott 4th gen.), but was negative on both confirmatory assays (BioRad Western Blot, BioRad Multispot). The other POC reactive sample was interpreted as negative for all five laboratory assays.

**Conclusion:** With the advent of better sensitivity and specificity and earlier detection times currently available in laboratory settings, the use of POC screening may not always be the most appropriate screening choice, especially in areas of low prevalence and/or situations involving recent exposures.
Use of the Abbott Architect HIV Antigen/Antibody Assay in a Low Incidence Population

Michael Pentella, T. DuBravac and T. Gahan, State Hygienic Laboratory at the University of Iowa

Objective: In 2010, Iowa reported 3.6 HIV diagnoses per 100,000 compared to the national average of 16.1. While incidence is low, the need to quickly identify and treat infected individuals is as great or greater because of the lack of public awareness. Detectable levels of antibody can take from two to eight weeks to develop, while HIV p24 antigen can be detected as early as one week after infection. This study was conducted to evaluate the performance of an HIV antigen/antibody combo (4th generation) assay compared to an EIA 3rd generation assay for routine screening in a low HIV incidence population.

Methods: Over a 4 month period, 2,037 specimens submitted for HIV screening were tested by BioRad HIV-1/2 Plus O EIA and the Abbott Architect HIV antigen/antibody combo assay. The performance characteristics of sensitivity, specificity, positive predictive value and negative predictive value were determined.

Results: Of the 2,037 specimens tested, there were 13 true positives detected. None of the positive test results were from patients who had not yet produced antibodies to HIV. The antigen/antibody assay had a sensitivity, specificity, positive-predictive value and negative predictive value of 100%, 99.85%, 81.25%, and 100% respectively. Three false positives were detected by the antigen/antibody assay and tested negative by EIA, western blot and HIV NAAT testing. For the three false positive tests by the antigen/antibody assay, the signal to cut off ratio was low at 1.86, 2.03 and 1.59 respectively. The EIA had a sensitivity, specificity, positive-predictive value and negative predictive value of 100%, 99.80%, 76.47% and 100%, respectively. The EIA had four false positive results which tested negative by the antigen/antibody assay and western blot.

Conclusion: In a low-incidence state where early infections are less commonly encountered, both the EIA assay and the antigen/antibody assay perform with near equivalency in detecting HIV infection. The antigen/antibody assay had one less false positive result. While no patients were detected in that early stage of infection, the use of the antigen/antibody assay presents the opportunity to detect an infected patient sooner.
Performance of a “Rapid Results” 4th Generation Immunoassay Algorithm

Chris Pilcher¹, L. Motta², R. Sperhacke², F. Hecht¹, R. Diaz³, L. Stanga⁴ and L. Borges²

1. University of California at San Francisco
2. University of Caxias do Sul, Brazil
3. Santa Marcelina Hospital, Brazil
4. UCSF Options Project

Objective: Several automated analysers can deliver 4th generation HIV immunoassay (4GIA) results within a few hours of specimen draw. We report a prospective trial of rapid testing for acute and chronic HIV infection using an all rapid results algorithm.

Methods: AMPLIAR Protocol 010 was a cross sectional study of HIV diagnostic testing, with a longitudinal component to follow patients with discrepant results to confirm HIV seroconversion and HIV status. Between 2007 and 2010, clients requesting HIV tests at 4 voluntary testing centers in South Brazil were enrolled. Site laboratories each performed 4GIA screening with confirmation by 3GIA and IFA/WB. Blood samples were obtained for pooled RNA (bDNA, 1:20), confirmatory testing as needed and BED-CEIA analysis. Beginning in 2009, samples were immediately tested using the VIDAS Duo Ultra 4GIA, Determine HIV 1/2 Ab rapid test and DPP HIV-1/2 Confirmatory Immunoblot rapid test. We evaluated performance of a serial rapid results algorithm (rapid 4GIA reflexed to Ab rapid test +/- confirmatory assay if 4GIA result is positive); a parallel algorithm (rapid 4GIA and Ab rapid test performed simultaneously, reflexed to confirmatory assay if either is positive); and standard batched 4GIA and pooled RNA-based algorithms.

Results: 3,617 subjects were studied; of those 521 were confirmed HIV+ (HIV prevalence: 14.4%). A subset of 1,829 (296 HIV+) subjects were tested with the all-rapid-results algorithm. With a pooled RNA/Ab algorithm as a reference standard we observed the following performance characteristics:

1. Algorithm: 4GIA-rapid (parallel,) Turnaround: 2.5 hours, Se of algorithm: 100.0 (98.8,100), Sp of initial screening test(s): 99.0 (98.4,99.4) 2. Algorithm: 4GIA-rapid (serial), Turnaround: 3.5 hours, Se of algorithm: 98.9 (97.1,99.6), Sp of initial screening test(s): 99.3 (98.8,99.6) 3. Algorithm: 4GIA-standard (serial), Turnaround: 1-3 weeks, Se of algorithm: 99.0 (97.8,99.6), Sp of initial screening test(s): 99.3 (98.9,99.5) 4. Algorithm: Rapid Ab only, Turnaround: 1.0 hour, Se of algorithm: 98.4 (97.0, 99.2), Se of initial screening test(s): 99.8 (99.6,99.9)

Conclusions: Rapid results from an automated 4G assay analyser provided similar sensitivity and specificity to batched 4G testing. We found furthermore that an algorithm with parallel rapid 4G/rapid Ab screening as the first step in the algorithm significantly reduced the time to final result, when compared to an algorithm with sequential rapid 4G screening and rapid Ab testing as the first steps. Parallel rapid 4G/rapid Ab testing also increased sensitivity. The parallel rapid test approach may be preferred when turnaround time is critical.
A Multicenter Performance Evaluation of the ADVIA Centaur HIV Ag/Ab Combo (CHIV) Assay on the ADVIA Centaur Immunoassay System

Lawrence Baker, Siemens Healthcare Diagnostics, Inc.

Objective: A multicenter study to evaluate the performance of the ADVIA Centaur® HIV Ag/Ab Combo (CHIV) assay* in terms of reproducibility and relative clinical specificity and sensitivity on the ADVIA Centaur systems compared to an FDA-approved HIV-1/2/O Antibody assay.

Methods: The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay was evaluated at three external sites. Reproducibility was evaluated with three reagent lots using an eight-member panel that consisted of four positive analyte types (anti–HIV-1, anti–HIV-1 group O, anti–HIV-2 and HIV-1 p24 antigen) and one HIV-negative sample. The standard deviation (SD) and percent coefficient of variance (%CV) were calculated for within-run, between-run, between-day, between-lot, and total (within-site) imprecision components for each site. The total study population consisted of 8954 specimens: 8539 prospectively collected, 311 retrospective and 104 contrived specimens. The patient specimens were obtained from HIV-1–positive and HIV-1 high-risk populations (adult, pediatric, and pregnant female). Individuals with risk factors such as intravenous drug use or sexually transmitted diseases, men who have sex with men (MSM), and others at high risk of HIV-1 infection were evaluated. Performance was also evaluated for low-risk apparently healthy individuals, hospitalized patients, individuals from an HIV-2–endemic region, and HIV-2–positive individuals. Specimens that were repeatedly reactive on one or both assays were confirmed by supplemental testing. Confirmation of positive results consisted of HIV-1 Western blot, HIV-2 EIA, HIV-2 Western blot, p24 Ag assay, and nucleic acid test. Seroconversion panels and known HIV antibody–negative, HIV p24 antigen–positive samples were also run to evaluate the detection of early acute HIV.

Results: Reproducibility estimates for the ADVIA Centaur assay were calculated across all lots and sites and for panel members with an Index value = 1.0. The within-run %CV estimates ranged from 2.1% to 4.4%, and the total %CV estimates ranged from 7.3% to 19.4%. The specificity for the low-risk/apparently healthy population was 99.72% (6121/6138). The sensitivity in individuals known to be infected with HIV-1 (including pregnant females and pediatric individuals) and in those known to be infected with HIV-2 was 100%. The ADVIA Centaur HIV Ag/Ab Combo assay demonstrated acceptable seroconversion sensitivity by detecting a greater overall number of reactive bleeds when compared to the FDA-approved HIV-1/2/O Antibody assay.

Conclusions: The results of this study indicate that the ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is a reliable and accurate, fully automated qualitative method to simultaneously detect the presence of HIV-1 p24 antigen and antibodies to HIV-1 (including group “O”) and HIV-2 in serum or plasma. *Not available for sale in the U.S. This assay is CE marked. ADVIA Centaur HIV Ag/Ab Combo assay is developed, manufactured, and sold by Siemens Healthcare Diagnostics Inc. for Ortho-Clinical.
Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay

Charles Noeson, Ortho Clinical Diagnostics

Objective: Assess the sensitivity, specificity and precision performance of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with MicroWell capability. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. p24 antigen detection is accomplished using monoclonal antibodies (MAbs) against HIV-1 p24. Biotinylated antigen or MAb are pre-bound to microwells coated with streptavidin. Sample is added to the coated wells in the first stage of the reaction and HIV analyte from the sample is captured by the biotinylated proteins. After washing HRP conjugated envelope proteins and anti-p24 MAbs are added. Following a final wash bound HRP conjugates are detected using the VITROS signal reagent. All testing was performed using two assay lots on three VITROS Systems. Assay specificity was assessed using 4766 blood donor samples and samples from 641 hospitalized patients. Assay sensitivity was evaluated by running 390 known HIV positive samples, including a commercially available panel of antibody subtypes, and 17 commercially available seroconversion panels. HIV-1 p24 antigen sensitivity was evaluated via serial dilution of AFSSAPS p24 standard. Each assay lot was evaluated for total within lab precision over 20 days in accordance with CLSI EP05-A2.

Results: Donor specificity was determined to be 99.85% (95% CI: 99.70% to 99.94%) for blood donors and 99.40% (95% CI: 98.4% to 99.8%) for hospitalized patients. All known HIV-1 group M, HIV-1 group O and HIV-2 positive samples generated positive results, including a commercially available panel of antibody subtypes. When used to test 17 commercially available seroconversion panels the HIV Combo assay was, on average, positive within one day of a commercially available 4th generation assay. The assay was reactive with all samples containing various subtypes of p24 and detects AFSSAPS p24 antigen at 13.2ng/mL. Precision of the assay ranged from 4.6 to 15.5% near the assay cut-off.

Conclusion: The VITROS HIV Combo Assay* enables earlier detection of HIV infection and provides comparable sensitivity and specificity performance to a commercially available 4th generation assay.

* Under Development
Performance of the Alere Determine™ HIV-1/2 Ag/Ab Combo Rapid Test with Specimens from U.S. HIV-1 Seroconverters and HIV-2 Positive Specimens from Ivory Coast

Silvina Masciotra, W. Luo, A. Youngpairoj, M. Kennedy and S. M. Owen, Centers for Disease Control and Prevention

Objective: FDA-approved 4th generation HIV immunoassays (IA) detect p24 antigen (Ag) and antibodies (Ab) but do not distinguish between them. These assays identify HIV-1 infections between 18 and 20 days before the Western blot (WB) becomes positive. FDA-approved rapid tests (RT) detect HIV-1 Ab 1 to 9 days before the WB becomes positive. Increased detection of acute HIV infections has been facilitated by using 4th generation IA in the laboratory. Given the advances in HIV testing technology, HIV diagnostic algorithms are changing in the U.S. to improve diagnosis of acute HIV-1 infection and HIV-2 infection. Currently, there is no 4th generation RT approved for use in the U.S. The Alere Determine™ HIV-1/2 Ag/Ab Combo (Determine Combo) is a non-FDA approved, rapid, lateral flow IA that detects HIV-1 p24 Ag and Ab to HIV-1 and HIV-2. The format of the assay allows distinction between p24 and Ab reactivity and thus has the potential to improve diagnosis of acute HIV infection. We evaluated the performance of Determine Combo with specimens from HIV-1 seroconverters from the U.S. and HIV-2 infected individuals from Ivory Coast to assess the ability of the assay to detect acute/early HIV-1 infections and HIV-2 antibodies.

Methods: Previously characterized, HIV-1 seroconversion panels (230 plasma specimens, 26 individuals) from the U.S. (SeraCare Life Sciences and ZeptoMetrix) were tested with Determine Combo according to package insert instructions. Data were used for a relative sensitivity analysis to ascertain the performance of Determine Combo relative to Western Blot. Sequence of reactivity for Ab only and combined reactivity for Ag+/Ab+/Ag+Ab+ was analyzed by comparing the calculated 50% cumulative frequency of positive Determine Combo results to HIV-1 WB positive results. Eighty-six HIV-2 plasma specimens from Ivory Coast (subtypes A and B in the integrase region) tested by Genetic System HIV-1/ HIV-2 Plus O, Multispot (Bio-Rad), and HIV-2 WB version 1.2 (MP Diagnostics) were tested to assess the ability of Determine Combo to detect HIV-2 antibodies and to evaluate the potential for detection of HIV-2 p27 Ag.

Results: The relative sensitivity analysis for HIV-1 early infections showed that the ranking for Determine Combo Ag+/Ab+/Ag+Ab+ or Determine Combo Ab-only was 15.5 and 9 days before WB positivity, respectively. All HIV-2 plasma specimens (86) were detected by Determine Combo with reactivity on the antibody detection line. Reactivity to the p24 Ag line was not observed among the HIV-2 specimens.

Conclusions: The relative sensitivity analysis places the combined (Ag/Ab) reactivity of the Determine Combo assay between FDA-approved 4th generation and 3rd generation laboratory IAs. Determine Combo Ab-only reactivity is similar to the FDA-approved RTs shown to be the most sensitive during early HIV-1 infection. The assay evolved from Ag reactivity to Ab reactivity in specimens from individuals known to be recently infected with HIV-1. Determine Combo performs well with HIV-2 specimens regardless of the subtype. These data indicate that this RT could be incorporated into alternative approaches for HIV diagnosis that improve detection of acute or early HIV-1 infection and reliably detect HIV-2 antibodies.
Performance of an HIV Diagnostic Algorithm Using the Architect HIV Ag/Ab Combo Assay and Potential Utility of Sample-to-Cutoff Ratio

Robert Coombs, University of Washington

**Background:** The ability to quickly and efficiently identify and confirm either acute or established HIV-1 infection is necessary to effectively manage and prevent the spread of this pandemic viral infection. In addition, distinguishing HIV-1 from HIV-2 infection is important as therapeutic monitoring and treatment regimens differ. The Architect HIV Ag/Ab Combo assay (a so-called 4th generation assay; Abbott Laboratories) can identify simultaneously p24 antigen (representing acute HIV-1 infection) and HIV-1/HIV-2 antibodies (representing early and chronic infection); however, a supplemental test is needed to discriminate between these two assay targets. Due to the rapid increase in p24 antigen associated with acute infection and the slower increase in IgM/IgG antibodies that follow, it should be possible to use the sample to cutoff ratio (S/CO) value to determine the assay reactivity for a false-positive versus true-positive test result for HIV-1 p24 antigen as confirmed by HIV-1 RNA testing.

**Objective:** Determine the performance of the Architect Ag/Ab combo assay to detect acute infection and the S/CO values to predict HIV infection status.

**Methods:** We performed a retrospective analysis of clinical test results derived using a modification of the proposed CDC algorithm for 4th generation HIV testing (1). Between May 2011 and July 2012, clinical samples were screened for HIV infection using the Architect HIV Ag/Ab Combo assay (Architect). All repeatedly reactive samples were tested using the Multispot HIV-1/2 rapid test (Bio-Rad) (MS) and depending on that result, were further tested using the Genetic Systems HIV-1 Western blot (WB) for confirmation, the Abbott m2000rt RealTime HIV-1 assay for HIV-1 RNA and/or referred for HIV-2 antibody testing (Focus Diagnostics) and for HIV-2 RNA quantification using a validated in-house assay (2). Presumptive clinical reports were issued based on the MS rapid test outcome and a final report was issued based on the appropriate confirmatory test results.

**Results:** A total of 15,076 clinical samples were tested by the Architect assay of which 394 (2.6%) were repeatedly reactive with a S/CO≥1 (Figure 1). From among the reactive samples, 337 were MS-reactive only for HIV-1, 4 were MS-reactive for both HIV-1 & HIV-2 and 53 were MS–non-reactive. Based on the MS HIV-1 reactive outcome, 337 samples were reported as “presumptive positive for HIV-1”; the WB resulted in 330 confirmed positive and 7 indeterminate samples. From these 7 WB-indeterminate samples, only 2 had sufficient sample volume for nucleic acid amplification testing (NAAT) and HIV-1 RNA was not detected; four patients were able to be tracked and confirmed to be HIV-1 positive. The Architect S/CO median value and interquartile range [IQR] was 793 [444-1,040] for the WB positives and 55 [46-349] for the WB-indeterminate samples.

With regard to the testing algorithm, for 4 specimens that were MS-reactive to both HIV-1 & HIV-2, one specimen was WB-indeterminate, Immunoblot (IB) for HIV-2 negative and was reported as HIV-1 infection not confirmed but with follow-up testing recommended. Two specimens were WB-positive and IB-negative, had an Architect S/CO mean value and range of 687 [635-739] and were both reported as HIV-1 infected. The last specimen was WB- and IB-positive with 17 HIV-2 RNA copies/mL and was reported as HIV-2 infection.
Finally, of 53 MS–non-reactive specimens that were evaluated for HIV-1 RNA, 10/53 specimens (18.9%) had a positive HIV-1 viral load (VL)>20,000 copies/mL with a median log copies/mL and IQR of 6.06 [5.1-7.0] and a median Architect S/CO value and IQR of 55 [2-76]. The remaining 43 specimens (82.7%) were NAAT not detected and had an Architect S/CO median value and IQR of 2.1 [1.4-5.0]. In this group, 33 specimens were WB-negative and were reported as “HIV-1 negative,” while 10 specimens were WB-indeterminate and were thus reported as “HIV-1 infection not confirmed.”

There was a log-linear relationship between the Architect S/CO value and HIV-1 RNA level in the acute infection group (Figure 2). The slope of the regression line for log-transformed HIV-1 RNA copies/mL and Architect S/CO was 0.9 with an intercept of -4.31, which showed that at an Architect S/CO value of 1.0, the HIV-1 RNA log copies/mL was 4.78 [95% confidence interval, 4.68-4.88]. From among 14,862 samples that were non-reactive by Architect (S/CO<1.0), the S/CO median value was 0.13 [IQR, 0.11-0.16]; however, for 55 samples the Architect S/CO values ranged between 0.70-0.99, a group that requires further investigation for low-level HIV-1 RNA.

Conclusions: The proposed algorithm based on 4th Generation serology testing detected 10 acute HIV-1 infections and one HIV-2 infection from among 15,076 clinical tests performed over a 13-month period by the Department of Laboratory Medicine, University of Washington. However, without further supplemental or confirmatory testing, the proposed testing algorithm would have misidentified 43 of 53 (81.1%) of the MS-negative samples as possible acute HIV-1 infection. There was a relationship among the Architect S/CO IQR values for antibody-negative acute infection [1.4-5.0], early infection [2-76] and established HIV-1 infection [444-1,040]. There was also a strong linear relationship (R2=0.91) between the Architect S/CO value and HIV-1 RNA level in acute infection that requires further investigation.

Reference:

![Graph showing the relationship between HIV-1 RNA copies/mL of plasma and the 4th generation Abbott Architect assay sample/control chemiluminescence (S/CO).](image)
Fig 1: Schematic diagram of the HIV testing algorithms using Architect assay.

15,076 Clinical Samples screened using Architect HIV Ag/Ab Combo

First test non-reactive or both duplicate re-tests non-reactive

- Non-reactives 14,682
  - S/CO (0.13) [0.11 - 0.16]

Reactives 394

Multispot Immunoperoxidase HIV-1 / HIV-2 differentation

First test reactive with at least one duplicate re-test reactive

55 had S/CO between 0.70 - 0.99

337 MS Reactive HIV-1

- WB Ind 7
  - WB Pos 330
    - S/CO (55) [46 - 349]
    - S/CO (793) [444 - 1,040]

Bands:
Weak other

HIV-1 S/CO, WB, VL

- WB Ind 10 (18.9%) > 20,000
  - 2 WB Ind
    - S/CO (76.5) [2.1 - 151]
  - 8 WB Neg
    - S/CO (19.0) [1.7 - 75]

53 MS Non-reactive

- VL (+) 43 (81.1%) = TND
  - WB Ind 10
    - S/CO (2.4) [1.3 - 4.2]
  - WB Neg 33
    - S/CO (2.1) [1.4 - 5.9]

365 MS Reactive HIV-1 & HIV-2

635 MS Reactive HIV-1 & HIV-2

4 WB Ind

WB Ind

S/CO, WB, VL

- 349, Ind, NA
- 53, Ind, NA
- 852, Ind, NA
- 55, Ind, NA
- 9, Ind, TND
- 46, Ind, NA
- 10, Ind, TND

WB Ind

S/CO, WB, VL

- 1.1, Neg, 25,000
- 2.1, Ind, 97,440
- 1.5, Neg, 133,300
- 2.1, Neg, 387,300
- 2.0, Neg, 397,600
- 36, Neg, 3,326,000
- 76, Neg, 8,924,000
- 74, Neg, >10,000,000
- 151, Ind, >10,000,000
- 208, Neg, >10,000,000

HIV-1 S/CO, WB, VL

VL (Log copies/mL) (6.06) [5.12 - 7.00]

Period of time
May 2011 – Jul 2012

Bands:
160, 120, 31, 24

43 (81.1%) = TND

4 WB Ind

S/CO, WB, VL

- HIV-1
  - 1.5, Ind, NA
  - 231, Pos, TND
  - 635, Pos, TND
  - 739, Pos, 5,589

- HIV-2
  - IB, VL
  - Neg, NA

S/CO: Sample / Cutoff ratio
WB: Western Blot
Ind: Indeterminate
VL: Viral load
TND: Target non-detected

S/CO: Sample / Cutoff ratio
IQR: Interquartile range
Neg: Negative
Pos: Positive
IB: Immunoblot
NA: Non-applicable
Evaluation of the Implementation of the 4th Generation HIV Combo/Multispot Assay Algorithm in Alaska, A Low HIV Prevalence State

Catherine Humphries, Alaska Public Health Laboratory

Objectives: To evaluate the effect of implementing the 4th generation APHL/CDC recommended HIV testing algorithm on result turn-around time (TAT), testing cost, and technician time in the public health laboratory system within Alaska, a low prevalence HIV state.

Methods: Comparative analyses were conducted on result TAT, testing costs, and technician time for 98 patient and validation sample results using the 3rd generation vs. 4th generation testing and confirmation algorithms.

Results: TAT for each repeat reactive EIA specimen confirmed by Multispot averaged 1.7 days, 9.9 days shorter than those sent for Western Blot (range 8-17 days from receipt of the specimen, average TAT 11.6 days). Unconfirmed EIA positives (repeat reactive on Combo, negative on Multispot) had the longest TAT (17 days), regardless of the final PCR result (Table 1). The cost to refer a specimen outside of Alaska for Western Blot testing is $185, including shipping. The Multispot costs $45 per patient, a savings of $140 for each confirmed positive ($2090/yr). The cost to send a discrepant specimen for NAAT testing is $257.78, which is an increase of $72.78 per specimen. Technician time did not significantly differ when considering the time it takes to perform and finalize the Multispot vs. packing and shipping a specimen for Western Blot or NAAT testing (22m; 20m respectively). There were overall 9% fewer false positives using the 4th generation vs. the 3rd generation algorithms in this data set. No window period positive patients have been detected thus far.

Conclusion: The 4th generation EIA/Multispot testing algorithm for HIV has significantly reduced the TAT and annual cost to confirm true positive results. True positive patients receive notification earlier than they would have under the old algorithm, which could potentially prevent further transmission of HIV. However, the 4th gen algorithm increased TAT (+9.4d) and cost for unconfirmed (false) positive specimens. Maintaining equipment, reagents, and proficiency on a NAAT test is cost prohibitive for our lab, deeming it necessary to refer plasma to an out-of-state lab for NAAT results. Additionally, a plasma specimen must be recollected from the patient, thereby increasing patient anxiety. As a low incidence state, false positive results are more likely than acute window period positives in Alaska, and the logistics of confirming with a NAAT test delays results and increases cost for each patient to a greater degree than the Western Blot algorithm. If the patient is a true window period positive, their results are dangerously delayed during a highly contagious period. With similar sensitivity and specificity values as the 3rd generation EIA, we have no reason to expect fewer false positives using the 4th gen EIA. Therefore, while the 4th generation algorithm decreases the TAT and cost for positive antibody patients, it dangerously delays TAT and increases cost and anxiety for false positives and window period Ag-only positives. For this algorithm to be truly effective in detecting and preventing the spread of HIV during the window period, NAAT testing needs to be more accessible and affordable for all public health labs, regardless of HIV incidence.
Table 1. Confirmatory Result TAT for Repeat Reactive HIV EIA Specimens

<table>
<thead>
<tr>
<th>Confirmation Test Performed</th>
<th>Result</th>
<th>Days From Receipt to Final Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multispot/Pos</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Multispot/Neg; NAAT Pos or Neg</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Western Blot/Pos</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Western Blot/Neg</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Western Blot/Indeterminate</td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>
Performance of the New HIV-1/2 Diagnostic Algorithm in Florida’s Public Health Testing Population: A Review of the First Five Months of Utilization

Dana Neumann, B. Bennett and L. Gillis, Florida Bureau of Public Health Laboratories

Objective: The Centers for Disease Control and Prevention and the Association of Public Health Laboratories have proposed a new HIV-1/2 Diagnostic Algorithm: a fourth-generation HIV-1/2 Ag/Ab immunoassay (IA) followed, when repeatedly reactive, by an HIV-1/HIV-2 antibody differentiation test, and if that is non-reactive, an HIV-1 nucleic acid amplification test (NAAT). The objective of the study was to evaluate performance of the new algorithm after five months of utilization in our high volume, high HIV-1 seroprevalence public health population.

Methods: Algorithm sensitivity and specificity was evaluated on 51,953 prospective serum or plasma specimens from individuals self-referring for HIV serostatus determination. Healthcare providers had to ensure specimens were submitted to the laboratory within 7 days of collection; specimens with anticipated delays were frozen and shipped to the lab as frozen. Specimens were tested on the day of receipt or maintained at 4 °C until next testing day. If the initial HIV-1/2 Ag/Ab IA (Abbott HIV-1/2 Combo) was nonreactive, a negative lab interpretation report would follow. If the initial IA was reactive, repeat screening in duplicate was immediately performed. Repeatedly reactive specimens were tested with an HIV-1/HIV-2 differentiation assay (Multispot HIV-1/HIV-2) on the same or next workday. If needed, the HIV-1 NAAT (APTIMA® HIV-1 RNA) was performed. In addition to the algorithm performance, we evaluated the laboratory “receipt to reporting” turn-around-time (TAT).

Results: The sensitivity of the Abbott HIV-1/2 Combo IA was 100% (922/922). The specificity of the IA was 99.86% (50961/51031). The new HIV-1/2 Diagnostic Algorithm specificity, including the differentiation and NAAT supplemental tests was 99.99% (51030/51031). Two acute HIV-1 infections (AHI) were detected with the new algorithm and no HIV-2 cases were identified. Only two of the 992 Multispot HIV-1/HIV-2 tests performed resulted in HIV-1 recombinant gp41 reactivity only (no gp41 synthetic peptide reactivity): one was HIV-1 NAAT reactive and one was HIV-1 NAAT nonreactive. Laboratory TAT for concordant reactive cases (i.e. Abbott Combo IA repeatedly reactive, Multispot HIV-1 reactive) was 1-2 workdays as compared to our most recent traditional algorithm (3rd generation IA and HIV-1 Western blot) 3-4 days.

Conclusions: Performance data from the first five months of utilization supports the continued use of the new HIV-1/2 Diagnostic Algorithm in our public health population. Sensitivity and specificity study data for the Abbott HIV-1/2 Combo IA is within the package insert sensitivity and specificity confidence intervals. Algorithm specificity continues to be an ongoing performance target, monitoring baseline viral load results on newly diagnosed individuals is a means to assess periodic specificity. The reduction in Laboratory TAT is beneficial for early HIV diagnosis and timely linkage to care. The identification of only two “algorithm-defined” AHIs from a study population of almost 52,000 individuals prompts a concern as to when individuals seek testing and current HIV screening practices. Additional research is needed to assess disease staging at initial diagnosis and the impact of high volume non-incidence based HIV rapid testing in a large public health population.
Clinical Performance of the Multispot HIV-1/HIV-2 Rapid Test to Identify HIV-2 Infection and Cross Reactivity with HIV-1 Western Blot

Eric Ramos, S. Harb, J. Dragavon and R. Coombs, University of Washington

Background: Infection with Human Immunodeficiency Virus type 2 (HIV-2) occurs mainly in West Africa; however, an increasing number of cases have been recognized world-wide, including in the United States. Currently available enzyme immunoassay (EIA) or chemiluminescence immunoassay (CIA) HIV-1/2 diagnostic tests do not distinguish between HIV-1 and HIV-2 antibodies and the confirmatory HIV-1 Western Blot (WB) assay may misclassify HIV-2 infection as HIV-1 due to cross reactivity. Thus, an efficient and rapid serologic method to differentiate HIV-1 from HIV-2 infection is required for confirmation.

Objective: Evaluate the performance of the Multispot assay in 3rd and 4th generation HIV-1/2 diagnostic assay algorithms to correctly classify HIV-2 infections.

Methods: Between Aug 2008 and Jul 2012, clinical samples were screened for HIV infection using the 3rd generation Genetic Systems HIV-1/2 plus O EIA (Aug 2008-Apr 2011) or the 4th generation Abbott Architect HIV Ag/Ab Combo (May 2011-Jul 2012). All repeatedly reactive samples were reflexed to the BioRad Multispot HIV-1/2 (MS) rapid test. MS HIV-1 positive samples were tested using the Genetic Systems HIV-1 WB assay. MS HIV-2 positive samples were tested using an HIV-2 Immunoblot (IB) assay (Focus Diagnostic). MS HIV-1 & HIV-2 positive samples were also tested with the Abbott m2000 HIV-1 RNA and an in-house HIV-2 RNA assays when possible. In the last two groups, the HIV-1 WB assay was also performed to evaluate cross reactivity.

Results: 53,970 samples were tested in the screening assays. 993 (1.84%) repeatedly reactive samples were tested using MS, yielding 882 samples reactive for only HIV-1, 3 samples reactive for only HIV-2 and 5 samples reactive for both HIV-1 & HIV-2. At the manufacturer’s recommended 1:100 dilution, three had strong HIV-1 spots and weak HIV-2 spots; one had strong spots for both HIV-1 and HIV-2; and one had weak spots for both HIV-1 and HIV-2. All three HIV-2-only positives and the sample with strong reactive spots for both HIV-1 and HIV-2 were IB positive for HIV-2. The last sample had an undetected HIV-1 viral load (VL) and 17 HIV-2 RNA copies/mL. These four samples were reported as positive for HIV-2 infection. The HIV-1 WB was also performed on these samples; 3 (75%) were classified as HIV-1 WB positive and 1 (25%) as HIV-1 indeterminate. The most common observed HIV-1 WB bands were Gag p24 and Pol p31 (100%), followed by Env gp160 (75%), Gag p55 (50%) and Env p120 and Gag p40 (25%).

The three samples with strong spots for HIV-1 and weak HIV-2 spots were HIV-2 IB negative and had all HIV-1 bands by WB. These samples were reported as HIV-1 infection. The sample with weak spots for HIV-1 and HIV-2 upon dilution was reactive in the HIV-2 spot and HIV-1 peptide spot and nonreactive in the HIV-1 recombinant spot. This sample was HIV-2 IB negative and HIV-1 WB indeterminate with only one weak band at gp160. This sample lacked sufficient volume for viral load testing and was reported as HIV-1 infection not confirmed.
Conclusions: Eight of 993 (0.8%) EIA/CIA repeatedly-reactive specimens were HIV-2 reactive by MS testing but only the four samples with strong reactions in the HIV-2 spot confirmed by HIV-2 IB. These 4 samples (0.04%) would have been otherwise misidentified as HIV-1 positive if only the HIV-1 WB was used to confirm the initially reactive screening test result. Weakly reactive HIV-2 spots likely reflect cross reactivity with HIV-1 antibody. The MS performed well as the confirmatory test for screening algorithms that used either 3rd or 4th generation HIV-1/2 assays.

Fig 1: Schematic diagram of the HIV testing algorithms using Multispot assay.

WB: Western Blot  
Neg: Negative  
Ind: Indeterminate  
VL: Viral load  
TND: Target non-detected
Evaluation of an Alternative Algorithm for HIV Diagnosis Among Men Who Have Sex with Men (MSM) in Five U.S. Cities - 2011

Silvina Masciotra, A. Smith, A. Youngpairoj, P. Sprinkle, I. Miles, C. Sionean, G. Paz-Bailey, J. Johnson and S. M. Owen, Centers for Disease Control and Prevention

Objective: Alternative algorithms for HIV diagnosis have been proposed by the Clinical and Laboratory Standards Institute and CDC. One algorithm specifies an initial screen with a 4th generation immunoassay (IA), followed by an HIV-1/2 discriminatory IA of initially positive-IA specimens. Discordant results are resolved by nucleic acid testing (NAAT). At CDC, we evaluated the performance of this alternative algorithm among men who have sex with men (MSM) in 5 US cities with different HIV prevalence.

Methods: A subset of specimens (n=992) from MSM who participated in the CDC National HIV Behavioral Surveillance survey (NHBS) recruited in 5 cities (sites) in the US in 2011 were used for algorithm evaluation. HIV testing was conducted at the CDC and the 5 sites. At CDC, plasma specimens were initially tested with the GS HIV Combo Ag/Ab IA (Bio-Rad, Laboratories). Supplemental testing of the positive-IA specimens (n=255) was conducted with the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories). Specimens with discordant results between the screening and supplemental test (n=1) were tested with the APTIMA Nucleic Acid Amplification Test (NAAT). To further identify acute infections, specimens negative on the screening assay (n=737) were tested with NAAT. At the sites, the different algorithms were a combination of initial screen with a 3rd generation IA from blood, or oral fluid (OF) or blood rapid test (RT), followed by OF- or blood- Western blot (WB). In some cases, self-reported positive (SRP) persons were only tested with WB. Performance of the alternative algorithm (infections identified/confirmed) by CDC testing was compared (McNemar’s test) to the number of infections confirmed at each site.

Results: Infections detected at CDC were 11/169, 11/98, 6/32, 77/332, and 149/361 for sites 1, 2, 3, 4, and 5 respectively. Of 992 specimens, WB-based algorithms failed to confirm a total of 19 infections, when compared to testing at CDC (p < 0.0001, McNemar’s test). Of the 19 infections incorrectly diagnosed, 4 were missed by OF initial testing among unaware individuals (sites 1 and 2). Supplemental testing with OF- or blood-WB at sites 4 and 5 accounted for the 15 remaining infections that were not identified in the field. Of these 15 infections, 12 had indeterminate-WB results (10 OF, 2 blood-WB), of which 8 (OF-WB) were from SRP individuals. The remaining three had negative-WB results, of which two were from SRP individuals (OF-WB, site 4) and one (blood-WB, site 5) from an acute infection later identified by the alternative algorithm. From the 737 4th generation IA negative specimens, one acute infection was identified by repeatedly positive-NAAT. No HIV-2 infections were identified in this population.

Conclusions: The proposed algorithm performed well in screening MSM for infection and confirmed more infections than algorithms which relied on WB as the supplemental assay. Discordant results between sites and CDC testing were associated with oral fluid testing (initial and supplemental). Testing with the alternative algorithm can increase the number of individuals diagnosed, including diagnosis of persons early in infection when they are likely the most infectious, which benefits early linkage to care and treatment.
Performance of the U.S. Army HIV Diagnostics Algorithm

Sheila Peel¹, M. Manak², K. Rice², E. Quick², J. Malia¹ and Y. Beale³

¹. Military HIV Research Program, Walter Reed Army Institute of Research
². Military HIV Research Program, Henry Jackson Foundation

Objective: An essential component of U.S. Army Force health operational and medical readiness is the capacity to detect and diagnose HIV infection with a single specimen so that personnel may be rapidly routed for HIV infection staging, and into appropriate care and treatment programs. From 1990 through 30 June 2011 the U.S. Army has conducted over 17,141,918 HIV screening tests and identified more than 3,584 HIV infected Soldiers within the Active Army, Army Reserve, and Army National Guard components. HIV infection surveillance is accomplished through a biennial testing program mandated by Army Regulation 600-110: Identification, Surveillance, and Administration of Personnel Infected with Human Immunodeficiency Virus (17AUG12). Additionally, HIV testing may be required for combat theater entrance for deploying forces as a further countermeasure for operational readiness protection of the “Walking Blood Bank” and thus, the safety of the battlefield blood supply and the War Fighter. Here we analyze the performance of the U.S. Army HIV Diagnostic Algorithm.

Methods: Specimens were screened with the 3rd generation EIA, HIV-1/HIV-2 Plus O EIA (BioRad Laboratories, Redmond Washington). Repeat reactive samples were reflexed to supplemental HIV-1 confirmatory testing using Genetic Systems HIV-1 Western Blot (WB) and Multispot HIV-1/HIV-2 Rapid Test (BioRad Laboratories, Redmond, WA). HIV-1 EIA, HIV-1 WB positive specimens were considered positive for HIV infection. EIA repeat reactive, HIV-1 WB negative or WB Indeterminate specimens were reflexed to the Aptima HIV-1 RNA Qualitative Assay (Gen-Probe, San Diego, CA) for Acute HIV-1 Infection (AHI) diagnosis. HIV-2 positivity was identified by reactivity on Multispot and by additional confirmatory nucleic acid testing. U.S. Army diagnosis of HIV infection was based upon a two independent positive HIV test events.

Results: From 01 Jan 2011 to 31 Aug 2012, the US Army conducted a total of 1,467,572 HIV screening tests yielding 1,752 EIA repeated reactive samples (0.12%). Of these 990 were HIV-1 WB positive (0.067%; known HIV infected and incident cases), 336 were WB indeterminate (0.02%), and 299 were WB negative (0.02%). Of 1,555 specimens tested on the Multispot HIV-1/HIV-2 Rapid test, 965 were HIV-1 reactive (0.065%); one sample was HIV-2 reactive. Of 299 WB Neg specimens, 4 were RNA reactive (0.0002%); of 336 WB IND specimens, 10 (0.0007%) were RNA reactive by Aptima qualitative assay. HIV-1 viral loads determinations for the 14 acute HIV infection case specimens at time of initial test ranged from 4.35 to 6.83 Log10 copies/ml. Mandated verification testing by acquisition of second independent specimen confirmed HIV infectivity in all acute/incident cases.

Conclusion: The US Army HIV Diagnostic algorithm resolves ambiguous HIV infection status classification. The Aptima HIV-1 RNA Qualitative Assay increased sensitivity for detection of acute HIV infection, enhanced specificity for HIV diagnosis, and prevented deployment of HIV infected personnel - mitigating risk to the “Walking Blood Bank.” The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.
Evaluation of Supplemental Testing with the Multispot HIV-1/HIV-2 Rapid Test and APTIMA HIV-1 RNA Qualitative Assay to Resolve Specimens with Indeterminate or Negative HIV-1 Western Blots

Laurie Linley¹, S. Ethridge¹, E. Oraka², S. M. Owen¹, L. Wesolowski¹, K. Wroblewski³, K. Landgraf³, M. Parker⁴, M. Brinson⁵, and B. Branson¹

1. Centers for Disease Control and Prevention
2. ICF International at the Centers for Disease Control and Prevention
3. Association of Public Health Laboratories
4. New York State Department of Health, Wadsworth Center
5. North Carolina State Laboratory of Public Health

Objective: Using the Western blot (WB) as a supplemental test after reactive results with sensitive initial assays leads to inconclusive HIV test results, which can delay diagnosis. An evaluation of specimens reactive by enzyme immunoassay (EIA) but indeterminate or negative by WB was conducted to determine the proportion that could be resolved by the supplemental tests recommended under the new HIV diagnostic testing algorithm.

Methods: Eleven public health laboratories de-identified and submitted remnant HIV diagnostic serum or plasma specimens that were reactive on 3rd generation HIV-1/2 EIA and either negative or indeterminate by HIV-1 WB. All were tested at either the New York Wadsworth Center Laboratory or North Carolina State Laboratory of Public Health with the Bio-Rad Multispot HIV-1/HIV-2 Rapid Test (Multispot) and the Gen-Probe APTIMA HIV-1 RNA Qualitative Assay (APTIMA). A Multispot result was considered positive for HIV-1 if both HIV-1 spots were reactive and indeterminate if only 1 spot was reactive. APTIMA-positive specimens were considered HIV-1 positive. Results from the testing were analyzed to evaluate the reclassification of specimens based on the new algorithm.

Results: Based on the APTIMA and HIV-2 antibody results, 570 (220 WB-indeterminate; 350 WB-negative) analyzable specimens were reclassified as follows: 512 (89.8%) as HIV-negative, 46 (8.1%) as HIV-1 positive (including 19 [3.3%] as acute HIV-1), 2 (0.4%) as HIV-2 positive, and 1 (0.2%) as HIV-positive, type undifferentiated. Nine (1.6%) specimens were positive for HIV-1 by Multispot but APTIMA-negative and WB-indeterminate. Of the 220 WB-indeterminate specimens, 38 (17.3%) were HIV-1 positive, of which 13 (34.2%) were identified as acute HIV; 1 (0.5%) was HIV-positive, type undifferentiated; 9 (4.1%) were positive for HIV-1 by Multispot but APTIMA-negative. Of the 350 WB-negative specimens, 8 (2.3%) were HIV-1 positive, of which 6 (75%) were identified as acute HIV-1; 2 (0.6%) were HIV-2 positive. Of 38 specimens with a single reactive Multispot HIV-1 spot, 27 (71%) were APTIMA-negative.

Conclusion: Applying the new HIV diagnostic algorithm to specimens tested retrospectively, HIV infection could be confirmed in nearly 9% of the EIA-reactive specimens: 18% of those that were WB-indeterminate and 3% of those that were WB-negative. The absence of HIV infection could be confirmed in nearly 90% of specimens. Possible explanations for the 9 Multispot-positive, APTIMA-negative results include: false-positive antibody results, errors in handling specimens not originally intended for RNA testing, or effective suppression of viral replication. These data demonstrate the new algorithm would correctly resolve the majority of specimens with a negative or indeterminate WB after a reactive 3rd generation EIA, and lend additional support for further testing of specimens with only a single reactive HIV-1 spot on Multispot.
The Multispot Rapid HIV-1/HIV-2 Differentiation Assay is Comparable with the Western Blot and an Immunofluorescence Assay at Confirming HIV Infection in a Prospective Study in Three Regions of the United States

Mark W. Pandori ¹, E. Westheimer², C. Gay³, N. Moss¹, J. Fu², L. Hightow-Weidman³, J. Craw⁴, L. Hall⁴, F. Giancotti², J. Embry³, B. Louie¹, P. Patel⁵, S. M. Owen⁵ and P. Peters⁵

Objective: To compare the effectiveness of Multispot (Bio-Rad), a rapid HIV-1/HIV-2 antibody differentiation assay, as a confirmatory test with the HIV-1 Western blot and HIV-1 immunofluorescence assays.

Methods: The STOP study is an on-going multi-site, prospective study evaluating methods to detect acute HIV infection. Participants (age > 12 years) from 12 HIV testing sites in sexually transmitted infection clinics and community-based HIV testing programs in New York City, San Francisco, and North Carolina are screened with the Architect (Abbott), an HIV-1/HIV-2 combination Antigen/Antibody (Ag/Ab) screening assay. Specimens with repeatedly reactive Architect results were tested with Multispot and either an HIV-1 Western blot (Bio-Rad) or an in-house immunofluorescence assay. Specimens with discordant screening and confirmatory results were resolved with an HIV-1 nucleic acid amplification test (NAAT).

Results: Between September 2011 and September 2012, 37,876 individuals were screened for HIV infection and 654 (1.7%) had a repeatedly reactive Architect combination Ag/Ab assay. On Multispot, 545 (83.3%) were HIV-1 reactive, 0 (0%) were HIV-2 reactive, 19 (2.9%) were reactive for both HIV-1 and HIV-2 (undifferentiated), and 90 (13.8%) were non-reactive [Figure]. Among HIV-1 reactive specimens, 536 (98.3%) had both recombinant HIV-1 and HIV-1 peptide spots, 6 (1.1%) had only a recombinant HIV-1 spot, and 3 (0.6%) had only an HIV-1 peptide spot. One HIV-1 reactive result (0.18%; exact 95% confidence interval 0.00% - 0.98%) with the HIV-1 peptide spot only was determined to be false-positive (Western blot and NAAT negative on same specimen, repeat Architect and NAAT negative on new specimen one week later). Among the 19 HIV reactive but undifferentiated specimens, 17 resolved as HIV-1 reactive at a 1:10 dilution and one resolved as HIV-1 at a 1:100 dilution. Among the 90 Multispot non-reactive specimens, 47 (52.2%) had a reactive NAAT assay consistent with acute HIV infection and 43 (47.8%) had a non-reactive NAAT assay consistent with a false positive screening result. Overall in this study HIV infection was confirmed in 610 (93.2%) of 654 repeatedly reactive Architect combination Ag/Ab assay specimens. Among those tested
with Western blot (n=429), the Multispot confirmed 407 (94.9%) infections and the Western blot confirmed 405 (94.4%) infections (exact McNemar’s p = 0.69). Among those tested with IFA (n=181), the Multispot confirmed 156 (86.2%) infections and the IFA confirmed 152 (84.0%) infections (exact McNemar’s p = 0.29).

**Conclusion:** In this prospective study, Multispot confirmed a comparable proportion of HIV infections as the Western blot and IFA although one of nine Multispot results with only one HIV-1 spot was false positive. Multispot also had a comparable frequency of false negative results as the Western blot and IFA highlighting that, regardless of the confirmatory assay used, NAAT is a necessary additional diagnostic test. NAAT successfully resolved all of the discordant screening and confirmatory results as either a false negative confirmatory test result (i.e., acute HIV infection) or a false-positive Architect combination Ag/Ab assay result.
The Route to Implementation of the New HIV Diagnostic Algorithm in New York State

Monica Parker1, B. Anderson2, L. Smith2, A. Muse2, L. Styer1 and J. Wikoff2

1. Wadsworth Center, New York State Department of Health
2. New York State Department of Health, AIDS Institute

Objective: The validity of the new CDC/APHL HIV Diagnostic Algorithm was affirmed through approval of the Clinical Laboratory Standards Institute (CLSI) guideline M53-A and a series of peer-reviewed, scientific publications. However, in order for clinical laboratories to begin using the revised algorithm, multiple programmatic modifications were necessary. Here we describe the steps taken by the New York State Department of Health (NYSDOH) to facilitate statewide implementation of the multi-test algorithm for the confirmation of HIV infection.

Methods: In January 2011, a department-wide HIV algorithm workgroup consisting of representatives from HIV policy, prevention, surveillance, and laboratory programs was formed to disseminate information on anticipated changes to HIV testing, identify issues and prioritize action items. As national efforts to promote the algorithm progressed, a laboratory-focused workgroup consisting of NYSDOH laboratory and surveillance staff was formed to address specific needs, execute programmatic changes and develop comprehensive guidance to assist clinical laboratories with implementing the multi-test algorithm and to facilitate continued public health reporting of HIV-related laboratory results.

Results: Outreach was formally initiated in January 2012 when the NYSDOH electronically disseminated a letter introducing the multi-test laboratory algorithm to clinicians, laboratory directors, and other relevant stakeholders in NYS. This letter discussed the rationale for changing the laboratory approach to HIV diagnosis, included descriptions of the tests at each step of the algorithm, and alerted laboratories that additional guidance on testing procedures and reporting requirements would be forthcoming. Additionally, a topic-specific email address (hivtesting@health.state.ny.us) was made available to which questions pertaining to the multi-test algorithm could be submitted and answered by appropriate DOH staff. In February 2012, the lab-focused workgroup continued the process by meeting monthly to identify and complete specific tasks. To facilitate laboratory reporting, workgroup members: 1) obtained a LOINC code for the HIV-1/HIV-2 differentiation test, 2) defined the specific test results to be provided to the NYSDOH for public health reporting, and 3) consulted with colleagues from the NYSDOH Electronic Clinical Laboratory Reporting System (ECLRS) to evaluate the feasibility of information technology systems used by laboratories to report results of the revised algorithm. The Wadsworth Center’s Clinical Laboratory Evaluation Program (CLEP): 1) reworked the categories under which clinical laboratories are issued permits to conduct HIV testing, 2) eliminated HIV-specific laboratory standards that would impede use of the algorithm, and 3) modified forms used by the NYSDOH Proficiency Testing (PT) Program to accommodate new initial and supplemental tests. Formal guidance directed to laboratories has been drafted. This document includes a detailed description of the revised algorithm and its testing components, guidance on how to report the test results to
healthcare providers, including interpretations for the different combinations of test results, and instructions for using ECLRS to meet public health reporting requirements.

Conclusions: Through the collaborative efforts of NYSDOH partners, an organized plan was developed to ensure appropriate laboratory testing and public health reporting as laboratories begin using the multi-test HIV testing algorithm. Several steps of the plan have been completed, but concerted efforts are still underway to complete all objectives of the plan.
Implementation of HIV Combo Ag/Ab EIA in a Public Health Laboratory

Pushker Raj, A. Saniesales, M. Coombs, D. Dutelle, and E. Delamater, Laboratory Services Section, Texas Department of State Health Services, Austin, Texas

Objective: We validated and implemented HIV Combo Ag/Ab EIA for routine screening test for detection of HIV at the Texas Department of State Health Services (DSHS).

Study Design: Beginning September 2012, sera submitted for routine HIV testing were screened with the Bio-Rad GS HIV Combo Ag/Ab EIA for repeated reactivity and confirmed with WB. All WB reactive, indeterminate, and nonreactive sera were tested with MS for the comparison study. WB indeterminate and nonreactive sera were also sent to Dallas for NAAT if enough volume was available for the testing.

Results: Serum samples routinely submitted to the DSHS laboratory for HIV testing were screened with Bio-Rad HIV Combo Ag/Ab EIA. Among the 8,544 sera screened, 101 sera were initially reactive. Among these, 91 were repeatedly reactive and 10 (0.12%) initially reactive were nonreactive when retested in duplicate. Repeatedly reactive 91 (1.06%) sera were confirmed with WB and 78 reported as reactive. Among the 78 WB reactive sera, 77 were tested with MS and the results were in 100% agreement for HIV-1. One WB-R sample could not be tested with MS due to quantity not sufficient (QNS) for testing. One WB-R, MS-R, was also tested NAAT-R. Twelve WB-NR samples were also tested MS-NR. Among the 12 WB nonreactive sera, 8 eligible samples were sent to Dallas for NAAT and 4 QNS sera could not be tested. Of the 8 NAAT samples, 3 were reactive and 5 were reported nonreactive. One indeterminate WB sample was tested nonreactive with MS and NAAT.

Conclusions: We will be compiling data from September to November and the results of HIV Combo Ag/Ab EIA and WB confirmation will be presented. The use of multispot rapid test (MS) for confirmation of HIV screening results is not established. We will also present the comparison of MS with WB for use as a confirmatory test in the new HIV testing algorithm for detection of HIV.
Indeterminate HIV-1 Serological Results and the Cost of Resolution

*Brock Neil, M. Landes, D. Smalley, Serology and Rabies, Tennessee Department of Health, Division of Laboratory Services*

**Objectives:** Early detection of HIV is crucial to control the spread of HIV. Public Health Clinics offer HIV diagnostic services at free or reduced prices to customers who do not have the ability to pay. The first aim of this study was to examine the resolution of indeterminate serological status of the Public Health Clinic patient population to determine if switching from the pre-2011 HIV diagnostic algorithm to the post-2011 algorithm makes sound clinical and financial sense. The second aim was to look at the cost associated with switching algorithms and make suggestions on how the Public Health Laboratories can absorb this cost.

**Methods:** The Public Health Laboratory performed a retrospective examination of HIV patient results from July 1, 2009 to June 30, 2011. All assays were performed using the pre-2011 HIV diagnostic algorithm.

**Results:** The pre-2011 HIV diagnostic algorithm has a window from approximately day 17 to day 32 post-infection where the clinician would expect an indeterminate result. The post-2011 CDC HIV testing algorithm has a similar window but it is from day 11 to day 24. A specimen that is EIA repeatedly reactive but Western Blot or Multispot negative is considered indeterminate. There were 257 specimens reported as indeterminate for HIV in this study. Out of these 257, only 44 had a subsequent specimen sent to the public health laboratory to resolve the indeterminate status. The remaining 213 patients had no known follow-up specimen sent to the Public Health Laboratory after report of the initial indeterminate status.

**Conclusions:** A 17% follow-up status is inadequate and demonstrates the need for a different testing algorithm to reduce indeterminate results. The new algorithm resolves indeterminate status with a qualitative RNA HIV-1 NAT assay which can detect the virus approximately 6 days post exposure. This confirmatory assay would have resolved most of the 257 specimens with indeterminate status. However, the NAT RNA HIV-1 assay is cost prohibitive for low and moderate level testing facilities due to short shelf life and large package volume. This fact makes it difficult for public health laboratories to implement the molecular assay. We suggest centralizing NAT RNA HIV-1 testing for Public Health Laboratories by region in order to control cost for this important public health issue.
Comparing the 3rd and 4th Generation HIV Algorithms in a High-risk Population

Brian Nefzger¹, J. Palm¹, G. Johnson¹, S. Vetter¹ and R. Wonderling²

1. Minnesota Department of Health
2. Abbott Diagnostics

Introduction: As public health laboratories and clinical laboratories consider switching from the 3rd generation to the 4th generation algorithm for HIV testing, it is important to consider the impact on workflow and cost as well as the benefits of earlier detection in the infection process that newer technologies may produce.

Objective: The Minnesota Department of Health-Public Health Laboratory (MDH-PHL) provides testing for local STD clinics that serve a high-risk subset of the population in the metropolitan area. We compared the use of two 4th generation combo immunoassays (IA), The Bio-Rad HIV Combo Ag/Ab enzyme immunoassay (EIA) and the Abbott ARCHITECT HIV chemiluminescent miroparticle immunoassay (CMIA), as part of the 4th generation HIV testing algorithm, with the Bio-Rad HIV 1,2 + O EIA using the 3rd generation algorithm. We assessed performance, cost and tech time for each IA along with the entire algorithm.

Methods: Specimens were tested with the 1,2+O EIA and both Combo IAs. For the 3rd generation algorithm, specimens that were Repeatedly Reactive (RR) with the 1,2+O EIA were confirmed with both the Bio-Rad Multispot HIV-1,HIV-2 rapid test for differentiation, and Bio-Rad HIV-1 Western Blot (WB). For confirmation with the 4th generation algorithm, RR specimens on either Combo IA were performed with the Multispot test only. Testing costs were tracked so the expense incurred following both algorithms could then be assessed.

Results: MDH-PHL tested 658 specimens in parallel with the 1,2+O EIA and the two Combo IAs. Using the 3rd generation algorithm as a ‘gold standard, 33/658 (5.01%) of the specimens were positive for HIV antibodies. The 4th generation algorithm showed 100% agreement with the 3rd generation algorithm. The ARCHITECT CMIA had one false positive that did not confirm as true with supplemental testing. Two specimens tested initially reactive with the 1,2+O EIA, but did not confirm as true positives after completing the remainder of the confirmatory algorithm. These two specimens were also non-reactive on both combo IAs.

To get a better idea about specificity of the combo IAs, seven previous false positive specimens (samples that were RR with 1.2 + O EIA, but negative Multispot and negative WB) were also tested with both combo IAs. All seven were non-reactive on the combo IA suggesting the combo IAs are more specific.

Reagent cost for the combo IAs was greater than the cost of the 1.2 + O EIA reagents. However, the total cost to perform all of the testing in the 3rd generation algorithm using the BioRad HIV 1,2+O kit and confirming with the BioRad Western Blot kit was greater than the cost of performing the 4th
Conclusions: Both the Bio-Rad Ag/Ab Combo EIA and the ARCHITECT HIV CMIA outperformed the Bio-Rad 1,2+O EIA due to improved specificity. Using either combo IA in conjunction with the 4th generation algorithm yields a cost savings over the 3rd generation algorithm. The ARCHITECT platform has a faster turnaround time (28 minutes/assay) for each test and will reduce labor costs compared to the Bio-Rad Combo test (2 hours/assay). However, the increased cost of purchasing the ARCHITECT platform may be prohibitive for laboratories that do not test a high volume of samples. Laboratories will need to consider their testing volumes and available budget in order to decide which platform best fits their needs.
Sensitivity and Type-Specificity of Multispot EIA Compared to Western Blot for Confirmatory Serodiagnosis of HIV

Lucia Torian and L. Forgione, New York City Department of Health

Objective: Recent improvements in the sensitivity of immunoassays (IA) used for HIV screening, coupled with increasing recognition of the importance of rapid point-of-care testing, have led to proposals to adjust the algorithm for serodiagnosis of HIV so that screening and confirmation can be performed using a dual or triple EIA sequence that does not require Western blot testing for confirmation. One IA that has been proposed as a second or confirmatory test is the BioRad Multispot® Rapid EIA. This test would have the added advantage of differentiating between HIV-1 and HIV-2 antibodies. We compared the sensitivity and type-specificity of an algorithm combining a 3rd generation EIA followed by a confirmatory Multispot with the conventional algorithm that combines a 3rd generation EIA (BioRad GS HIV-1-2+0 EIA) followed by confirmatory Western blot (BioRad GS HIV-1 WB).

Methods: 8,760 serum specimens submitted for HIV testing to the New York City Public Health Laboratory between May 22, 2007 and April 30, 2010, and testing repeatedly reactive on 3rd generation HIV-1-2+0 EIA screening received parallel confirmatory testing by WB and Multispot (MS).

Results: 8,678/8,760 (99.1%) specimens tested WB-positive; 82 (0.9%) tested WB-negative or indeterminate (IND). 8,690/8,760 specimens (99.2%) tested MS-positive, of which 14 (17.1%) had been classified as negative or IND by WB. Among the HIV-1 WB-positive specimens, MS classified 26 (0.29%) as HIV-2. Among the HIV-1 WB negative and IND, MS detected 12 HIV-2.

Conclusion: MS detected an additional 14 HIV-1 infections among WB negative or IND specimens, differentiated 26 HIV-1 WB positives as HIV-2, and detected 12 additional HIV-2 infections among WB negative/IND. A dual 3rd generation EIA algorithm incorporating MS had equivalent HIV-1 sensitivity to the 3rd generation EIA-WB algorithm and had the added advantage of detecting 12 HIV-2 specimens that were not HIV-1 WB cross-reactors. In this series an algorithm using EIA followed by MS would have resulted in the expedited referral of 38 specimens for HIV-2 testing and 40 specimens for nucleic acid testing to rule out acute HIV-1 infection. Further testing using a combined gold standard of nucleic acid detection and WB is needed to calculate specificity and validate the substitution of MS for WB as the supplemental confirmatory antibody test in the diagnostic algorithm used by a large public health laboratory.
Detection of a Rare HIV-1/HIV-2 Co-Infection Using the New Supplemental Testing Strategy

Linda Styer, T. Sullivan and M. Parker, Wadsworth Center and New York State Department of Health

Objective: One of the stated benefits of the new CDC HIV testing algorithm is that it will detect more HIV-2 infections. Here we present a case report that highlights how the supplemental tests of the new algorithm can detect HIV-1/HIV-2 co-infections.

Methods: We received a whole blood specimen for confirmation of a reactive rapid HIV test. According to our standard referral protocol, we tested the plasma with the GS HIV-1/2 plus O enzyme immunoassay (EIA), Multispot HIV-1/HIV-2 Rapid Immunoassay (MS) and the GS HIV-1 Western Blot (WB) (all Bio Rad, Inc). RNA testing was performed using the Aptima HIV-1 RNA Qualitative Assay (Gen-Probe) and a laboratory-developed, real-time RT-PCR assay for qualitative HIV-2 RNA detection.

Results: The specimen was strongly reactive by EIA and all bands were positive on the HIV-1 WB, confirming HIV-1 infection. However, the HIV-1 and HIV-2 spots on the MS were reactive (an ‘Undifferentiated’ result) for undiluted plasma as well as the 1:10 and 1:100 dilutions; dilutions were made following the manufacturer’s dilution protocol. The HIV-2 reactivity of previous specimens in our lab with an ‘Undifferentiated’ MS result has always been resolved by the dilution protocol. In this case, the intensity of the HIV-1 and HIV-2 spots decreased proportionally in the diluted samples, suggesting a possible co-infection. To confirm this, we tested the specimen with our HIV-2 qualitative RNA detection assay and obtained a low positive result (Ct = 41). Because this result was so weak and there was no indication on the requisition form that this individual was at risk for HIV-2 infection, we were unsure if this was a true HIV-2 positive result. We contacted the submitter and learned that this person reported sexual contact with a person from Gambia in 2006. HIV-2 prevalence is highest in West Africa and the reported connection with Gambia suggested that a HIV-1/HIV-2 co-infection was possible. We requested a follow-up specimen and repeated the HIV-2 qualitative RNA detection assay. The newly collected specimen produced a positive HIV-2 RNA result with a stronger signal (Ct ~ 35), confirming HIV-2 infection. The follow-up specimen also produced similar EIA, HIV-1 WB and MS results. Additionally, HIV-1 RNA was detected in this specimen using the Aptima HIV-1 RNA assay.

Conclusion: Under the conventional EIA-WB testing algorithm, a positive result on the HIV-1 WB confirmatory assay would lead to referral to care for a HIV-1 infection. HIV-2 testing is only recommended for persons with a reactive EIA and negative or indeterminate HIV-1 Western Blot or when HIV-2 risk factors are present. Co-infection with HIV-2 would be suspected only if the person’s immunologic status continued to deteriorate after their HIV-1 viral load was controlled. In this case, we were able to detect a rare HIV-1/HIV-2 co-infection using the original specimen submitted for rapid test confirmation. This was due to the results of the Multispot assay, which provided the only clue to the presence of HIV-2 in this person. This case illustrates another benefit of the new HIV testing algorithm, detection of HIV-1/HIV-2 co-infections.
APHL/CDC Demonstration Project for Referral of HIV Nucleic Acid Amplification Testing (NAT) for U.S. Public Health Laboratories (PHLs) Using the Proposed HIV Diagnostic Testing Algorithm

Kelly Wroblewski1, L. Wesolowski2, S. Ethridge2, M. Parker3, B. Bennett4, B. Werner5 and S. M. Owen2

Objective: For the majority of U.S. PHLs implementing the proposed Diagnostic Testing Algorithm, only a small number of specimens per year will require resolution with NAT, making the cost of bringing the assay in-house prohibitively high. The objectives of the HIV NAT Referral project are to provide PHLs using the proposed algorithm with access to the GenProbe APTIMA HIV-1 RNA Qualitative assay (APTIMA) and to document the feasibility and performance of the algorithm when reference laboratories provide testing. The data from the project will be used to compare the performance of the qualitative assay with commercially available HIV-1 viral load assays using de-identified remnant specimens. This comparison should assist laboratories that plan to conduct validation of viral load assays for off-label diagnostic use.

Methods: The Association of Public Health Laboratories (APHL) has contracted with the New York State Department of Health’s Wadsworth Center and the Florida Department of Public Health, Bureau of Laboratories to serve as referral laboratories offering qualitative and quantitative HIV-1 NAT. To date, 11 PHLs have enrolled in the project as submitting sites and PHL enrollment is ongoing. The PHLs submit specimens that are repeatedly reactive using an antigen-antibody combo HIV-1/ HIV-2 IA and either non-reactive or indeterminate using a supplemental antibody assay, including an HIV-1/HIV-2 differentiation assay, Western blot or IFA, to one of the two referral laboratories. The referral laboratories perform the APTIMA and report the results to the submitting laboratory. Subsequently, the specimens are de-identified, assigned a project identifier and tested using an FDA-approved quantitative HIV-1 NAT assay. Data are analyzed to determine the proportion of specimens meeting package insert requirements for APTIMA, and to describe reasons that specimens did not meet such requirements. Data are being recorded to calculate the turn-around-time (TAT) from specimen collection to reporting of APTIMA results and, by submitting laboratory, the specificity of the fourth-generation immunoassay and the proportion of acute infections identified. The project will also assess the concordance of APTIMA and detectable quantitative NAT results.

Results: To date, four specimens have been submitted for testing from two states. Three met APTIMA package insert requirements, but one did not, due to storage temperatures. For the three eligible specimens, median TAT for APTIMA results following submission from the referral laboratory was 3 days (1 day transport, 2 days NAT). The submitting laboratory had acquired these specimens before the beginning of this project, so TAT from when specimens were collected was not assessed. These three specimens were HIV-1 RNA negative using APTIMA. Data collection and analysis are ongoing.

Conclusions: This project will provide a timely source of NAT for US PHLs using the proposed HIV Diagnostic Testing Algorithm. Reference laboratories were selected and have protocols in place and submitting laboratories are being enrolled. Additional specimens must be acquired for study objectives to be evaluated.
Multispot Test Results in Two Different HIV Diagnostic Algorithms


**Objective:** The Massachusetts Department of Public Health [MDPH] Hinton State Laboratory Institute [HSLI] has been proactive in implementing an HIV algorithm that is highly sensitive and specific, cost effective, eliminates indeterminate results, and identifies HIV-2 infections. The HSLI previously followed the CDC/APHL recommended algorithm for HIV antibody detection using EIA and WB. This protocol yielded a limited number of indeterminate or negative WB results requiring further elucidation. The BioRad Multispot HIV-1/HIV-2 Rapid Test was therefore incorporated into our algorithm following indeterminate and negative WB, and since June 21, 2012, as the initial supplemental test in place of the WB following reactive combo Ag/Ab screening. Our objective in choosing the Multispot assay was to improve overall sensitivity and specificity and to reduce the number of indeterminate reports.

**Methods:** The Multispot assay was validated primarily as a test for HIV-2 confirmation and evaluated in two testing strategies: **Algorithm 1** employed the 3rd generation BioRad HIV-1/HIV-2 plus O EIA followed by WB for all specimens testing reactive by EIA. Multispot testing was performed for specimens testing negative or inconclusive by WB. **Algorithm 2** utilized 4th generation BioRad HIV Combo Ag/Ab EIA followed by the Multispot. Specimens testing nonreactive or undifferentiated by Multispot were tested for HIV-1 RNA.

![Algorithm 1 diagram]
**Results:** Since the initiation of Multispot testing in 2006, 204 specimens tested either WB negative (48) or WB indeterminate (156) and were followed by Multispot. Two additional WB+ specimens, suspect for HIV-2 were also tested by Multispot. From these 206 Multispot tests, 179 tested negative, 19 reactive for HIV-1, and 8 reactive for HIV-2. HIV-1 Multispot reactive specimens yielded 10 results with reactivity with both recombinant and peptide spots (R+, P+), 6 with recombinant reactivity only (R+), and 3 with peptide (P+) reactivity only. WB for samples which tested R+P+ and R+ by Multispot exhibited reactivity at several HIV-1 specific locations. WB for samples which tested P+ only were negative (2) or exhibited equivocal reactivity at p24 only (1). No samples tested by Multispot were undifferentiated. Since the start of 4th generation screening, 100/5097 (2.0%) screened reactive and Multispot testing yielded 100 R+P+ reactive, 1 R+ reactive. Four specimen, reactive by the 4th gen EIA, were negative by Multispot. Three of these tested nonreactive by HIV-1RNA, one sample was HIV-1 RNA+.
**Conclusions:** Multispot results obtained with specimens that tested negative or indeterminate by WB underscore the improved performance of Multispot testing when compared to WB. From the 156 WB indeterminate results obtained using Algorithm 1, 142 (91%) were identified as Multispot nonreactive. This significant reduction in possible false reactivity, together with its ability to detect HIV-2, streamlined testing and reporting processes and provided a more sensitive and specific testing algorithm, although limited follow-up information was available. Specimens tested by 4th generation screening to date are few. Multispot results obtained exhibit reactivity with both recombinant and HIV-1 peptide spots. The combination of these assays, including NAAT for undifferentiated or nonreactive Multispot, has eliminated the reporting of indeterminate results.

<table>
<thead>
<tr>
<th></th>
<th>Algorithm 1</th>
<th>Algorithm 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>time period</td>
<td>3/31/06-6/20/12</td>
<td>6/21/12-11/30/12</td>
</tr>
<tr>
<td>total tested</td>
<td>131,009</td>
<td>5,097</td>
</tr>
<tr>
<td>WB total</td>
<td>1,731</td>
<td>N/A</td>
</tr>
<tr>
<td>MS total</td>
<td>206</td>
<td>105</td>
</tr>
<tr>
<td>MS R-,P-</td>
<td>179</td>
<td>4</td>
</tr>
<tr>
<td>MS R+, P+</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>MS R+</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>MS P+</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>MS 2P+</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>HIV-1 RNA-</td>
<td>N/A</td>
<td>3</td>
</tr>
<tr>
<td>HIV-1 RNA+</td>
<td>N/A</td>
<td>1</td>
</tr>
</tbody>
</table>

*MS = Multispot, R = Recombinant HIV-1, P = HIV-1 Peptide, 2P = HIV-2 Peptide*
HIV Testing Practices in Washington, DC and Bronx, New York Hospitals Participating in the HPTN 065 (TLC-Plus) Study

Steven Ethridge1, T. Gamble2, E. Greene2, L. Wesolowski1, P. Chavez1, E. DiNenno1, W. El-Sadr3 and B. Branson1

1. Centers for Disease Control and Prevention
2. FHI360
3. Columbia University

Background: The HPTN 065 Test, Link to Care, Plus Treat (TLC-Plus) Study in Washington, D.C. and the Bronx, NY is designed to determine the feasibility of a combination HIV prevention strategies, including expanded HIV testing with universal screening for emergency department and inpatient admissions at 14 participating hospitals. These hospitals serve populations with HIV prevalence above the 0.1% threshold for HIV screening according to the 2006 CDC guidelines. HPTN 065 encourages laboratory-based HIV testing to increase testing capacity and decrease costs. An analysis was conducted to evaluate (1) baseline practices, (2) ability to implement screening on-site with a third- or fourth-generation immunoassay and (3) ability to conduct supplemental testing using the CLSI testing algorithm (HIV-1/HIV-2 antibody differentiation test and a nucleic acid test (NAT).

Methods: In January 2012, all 14 participating hospital laboratories completed a survey about their HIV testing practices and impediments to expanding HIV testing within the laboratory. Descriptive analyses were performed.

Results: Most hospitals screened patients with CLIA-waived point-of-care rapid tests (n=5) or 3rd generation HIV assays (n=7) (Table 1). Of those hospitals that conduct in-house laboratory testing, five use fully automated 3rd generation random access HIV assays, and two use the 3rd generation Bio-Rad HIV-1/2 Plus O assay. None use a fourth-generation assay. One laboratory currently performs the Multispot HIV 1/HIV-2 differentiation test, but uses it only for screening persons who are not tested with a rapid test at point of care. All hospitals rely on the HIV-1 Western blot (WB) for supplemental testing, but only one conducts WB in-house. Four hospitals wait for completion of the WB before reporting any laboratory results. Half of the institutions (n=7) conduct in-house NAT with a quantitative viral load test (Table 1). The seven hospitals that had not yet adopted third- or fourth-generation laboratory-based testing were in the process of doing so, and five hospitals are planning to implement the Multispot differentiation test as a supplemental test. In terms of barriers to implementing on-site automated testing or supplemental testing using the CLSI algorithm, three hospitals indicated that fully automated HIV testing is more expensive than rapid testing, two did not see the need for on-site automated HIV screening, and three did not see a need for HIV differentiation testing (Table 2).

Conclusion: Many hospitals continue to rely on labor-intensive point-of-care rapid tests, even for laboratory-based testing. Fully automated third- or fourth-generation HIV testing could help screen larger numbers of patients, improve turn-around-times, reduce overall HIV testing costs and improve sensitivity for early HIV infection. All hospitals still rely on the HIV-1 WB for confirmation. The lack of perceived need and misconceptions about the overall cost of automated HIV screening tests are barriers to adoption of third- or fourth-generation testing and alternatives to the WB.
### Table 1

**Current HIV testing practices in Bronx and Washington D.C. hospitals during HPTN 065**

<table>
<thead>
<tr>
<th>Screening tests</th>
<th>Total (N = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laboratory testing</strong></td>
<td></td>
</tr>
<tr>
<td>ADVIA Centaur HIV 1/O/2</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Ortho VITROS Anti-HIV-1/2</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Bio-Rad HIV-1/2 Plus O</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>CLIA-waived rapid test only</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Multispot HIV-1/HIV-2</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Sent out</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Oversees CLIA-waived rapid tests outside laboratory</td>
<td>10 (71%)</td>
</tr>
</tbody>
</table>

#### Supplemental Testing and Viral Load Assays

<table>
<thead>
<tr>
<th>HIV-1 Western Blot</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Sent out</td>
<td>13 (93%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HIV-1 NAT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime HIV-1</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>COBAS Ampli-prep/Taqman HIV-1</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Roche Amplicor HIV-1</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Versant HIV-1 RNA 3.0 (bDNA)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Sent out</td>
<td>7 (50%)</td>
</tr>
</tbody>
</table>

### Table 2

**Impediments to implementing NAT for hospitals that currently send out for NAT**

<table>
<thead>
<tr>
<th>Impediments</th>
<th>Total (N = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of physical space</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Workforce/staffing</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Low volume</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>No perceived need</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>None</td>
<td>2 (14%)</td>
</tr>
</tbody>
</table>

John Kim, L. Malloch, B. Calder-Kent, Y. Adonsu-Hoyi, National Laboratory for HIV Reference Services, Public Health Agency of Canada, Ottawa, Canada

Objective: Algorithm I of the recently published CLSI M53 guidelines recommends the implementation of an HIV-1/HIV-2 discriminatory assay. This study evaluated the performance of the FDA-approved Bio-Rad Multispot HIV-1/HIV-2 rapid HIV test and the recently CE-marked Bio-Rad Geenius HIV 1/2 confirmatory assay.

Methods: One hundred sixty-one (43 HIV-1, 52 HIV-2, 66 negative) samples from the NLHRS repository were chosen. The source of these samples included (mainly) reference samples from Canadian stakeholder labs previously confirmed by the NLHRS, commercial panels and negative samples obtained from low-risk settings. The Bio-Rad HIV-1/2 Multispot is a rapid HIV test incorporating separate HIV-1 and HIV-2 antigens that is read visually. The Bio-Rad Geenius HIV-1/2 Geenius assay also incorporates separate HIV-1 and HIV-2 antigens applied as distinct lines similar in fashion to a line immunoassay. Reading and interpretation is performed using an automated reader.

Results: To facilitate 2 x 2 table analysis, the indeterminate samples were classified according to their true infection status. Using this approach, sensitivity and specificity were very high and identical between the two assays (sens/spec, 98%/97%). However, further analysis of the 3 categories of samples (HIV-1, HIV-2 and negative) revealed differences between the two assays. Of 43 HIV-1 samples, each assay had three false negatives. Two were common to both assays; a clinical sample from the NLHRS and a member of a combo panel which was HIV positive but untypeable by both assays. The Geenius had a third false-negative, a member of an HIV antigen/Ab panel and the Multispot also had a third which was from an HIV-1 combo panel. Of 52 HIV-2 samples, the Geenius assigned an ‘indeterminate HIV-2’ status to 2 samples. The Multispot correctly identified all 52 HIV-2 samples. Of 66 HIV Ab negative samples, the Geenius assigned an ‘indeterminate-HIV-1’ status to one sample. The Multispot completely identified all negative samples.

Conclusion: Both assays had very high sensitivity and specificity. Based on testing of the HIV-2 and negative samples, it could be argued that the Multispot performed slightly better than the Geenius assay; however, continued use and developing experience in performing both assays may impact on this. The Bio-Rad Geenius HIV-1/2 assay has operational advantages including standardization through the use of an automated reader and traceability features required of quality management systems. This assay was recently CE-approved and Bio-Rad has indicated they will file for licensure of this kit in Canada in the very near future.
HIV/Hepatitis Co-infection Rate in an Anonymous Unlinked Seroprevalence Survey in an Urban Hospital

Debbie Mohammed\textsuperscript{1}, E. Martin\textsuperscript{2}, S. Paul\textsuperscript{3}, C. Sadashigie\textsuperscript{3} and S. Scott\textsuperscript{1}

1. University of Medicine and Dentistry of New Jersey (UMDNJ)
2. University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School
3. New Jersey Department of Health

**Objective:** Newark, New Jersey had an HIV seroprevalence rate of 2.1\% in 2011 with 29\% of living cases attributed to injection drug use. In the United States, the reported HIV/HEP C co-infection rate is about 25\% among HIV–infected individuals. An Anonymous Unlinked HIV Survey (AUS) which reduces participation bias was conducted in 2008 to determine the rate of HIV and HEP C co-infection in an Urban Emergency Department (ED).

**Methods:** Discarded blood specimens from the ED were collected from patients >18 years of age. Data were abstracted from the medical record and a study number was generated for each specimen. Patient identifiers were removed from the database and specimens. De-identified blood specimens were tested for HIV by ELISA and confirmed by Western Blot. Confirmed HIV positive specimens were tested for Hepatitis C. This study was approved by the Institutional Review Board.

**Results:** A total of 3,488 specimens were obtained; 6.5\% of patients (226/3,488) tested positive for HIV. Thirty-nine percent (89/226) of HIV seropositive patients were co-infected with Hepatitis C. Independent predictors for HIV/HEP C co-infection included persons >45 years compared to persons <34 years (OR 8.3, 95\% CI 2.8-24.5) and persons who tested positive for either cocaine (OR 2.2, 95\% CI (1.2-3.9) or heroin (OR 2.9, 95\% CI 1.6-5.3).

**Conclusion:** A high rate of HIV/HEPC co-infection was noted in this sample of patients. ED patients tested for HIV are candidates for hepatitis C testing as these patients may engage in behaviors such as illicit drug use that would put them at high risk for contracting HIV and HEP C. Additionally, CDC's 2011 recommendation for routine Hepatitis C testing among persons born between 1945 and 1965 is supported as higher proportions of persons > 45 years tested Hepatitis C positive.
Performance Evaluation of the DPP® HIV-SYPHILIS Assay: A Novel, Point-of-care Rapid HIV 1/2 and Syphilis Treponema pallidum Antibody Combination Test


Objective: Syphilis commonly co-exists in patients with HIV. The patented DPP® HIV-SYPHILIS Assay, under development for the simultaneous detection of HIV 1/2 and Syphilis Treponema pallidum antibodies in blood, serum, or plasma samples, uses Chembio’s new, innovative technology. These studies examined the sensitivity and specificity of the Assay.

Methods: A total of 398 retrospectively collected, pre-screened, banked clinical trial serum and plasma samples belonging to a known-HIV positive population at high risk for syphilis infection from a US population; serum obtained by blood collection at two sites in Brazil (Site 1 n= 33; Site 2 n= 108); 330 blood, 202 sera and 407 plasma samples found to be HIV 1/2 EIA and Syphilis RPR Negative; and numerous well characterized qualified-vendor purchased performance panels were all tested on the Chembio DPP HIV-Syphilis Assay. Results were compared to an FDA approved HIV EIA, a Western blot for HIV-1 and HIV-2 antibodies, a Treponemal specific EIA and/or TP-PA. In some cases, as part of resolution testing, a different-branded, Trep EIA was performed followed by RPR.

Results: 398 samples positive for HIV via EIA and WB caused a reactive HIV band to develop on the DPP Assay making the sensitivity of the HIV band 398/398 = 100% (95% CI= 99.1 to 100%). Of these 398 specimens, after discordant testing (i.e. comparison to EIA followed by RPR confirmation), the sensitivity of the Trep band is 83/83= 100% (95% CI= 95.6 – 100%). On the Brazilian sera samples, the results of the DPP Assay HIV test line agreed with the results of the HIV EIA 100% of the time (161/161 = 100% with 95% CI = 97.7 to 100%). There was 100% agreement in the reactivity of the DPP Assay HIV test line when tested on performance panels in comparison to reference assays. On the Brazilian sera samples, from site one, the DPP Assay Trep line agreed with TP-PA and Trep-EIA in all cases (33/33 = 100% with 95% CI = 89.4 – 100%). At Brazil site 2, after discordant resolution, there were 3 samples found to be false negative on the DPP Assay Trep line (105/108 =97.2% with 95% CI = 92.1 to 99.4). All 939 HIV 1/2 EIA and Syphilis RPR Negative samples were nonreactive for HIV on the DPP Assay, making the specificity of the DPP HIV test line 939/939 = 100% with the 95% CI = 99.6 – 100%. In comparison to Trep EIA, the overall specificity of Trep line on the DPP Assay with these samples was 911/939 = 97.0% with the 95% CI = 95.7 – 98.0%.

Conclusion: The Chembio DPP® HIV-Syphilis Test is unique as an aid in the simultaneous diagnosis of infection with HIV and/or Syphilis. The DPP rapid test provides a superior advantage to both patient and physician over other tests in that it saves time and money without a sacrifice in performance.
HIV-1 and HBV Co-infections in the State of Tennessee

Michelle Landes¹, R. Neil² and D. Smalley²

¹. APHL/CDC and Tennessee Department of Health, Laboratory Services
². Tennessee Department of Health, Laboratory Services

Objective: The leading cause of hospital admission and death in the HIV-infected population are conditions associated with Hepatitis B (HBV) and Hepatitis C. Approximately 10% of the HIV-infected population worldwide is infected with HBV. The co-infection rate is 5% in North America and Western Europe. Our objective is to determine the incidence of HIV and HBV co-infection in Tennessee (TN).

Methods: Chronic HBV positive serum specimens and HIV-1 positive specimens were acquired from residual diagnostic specimens across the state of TN. Specimens were de-identified and banked. For the study, specimens were chosen from each geographic region or metropolitan county in proportion to 2010 census data. 196 HBV positive specimens were tested for HIV-1 using the Bio-Rad EIA HIV-1/2 test system and confirmed using the Multispot Rapid Test by Bio-Rad. 118 HIV-1 positive specimens were tested for HBV core total antibody and HBV surface antigen. 5 of these specimens were tested for HBV IgM antibody.

Results: When testing chronic HBV specimens for HIV-1/2; 13 out of 196 patients (6.6%) were preliminarily positive for HIV-1/2 by EIA. Our HIV-1/2 confirmatory testing by Multispot confirmed that 9 of the 13 patients (4.6% of total) were true HIV-1 positive specimens. Next we tested previously positive HIV-1 specimens for HBV. We found that 29 out of 118 specimens (24.6%) were positive for HBV core total antibody and 6 out of 118 (5.0%) specimens were positive for HBV surface antigen. 5 out of the 6 surface antigen positive specimens were also positive for HepB core total, indicating one was likely a false positive. This indicates that 5 out of 118 (4.2%) specimens are current active acute/chronic infections and 24 out of 118 (20.3%) were resolved acute infections. To determine if the active infections were acute or chronic, we tested the specimens for HBV IgM antibody. We found 1 out of 5 specimens (20%) to be positive for IgM antibody; indicating an acute infection. This means 4 out of 118 specimens (3.4%) are chronic infections.

Conclusions: The HIV-1 and chronic HBV co-infection rate of 4.6% in the known HBV positive population and 3.4% in the known HIV-1 population in TN is below the average in North America, 5%. In the United States, approximately 5% of the adult population has been infected with HBV and resolved the infection leaving them with antibodies to HBV core antigen. However, the HIV-1 positive sample population in TN has 24.6% exposure to HBV according to HBV Core total antibody assay. 3.4% of these are active chronic infections, which may lead to complications. The finding that 20% of the active infections in our study are newly acquired reinforces that HIV-1 patients in TN be vaccinated against HBV due to their exposure rate, in order to prevent infection and the complications that come with co-infection. These results also support the need to offer HBV vaccines to high-risk populations (injection drug users, sexually active heterosexuals, men having sex with men) in the state of Tennessee.
Accuracy of Anti-HIV 1+2 Signal-to-Cutoff Ratio in Predicting HIV-1 Confirmatory Test Results

Lixia Liu, J. Gentry and J. Lovchik, Indiana State Department of Health Laboratories

Objective: To evaluate the correlation between the signal to cutoff ratio (S/CO) of the VITROS anti-HIV 1+2 chemiluminescent immunoassay and the result of confirmatory testing.

Methods: In the Indiana State Public Health Serology Laboratory, the VITROS anti-HIV 1+2 chemiluminescent immunoassay (CIA) is used as the screening test, and all of the CIA reactive serum samples are reflexed to a confirmatory test. Prior to mid April 2011, the HIV-1 Western Blot (WB) was used as the confirmatory test, and since late April 2011, the APTIMA HIV-1 RNA Qualitative Assay (NAAT) has been used as the confirmatory test. In this data mining study, the data collected between January 2010 and August 2012 was analyzed. The HIV-1 confirmatory test results were categorized as confirmed or non-confirmed based on the WB or NAAT result interpretations. Receiver-operating characteristic (ROC) curve analysis was performed to determine the anti-HIV 1+2 S/CO that can accurately predict the positive/reactive results of the HIV-1 confirmatory tests.

Results: A total of 733 serum specimens (representing individual patients) out of 73,000 specimens were reactive by CIA; they were subjected to HIV-1 WB or HIV-1 NAAT confirmatory testing. 526 (71.8%) samples and 207 (21.8%) samples were in the confirmed and non-confirmed categories, respectively. The mean anti-HIV 1+2 S/CO ratio was significantly different (p<0.0001) between these two categories. By ROC analysis, a S/CO value of 23.9, which gives both sensitivity and specificity of 97.7%, provided high accuracy, with the area under the curve of 0.995 (area under curve =1.0 as perfect prediction) for predicting positive/reactive confirmatory test results.

Conclusion: The anti HIV1+2 CIA S/CO ratio was found to be highly predictive of a positive HIV-1 confirmatory result in a population with rare HIV-2 infections. The anti-HIV 1+2 S/COS/CO ratio (23.9) can be used to determine whether a confirmatory test should be performed to confirm the initial reactive screening results. That is: when the VITROS anti HIV 1+2 CIA S/CO ratio is <23.9, a HIV-1 confirmatory testing should be performed; and when the anti HIV 1+2 S/CO ratio is =23.9, a HIV-1 confirmatory testing does not add value.
Utility of Multispot HIV-1 Spot Reaction Intensity and Immunoassay S/CO Ratio for Detecting HIV Infection in Clinical Samples

Linda Styer, T. Sullivan and M. Parker, Wadsworth Center, New York State Department of Health

Background: The CDC HIV testing algorithm includes a 3rd/4th generation enzyme immunoassay (EIA) followed by a HIV-1/HIV-2 differentiation immunoassay. Although final results of these immunoassays are qualitative (reactive/non-reactive), additional quantitative or semi-quantitative information can be obtained from both assays. For example, an EIA produces a quantitative signal-to-cutoff ratio (S/CO) and the reaction intensity of the differentiation immunoassay spots can be visually scored to indicate the relative amount of reactivity.

Objective: The objective of this analysis was to determine if EIA S/CO and the reaction intensity of the HIV-1 spots on the differentiation immunoassay can provide useful diagnostic information.

Methods: Diagnostic test results were retrospectively analyzed from specimens received between January 2007-June 2010. Specimens were tested using our standard clinical testing algorithm, which includes the GS HIV-1/2 plus O EIA, Multispot HIV-1/HIV-2 Rapid Immunoassay (MS), GS HIV-1 Western Blot (WB) and Aptima HIV-1 RNA Qualitative Assay (RNA). The MS contains 4 spots: an IgG control, two HIV-1 (gp41 recombinant protein, gp41 peptide antigen), and HIV-2 (gp36 antigen). In addition to recording each MS spot as reactive/non-reactive, color intensity was recorded based on a subjective 4-point scale, ranging from weak (intensity=1) to strong (intensity=4). EIA S/CO results were classified as high (>8.0) or low (<8.0). Our dataset included 1,646 EIA-reactive specimens that were MS tested and had complete results that enabled classification as HIV-1 positive (n=1,578) or HIV-negative (n=68).

Results: Data were split into four groups. Group 1 included 1,575 specimens with high EIA S/CO and a HIV-1 positive MS; all (100%) were HIV-1 positive. MS intensity scores in this group correlate with WB result. Two WB negative specimens had the lowest scores (peptide = 1, recombinant=1). Twenty-eight WB indeterminate specimens had higher scores (mean peptide = 2.3, mean recombinant = 3.4). The highest scores (mean peptide = 3.9, mean recombinant = 4.0) occurred in WB positive specimens (n=1,545). Group 2 included 3 specimens with low EIA S/CO and a HIV-1 positive MS; 1 (33%) was HIV-1 positive (WB+). The true positive specimen in this group had higher MS intensity scores (peptide=1, recombinant=4) than did the negative specimens (peptide=0, recombinant=1). Group 3 included 8 specimens with high EIA S/CO and a negative MS; 1 (13%) was HIV-1 positive (WB-, RNA+). Group 4 included 60 specimens with low EIA S/CO and a negative MS; 1 (2%) was HIV-1 positive (WB- , RNA+).

Conclusions: The CDC HIV testing algorithm uses the MS as the supplemental test instead of the WB. Previous studies showed that the MS can produce false positive results, particularly when only one HIV-1 spot is reactive. Here we show that EIA S/CO and MS intensity scores can also help to identify false positive results. The WB status of an individual (negative, indeterminate, positive) has long been clinically useful to determine recency of infection. Our data show that MS intensity scores are correlated with WB status. Additional analysis will be performed using specimens submitted after June 2010 and to determine if MS intensity scores are correlated with reactivity of specific WB bands.
Cost and Effects of the APHL/CDC Proposed Laboratory-based Algorithm for the Detection of HIV

Angela Hutchinson, S. Ethridge, L. Wesolowski, P. Farnham, R. Shrestha, P. Patel and B. Branson, Centers for Disease Control and Prevention

Objective: We conducted a decision analysis to compare the cost-effectiveness of the proposed HIV testing algorithm (3rd generation [3G] or 4th generation [4G] immunoassay [IA], HIV-1/HIV-2 differentiation assay and nucleic acid test [NAT]) with the current testing algorithm (3G IA and Western blot [WB]).

Methods: We constructed a decision-analytic model (Treeage Pro, 2009) to compare the cost and outcomes of the proposed and current laboratory HIV testing algorithms. Because costs are volume dependent, we modeled testing a cohort of 30,000 specimens using two scenarios, a medium volume laboratory (<50,000 IA specimens annually) and a high volume laboratory (≥50,000 IA specimens annually). For each scenario, we estimated the cost per HIV-infected specimen correctly identified by the proposed algorithm with a 3G or 4G IA and by the current algorithm with a 3G IA. The model included test sensitivity and specificity, HIV prevalence (WB-positive), and HIV incidence (acute infections, defined as repeatedly reactive IA, reactive NAT, non-positive Western blot). Cost data included test kit costs, number of tests per kit, frequency of running controls, batch size, and labor. The base case assumed a 1% HIV prevalence and 0.1% annual incidence. We conducted sensitivity analyses on incidence, prevalence and HIV test costs.

Results: For the base case in both medium and high volume laboratories, the current algorithm more costly and less effective (is dominated) by the proposed algorithm. In medium volume laboratories, the proposed algorithm using a 4G IA costs $93,900 more than a 3G IA, but identifies 10 additional HIV infections, for an incremental cost per HIV infection identified of $9,485. For high volume laboratories, the proposed algorithm with 4G IA dominates 3G IA (costs $3600 less, identifies 10 more infections). In sensitivity analysis of medium volume laboratories, 4G IA becomes the most cost-effective strategy if 4G IA costs drop 30%; there was no HIV incidence, prevalence threshold at which the current algorithm becomes favored. In high volume laboratories, the incidence threshold at which the current algorithm is most cost-effective is 0.001% with HIV prevalence at 0.1%.

Conclusion: For both medium and high volume laboratories, the current algorithm is more costly and less effective than the proposed algorithm with either a 3G or 4G IA. The additional cost of 4G over 3G IA in medium volume laboratories might be justified by the additional cases of HIV detected and transmissions averted due to earlier detection.
Table. Cost and Effectiveness of the Proposed versus Current Laboratory HIV Testing Algorithm for a Cohort of 30,000 Specimens with 1% HIV Prevalence, 0.1% Incidence

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>HIV Test Costs, $</th>
<th>HIV Infections Detected</th>
<th>Cost/Effectiveness, $</th>
<th>Incremental Cost-effectiveness Ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium Volume Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current: 3G IA</td>
<td>273,000</td>
<td>299.4</td>
<td>912</td>
<td>Dominated</td>
</tr>
<tr>
<td>Proposed: 3G IA</td>
<td>232,200</td>
<td>314.4</td>
<td>739</td>
<td>---</td>
</tr>
<tr>
<td>Proposed: 4G IA</td>
<td>326,100</td>
<td>324.3</td>
<td>1006</td>
<td>9,485‡</td>
</tr>
<tr>
<td>High Volume Laboratory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current: 3G IA</td>
<td>165,570</td>
<td>299.4</td>
<td>553</td>
<td>Dominated</td>
</tr>
<tr>
<td>Proposed: 3G IA</td>
<td>167,130</td>
<td>314.4</td>
<td>532</td>
<td>Dominated</td>
</tr>
<tr>
<td>Proposed: 4G IA</td>
<td>163,530</td>
<td>324.3</td>
<td>504</td>
<td>---</td>
</tr>
</tbody>
</table>

† Dominated options are excluded from calculation of the incremental cost-effectiveness ratio.
‡ Represents cost per additional HIV infection identified

Word count 499/500
Laboratory Costs of the APHL/CDC Proposed Laboratory Algorithm for the Diagnosis of HIV

Angela Hutchinson\textsuperscript{1}, S. Ethridge\textsuperscript{1}, L. Wesolowski\textsuperscript{1}, B. Bennett\textsuperscript{2}, T. Sullivan\textsuperscript{3}, M. Pentella\textsuperscript{4}, P. Farnham\textsuperscript{1}, R. Shrestha\textsuperscript{1}, P. Patel\textsuperscript{1} and B. Branson\textsuperscript{1}

1. Centers for Disease Control and Prevention
2. Florida Bureau of Laboratories
3. Wadsworth Center, New York State Department of Health
4. State Hygienic Laboratory at the University of Iowa

**Objective:** The proposed new laboratory HIV testing algorithm is designed to improve the detection of acute infections and to differentiate between HIV-1 and HIV-2 antibodies. The proposed algorithm promises greater sensitivity, but concerns about increased cost may hinder its adoption. We conducted a cost analysis to compare the laboratory costs of the proposed (3rd generation [3G] or 4th generation [4G] immunoassay [IA], HIV-1/HIV-2 differentiation assay and nucleic acid test [NAT]) with the current testing algorithm (3G IA and Western blot [WB]).

**Methods:** We elicited HIV test cost information (including test kit costs, number of tests per kit, the frequency of running controls, batch size, labor costs, supplies and HIV testing volume) for the FDA-approved tests necessary to conduct each algorithm from 17 laboratories, including regional medical centers, hospital laboratories, and state and local public health laboratories. Cost data were collected May 2011-March, 2012. We calculated median test costs adjusted for controls, in 2011 US dollars, for 3G (Advia, Bio Rad, Vitros) and 4G (Architect, Bio-Rad), Multispot HIV-1/HIV-2 differentiation assay (MS), Western blot, and NAT (APTIMA). Because reagent costs varied widely depending on the laboratory’s volume, we summarized cost estimates by laboratory IA testing volume (high: ≥50,000/year and medium: <50,000/year). We then calculated costs by test result for each step of the testing algorithms.

**Results:** For specimens that are confirmed positive for HIV antibody, the proposed algorithm (IA, MS) is less costly than the current algorithm (IA, WB) (Table). Costs are lower in high volume laboratories regardless of testing algorithm; the difference is most evident for WB, for which the cost is over 3 times as high in medium ($154.10) than high ($41.39) volume laboratories (Table). On the other hand, in both medium and high volume laboratories, for specimens with a positive IA but a negative MS, the cost of the proposed algorithm with NAT (to identify acute infection or to resolve false-positive IAs) is greater than the cost of the current algorithm with only WB, but the current algorithm would not identify acute infections. Ultimately, costs will depend on the need to resolve IA discrepancies and the presence of AHI in the population.

**Conclusion:** HIV testing costs varied with IA testing volumes. For confirmed antibody-positive specimens, the proposed HIV testing algorithm costs less than the current algorithm. Costs for the proposed algorithm are higher than those for the current algorithm when NAT is necessary to resolve false-positive IAs or identify acute infections.
Table. Median Per Specimen Laboratory Costs for the Proposed and Current HIV Testing Algorithms by Lab Volume, $

<table>
<thead>
<tr>
<th>Proposed Algorithm</th>
<th>Medium Volume 3rd Gen IA, $</th>
<th>Medium Volume 4th Gen IA, $</th>
<th>High Volume 3rd Gen IA, $</th>
<th>High Volume 4th Gen IA, $</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA-</td>
<td>7.04</td>
<td>9.08</td>
<td>4.91</td>
<td>3.84</td>
</tr>
<tr>
<td>IA+ MS+</td>
<td>36.20</td>
<td>44.77</td>
<td>30.73</td>
<td>29.14</td>
</tr>
<tr>
<td>IA+, MS-, NAT$</td>
<td>194.04</td>
<td>202.61</td>
<td>188.57</td>
<td>186.98</td>
</tr>
</tbody>
</table>

| Current Algorithm  |                                |                               |                               |                               |
|--------------------|                                |                               |                               |                               |
| IA -               | 7.04                           | 9.08                          | 4.91                          | 3.84                          |
| IA+, WB            | 171.97                         | 180.54                        | 53.79                         | 52.21                         |

Cost represents a single NAT, not run in duplicate
Serial Plasma/PBMC Collections from Recently HIV Infected Individuals

Patricia Garrett¹, M. Dowell¹, C. Barrett¹, H. Hjord², C. Shayevich² and S. Philip²

1. SeraCare Life Sciences
2. San Francisco City Clinic, San Francisco Department of Public Health

Objective: SeraCare partnered with a clinical site to recruit three recently HIV-infected individuals for a one year collection program. Serial plasma and cell samples from recently-infected individuals, collected early in infection and over at least a year, are an unmet need for HIV early infection research, for HIV incidence/prevalence studies, and for Precision Medicine targeted to the appropriate stage of infection. HIV seroconversion panels and the Fiebig staging algorithm provide understanding of the profile of recent infection, and tests for HIV RNA among high risk, HIV antibody negative individuals provide a means to detect very recent infections.

Methods: A protocol was submitted, and approval was granted by SeraCare and clinical site IRBs. As standard care, the clinical site pool-tests samples from HIV antibody negative STD clinic patients. The clinical site identified, recruited, and obtained consent from three RNA Pos/Ab Neg patients. Each patient will provide 14 whole blood collections over 11 months; four in the first two weeks with monthly collections for ten months thereafter. The collected whole blood is shipped to SeraCare for processing into plasma and PBMCs. Aliquots (0.5 mL plasma, 10M cells/mL PBMCs) with clinical and demographic data, including estimated infection date, will be commercially available as individual samples or series.

Results: Three recently HIV-infected individuals have been recruited: to date, two have had nine collections and the third has had 10. Samples from the first collections are being prepared for offer to researchers studying early HIV infection and to incidence/prevalence test developers. DATA WILL BE PRESENTED IN POSTER.

Conclusions: HIV RNA pool testing of high-risk anti-HIV negative individuals can detect very early infection. Recruitment of these patients can yield the long seroconversion series required for early infection research, evaluation of HIV incidence/prevalence tests, and potentially, targeted Precision Medicine treatments.
A Tool for Study of Early HIV Infection

*Patricia Garrett, B. Weiblen, B. deFreitas and J. Marchand, SeraCare Life Sciences*

**Objective:** The Fiebig staging algorithm provides a mechanism for classifying very early HIV infection (within the first three months) and for closely estimating HIV infection dates based on a single sample. Using this algorithm, we assembled 24 retrospective samples from 13 individuals recently infected according to the Fiebig algorithm into an Early HIV Infection Panel.

**Methods:** The Fiebig algorithm classifies early HIV infection according to HIV RNA, HIV antigen, anti-HIV ELISA and anti-HIV Western blot results. The chart below describes the Fiebig stages, the corresponding test results, the average days (with confidence interval [CI]) for each stage, and the cumulative days since infection (with CI). DATA WILL BE PRESENTED IN POSTER.

**Results:** Retrospectively found serial bleeds and single units from deferred plasma donors were characterized and found to correspond to Fiebig stages Eclipse through V, which describe the course of HIV infection through the first three to five months. Four units representing each of these Fiebig stages were chosen and further characterized with multiple test methods for each analyte. Units chosen to represent the eclipse stage, when no HIV markers are positive, were paired with at least one other unit from the same donor that was at Fiebig stage 1. HIV infection dates were estimated based on the cumulative duration of the highest Fiebig stage from a given donor.

**Conclusion:** The Early HIV Infection panel provides 24 samples collected from 13 different individuals when each was in the first few months of HIV infection, and their estimated dates of infection. These samples should be useful for those studying early HIV infection, and for those evaluating the performance of test methods for HIV, especially with respect to sensitivity.
HIV Single Stage Testing: Integration and Maximization of Resources by Reducing Time Between HIV Diagnosis and Treatment

Berry Bennett and S. Fordan, Florida Bureau of Public Health Laboratories

Objective: Early HIV diagnosis is essential for timely linkage to care and treatment to improve personal health outcomes. Early diagnosis presents an opportunity to take advantage of treatment as prevention, an essential public health strategy to protect individual partners. However, in the U.S. only 37% of those HIV-1 infected individuals are retained in care (Hall 2012). Newer laboratory and point of contact rapid tests provide the ability to detect early as well as chronic HIV infections and may expedite the “test-to-treat” process. The timeliness and benefit of new diagnostic algorithms can be further maximized by including baseline HIV-1 clinical management tests on newly diagnosed individuals. The objective of this study was to evaluate the feasibility of conducting diagnostic and baseline clinical management tests (Single Staging) on newly diagnosed individuals within the traditional pre-posttest counseling period (14 days).

Methods: Baseline CD4 and HIV-1 viral load (VL) tests were performed concurrently with an HIV-1/2 immunoassay (IA) and HIV-1 Western blot (WB) supplemental test on 105 rapid test preliminary positive individuals, provided an adequate sample volume was received. Participating study sites (3) were located in high-risk, high-morbidity areas. The data analysis assessed the median time between preliminary HIV diagnosis by a rapid test with subsequent reporting of the IA, WB, CD4/VL and entry into HIV medical care (per medical record review). The laboratory objective was to complete testing and ensure that all results were received by the participating provider prior to posttest counseling.

Results: Of the 105 individuals with HIV-1 rapid preliminary positive results, 102 were confirmed HIV-1 positive by WB and one was confirmed by an HIV-1 nucleic acid amplification test (an algorithm-defined early infection). Ninety-two baseline viral loads and 78 baseline CD4 absolute counts were performed and made available to the provider prior to posttest counseling. The mean return rate for the posttest counseling at 14 days was 44.7% (range 37.9%-56.5%) with an additional 31.1% (range 22.7%-37.9%) returning within a total of 30 days. Overall 13.6% (range 9.1%-17.2%) did not return for the confirmatory and clinical management test results. A subsequent appointment provided the client access to a medical provider. The mean non-return rate for the medical provider appointment was 44.6% (range 34.5%-50%).

Conclusions: HIV Single Stage testing and reporting within the traditional pre-posttest counseling appointments is feasible and supported by data. A mean of 75.8% of the participating clients returned to a post-test appointment within 30 days of the pre-test date whereas the non-return rate for a subsequent appointment was a mean of 44.6%. The decrease in client return rates supports a process to ensure that the newly-diagnosed HIV client has sufficient laboratory findings to access clinical services and commence antiretroviral therapy, if indicated by the physician. The integration of laboratory and clinical care services is the first phase in the continuum of care that ultimately leads to retention and successful medical oversight.
Unique Testing Experience for Acute HIV Infection: The Dallas County NAAT Program

Brian Emerson, Dallas County Health and Human Services Laboratory

**Background:** Routine antibody assays for HIV-1 (Enzyme Immunoassays) are usually nonreactive during the first four to five weeks after infection. Dallas County Health and Human Services Laboratory (DCHHS) integrated HIV-1 RNA Qualitative Assay (Aptima Gen-Probe Inc.), an HIV-1 Nucleic Acid Amplification Test (NAAT), in July 2009 making possible detection of HIV-1 within two weeks of infection. Dallas County Laboratory defines acute HIV-1 infection (AHI) as an antibody negative and RNA positive specimen. This testing methodology is critical in HIV prevention as individuals are the most infectious during the acute stage.

**Objectives:** To describe how DCHHS implemented the NAAT assay alongside the standard HIV antibody assays for detecting an Acute HIV infection (AHI).

**Methods:** DCHHS laboratory tested patients for AHI using the NAAT assay. The specimens are pooled together in pools of 10. A reactive pool is broken down and each specimen is tested individually. A reactive result is available within 3 days of collection.

**Results:** A total of 119 HIV-1 RNA reactive specimens were detected out of 113,843 specimens analyzed. In 2009 18,957 specimens were tested, with 12 HIV-1 RNA reactive specimens detected. In 2010, 36,760 specimens were tested, with 44 HIV-1 RNA reactive specimens detected. In 2011 58,126 specimens were tested, with 63 HIV-1 RNA reactive specimens detected. Additional STDs occurring with RNA reactive: 29% no other STDs; 22% Syphilis; ~12% other STDs excluding Syphilis (example: Neisseria gonorrhoeae). The antibody assay (EIA) did not detect any antibodies for HIV on 51% (61/119) of the HIV-1 RNA reactive specimens. The remaining 49% (58/119) HIV-1 RNA reactive specimens were reactive on the antibody assay with a nonreactive or indeterminate on the Western Blot.

**Conclusions:** Improving the detection of an AHI is crucial for HIV prevention, because without the advancement in technology 119 patients could have received a negative or indeterminate test result prior to the HIV-1 RNA qualitative assay. We feel the increase in detection of an AHI warrants implementing the new technology for HIV-1 detection.
Validation and Clinical Use of a HIV-2 Viral Load Assay

Linda Styer, T. Miller and M. Parker, Wadsworth Center, New York State Department of Health

Background: HIV-2 was discovered in 1987 as a second virus that causes AIDS. Although HIV-2 infection is rare in the United States (166 cases reported from 1987-2009), cases are concentrated in the Northeast with a majority residing in New York State (NYS). No FDA-approved or research-use HIV-2 viral load test kits are available in the U.S. Consequently, physicians cannot monitor a patient’s HIV-2 viral load during treatment.

Objective: Our objective was to develop a viral load assay to quantify HIV-2 RNA in plasma and to validate the test for clinical use. Because HIV-2 RNA levels are typically low, we aimed to develop a highly sensitive assay that includes a whole virus internal control to monitor assay performance and detect PCR inhibition in patient samples.

Methods: The HIV-2 viral load assay is based on a real-time RT-PCR assay that simultaneously amplifies a conserved region of the HIV-2 5’ LTR and a portion of mouse hepatitis virus (MHV), an enveloped RNA virus. MHV is added during the lysis step and serves as an internal control throughout the assay. Detection of MHV within specified parameters signifies proper assay function. Two extraction volumes (200ul or 900ul) can be used in the assay. Each run includes calibrators which were assigned International Unit (IU) values by comparison to the HIV-2 RNA International Standard. The run is valid if results of controls and calibrators are within specified limits. We validated the assay according to clinical validation guidelines to establish its technical limitations and to ensure that the assay was sufficiently sensitive, specific and accurate for clinical use.

Results: Validation studies demonstrated that the assay is sensitive, with a limit of detection of 7 IU/ml and a lower limit of quantification of 29 IU/ml. It can detect multiple HIV-2 strains of subtype A and B. Assay results (IU/ml) are reproducible when samples are repeated within the same run (CV < 20%) and in different runs (CV < 40%). Samples exchanged with a comparator laboratory produced similar viral load results, with 63% (19/30) differing less than 0.5 log IU/ml and 93% (28/30) differing less than 1.0 log IU/ml. Two samples with differences >2.0 log IU/ml were undetectable in the comparator laboratory and detected at a low level (~100 IU/ml) in our assay. Since we received approval from the NYS Clinical Laboratory Evaluation Program to use the assay on clinical samples, we have tested 19 specimens from 11 individuals. Twelve (63%) specimens had measurable HIV-2 viral loads (range: 2.38 to 4.88 log IU/ml), 2 had viral loads below the limit of quantification, and 5 had undetectable levels of HIV-2 RNA. HIV-2 RNA was detected in at least one specimen from 9 of 11 (81%) individuals tested. All HIV-2 viral load results are reported electronically to NYS Department of Health for surveillance purposes.

Conclusions: We developed a sensitive and accurate assay to quantify HIV-2 RNA. Validation data indicate the assay is suitable for clinical use and its availability will improve clinical monitoring capabilities for HIV-2 infected patients.
Detection of HIV-2 Proviral DNA by Using Single or Multiplex Real-time PCR

Tim Granade, C. Pau, S. Wells, S. M. Owen and A. Youngpairoj, Centers for Disease Control and Prevention

Objectives: Although HIV-2 infections primarily occur in West Africa, the ability to identify HIV-2 infections and to differentiate them from HIV-1 is needed since treatment options for the two viruses differ significantly. Identification and confirmation of HIV-2 infections in low prevalence areas outside of West Africa is difficult since there are few commercially available products for the detection of HIV-2 nucleic acids and most such tests were developed by individual testing laboratories.

Methods: A real-time PCR test for the detection of HIV-2 proviral DNA from two distinct regions of the HIV-2 genome was developed. Twenty-four HIV-2 plasmids covering the LTR and pol regions from 12 HIV-2 isolates representing HIV-2 subtypes A and B were used as reference materials for optimizing and assessing the sensitivity of the assays. The plasmids were quantified using the Qubit dsDNA HS assay kit and were diluted in ten-fold serial dilutions in TE buffer to approximate 4 x 10^6 to 4 plasmid copies/reaction. PCR primers and TaqMan probes were selected from consensus sequences of the three regions, LTR, protease and integrase. PCR using the human RNAse P gene was also optimized and was used as an internal control for nucleic acids extracted from whole blood or cell lines. Specificity of the HIV-2 PCR was assessed using nucleic acid extractions from HIV serologically nonreactive fresh whole blood specimens (n=52), HIV-1 infected frozen whole blood (in-house whole blood PCR)(n=36), HTLV-I and HTLV-II infected human cell lines (n=5 and 5, respectively).

Results: All of the HIV-2 plasmids were detected by the three real-time PCR assays with limits of detection between 5 and 10 input copies. None of the primer/probe combinations amplified genetic material extracted from the HIV-1-infected specimens, the HIV negative samples or the HTLV-I, HTLV-II infected cell lines. Multiplexing the assays into a single reaction retained the overall sensitivity and specificity for the sample set although the integrase primers had reduced sensitivity when multiplexed.

Conclusions: HIV-2 proviral DNA contained within characterized plasmids was detected with excellent sensitivity from multiple gene regions. The assay was specific for HIV-2 DNA as genetic material from related viruses was not amplified. More work with HIV-2 infected whole blood is needed to further characterize the performance characteristics of the primer sets and to assess the diagnostic utility of the single and multiplexed assays.
Development of Real-Time PCR Assays for Detection of Integrated and Total HIV-1 DNA

Sheila Peel 1, L. Jagodzinski 1, C. Kibirige 2, H. Hack 3, Y. Liu 3 and M. Manak 3

1. Military HIV Research Program, Walter Reed Army Institute of Research
2. Johns Hopkins University

Background and Objective: HIV-1 detection assays based on measurement of HIV-1 antibodies in serum or plasma may result in false positive test results in cases where antibody may be present in the absence of infection. Examples include infants born to HIV-1 positive mothers and HIV-1 vaccine recipients. Serological testing is ineffective in these cases and diagnosis must be made based on nucleic acid detection. Since HIV infection can persist even in the absence of detectable plasma RNA, a more rigorous test for infection in such cases would be sensitive detection of HIV DNA in whole blood or PBMCs. A laboratory developed assay for integrated HIV-1 DNA was designed to permit detection and quantification of the viral reservoir from which continued infection persists. A total HIV-1 DNA assay was also developed for quantification and detection of all forms of HIV DNA.

Methods: Primer/probe sets were designed for amplification of the HIV-1 LTR (R/U5 region) using conserved sequences capable of detecting all major HIV-1 subtypes. A ‘nested’ PCR amplification approach was used for the integrated HIV-1 DNA assay in which a first round amplification was performed for 12 cycles using human repeat primers Alu 1 and 2 and a bacteriophage lambda tagged HIV-1 primer. This first round amplifies sequences spanning the integrated HIV-1 and human genomic DNA. A lambda primer and HIV-1 LTR primer and probe were used in a second round of amplification for 43 cycles to detect and quantify the integrated DNA. Total HIV-1 DNA real-time PCR was performed using an HIV-1 LTR primer/probe set. The use of DNA polymerase in the absence of reverse transcriptase ensured only DNA sequences are amplified. ACH-2 cells containing one integrated HIV-1 copy per cell were mixed with either whole blood or HIV uninfected A.301 cells and used to determine the performance range of the assays.

Results: The in-house developed quantitative PCR assays for integrated and total HIV-1 DNA detected and quantified all major HIV-1 subtypes tested (A, B, C, D, CRF01_AE, and CRF02_AG). The linear performance range of the quantitative Integrated HIV-1 DNA assay was from 3 to 30,000 input copies. The performance range was broader for the quantitative total HIV-1 DNA assay (10 to 1,000,000 input copies). The integrated and total HIV-1 DNA assays are capable of reproducibly detecting extracts from ACH2 cells, equivalent to three copies of integrated HIV-1 DNA.

Conclusion: The HIV-1 DNA assays described here are capable of detecting all major HIV-1 subtypes, are extremely sensitive, and can be used to differentiate between integrated and non-integrated forms of HIV-1 DNA. These assays will be useful for detecting HIV-1 DNA in cells and tissues in circumstances where HIV-1 RNA may be negative (patients on antiretroviral therapy or elite controllers) or HIV-1 serology is confounded (HIV vaccinees and babies born to HIV-1 infected mothers). The views expressed are those of the authors and should not be construed to represent the positions of the U.S. Army or the Department of Defense.
VERSANT HIV1 RNA 3.0 Assay Validation by System 340 bDNA Analyzer and VERSANT TM440 Molecular System

Marcia Toth, R. Valiathan, M. Ashman and D. Asthana, University of Miami

Objective: Quantitation of viral load is an important part of prognosis and effective clinical management of HIV-1 infected individuals. Introduction of new antiretroviral drugs, improved treatment regimens and increase in access to diagnostic services have led to an increase in life span of HIV-1 infected individuals worldwide. Currently various methods are available for measurement of HIV-1 RNA levels with sensitivity limits ranging from 20 to 400 copies per ml; there are various ongoing studies that are evaluating the clinical significance of viral load levels in this range. In this study, we compared 2 systems for HIV-1 RNA quantitation, the VERSANT HIV-1 RNA 3.0 assay by System 340bDNA analyzer and VERSANT TM440 Molecular system in parallel from plasma specimens of 48 HIV-1 infected individuals.

Methods: Whole blood EDTA specimens from 12 HIV-1 infected individuals submitted to the Laboratory for Clinical and Biological Studies, University of Miami, FL, for routine quantitation of plasma HIV-1 RNA and 4 test panels (36 samples) run in duplicate were used for the study. Quantitation of plasma HIV-1 RNA was performed in parallel using the two technologies according to protocols provided by the manufacturers. All statistical evaluations were carried out using Prism software (version 5).

Results: Among 48 plasma specimens, HIV-1 RNA was detected in 32 by VERSANT TM440 Molecular system (sensitivity, 66.6 %) and 31 by VERSANT HIV-1 RNA3.0 assay by System 340 bDNA analyzer (sensitivity, 64.6%). For one sample <75 copies were detected by 340 bDNA analyzer but 440 Molecular system detected 198 copies/ml. On comparing the technologies on a one to one basis we observed that HIV-1 RNA levels obtained with VERSANT TM440 Molecular system and VERSANT System 340 bDNA analyzer had a significant positive correlation (Pearson Correlation, P<0.0001, r=0.9829).

Conclusions: Viral load measurement at low copy numbers is subject to constraints imposed by the inherent variability of the assay technology. Though the difference is not significant, minor variation was observed in the sensitivity of results between the two systems with VERSANT TM440 Molecular system being more sensitive. This needs to be considered carefully for a better prediction of the disease prognosis and treatment outcome on an individual level.

Ae Youngpairoj, K. Curtis, S. Wells, C. Pau, T. Granade, and S. M. Owen, Centers for Disease Control and Prevention

**Background:** Although human immunodeficiency virus type 2 (HIV-2) infections occur mostly in West Africa, cases have spread to other parts of the world such as Europe, India, and the United States making accurate diagnosis of HIV-2 infection essential. Currently in the US, no FDA-approved commercial nucleic acid assays for HIV-2 are available and several laboratories have developed in-house assays to confirm HIV-2 infections. However, the HIV-2 samples needed to develop, optimize, evaluate and monitor the performance of HIV-2 nucleic acid assays are also severely limited. Thus, there is an acute need for the development and characterization of specialized reagents that could be used for these activities.

**Objectives:** To develop a panel of plasmid HIV-2 DNA standards containing cloned sequences covering two distinct gene regions known to be useful for various nucleic acid amplification assays. The plasmids represent multiple HIV-2 subtypes from well-characterized isolates obtained from HIV-2 endemic West African countries including the Ivory Coast, Senegal, and Guinea-Bissau.

**Methods:** Viral stocks from previously described HIV-2 isolates, which included HIV-2 subtype A (n=9), subtype B (n=2), and subtype AB (n=1) were used as the source material for clone generation. RNAs were extracted from all viral stocks using the QIAamp Viral RNA Mini Kit (Qiagen) and were amplified using HIV-2 LTR and pol specific primers by reverse transcriptase-PCR. Amplicons were inserted into TOPO TA plasmids (Life Technologies) using standard cloning techniques and were transformed into E. coli (TOP10 Chemically Competent). Additional sub-cloning modifications such as digesting the insert with EcoR I and reinsertion into the same vector, to prevent insert expression were performed to stabilize clones. Plasmids were purified from each clone using the QuickLyse Miniprep Kit (Qiagen). Plasmids were sequenced and phylogenetic trees (neighbor-joining method) were done to confirm the HIV-2 subtype in each gene region.

**Results/Conclusions:** The entire LTR (~849 bp) and pol (~2995 bp) regions were successfully cloned and sequenced; and subtype designation was confirmed for all 12 isolates. This reference panel of HIV-2 plasmid clones provides an easily quantifiable, renewable, and non-infectious set of reagents which will be valuable for the development and evaluation of HIV-2 molecular diagnostic assays as well as reagents for Quality Assurance and Quality Control.
Evaluation of the Accuracy and Ease of Use of a Rapid, 60 Second HIV Test Performed by Untrained Operators in POC Test Centers in the U.S.

Rick Galli¹, K. Green¹, A. La Marca², L. Waldman³ and R. Powers⁴

¹. bioLytical Laboratories
². Therafirst Medical Center
³. Southwest Center for HIV/AIDS
⁴. Mazzoni Center

**Background:** Diagnostic HIV testing in the US, Canada and global regions is often conducted in clinical and non-clinical Point of Care (POC) settings such as outpatient clinics, medical offices, Voluntary Counseling and Testing (VCT) Centers, testing outreach/community services and sexual health programs. These POC testing programs tend to rely upon the use of samples of convenience, i.e. fingerstick blood, and rapid test technologies that are frequently performed by non-professional untrained operators. It is therefore important to demonstrate that rapid test procedures and interpretation of results in these settings are simple enough to be used by all types of intended use operators (trained and untrained) without increased risk of erroneous results.

**Objective:** The purpose of this prospective Clinical Study was to determine that the 60-second INSTI HIV antibody test employs a methodology that is so simple and accurate as to render the likelihood of erroneous results by untrained intended use operators in point of care (POC) test settings negligible.

The study was designed in accordance with the FDA guidance document Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA) Waiver Applications for Manufacturers of In Vitro Diagnostic Devices, Jan 30, 2008, and with additional guidance from the US FDA Center for Devices and Radiological Health (CDRH). Prior to initiation of the study, the clinical study protocol and the English version of the informed consent document were reviewed and approved by an Institutional Review Board (IRB) operating in accordance with Title 21 of the Code of Federal Regulations (CFR), Part 56. Three geographically distinct US study sites representing diverse HIV testing facilities (medical office, Ft. Lauderdale, FL; HIV/AIDS community service organization, Phoenix, AZ; HIV testing outreach program, Philadelphia, PA), and one central laboratory were utilized. The study site portion included recruitment of high risk subjects with unknown HIV status, who had consented to be tested for HIV per the study site’s standard of care procedures, and known HIV-1 positive subjects.

This was a blinded, controlled study to evaluate the ability of untrained operators to correctly perform and interpret the results of the INSTI™ HIV-1 Antibody Test. The test was conducted by untrained operators at three (3) clinical and non-clinical study sites in the U.S. For each study subject sample collection and testing of fingerstick whole blood with the INSTI™ HIV-1 Antibody Test occurred on a single study day. Results of the INSTI™ HIV-1 Antibody Test were compared with the Patient HIV Status as determined by a pre-defined algorithm of current United States (U.S.) Food and Drug Administration (FDA) approved Comparator Method (CM), ADVIA Centaur HIV-1/2/O Assay, and HIV-1 Western blot. For known HIV positive subjects, the Patient HIV Status was determined from the site source documentation as indicated in the Study Protocol.
**Inclusion/Exclusion Criteria:**

Subjects had to meet all of the following criteria to be enrolled in the study:

1. Subjects were either of unknown HIV status who were undergoing voluntary testing for HIV infection in the clinic setting or known HIV-1 positive subjects willing to be re-tested in a blinded manner and had documentation of HIV-1 infection.
2. Ability to give proper informed consent.
3. Had a self-reported history at Visit 1 of increased risk of HIV infection, including any of the following:
   a. Men who have/had sex with men,
   b. Ever injected illegal drugs,
   c. Was born to an HIV positive mother,
   d. Had sex with an injection drug user,
   e. Had sex with a partner positive for HIV infection,
   f. Had sex with multiple partners (defined as two or more within the preceding three months),
   g. Had a current or prior history of sexually transmitted disease (STD) or had sex with a partner with a STD.
4. Willingness to participate in the study site’s standard of care HIV counseling and testing program and receive the study site’s standard of care test results.
5. Willingness to provide the necessary volume of whole blood collected through venous blood draw and finger stick (approximately 10 ml) for use in the study protocol testing methods.
6. Male or female subjects at least 18 years old.

Subjects enrolled with unknown HIV status were to be excluded from the study if any of the following conditions applied:

1. Subject participated previously in the current clinical trial.
2. Were employees or immediate family members of bioLytical Laboratories or the study sites.
3. Self-reported having a history of multiple myeloma (listed as a limitation in the INSTI package insert).
4. Self-reported a history of known HIV infection.
5. Self-reported history of long-term anti-retroviral therapy of greater than ten (10) years (listed as a limitation in the INSTI package insert).

Subjects enrolled with a known HIV status were to be excluded from the study if self-reported a history of long-term anti-retroviral therapy of greater than ten (10) years.

**Methods:** For subjects with unknown HIV status a venous blood sample was obtained for testing at a central laboratory by the Siemens ADVIA Centaur HIV-1/2/0 Assay (CM), and Western Blot if necessary. From the same subject, a fingerstick sample was obtained for performance of INSTI by a blinded untrained operator at the study site. Based on an assumption of 2% HIV prevalence in the study site populations, enrollment of subjects with unknown HIV status across the three sites was to continue until a minimum of 30 de novo confirmed HIV positives were identified.

For known HIV-1 positive subjects a fingerstick sample was obtained for performance of INSTI by a blinded intended use operator at the study site. Any known HIV-1 subjects with non-reactive INSTI had a blood sample obtained for testing at a central laboratory (ACM Global, Rochester, NY) by the CM. The central laboratory portion of the study conducted CM testing on matching venous blood...
samples collected from study subjects using the FDA-approved CM, and HIV-1 Western blot (WB) if necessary. A minimum of 480 subjects known to be positive for HIV-1 antibodies were to be enrolled in the study. Results of INSTI obtained by untrained operators were compared to the results of the CM and WB performed by laboratory professionals.

Blinded untrained operators who participated in INSTI testing completed a demographics questionnaire prior to participating and an ease-of-use assessment of INSTI at the study conclusion.

INSTI Procedure:

Untrained, intended use operators performed the INSTI test on fingerstick blood by following the instructions in the Package insert, and a Quick Reference Guide that was provided to each study site. No operator training was provided. The operators from the 3 sites collected 50µL of fingerstick blood from each study subject using the lancet and capillary pipette provided with the INSTI kits, and added the blood to Solution 1 (Sample Diluent). Each operator then followed the sequence of test procedure steps as described in the INSTI package insert and Quick Reference Guide, outlined below:

1. Tear open the pouch and carefully remove the Membrane Unit without touching the center well. Place the unit on a level surface. For specimen identification purposes the tab of the Membrane Unit may be labelled with the patient identifier.
2. Mix the Sample Diluent-specimen mixture by inverting several times and pour the entire contents to the center of the Membrane Unit well. (Note: Do this within 5 minutes after the specimen has been added to the Sample Diluent vial). The Sample Diluent-specimen mixture should be absorbed through the membrane in less than 30 seconds; however, absorption times will vary slightly depending upon specimen type. (see Note, below)
3. Re-suspend the Color Developer by slowly inverting to mix the solution thoroughly, until the reagent is evenly suspended. Open the Color Developer and add the entire contents to the center of the Membrane Unit well. The colored solution should flow through completely in about 20 seconds.
4. Open the Clarifying Solution and add the entire contents to the center of the Membrane Unit well. This will reduce the background color and facilitate reading. Immediately read the result while the membrane is still wet. Do not read the results if more than 5 minutes has elapsed following the addition of Clarifying Solution.

In as little as 60 seconds following addition of the blood sample to solution 1, the results were ready for interpretation. INSTI results were recorded as Reactive (test and control spots visible), Non-Reactive (only the control spot visible), or Invalid (no spots visible) on individual Case Report Forms.

**Results:** The first subject was enrolled and tested on September 14, 2011 and the last subject on January 4, 2012. The results of the INSTI™ HIV-1 Antibody Test were not disclosed to the subject or used to determine the subject’s HIV infected status.

**Overview of the Study Population for Analysis**

<table>
<thead>
<tr>
<th>Subject Parameter</th>
<th>Result, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects who signed informed consent</td>
<td>1426</td>
</tr>
<tr>
<td>Number of subjects who met study eligibility criteria</td>
<td>1418</td>
</tr>
<tr>
<td>Number of subjects with no major protocol violations</td>
<td>1416</td>
</tr>
<tr>
<td>Number of subjects with known HIV positive status</td>
<td>484</td>
</tr>
</tbody>
</table>
Subject Parameter | Result, n
--- | ---
Number of subjects with unknown HIV antibody status | 934
Subjects who have provided fingerstick blood sample | 1416
Subjects who provided venous blood sample | 909
Subjects who provided both venous and fingerstick blood | 909
Subjects who discontinued prior to completing visit | 301
Subjects included in the per protocol analysis | 1388

One Reason for Discontinuation:
- Subject request/consent withdrawal: 1
- Inability to collect a venous blood sample: 23
- Adverse event/adverse device effect: 1
- Other (shipping issue): 1
- Missing INSTI results (results not recorded on CRF): 2
- Subjects with major protocol violations: 2

Baseline Subject Demographics

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>1009 (71.2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
<td>409 (28.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Race</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Indian/Alaskan Native</td>
<td>33 (2.3%)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (0.4%)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>594 (41.9%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>766 (54.0%)</td>
</tr>
<tr>
<td>Native Hawaiian/Pacific Islander</td>
<td>2 (0.1%)</td>
</tr>
<tr>
<td>Other</td>
<td>15 (1.1%)</td>
</tr>
<tr>
<td>Declined to answer</td>
<td>2 (0.1%)</td>
</tr>
</tbody>
</table>

The following tables summarize the results from the 1388 per protocol study population, including the prospective, voluntary testing population of unknown HIV status (n=905) and those with known HIV-1 infection (n=483) tested in the study sites and central laboratory:

Table 1 provides the percent positive agreement and the percent negative agreement between INSTI results obtained by intended use operators with fingerstick blood for the per protocol population compared to the subjects’ HIV status as determined by the CM results on matching plasma from venous blood tested at the central laboratory by trained laboratory professionals, or to the source documented confirmed HIV antibody positive status for the known HIV positives. For each calculation of the percent agreement, the 95% one-sided and two-sided confidence intervals are provided, indicating a high degree of agreement between INSTI results and the subjects’ HIV status: percent positive agreement was 100% (517/517) and percent negative agreement was 99.8% (869/871). There were no INSTI invalid results obtained by any intended user for the per protocol population.

Table 2 summarizes the data for the prospective, HIV status-unknown subjects in the per protocol population. There were 34 previously undiagnosed HIV infections identified by INSTI and the CM in the study from the total of 905 prospective subjects who provided fingerstick blood and venous
whole blood. There were no INSTI invalid results obtained by any intended user for the per protocol population.

Table 3 provides a summary of the untrained, intended use operators that participated in the conduct of the study.

Safety Analysis: There were 2 subjects who experienced adverse events (AE) at a study site, described as mild to moderate vasovagal event following. There were no Adverse Device Events or Unexpected Adverse Device Effects reported for this study.

Conclusions and Discussion: Based on the results of this evaluation, intended use operators with varied background and no prior experience with INSTI, from clinical and non-clinical settings, were able to collect fingerstick blood and perform the test on correctly by following only the instructions provided in the INSTI Package Insert and Quick Reference Guide, without any additional training. Their results were highly concordant with FDA approved laboratory based comparator method results produced by trained professionals. There were no false negative results obtained by the untrained, intended use operators for the 517 HIV-1 antibody positive fingerstick blood samples collected from subjects tested in the study, for a positive percent agreement (equivalent to a sensitivity) of 100% (95% CI 99.3-100%). Each operator was required to test a minimum of five HIV positive subjects during the study duration. The same operators interpreted a total of two (2) false positives from the 871 study subjects with HIV Negative status, for a negative percent agreement (equivalent to a specificity) of 99.8% (95% CI 99.2-99.9%). Each operator was required to test a minimum of five HIV negative subjects during the study duration.

In comparison, this data is also highly concordant with data from the US device trials to determine safety and effectiveness of INSTI for the FDA Premarket Approval (granted in November, 2010) that was conducted by trained operators in monitored clinical trial settings. In that study, which used the same CM, overall INSTI sensitivity for fingerstick blood was 99.8% (1095/1097 HIV positives correctly identified, 95% CI= 99.3-99.9%), and specificity was 99.5% (1375/1382 HIV negatives correctly identified, 95% CI=99.0-99.8%). Data source: INSTI HIV-1 Antibody Test Package Insert, 50-1080C. As shown in this study, the INSTI HIV-1 Antibody test employs a methodology that is so simple and accurate as to render the likelihood of erroneous results by untrained intended use operators in point of care (POC) test settings negligible. Based on responses to the ease of use questionnaire completed by each operator at the conclusion of all INSTI testing for the study, the untrained operators participating in this study found the INSTI procedure simple to perform as described in the package insert and quick reference guide.

### Table 1: Measures of Agreement of INSTI with CM and Respective 95% Score Confidence Intervals, Per Protocol Analysis Dataset, n=1388.

<table>
<thead>
<tr>
<th>Measure of Agreement</th>
<th>Point Estimate</th>
<th>95% one sided CI</th>
<th>95% 2 sided CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent positive agreement</td>
<td>517/517 (100%)</td>
<td>99.48-100</td>
<td>99.26-100</td>
</tr>
<tr>
<td>Percent Negative agreement</td>
<td>869/871 (99.77%)</td>
<td>99.31-99.92</td>
<td>99.17-99.94</td>
</tr>
</tbody>
</table>
Table 2: 2x2 Summary of Results of INSTI and Subject HIV Status (CM) for Prospective, HIV status unknown subjects in the Per Protocol Analysis (n=905).

<table>
<thead>
<tr>
<th>INSTI RESULT</th>
<th>POSITIVE</th>
<th>NEGATIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>34</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>0</td>
<td>869</td>
<td>869</td>
</tr>
<tr>
<td>TOTAL</td>
<td>34</td>
<td>871</td>
<td>905</td>
</tr>
</tbody>
</table>

1 confirmed by FDA approved Western Blot Assay

Table 3: Operator Demographics:

<table>
<thead>
<tr>
<th>Operator ID</th>
<th>Site Description</th>
<th>Operator Initials</th>
<th>Experience at site</th>
<th>Education</th>
<th>Currently Use Rapid Tests</th>
<th>Previous INSTI HIV Experience</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site1-001</td>
<td>Outreach CS</td>
<td>CS</td>
<td>3 months</td>
<td>4 yr. BA/BS</td>
<td>Yes</td>
<td>No</td>
<td>47</td>
</tr>
<tr>
<td>Site1-002</td>
<td>Outreach SH</td>
<td>SH</td>
<td>3.5 years</td>
<td>Some college</td>
<td>Yes</td>
<td>No</td>
<td>30</td>
</tr>
<tr>
<td>Site1-005</td>
<td>Outreach TR</td>
<td>TR</td>
<td>1 year</td>
<td>Some college</td>
<td>Yes</td>
<td>No</td>
<td>33</td>
</tr>
<tr>
<td>Site1-006</td>
<td>Outreach JJ</td>
<td>JJ</td>
<td>10 years</td>
<td>GED/high school</td>
<td>Yes</td>
<td>No</td>
<td>44</td>
</tr>
<tr>
<td>Site2-003</td>
<td>Medical office JR</td>
<td>JR</td>
<td>1yr., 7 mo.</td>
<td>Technical school</td>
<td>Yes</td>
<td>No</td>
<td>47</td>
</tr>
<tr>
<td>Site2-005</td>
<td>Medical office CEC</td>
<td>CEC</td>
<td>7 years</td>
<td>Some college</td>
<td>Yes</td>
<td>No</td>
<td>63</td>
</tr>
<tr>
<td>Site3-001</td>
<td>CSO KLC</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>Site3-004</td>
<td>CSO JK</td>
<td>4 yr. BA/BS</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Yes</td>
<td>No</td>
<td>28</td>
</tr>
<tr>
<td>Site3-008</td>
<td>CSO JN</td>
<td>Technical school</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Yes</td>
<td>No</td>
<td>66</td>
</tr>
<tr>
<td>Site3-009</td>
<td>CSO BDT</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Not provided</td>
<td>Yes</td>
<td>No</td>
<td>44</td>
</tr>
</tbody>
</table>

1 Community Service Organization
Expansion of HIV Screening to Non-clinical Venues is Aided by Use of Dried Blood Spots for Western Blot Confirmation

Timothy Sullivan, M. San Antonio-Gaddy, L. Styer, A. Richardson-Moore, D. Bigelow-Saulsbery and M. Parker, Wadsworth Center, New York State Department of Health

**Objective:** Community-based organizations and other non-clinical venues facilitate access to HIV screening for individuals who may not seek testing in clinical settings. HIV rapid testing programs in New York State (NYS) are required to collect a specimen for laboratory confirmation of a preliminary positive result; however these venues have limited options to collect a blood specimen and oral fluid is not economical. This project evaluates the feasibility of using dried blood spots (DBS) at non-clinical HIV rapid testing sites in NYS for Western blot (WB) testing.

**Methods:** All test sites are registered with the NYS Clinical Laboratory Evaluation Program (CLEP) as a limited service laboratory. NYS staff developed procedures and conducted training for DBS collection and storage and, in some cases, fingerstick training. Proficiency in collection was established during the training sessions. Following a reactive rapid test, DBS were collected by fingerstick onto filter paper cards, dried and sent by standard mail to the NYS DOH Bloodborne Viruses Laboratory at the Wadsworth Center. A ¼-inch punch of the DBS was eluted and tested using the GS HIV-1 Western blot kit (Bio-Rad Inc.) according to the manufacturer’s instructions.

**Results:** From October 2010 to August 2012, a total of 38 HIV rapid testing sites completed DBS training. During this period, 208 DBS specimens were submitted to the laboratory for confirmation. There were 4 DBS specimens submitted the 1st month after implementation and 31 in the most recent month, demonstrating increased uptake by the submitting sites. Of these 208 DBS specimens, 2 (0.95%) were unsatisfactory for testing. A total of 206 DBS were tested by WB; 174 (84.5%) were positive, 29 (14.1%) were negative and 3 (1.5%) were indeterminate. The average time from date of receipt in the laboratory to date the report was sent out was 4 days (range 1 to 13 days). Specimens that did not confirm as positive were reported along with a request to collect a whole-blood follow-up specimen to allow for additional tests for HIV RNA detection or HIV-1/HIV-2 antibody differentiation to be performed, if needed, to resolve infection status. During this period, we also received 902 blood specimens collected by venipuncture for rapid test confirmation. In comparison, 21 (2.3%) of these were unsatisfactory for testing. Of the 881 specimens tested by WB, 689 (78.2%) were positive, 178 (20.2%) were negative and 14 (1.6%) were indeterminate.

**Conclusions:** The WB confirmation rate for DBS was greater and the frequency of unsatisfactory DBS specimens was less than that of standard blood specimens submitted to our laboratory for rapid test confirmation. Our results indicate that DBS are a suitable alternative to venous blood for Western blot confirmation. However, very few HIV diagnostic tests are FDA approved for use with DBS which limits the laboratory’s ability to resolve discrepant results using other test methods.
Rapid-2-Rapid - A Collaborative Approach to Linkage

Eugene Martin¹, S. Paul², G. Salaru¹, E. Cadoff¹, L. Berezny², L. Dutton², S. Saudners² and J. Corbo¹

1. University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School
2. New Jersey Department of Health

Objective: Engagement and retention in healthcare remains problematic for many HIV infected persons. We assessed the feasibility of combining the advantages of rapid testing algorithms (RTAs) with a collaborative strategy to engage recently identified clients into HIV care with an ultimate goal of viral load (VL) suppression. The RTA program was implemented throughout NJ in 2009. The NJ RTA relies primarily on an initial screen utilizing the Clearview® HIV 1/2 STAT-PAK®. Preliminary positive screens are followed by a second rapid HIV test, the Trinity Uni-GoldTM Recombigen® HIV. There are two negatives to the NJ RTA program: the cost of quality assurance to run the second rapid test and the varying success of different screening entities in linking clients effectively to healthcare. Medical facilities are nearly twice as effective at engaging and retaining clients in healthcare as other screening entities (health departments and community based organizations).

Methods: In 2012, 11 regional collaborations were established encompassing all 21 NJ counties. Seven patient navigators (PN) were hired and housed in collaborating clinical sites. PN were trained in comprehensive prevention and intervention strategies. Their primary goal was to facilitate connection between clients found HIV + at initial screening facilities and HIV specialty medical clinics in nearby communities. The PN assists clients in the entry, maintenance and re-entry into care. Formally established relationships are negotiated among the partners. The process is coordinated by the NJ Department of Health, Division of HIV, STD and TB Services (DHSTS). QA monitoring of treatment sites includes VL suppression, of < 200 copies, thereby evaluating cascade completion from identification through VL suppression is evaluated.

Results: We describe a process for the currently evolving collaborative program that connects screening facilities to healthcare facilities using the PN and a second rapid test performed at the intake facility. The PN serves as a concierge, facilitator and ensures the entry of the infected into care. In New Jersey, single, rapid HIV screens in lower prevalence populations are associated with false positive screening results at a rate of ~1:10. When combined with a second, orthogonal rapid assay the frequency of false positive results is reduced to 7% who refuse Western blot confirmation. Using an RTA 20% more clients are linked to care each day. VL suppression in New Jersey Ryan White funded treatment sites is 70%, slightly less than 72% observed nationally. Data to be discussed includes performance data on the multi-facility orthogonal process, entry and linkage to care, as well as retention in care.

Conclusions: According to one estimate, only 61% of HIV-infected individuals are linked to care in the United States and only 28% are reported to have a suppressed viral load < 200 copies. Efforts to engage and retain individuals in care need to recognize the importance of testing broadly and transitioning HIV + clients into care without delay and the need to schedule multiple appointments.
Routine HIV Testing and Linkage to Care Services Offered at Public Aid Offices Can Help Identify Undiagnosed HIV Infections and Facilitate Linkage to HIV Care in Urban High Risk Minority Communities

**Tomas Soto and D. Clay, AIDS Foundation of Chicago, Brothers Health Collective**

**Background:** Strategies to expand HIV testing and facilitate linkage to HIV care services are needed to reduce HIV infection. We evaluated the impact of offering HIV testing and linkage to care services at public aid offices in minority Chicago neighborhoods with high HIV prevalence rates. Our primary objectives are to describe the key components of our intervention model, share lessons learned, and report process and outcome evaluation findings.

**Methods:** As part of a national initiative to increase routine HIV testing (HIV Focus), we formed a collaboration of 3 organizations (1 lead and 2 minority-based agencies) and developed a coordinated HIV screening and linkage to care program model entitled the Bridge Project. Using HIV surveillance data, we identified 3 low-income community neighborhoods with high HIV prevalence rates. We then partnered with public aid offices located in these neighborhoods to provide HIV prevention services across settings. From May 2011 through July 2012, the testing collaborative provided 199 days of HIV testing and linkage to care (LTC) services and administered 6,182 HIV screenings. We began in year II piloting a new testing algorithm providing two CLIA waived manufactured products in order to increase our linkage to care outcomes. Preliminary data shows a slight increase in our LTC rates.

**Results:** Of those screened, 47.7% were first time testers, 63.5% women, 84.7% African American, 10.9% Hispanic, and 3.2% white, with an average of 34. We identified 35 positives for an overall seropositivity rate of 0.6%. HIV seropositivity rates varied by gender (1.0% for males vs. 0.3% for women, p = .001). Of those positive, 24 (68%) self-reported being newly diagnosed and 51.4% were successfully linked to HIV primary care services. Contextual factors at sites (e.g. client volume and space) impacted HIV testing acceptance rates, yet across sites, 90.5% reported being “very satisfied” with services. Linkages to care activities proved more challenging to initially implement without established mechanisms to quickly link clients to HIV primary care services. We also found that the presence of these services can serve as an outreach mechanism to “re-link” those HIV+ individual disengaged from HIV care.

**Conclusions:** Routine HIV testing and linkage to care services in public aid offices can reach high numbers of first-time testers and at risk individuals who might otherwise not be tested. Evaluation findings suggests that with administrative buy-in from public aid offices it’s feasible to implement routine community based HIV testing and linkage to care programs in non-clinical settings. Established processes and working relationships with area HIV primary care providers are essential to facilitate quick linkage of HIV+ clients screened at non-clinical settings with medical services.
The Third Wave of HIV Infection in an Urban Hospital

Debbie Mohammed, S. Paul, E. Martin, U. Blanc, C. Sadashigie, S. Scott University of Medicine and Dentistry of New Jersey

Objective: Seroprevalence of HIV infection in Newark, New Jersey (NJ) was 2.1% in 2011. Among patients living with HIV/AIDS, 14.0% can be attributed to males who have sex with males (MSM). We will describe characteristics of patients testing for HIV in the past 10 months at a Newark, NJ, academic medical center which offers routine HIV screening in the Emergency Department (ED), in addition to patients who request testing at a WalkIN site and evaluate the proportion of patients tested who were successfully linked to medical care.

Methods: A retrospective chart review of patients receiving rapid HIV testing from 10/1/2011 to 8/31/2012 at a Newark, NJ, academic medical center was performed. Rapid testing was conducted by trained counselors after routine opt-out screening in the Emergency Department (ED) and for patients requesting testing. Linkage to care was defined as a kept medical appointment. Descriptive statistics were evaluated. This study was approved by the Institutional Review Board.

Results: A total of 7,988 patients were tested: 7,307 (42.4%) of 17,241 patients screened in the ED and 681 patients who walked in and requested testing. The majority of patients tested were aged 20-39 years (37.7%) with equal numbers of males and females. Overall, 73(0.9%) tested HIV positive, with a slightly higher rate among males vs. females (1.2% vs.0.7%). More than half (52.2%) of patients tested positive in the WalkIn site were 13-29 years with more than two-fifths of younger males and females reported having sex with an HIV infected patient (43.5%), primarily male (69.6%) and self-reported MSM (39.0%). Two thirds of patients were successfully linked to medical care.

Conclusion: Young males aged 13-29 years who self-reported MSM accounted for 30% of patients testing positive at this site which is higher compared to prevalence reports in Newark from 2010. At that time, higher proportions of reported risk factors were injection drug use (29.0%) and heterosexual contact (42.0%) which included 40% of women. The normalization of HIV testing at this site over the past 7 years accounts for low proportions of patients with CD4+ counts < 200 cells (12.3%) compared to national reports of 38.3% of patients with an AIDS diagnosis, within one year of testing HIV positive. Strategies for improvement in linkage rates are systemic and include the introduction of Rapid-Rapid HIV testing, same day appointments for medical care, aggressive patient navigation and specialty services available in the ED after hours and on the weekends. This site is strategically positioned to engage and provide high impact prevention services to a transient high risk population who choose to access services via the ED and as a walk-in.
Variable Temperature Conditions in Laboratories and Clinics Located in Low Resource Countries and Their Potential Impact on HIV Rapid Diagnostic Tests


Objective: Rapid Diagnostic Tests (RDTs) for HIV diagnosis are commonly specified by the manufacturer for storage between 15°C to 30°C. High temperatures and humidity have been identified as key contributors to the decline in sensitivity of RDTs over time. In low resource settings (LRS) where refrigerators or incubators and consistent power supply to run such equipment are all typically absent or inconsistent, diagnostic kits are typically stored at ambient temperature until use. We tracked actual temperature occurrences over one to two years by placing temperature data loggers in laboratory and clinic locations at eight locations in three developing countries (India, Uganda, and Nicaragua). Data collected will provide an understanding of any temperature-related problems which may potentially affect the integrity of HIV rapid diagnostics and the use of the RDTs.

Methods: Temperature data recorded by temperature loggers varied from every two minutes to hourly. Two types of temperature data loggers were used; Temperature Data Logger (SM300, Dickson Data) and Track-It Data Logger (5396-0101, Monarch International). One temperature monitor was placed in a laboratory where diagnostic tests are performed and another logger in the clinic where patients are treated and samples collected. All data were analyzed by a statistician consultant (Tsuga Stats, LLC).

Results: All target locations are subject to sustained, elevated temperatures. Over 2.5 million data points were analyzed. Temperatures ranged from a minimum of 8°C to a maximum of 40°C. All locations demonstrated a diurnal pattern and a slow cooling of temperatures lasting, for some sites, well past midnight. Sites in India consistently showed prolonged periods of time where the temperatures remained greater than the recommended upper limit of 30°C for consecutive days and nights.

Conclusion: Previous evidence suggests that improperly stored RDTs may underperform in the field and fail to accurately diagnose patients. Manufacturers aim for storage and use of RDT kits with temperature requirements at controlled room temperature (15°C to 30°C). However, ambient temperature in sub-Saharan Africa frequently and routinely goes above the recommended storage temperature for the tests. Potential degradation of RDTs may occur at distribution centers and during transport. The danger of underperforming diagnostic tests is grave, as a 1% error rate in testing 1 million people could result in misdiagnosis of 10,000 cases. Our preliminary data demonstrate that temperatures not only reach greater than manufacturers’ recommended upper limit of 30°C but can also remain above 30°C for prolonged periods of time. Furthermore, the data show that cyclical fluctuations of temperatures are common in these settings. Stability testing and product claims for diagnostic tests intended for use in low resource settings should be expanded to include temperature cycling and elevated temperatures (e.g. 40°C - 45°C).
Detection of HIV infection by HIV Rapid Tests in Elite Suppressors and Individuals with Antiretroviral Drug-induced Viral Suppression


Objective: HIV rapid tests are commonly used to screen for HIV infection. In this study, we evaluated the performance of HIV screening tests for detection of HIV infection in HIV-infected adults who were virally suppressed.

Methods: Three sets of samples were tested (plasma): (1) 1,155 enrollment samples from men in the HPTN 061 study who reported no prior HIV diagnosis and had non-reactive OraQuick ADVANCE HIV-½ Antibody Test results (whole blood) obtained at study sites, (2) 22 samples from adults with natural viral suppression (elite suppressors who had undetectable HIV RNA for several years in the absence of antiretroviral treatment [ES]), and (3) 79 samples from HIV-infected adults on suppressive ART (S-ART). Samples from the HPTN 061 study were screened using the Abbott ARCHITECT HIV-1 Ag/Ab COMBO test; a subset of the samples, including all samples that were reactive using the COMBO test, were tested using additional assays, as described below. Samples from ES and adults in the S-ART group were tested using the following assays: the Uni-Gold Recombigen HIV Test, the OraQuick ADVANCE HIV-½ Antibody Test, the INSTI Rapid HIV Test, and the VITROS Anti-HIV 1+2 Test.

Results: One (0.09%) of the 1,115 samples from HPTN 061 that had a negative OraQuick rapid HIV test had a reactive COMBO test and a reactive enzyme immunoassay (EIA). A second HPTN 061 sample that had a negative OraQuick rapid HIV test had a non-reactive COMBO test with a reactive EIA. Both samples had positive Western blots and had undetectable HIV RNA using two sensitive assays. Antiretroviral (ARV) drugs were detected in one sample that were consistent with ARV treatment. No ARV drugs were detected in the other sample (this study participant was presumed to be an ES). Identical test results (for COMBO, EIA, Western blot, HIV RNA, and ARV detection) were obtained in both cases for samples collected 6 and 12 months after enrollment. All 22 samples from the ES group had reactive/positive tests with all assays. All samples from the S-ART group...
had reactive/positive test results with these exceptions: one was non-reactive with the OraQuick test and one was non-reactive with the Unigold test, (note: one ES sample did not have sufficient volume to perform all four tests). Overall, two (2%) of 101 samples from individuals with known viral suppression tested were missed using one of the HIV screening assays.

**Conclusion:** Individuals with viral suppression may have false-negative HIV rapid test results. In some settings, it may be appropriate to use two screening tests in parallel to screen for HIV infection.
Rapid Point of Care HIV Testing in Ontario, Canada 2007 - 2011

Ashleigh Sullivan¹, Carol Major², K. English³, C. Swantee⁴, J. Maregman⁴, T. Mazzulli⁴, R. Remis⁴,⁵ and F. McGee³

1. Public Health Agency of Canada
2. Ontario HIV Treatment Network
3. AIDS Bureau, Ontario Ministry of Health and Long-Term Care
4. Public Health Laboratories, Ontario Agency for Health Protection and Promotion
5. University of Toronto, Ontario, Canada

Background/Objective: In 2007, the Ministry of Health and Long-Term Care launched an HIV POC testing program to increase access to testing for at risk and vulnerable populations. In partnership with the Public Health Laboratories, HIV POC testing is now available in 43 sexual health and community health centres across the province and has more than 200 trained providers. Many sites offer anonymous HIV testing and outreach services. A comprehensive quality assurance program includes training, competency assessment, kit lot validation, quality control, environmental monitoring and external quality assessment. All reactive POC results are confirmed by whole blood tested at the PHL. In addition, any POC non-reactive clients likely to be in the window period are advised to submit a whole blood sample for p24 antigen testing. Data on all POC and confirmatory testing are captured by the PHL information system and extracted for analysis.

Results: To the end of 2011, 84,085 POC tests had been performed using the bioLytical Insti Assay. 777 were reactive and of these 722 provided samples for confirmatory testing with 547 (76%) confirmed HIV positive, 162 negative and 13 inconclusive. Of 707 suspected window period clients who provided samples for p24 antigen, 696 were negative for both HIV antigen and antibody and 11 were inconclusive at the PHL. The overall positivity rate has declined from 1.2% to 0.6% as testing volume has increased. The highest positivity rates are MSM (1.85%), IDU (1.77%), people from HIV endemic areas (1.39%). The specificity of the Insti assay is 99.8%, with no evidence of any “missed” cases during window period. The positive predictive value of the Insti assay is MSM-IDU (100%), MSM (93%), IDU (77%), Endemic(62%), high-risk heterosexual (69%), low-risk hetero (33%).

Conclusions: The POC program attracts more high risk clients than the routine testing program (32% vs 16%) and the positivity rates are 3 times higher (0.64% vs 0.22%). Test performance has been excellent to date.
Evaluation of Rapid HIV Self-Testing Among Men Who Have Sex with Men (MSM) in High Prevalence Cities: The eSTAMP Project

Pollyanna Chavez1, R. MacGowan1, L. Wesolowski1, A. Freeman1, D. Higa1, J. Raiford1, W. Johnson1, A. Margolis1, L. Belcher1, S. Ethridge1, C. Borkowf1, M. S. Owen1, A.D. McNaghten2 and B. Branson1

1. Centers for Disease Control and Prevention
2. Emory University

Objectives: With the approval of the OraQuick® In-Home HIV Test (OraQuick) and the manufacturer of the SURE CHECK® HIV 1/2 Assay (Sure Check) seeking approval for over-the-counter (OTC) use, rapid self-testing is now a reality in the United States. The high prevalence of undiagnosed HIV infection among MSM and the potential need for more frequent and timely testing suggests that MSM might benefit from an OTC rapid test. The CDC eSTAMP project will recruit MSM to 1) assess the testing frequency, impact on risk behaviors and field performance of rapid HIV self-tests, 2) evaluate the degree to which persons with a reactive rapid self-test result are linked with medical care, and 3) evaluate the extent to which MSM distribute self-test kits within their social and sexual network associates.

Methods: MSM will be recruited from social networking and/or sex-seeking internet sites for all 4 sequential parts of the project. Through individual qualitative interviews and focus groups, Part 1 will evaluate the written and video instructions for conducting self-testing with OraQuick and Sure Check and for self-collection of dried blood spot (DBS) specimens. In part 2, untrained users’ proficiency will be evaluated by observing participants using OraQuick and Sure Check and collecting, drying and packaging their own DBS specimen according to written and video instructions. In part 3, the performance of user-administered and interpreted OraQuick and Sure Check tests in real-world settings will be evaluated by a comparison to self-collected DBS specimens tested by immunoassay. Part 3 participants will receive one OraQuick rapid test, one Sure Check rapid test, a DBS collection kit and instructions in the mail. They will be asked to use both rapid tests, report their results, complete a brief survey, and mail back the DBS specimen. Part 4 includes a two-arm randomized controlled trial for MSM of self-reported negative/unknown HIV status. Participants will be followed for 12 months, and will complete surveys every 3 months on demographic characteristics, HIV testing, and sexual behaviors. Intervention participants will be mailed two OraQuick and two Sure Check tests, and may request up to 4 rapid tests at each follow-up. Control participants will receive HIV prevention messages and information about HIV testing and links to resources to locate HIV testing and care. At 12 months both rapid tests and a DBS collection kit will be sent to all study participants. Additionally, in-person focus groups will be conducted among intervention participants completing the randomized controlled trial to assess the use of test kits, attitudes about self-testing, benefits and drawbacks of self-testing, unintended consequences, and test preferences. A separate Part 4 activity will recruit HIV-positive MSM to evaluate self-test kit distribution to their social and sexual networks.

Results/Conclusion: Home rapid HIV tests are available and better information is needed to determine how they might be used by populations for whom frequent testing is a high priority. Data obtained through this project will inform policies to guide the optimal use of OTC rapid HIV tests for MSM.
Finding Those At Risk: AHI in Newark, New Jersey

**Eugene Martin**, **S. Paul**, **D. Mohammed**, **G. Salaru** and **E. Cadoff**

1. University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School
2. New Jersey Department of Health

**Objective:** Current HIV screening techniques result in a small number of screen negative individuals who were recently infected and lack antibodies indicative of HIV infection. This serologic window may last up to 6 weeks. RNA testing of pooled, HIV antibody negative specimens’ permits identification of some of those recently infected with HIV and narrows the window to approximately 2 weeks. The importance of this seronegative window is the risk of transmitting HIV to others prior to the appearance of HIV antibodies. By combining rapid HIV testing assays with pooled NAAT, additional infected individuals can be identified and brought to treatment and effective prevention counseling during the early acute stage of HIV infection. We employed rapid HIV testing and NAAT pooling in order to assess the relative likelihood of falsely negative HIV screening in an emergency room and an outpatient high risk clinic in Newark, NJ.

**Methods:** In addition to rapid HIV screening, between Feb 2010 and Aug 2011, pooled NAAT testing was offered to emergency department (ED) patients and outpatients (OP) seen at University Hospital, a large, urban hospital in Newark, NJ. Rapid HIV antibody screening (12,390) was performed using Clearview HIV 1/2 STAT-PAK rapid HIV test (Alere North America, Inc. Princeton, NJ). For those negative by rapid HIV and agreeing to NAAT testing (6785 – 54.7%), plasma samples were collected, centrifuged and stored frozen until a 27 sample batch could be pooled and transported, frozen to the Univ. of Washington Department of Laboratory Medicine where real-time reverse transcription-polymerase chain reaction (RT-PCR) amplification was performed to assess HIV RNA (dynamic range for HIV RNA detection by Real-Time RT-PCR, 30 to 1,000,000 copies/mL).

**Results:** Of 12,390 individuals screened, 5605 (45.3%) had rapid HIV testing, (3139 female, 2466 male) alone, while 6785 (54.7%) (3259 female, 3524 male) agreed to both rapid HIV testing and NAAT screening. Rapid testing identified 124 antibody positive individuals (1.0 %). Pooled NAAT increased HIV case detection by 6.9% identifying 8 additional cases. Overall, AHI yield was 0.11%. An additional 8.1 individuals would have been identified by NAAT testing in the Rapid Only group had they agreed to NAAT testing. While representing 48.4% of those tested, all NAAT positive screens were male.

**Conclusions:** RNA testing of pooled, rapid HIV antibody-negative specimens permits identification of individuals recently infected with HIV. In Newark, pooled NAAT increased HIV case detection substantially and provided an important opportunity to focus attention on treatment and prevention messages for those most at risk of transmitting an HIV infection. Logistic issues limit the willingness to await NAAT results and raise the possibility of using an enhanced rapid HIV test to achieve better acceptance. The increase in case identification is consistent with that reported in other high-risk communities including Los Angeles, Atlanta and Seattle-King County.
False Positive HIV Results Using a Tie Breaker Testing Algorithm

Leslie Shanks, J. Kliescikova, C. Kosack, E. Pilou and R. Siddiqui, Médecins Sans Frontières

Objective: Point of care HIV tests are essential tools in antiretroviral treatment scale up. They allow an increase in the uptake of testing through decentralization of services and performance by non-medical staff and provide same day results. An important drawback to the use of POC in algorithms is the risk of false positive results. Confirmatory tests can be difficult to implement as the gold standard, Western blot (WB), is rarely feasible in peripheral or decentralised settings. MSF has adopted a simple confirmatory test that gives results while the patient waits for use in its POC testing algorithms.

In Ethiopia, the national testing protocol is a tie breaker algorithm using 3 rapid diagnosis tests (KHB®, Stat-Pak® and Unigold®) in series. MSF uses an alternate algorithm of serial testing with 2 RDTs (KHB®, Stat-Pak®) followed by a confirmation test for all double positive RDT results. The objective of the research was to compare the performance of the tie-breaker regimen against a serial regimen, and to evaluate the addition of a confirmation test to both algorithms.

Methods: The study site is in a MSF supported health centre in Abdurafi and a zonal hospital in Humera. All clients, aged 5 years and older, presenting to the voluntary counselling and testing center in either site were offered participation in the study. The first 200 positive samples on the RDT testing algorithm, and a random selection of 200 negative RDT samples were selected. Each sample was tested using the Orgenics Immunocomb Combfirm® (OIC). The gold standard test was the Western Blot. Indeterminate Western Blots were resolved by PCR testing.

Results: 2621 samples were tested, with 204 double RDT positive samples and 204 RDT negatives included in the study. One sample was excluded due to missing results. The HIV prevalence was 7.5%. The tie-breaker algorithm resulted in 16 false positive results giving a PPV of 92.7% (95% CI 90.2-95.2%) and a no false negatives for a NPV = 100% (95% CI: 100%-100%). Adding the OIC confirmatory test to the algorithm eliminated the false positives for a PPV = 100% (95% CI: 100%-100%).

A serial test algorithm with 2 RDTs and no tie-breaker, had a single false positive result (1 out of 203) to give a PPV = 99.51% (95% CI: 98.8%-100%). Adding the OIC confirmatory test to the algorithm gave a PPV = 100% (95% CI: 100%-100%).

The median value of CD4 for the false positive samples is 526 with a range of 191-992.

Conclusions and Recommendations: The risk of false positive HIV diagnosis based on double positive RDT results in a tie-breaker algorithm is significant, with more than one in 13 people falsely diagnosed with HIV. The addition of a simple and feasible confirmation test improved the diagnostic algorithm to 100% PPV.
Status of HIV Surveillance-related Laboratory Reporting in the United States and U.S. Dependent Areas

Kristen Mahle Gray, C.J. Hall, M. Rein, P. Sweeney and H. I. Hall, Centers for Disease Control and Prevention

Objective: To provide an update on the status of HIV-related laboratory reporting laws and the use of electronic laboratory reporting (ELR) methods by health departments conducting HIV surveillance.

Methods: We summarize information reported from HIV surveillance programs in 59 jurisdictions that provide updates to CDC on the status of state laws, regulations, and practices for the reporting of HIV-related CD4 and viral load (VL) results. We also present data from the Epidemiology and Laboratory Capacity for Infectious Diseases (ELC) program at CDC, which supports ELR infrastructure, implementation, and use. In 2012, the ELC program began monitoring the progress of ELR. One phase of the project assessed surveillance information systems and infrastructure to determine how ELR are received, managed and used. Health departments reported on the specific laboratories submitting reports, the volume of reports received and the percentage of ELR.

Results: While the majority of jurisdictions (62.7%, n=37) require all CD4 and VL results to be reported, 17 jurisdictions require limited CD4 and/or VL reporting, and 5 jurisdictions do not have regulations in support of CD4/VL reporting to HIV surveillance. Of the 17 jurisdictions, 8 (13.6%) require CD4 and VL reporting but not at all reportable levels (e.g., CD4 <200 or only detectable VLs), 6 (10.2%) require all VL results but limited CD4 results (e.g., <200 or <14%), 2 (3.4%) require no CD4 reporting and limited VL results (e.g. detectable VLs), and 1 (1.7%) requires all CD4 results but limited VL results (e.g. detectable VLs).

Among the 57 ELC jurisdictions, an estimated 23% (range: 0-75%) of laboratories send ELR feeds (HIV and non-HIV related) to health departments. A total of 51 areas (45 states and 6 cities) are receiving ELR, while 6 areas (5 states and 1 territory) have not received ELR. When the data were limited to HIV records, the survey indicated that approximately 49% (range: 0-100%) of all HIV laboratory results are received electronically by health departments and the volume of HIV-related laboratory results (paper or electronic) sent to the health departments every year is estimated to be ~3,250,000. This estimate includes results from tests that diagnose and monitor HIV infection and negative HIV test results from counseling and testing sites.

Conclusion: Our data suggest that many jurisdictions do not have laws in place that support CD4 and VL reporting. Jurisdictions without adequate CD4 and VL reporting may be limited in their ability to track stage of disease and maximally use surveillance data to monitor care and treatment. Additionally, health departments are dealing with large volumes of HIV-related laboratory results and only half of HIV laboratory test results are received electronically. Use of ELR methods can assist in management of large numbers of laboratory tests, and may increase completeness and timeliness of HIV surveillance data. Programs should continue to work toward implementing state laws that support the reporting of HIV-related test results and implementing ELR to ensure surveillance data can be maximally used for public health action and monitoring progress toward the goals of the National HIV/AIDS Strategy.
Assuring the Quality of Dried-Blood Spot Assays for Anti-HIV-1 Antibodies; Laboratory and Method Performance

Joanne Mei, L. Li, C. Bell and N. Meredith, Newborn Screening Quality Assurance Program, Centers for Disease Control and Prevention

Objective: To track the long-term laboratory and method performance of Anti-HIV-1 dried-blood spot (DBS) serological assays.

Methods:

1. The Newborn Screening Quality Assurance Program (NSQAP) provides quality control (QC) and proficiency testing (PT) DBS materials for HIV serology
2. DBS materials were made by:
   a. Blending HIV-positive donor serum with HIV-negative serum and washed red blood cells to create whole blood matrices of varying HIV reactivities
   b. Blood pools were spotted onto FDA-approved filter paper, dried overnight, and stored at -20 ºC with desiccant until shipment
   c. QC materials were sent to laboratories 2 times per year
   d. PT materials were sent 4 times per year
   e. False-positive and false-negative results were tracked over time

Results: Participant and Method Demographics

1. PT participation averaged 58 laboratories over an 8-year period from 2005-2012 and ranged from 28 (2012 enrollment) to a high of 83 (2009 enrollment) laboratories (Figure 1)
   a. Domestic laboratories ranged from 13 to 18 laboratories; 15 laboratories currently participate
   b. Foreign laboratory participation reached a high of 70 laboratories in 2009 compared to the current participation of 13 laboratories
2. The false-positive rate for domestic laboratories for the years 2009-2011 was 0.0% and the false-negative rate ranged from 0.0% to 2.1% (Figure 2)
3. The false-positive rate for foreign laboratories for the same years ranged from 1.4% to 3.5% and the false-negative rate ranged from 0.0% to 5.8% (Figure 3)
4. The greatest number of errors came from foreign laboratories and correlated with the years of the greatest number of foreign enrollments (Figures 1 and 3)
5. Tables 1 and 2 list the HIV enzyme immunoassay (EIA) and western blot (WB) methods reported to NSQAP in 2011
   a. Only one domestic EIA method is FDA-approved for DBS
   b. Only one WB is FDA-approved for DBS
6. Table 3 lists the HIV algorithms used by laboratories testing DBS
Results: Method Performance

1. Method reproducibility for QC and PT materials varied within and between methods, and between laboratories using the same method (Figures 4-6)
   a. Bio-Rad (Genetic Systems) HIV-1/HIV-2 Plus O method had the greatest variability for QC materials (Figure 4)
      i. Low-Positive and High-Positive Bio-Rad QC values were close, indicating a lack of optimization for use with DBS
   b. Reproducibility for HIV-Reactive PT specimens also showed greater variability for the Bio-Rad method (Figure 5)
   c. Absorbance values for HIV-Nonreactive PT specimens should more uniformity between methods (Figure 6)

2. All Western blot (WB) methods for HIV-reactive DBS consistently detected gp160 and gp 24 over the period from 2009-2011
   a. Bio-Rad (Genetic Systems) HIV-1 WB method varied in its ability to detect gp120, p66, p51, p41, and p18 as shown by the reproducibility of the same PT specimen in 2011 (Figure 7)
      i. Twelve laboratories reported using this method in Quarters 2 and 4
      ii. Thirteen laboratories reported using this method in Quarter 3

Conclusion:

1. NSQAP provides evaluations of laboratory PT performance and tracks yearly error rates
2. It is commendable that domestic laboratories reported no false-positive errors for 2009-2011 and no false-negative errors in 2009 and 2011
3. Using both DBS PT and QC materials, differences in EIA and WB method performance was observed
4. PT and QC are important parts of a quality management system
5. PT provides a snap shot of laboratory performance at one point in time while QC illustrates method performance over time
6. Continued independent assessments of laboratory performance are needed for certification and for continuous quality improvement
Table 1. HIV EIA Methods Used to Test Dried Blood Spot Quality Assurance Materials in 2011.

<table>
<thead>
<tr>
<th>Kit Source</th>
<th>Number of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FDA Licensed for DBS-Avioq HIV-1 Microelisa Systems</strong></td>
<td>11</td>
</tr>
<tr>
<td>Genetic Systems mRNA HIV EIA (Bio-Rad) (Discontinued)</td>
<td>5</td>
</tr>
<tr>
<td>Bio-Rad HIV-1/HIV-2 plus O EIA</td>
<td>3</td>
</tr>
<tr>
<td>Fujirebio Serodia-HIV 1,2</td>
<td>2</td>
</tr>
<tr>
<td>Tecnosuma (Cuba) UMEELISA HIV 1+2</td>
<td>3</td>
</tr>
<tr>
<td>Q-Preven HIV 1+2, DBS, Brazil</td>
<td>1</td>
</tr>
<tr>
<td>In House</td>
<td>1</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td><strong>7</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>33</strong></td>
</tr>
</tbody>
</table>

Table 2. WB Methods Used to Test Dried Blood Spot Quality Assurance Materials in 2011.

<table>
<thead>
<tr>
<th>Kit Source</th>
<th>Number of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FDA Licensed for DBS - Genetic Systems HIV-1 WB (Bio-Rad)</strong></td>
<td>12</td>
</tr>
<tr>
<td>Cambridge Biotech HIV-1 WB Kit (Maxim)</td>
<td>1</td>
</tr>
<tr>
<td>OraSure HIV-1 WB Kit</td>
<td>1</td>
</tr>
<tr>
<td>New LAV Blot I (Bio-Rad)</td>
<td>1</td>
</tr>
<tr>
<td>Genelab diagnostics HIV 2.2 WB</td>
<td>1</td>
</tr>
<tr>
<td>MP Diagnostics HIV Blot 2.2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td><strong>1</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>18</strong></td>
</tr>
</tbody>
</table>
Table 3. HIV Algorithms used by Laboratories Testing DBS, Quarter 4, 2011

<table>
<thead>
<tr>
<th>Testing Combination</th>
<th>Number of Laboratories Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutination/WB</td>
<td>1</td>
</tr>
<tr>
<td>Agglutination Only</td>
<td>1</td>
</tr>
<tr>
<td>EIA/Agglutination/WB</td>
<td>1</td>
</tr>
<tr>
<td>EIA/WB</td>
<td>13</td>
</tr>
<tr>
<td>EIA Only</td>
<td>5</td>
</tr>
<tr>
<td>EIA/EIA</td>
<td>2</td>
</tr>
<tr>
<td>EIA//EIA/WB</td>
<td>1</td>
</tr>
<tr>
<td>WB Only</td>
<td>2</td>
</tr>
<tr>
<td>Luminex Multiplex</td>
<td>2</td>
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Comparative Performance of the Avioq HIV-1 Microelisa System on Oral Fluid Specimens

Berry Bennett, S. Fordan and D. Neumann, Florida Bureau of Public Health Laboratories

Objective: In 2008, the sole FDA approved laboratory-based oral fluid HIV-1 immunoassay (IA), Vironostika HIV-1 Oral Fluid EIA, was withdrawn from the U.S. market, leaving public health prevention programs and laboratories straining to maintain vital outreach activities dependant upon “alternatives to blood collection.” Until late 2009, laboratories that continued to screen for HIV-1 antibodies in oral fluid specimens had to validate a blood-based IA for that purpose. On Sept. 21, 2009, the Avioq HIV-1 Microelisa System® was FDA approved for diagnostic use and included an oral fluid (obtained with OraSure® HIV-1 Oral Specimen Collection Device) claim in the Intended Use statement. The objective of this study was to compare performance of the Avioq HIV-1 immunoassay with oral fluid specimens to the 3rd generation blood-based Bio-Rad HIV-1/2 Plus O EIA (in-house verified for oral fluid screening). The latter verification process was performed in 2007-2008, prior to the loss of the Vironostika HIV-1 EIA.

Methods: Sensitivity and specificity data on prospective fresh OraSure® specimens for the first 4 months of the Avioq HIV-1 EIA (n=9,543) implementation was compared to the preceding 4 months of screening using the oral fluid validated Bio-Rad HIV-1/2 Plus O EIA (n=10,620). Orasure Technologies’ HIV-1 Oral Fluid Western Blot (WB) was performed on all repeatedly reactive screening results from both assays.

Results: Of the 9,543 OraSure specimens screened with the Avioq immunoassay, 216 were repeatedly reactive, WB positive (2.3% seropositivity). The sensitivity of the assay was 99.5% (216/217) and the specificity was 99.9% (9317/9327). The one false negative (FN) Avioq case was based on an indeterminate WB, OraQuick® positive and proof of seroconversion within 30 days. Of the 10,620 Orasure® specimens screened with the Bio-Rad immunoassay, 224 were repeatedly reactive, WB positive (2.1% seropositivity). The sensitivity was 100% (224/224) and the specificity was 97.1% (10085/10396). The 10 false positive (FP) Avioq and 311 FP BioRad cases were based on WB non-reactive or indeterminate results, however due to limited follow up in our public health setting, it is unclear how many, if any, of these cases may be early HIV-1 infections.

Conclusions: Data from this initial 4 month review of the Avioq HIV-1 Microelisa System supports its continued use to screen OraSure® specimens. The assay performance meets or exceeds the manufacturer’s stated performance characteristics based on screening for HIV-1 antibodies in high-risk subjects. It is important to note that the Avioq HIV-1 test is a second generation immunoassay containing HIV-1 viral lysate and purified gp160 for the detection of HIV-1 antibodies. Although these antigens provide extensive cross reactivity to HIV-2 antibodies the manufacturer does not have a claim for the detection of HIV-2. The Avioq HIV-1 test is consistent with a Fiebig stage V laboratory diagnostic methodology (Fiebig 2003).
Session 10: Testing Oral Fluid Specimens | Oral Abstract

Comparison of HIV Oral Fluid and Plasma Testing During Early Infection in a Longitudinal Nigerian Cohort

Wei Luo1, S. Masciotra1, K. Delaney1, M. Charurat2, T. Croxton2, N. Constantine2, A. Nasidi3, W. Blattner2, L. Wesolowski1 and S. M. Owen1

1. Centers for Disease Control and Prevention
2. Institute of Human Virology, University of Maryland School of Medicine
3. Nigerian Federal Ministry of Health/Reach Care Foundation

Objectives: HIV oral fluid (OF) testing is a non-invasive alternative to blood based testing. However, the performance of OF tests in longitudinal cohorts containing acute/early infections has not been extensively evaluated. The objective of this study was to compare OF and plasma testing in a cohort of HIV seroconverters from Nigeria.

Methods: Sixteen individuals (subtypes G and CRF02_AG viruses) from the Nigerian acute HIV infection cohort (REACH Study) were included in this study. These individuals were initially antibody negative and became antibody positive during subsequent testing. Of these 16 individuals, 14 had multiple sequential follow-up specimens. Paired (150 each) plasma (EDTA) and OF specimens collected at 7-10 days, 3, 5, 7, 9 weeks, and then 3, 4, 6, 8, 10, 12, 15, 21, 24 months after the initial HIV screen were available for testing. Two individuals had only 1 time point for paired OF and plasma specimens. OF specimens were collected using the OraSure device (OraSure Technologies, Inc.) in accordance with the manufacturer’s instructions and stored at -80°C (> 3 years) until use. Plasma and OF (n = 152 each) were tested using Avioq HIV-1 Microelisa System (Avioq, Inc). OF specimens reactive by Avioq were tested by OraSure HIV-1 Western blot (WB). Plasma WB (Genetic Systems HIV-1 Western blot) was performed on the corresponding plasma of the first 2 Avioq-OF positive time-points.

Results: From the 14 individuals with longitudinal specimens, 5 (35.7%) were concordant between plasma and OF for all time points tested, whereas 9 (64.3%) showed discordant results during the early course of infection. One of the discordant was a HIV-1 confirmed pregnant woman who remained negative by OF-Avioq for up to 20 months, but had intermittent positive and negative results with plasma testing. The remaining 8 individuals, showed a delayed antibody response with OF testing and the median delay for concordance of reactivity between OF and plasma Avioq testing was 44 days (range: 5 to 174 days). The 2 individuals with only 1 time point specimens had concordant Avioq results between OF and plasma. Of 152 specimen, 41 (26.9%) corresponding OF specimens were negative. There was concordance between OF Avioq and OF WB results with 98.2% of samples positive on both assays. Two Avioq- positive OF specimens were OF WB indeterminate. OF and plasma WB results were concordant for final interpretations, but there were fewer bands present on OF WBs compared to plasma WBs. There was no association between HIV-1 subtype and delayed antibody response in OF-testing.

Conclusion: We show decreased sensitivity of OF testing with the Avioq HIV-1 compared to blood-based testing with specimens obtained early after HIV infection. Programs that utilize OF testing in populations with increased risk of acute /early HIV infections should be cautious about false-negative results and alternative testing may be needed. Individuals should be aware of the limitations of OF testing to detect HIV antibodies within the first 3-6 months after infection. The effects of long-term storage of OF at -80°C prior to testing have not been reported by the manufacturer, and may be a potential limitation of our study. Further studies are needed to evaluate the sensitivity of OF tests during early HIV infection.
Oral Fluid Is Inferior to Fingerstick Point-of-Care HIV Tests Among Seattle MSM

Joanne Stekler¹, A. Lane¹, J. O’Neal² and M. Golden¹

¹. University of Washington and Public Health - Seattle and King County HIV/STD Program
². San Francisco State University

Objective: The Rapid Test Study is an ongoing, real time comparison of four point-of-care (POC) HIV tests designed to determine their relative abilities to detect early HIV infection.

Methods: HIV-negative men who have sex with men (MSM) and transgender persons seeking HIV testing were recruited at the Public Health - Seattle & King County (PHSKC) STD Clinic, Gay City Health Project Wellness Center, and University of Washington Primary Infection Clinic (PIC). Study procedures included one POC test performed on oral fluids (OraQuick, Orasure Technologies) and two or three POC tests performed on fingerstick whole blood specimens: OraQuick (5µL), Uni-Gold Recombigen HIV Test (Uni-Gold, Trinity Biotech, 50µL), and Determine HIV-1/2 Ag/Ab Combo (Determine, Alere Inc., 50µL). Serum specimens from subjects with negative POC results were sent for EIA and pooled NAAT. McNemar’s exact tests were used to compare the numbers of HIV-infected subjects detected by the different POC HIV antibody tests.

Results: Between February 2010 and June 2012, 1822 subjects were enrolled. Of 1806 MSM seen at the STD Clinic and Wellness Center, 64 (3.5%) were newly diagnosed with HIV infection. Only 48 (75%) had reactive results on all POC tests, and 4 (6%) additional subjects had discordant results with at least one reactive and one non-reactive POC test. Data comparing test performance were analyzed for these 64 HIV-infected subjects plus 16 HIV-infected men enrolled at the PIC. Of these 80 total subjects, 57 (71%) had concordant reactive POC test results, 5 (6%) had concordant non-reactive POC tests but a reactive 3rd generation EIA, and 8 (10%) of subjects had acute HIV infection. Ten (12%) subjects had discordant POC test results, including one subject with a reactive Determine p24 antigen and an HIV RNA level of 5.7 million copies/mL. OraQuick performed on oral fluids identified fewer men with discordant results compared to both OraQuick performed on fingerstick (0 versus 6, p=.03) and Uni-Gold (1 versus 7, p=.07).

Conclusion: Our data show that oral fluid POC testing is inferior to fingerstick and should be the specimen collection method of choice only in rare circumstances. These data also reinforce published data from the PHSKC Pooled HIV NAAT Program that have shown that rapid HIV antibody tests correctly diagnose fewer than 80% of HIV-infected MSM in Seattle. In high HIV incidence populations like ours, currently approved POC tests are inadequate and must be supplemented with pooled NAAT or 4th generation assays.
DPP Supplemental Rapid Test Data Can Discriminate Recent from Longstanding HIV Infection

Chris Pilcher1, R. Kassanjee2, L. Motta3, S. Facente4, S. Keating5, F. Hecht1, R. Sperhacke3, M. Busch5, G. Murphy6, J. Esfandiari7 and A. Welte2

1. University of California, San Francisco
2. South African Center for Epidemiological Modelling and Analysis
3. Universidade de Caxias do Sul
4. Facente Consulting
5. Blood Systems Research Institute
6. Health Protection Agency-Virus Reference Department
7. Chembio Diagnostic Systems Inc.

Objective: HIV-1/2 discriminatory rapid tests using dual path platform (DPP) technology have replaced Western blot and IFA as preferred supplemental testing in many laboratories in Brazil, and a derivative assay (Geenius) has been developed by Biorad that is now being introduced to ex-US markets and in clinical trials for US FDA clearance, beyond the claims DPP has for high sensitivity and specificity, this class of rapid tests is also unique in that it generates reproducible, quantitative results for antibody binding (i.e., band intensities) against multiple HIV antigens. We investigated the potential performance of the existing Chembio DPP HIV-1/2 Confirmatory Test as a test for recent HIV infection.

Methods: 234 specimens from antiretroviral-naïve subtype B or C HIV-infected individuals were collected in US, South Africa, and Brazil. Samples were selected from subjects in whom the date of seroconversion was known to within 45 days; duration of time from seroconversion to sample collection varied from 8 days to 7 years (mean 1.5 years). Intensity of gp36, gp41, p24, gp120 and gp160 bands was measured as light reflectance units, using the DPP reader at 20 minutes. Optimal band intensity value thresholds were selected using ROC analysis, based on their ability to correctly classify individual samples as being within 90, 120 or 180 days of seroconversion. To assess potential utility of band intensity thresholds for cross-sectional HIV incidence estimation, we further calculated window periods (or, mean durations of recent infection, MDRI) based on linear interpolation of the observed data. The “false-recent rate” was calculated as the proportion of samples classified as recent among those >365 days from seroconversion. MDRI and FRR were calculated for both single band intensity thresholds and for combination algorithms using data from multiple bands.

Results: Individual staging analysis: Different single band intensity thresholds were selected as optimal for classifying infections as being < 90, < 2% with only very slight reduction in the MDRI.

Conclusions: In addition to confirming HIV-1 and HIV-2 status, DPP supplemental tests could also be used to routinely stage individual’s infections as being acute, recent or longstanding. The method we describe is novel and must be further evaluated, but our data suggest that the risk of misclassifying longstanding (>1 yr) infections as ‘recent’ using routine DPP data could be very low. The possibility of using routinely collected DPP data for surveillance of recent infection and incidence estimation are being further investigated.
Session 11: Assays Under Development | Oral Abstract

Development of Global HIV Subtype Reference Panels for Molecular Testing

Michael Busch¹, T. Denny², F. Gao², A. Sanchez³ and I. Hewlett⁴

1. Blood Systems Research Institute
2. Duke Human Vaccine Institute
3. Immunology and Virology Quality Assessment Center
4. Laboratory of Molecular Virology, CBER, U.S Food and Drug Administration

Objective: The extensive diversity and rapid evolution of HIV pose serious challenges for maintaining reliable nucleic acid tests for blood screening, epidemiological surveillance, diagnosis and clinical management of infected persons. The panels available for assay development and evaluations were isolated more than a decade ago and don’t represent the currently circulating viruses. This has two important implications. First, the viruses that are currently in circulation have significantly evolved and recombined in the human population over the past decade. Second, the current panels are incompletely characterized in molecular terms which make it difficult to know if assay comparisons are valid. To meet this challenge, NIH established a Viral Diversity Program through the NIAID External Quality Assurance Program Oversight Laboratory (EQAPOL) at Duke University.

Methods: Plasma samples, screened positive for HIV infection, were procured from blood centers around the world with additional viruses captured through surveillance and clinical studies. Preference was given to acute/early stage infection samples which are more relevant to detection and intervention studies. All virus sources were cultured to high titer/high volume and stored as neat culture supernatant or spiked into defibrinated plasma. Viruses are then evaluated, characterized, and fully sequenced in a GCLP compliant laboratory facility before adding them to a central repository.

Results: The table summarizes the countries and subtypes/CRFs isolates currently represented in the EQAPOL repository. The viruses will be used to construct several panels based on the needs of the scientific community, regulatory agencies, researchers, diagnostic and pharmaceutical manufacturers.

Conclusions: It is critically important to identify and quantify extent and spread of new recombinants and to quantify viral load across diagnostic platforms for several reasons. First, blood screening and diagnostic assays are sold in the global market by a limited number of test vendors. Second, trials for assessing vaccine and microbicide products are conducted in regions that are a hot bed of viral diversity. Finally, monitoring transmitted drug resistance, especially with the expanded use of therapies in resource-limited settings and the introduction of new treatments with novel mechanisms of action will need to be assessed more frequently. Laboratories wishing to participate as contributors of qualified viruses or recipients of pre-developed or customized plasma panels or viral isolates should contact EQAPOL (EQAPOL@duke.edu).
New HIV Peptide-based Immunoassay Resolves Vaccine Induced Seropositivity in HIV Vaccine RV144 and HVTN204 Trials

Oksana Penezina1, D. Clapham1, V. Kovalenko1, I. Rodriguez-Chavez2, M. Busch3, K. Hennessy1, D. Crane1, N. Krueger1 and A. Levin1

1. Immunetics Inc.
2. National Institutes of Health (NIH), NIDCR
3. Blood Systems Research Institute

Objective: Recipients of complex HIV vaccine preparations tested in phase I-III trials are increasingly exposed to vaccine-induced HIV seropositivity (VISP) risks, resulting in health and life insurance access issues and other financial and socially related negative consequences. We have redesigned the peptide-based HIV Selectest EIA (J.Virol 2006;80:2092-2099) to increase its sensitivity and specificity in discriminating VISP from true HIV infection in a format suitable for routine laboratory screening of vaccine trial participants.

Methods: The HIV Selectest incorporated five synthetic peptides in a single well microplate EIA. Serum panels evaluated comprised well-characterized HIV-positive sera from clades A, B and C, worldwide panels including all major clades, blood donor controls, and sera from vaccine and placebo recipients in RV144 and HVTN204 trials.

Results: The HIV Selectest yielded an overall sensitivity of 98.3% when tested on a total of 648 HIV positive sera representing all major HIV clades. Specificity as determined by testing a group of 400 U.S. blood donors was above 99.5%. 184 serum samples from uninfected subjects in the HVTN204 vaccine trial were negative on the HIV Selectest EIA. These specimens included 92 sera from vaccinees at the peak of immune response and 92 sera from the placebo group. Out of the 360 sera from uninfected subjects in the RV144 vaccine trial which were tested by the Selectest EIA, one vaccinee (0.6 %) and one pre-immune serum (0.8%) yielded false-positive results, while none of the placebo recipients (0%) tested positive. All false-positive sera demonstrated broad non-specific cross-reactivity that was not restricted to a particular HIV-specific peptide. The RV144 specimens included 170 samples from vaccinees at the peak of immune response, 120 pre-immune sera, and 70 placebo sera.

Conclusion: The HIV Selectest EIA demonstrated sensitive and specific detection of true HIV infection with effectively no VISP in participants from two HIV vaccine trials involving different vaccine prototypes. This EIA could thus provide a useful tool to monitor participants in future HIV vaccine trials.
Isothermal Amplification of Diverse HIV-1 Subtypes by RT-LAMP

Kelly Curtis, D. Rudolph and S. M. Owen, Centers for Disease Control and Prevention

Background: A rapid NAAT is highly desired for HIV-1 detection in resource-limited or point-of-care (POC) settings, especially for facilitating the diagnosis of acute infection and infants born to HIV-infected mothers. Isothermal amplification techniques, such as reverse-transcription loop-mediated isothermal amplification (RT-LAMP), offer an attractive alternative to traditional PCR or RT-PCR, as they are typically quicker, easier to perform, allow for greater versatility in terms of heating devices, and do not necessarily require nucleic acid extraction of clinical specimens. While previous studies have demonstrated the development and performance of HIV-1-specific RT-LAMP, extensive validation studies using diverse subtypes and clinical specimens have yet to be performed.

Methods: RT-LAMP primers were designed against conserved regions within the HIV-1 reverse transcriptase (RT) and integrase (INT) gene regions. Given the subtype diversity in the INT gene region, subtype B and non-B specific primer sets were generated. All three primer sets were evaluated using an international panel of HIV-1 isolates, representing subtypes A, B, C, D, CRF01_AE, and CRF01_AG (n=6 per subtype). For comparison purposes, time to positivity for each panel member was determined based on real-time data obtained from RT-LAMP performed in a Stratagene Mx3000P (Stratagene Corp., La Jolla, CA). The virus concentration of each panel member was normalized based on p24 levels determined by the Zeptometrix HIV-1 p24 Antigen kit (Zeptometrix Corp., Buffalo, NY). For further validation, whole blood specimens (n=20) from HIV-1 subtype B-infected individuals were tested directly or following nucleic acid extraction and compared to the results from in-house PCR.

Results: The RT and subtype B-specific INT primers detected subtype B isolates with a median time to positivity of 15 and 9.5 minutes, respectively. The increased sensitivity of the INT primers was further validated with whole blood specimens, since the RT-LAMP results closely matched those provided by gp41-specific in-house PCR. All three primers sets detected all of the subtype isolates; however, the non-B INT primers exhibited the shortest times to positivity, detecting all subtypes, except subtype C, with a median time < 15 minutes. Subtype C exhibited the longest median time to positivity for all primer sets (= 22 minutes); however, slight modification to both the RT and INT-specific primer sequences reduced the median detection time to < 15 minutes.

Conclusions: HIV-1-specific RT-LAMP offers a rapid, low-cost alternative to PCR with the potential to facilitate HIV-1 testing in resource-limited and POC settings. We demonstrate the ability of RT-LAMP to detect multiple HIV-1 subtypes, further expanding the utility for widespread use as a rapid HIV-1 NAAT.
Objective: We have previously used Europium (III) nanoparticles as probes for HIV-1 antigen/antibody detection at subpicogram per milliliter level. By miniaturizing the assays to microfluidic format, we further decrease sample volume, assay time, and overall footprint of the instrument, thus allowing adaptation of the assays to a broad range of settings for rapid and sensitive detection of HIV infection, a major public health challenge that still remains as of today.

Methods: Plastic microfluidic cartridges with 12 microreactors were fabricated using soft lithography. Depending on assay types, either p24 specific monoclonal antibody, recombinant p24 or its segment peptides were coated on microreactor surface for on-chip Europium nanoparticle immunoassays (ENIA). The fluorescence intensity of the specifically retained Europium nanoparticles in the on-chip assay was recorded using time-resolved fluorometry. We evaluated performances of microfluidic antigen/antibody assays using serial dilutions of recombinant p24. Human serum panel and plasma samples were used in real-sample assays, and cell culture was used in quantitation test.

Results: ENIA of p24 serial dilutions indicates that on the microfluidic cartridges, linear dynamic range was found to extend from 0 to 500 pg/mL, with a detection limit of 5 pg/mL. Chip-to-chip variation was about 10%, and overall assay time was 45 minutes. In real-sample tests, randomly selected 10 serum samples from a serum panel were screened using microfluidic antigen assay. 9 out of 10 serums were found positive, comparable with the results from microtiter plate-based assays and nucleic acid tests. Evaluation of microfluidic cartridges for HIV antibody assay was performed using human plasma samples. S/N values up to 12 were routinely obtained in positive plasma, comparable to microtiter plate assays. To determine p24 abundance in a cell culture, we first established a p24 calibration curve using microfluidic ENIA, followed by deducing p24 concentration from signal intensity of the sample. The p24 concentration was found to be 18 ± 0.4 µg/mL (3 tests), agreeing well with the value (18 µg/mL) obtained from NanoDrop UV-Vis spectrometry. We performed all assays in parallel using microtiter plates and microfluidic cartridges, and found that the microfluidic platform has a comparable sensitivity to that of the microtiter plate, with a 4-fold reduction in reagent consumption and a 2-fold reduction in assay time. Additionally, no electrically powered plate-washers and pumps were required in all microfluidic ENIA assays. These features enable their use in resource-limited settings.

Conclusion: We successfully demonstrated Europium nanoparticle immunoassays on a dedicated microfluidic cartridge for rapid and sensitive detection of HIV infection. Based on needs, the microfluidic device can be adapted to high-throughput singleplex or multiplex assays for routine detection of HIV infection or multiplex assays for discrimination of acute and chronic infections. The microfluidic ENIA is suitable for de-centralized laboratories and remote regions because they have advantages such as low sample/reagent/energy consumption, low waste generation, small footprints, high speed, high sensitivity and ease of use.
Performance Evaluation of a Novel HIV-1/2 Rapid Test for the Detection of HIV-1/2 Antibodies in Oral Fluid and Whole Blood

Louise Sigismondi, J. Esfandiari and D. Gunasekera, Chembio Diagnostic Systems

Objective: Rapid HIV antibody tests that are approved for use with oral fluids have become more acceptable to patients because of their non-invasive and pain-free specimen collection and rapid turnaround time. In addition, healthcare workers face a much lower risk of exposure to infectious diseases from oral fluid than from blood. The objectives of this investigation was to assess the performance the Chembio Diagnostics Systems DPP® HIV 1/2 Assay (DPP HIV) as a single-use, disposable, visually read qualitative rapid immunochromatographic lateral flow test in the detection of HIV antibodies in a head-to-head comparison of paired capillary (fingerstick) whole blood and oral mucosal transudate (OMT) samples.

Methods: Comparative data included 2808 participants across five geographically diverse clinical sites, all in the United States, from three risk groups: 868 individuals known to be positive for HIV-1 (PS), 976 individuals at high risk for HIV infection (HR) and 964 individuals at low risk for HIV infection (LR). Each participant was tested with the DPP HIV at the clinical sites on oral fluid and fingerstick capillary whole blood. Each sample was dispersed into premeasured buffer in dropper bottles (SampleTainer) and added to the Sample Well of the device. After testing with DPP HIV, serum and plasma samples were prepared from venous whole blood and sent to a reference laboratory for confirmatory and discrepancy resolution testing. Reference testing and discordant resolution testing included FDA approved EIAs repeated in duplicate, HIV-1 WB and a HIV-1 NAT.

Results: The sensitivity of the DPP HIV to detect infection with HIV-1 was evaluated using 868 PS specimens and from 976 HR specimens. 964 individuals reported were identified positive for infection with HIV-1 using a WB and/or NAT assay. The calculated sensitivity for the OMT samples was 953/964 = 98.9% (95% CI= 98.0 to 99.4%). The calculated sensitivity for the fingerstick samples was 962/964 = 99.8% (95% CI = 99.2 to 99.9%). The specificity of the DPP HIV was evaluated by testing LR and HR specimens. Based on these studies, the specificity of DPP HIV in OMT specimens was calculated to be 1815/1816 = 99.9% (95% CI = 99.7 to 99.9%). The specificity in fingerstick specimens was calculated to be 1815/1815 = 100% (95% CI = 99.8 to 100%).

Conclusion: Assay sensitivity (oral 98.9%, 98.0 -99.4; blood 99.8%, 99.2 to 99.9) and specificity (oral 99.9%, 99.7 -99.9; blood 100%, 99.8 to 100) obtained for both matrices was similar. The DPP HIV offers a new alternative as an oral fluid-based HIV test, especially with at-home self testing on the horizon. The assay is especially unique and advantageous in that the original sample is collected in a closed vial, available for re-test for 7 days.
Evaluation of the DPP HIV-HCV-Syphilis Assay: A Novel, Point-of-care Rapid HIV 1/2, HCV and Syphilis Treponema pallidum Antibody Combination Test


Objective: HIV, HCV and Syphilis are all sexually transmitted infections. The Chembio DPP HIV-HCV-Syphilis Assay, currently under development, is a single-use, point-of-care, rapid screening test for the detection of antibodies to HIV 1/2 and/or HCV and/or Syphilis Treponema pallidum in whole blood, serum, or plasma samples. Testing was aimed at ensuring that this developmental product would be suitable for use in multi-test algorithms designed for the statistical validation of rapid HIV, HCV and Syphilis test results.

Methods: The initial design validation of the Chembio DPP HIV-HCV-Syphilis Assay was tested on numerous well characterized qualified-vendor purchased performance panels that were assembled from highly characterized natural patient samples. They were used as a vital part of assay development and regulatory compliance by providing a common reference for assay comparison, evaluation and validation of test methods, method comparison and documentation of laboratory proficiency. Each test was performed by trained laboratory staff on sera or plasma specimens according to manufacturer’s directions. The results of both the reference tests and rapid tests performed by laboratory personnel are presented here.

Results: A total of 200 negative plasma samples were tested on the DPP HIV/HCV/SYP assay. All of these 200 samples were found to be negative on FDA-licensed HIV1/2 EIA, HCV EIA and Syphilis RPR assays. All 200 samples were nonreactive for HIV on the DPP Assay, making the specificity of the DPP HIV test line 200/200 = 100% with the 95% CI = 98 – 100%; the specificity for the DPP HCV test line was 198/200 = 99% with the 95% CI = 96.4 to 99.9%; the specificity for the DPP SYP test line was 193/200 = 96.5% with the 95% CI = 92.9 to 98.6 %. The DPP HIV/HCV/SYP assay was tested on a total of 11 performance panels consisting of 183 individual members. Preliminary results revealed that the sensitivity of the DPP HIV test line was 143/143 = 100% with the 95% CI = 97.5 – 100%; the sensitivity for the DPP HCV test line was 50/57 = 87.7% with the 95% CI = 76.3 to 94.9 %; the sensitivity for the DPP SYP test line was 35/35 = 100% with the 95% CI = 90.0 to 100 %.

Conclusion: The Chembio DPP HIV-HCV-Syphilis Assay is unique as an aid in the simultaneous diagnosis of infection with HIV, HCV and Syphilis. With further design and development planning, the DPP Assay is capable of achieving desired safety and performance characteristics.
**Session 11: Assays Under Development | Poster Abstract**

**Novel Diagnostic Peptide Epitope Biomarkers for Detection of Recent HIV Infection Using the Europium Nanoparticle Immuno Assay (ENIA)**


**Background:** Early and accurate detection of HIV is critical for epidemiological survey, incidence estimation and targeted intervention of HIV infection. However, current serology assays are limited by poor sensitivity, high false positive and discordance rates leading to misclassification. In addition, genetic variability poses a major challenge for accurate diagnosis. To further develop accurate and sensitive assays that can both identify incident infections and are broadly inclusive for diverse HIV strains, we performed studies to identify epitopes in the HIV p24 proteins that could potentially serve as diagnostic biomarkers for changes in epitope recognition during acute and chronic infection.

**Methods:** Peptides that span the p24 and Gp41 proteins were coated on to microtiter plate wells to capture anti-p24 and anti-gp41 antibodies followed by binding to an anti-human antibody labeled with biotin molecules and streptavidin (SA)-conjugated Eu3+ nanoparticles (NPs) through biotin-SA interaction. After extensive washing between steps to remove unbound or nonspecifically bound conjugates, fluorescence from the resulting complex was directly measured by a fluorometer.

**Results:** HIV positive plasma, minimum of 10 and maximum of 20 samples for both recent and chronic with appropriate negative controls were used. Immune response changes from polyclone-like during acute infection to monoclone-like or non-response to linear epitopes during chronic infection were observed. All 16 peptides spanning the p24 protein reacted with all samples from the acute phase of infection to a significant extent. In contrast, no significant reactivity was observed with samples from chronically infected individuals. A polyclone-like response during acute infection and monoclone-like or non-response during chronic infection was observed using linear epitopes of p24. The peptides reacted with multiple HIV strains indicating cross-clade reactivity. New p24 and gp41 peptide epitopes were identified that distinguish recent and chronic infection.

**Conclusion:** Novel p24 and gp41 antigen epitopes that could serve as diagnostic biomarkers for recent HIV infection have been identified. Together with HIV p24 antigen, the inclusion of appropriate peptide epitopes could enhance the accuracy and specificity of identifying recent HIV infection. The Time Resolved Fluorescence Europium Nanoparticle Immuno assay platform eliminates background fluorescence thus enhancing sensitivity. It will be useful to evaluate other HIV epitopes to improve the sensitivity of the assay to identify recent infection and a suitable testing format for use in a variety of settings. This work will result in new assays for distinguishing recent vs. chronic infection which is currently an unmet public health need in HIV/AIDS. This will contribute to accurate, sensitive specific HIV incidence estimation and thus promote public health through early diagnosis of disease.

**Future Directions:** The whole HIV proteome will be scanned for immunogenic differentiating peptide epitopes for recent and chronic infection to detect earliest immune responses that could serve as specific and accurate biomarkers for incidence estimation assays. The identified differentiating peptide epitopes will be multiplexed on lab-on-a-chip and lateral flow immunoassay platforms, and applied to rapid, point-of-care and laboratory diagnosis of recent/chronic HIV infections in resource limited and other settings. The findings and conclusions in this poster have not been formally disseminated by the Food and Drug Administration.
Assessment of a Novel Lateral Flow Test for the Simultaneous Detection of Recent or Long-term HIV-1 Infection

Timothy Granade, K. Ambrose, B. Parekh and S.M. Owen, Centers for Disease Control and Prevention

Objectives: The monitoring of incident HIV infections is important for targeting prevention efforts. Current assays for detecting recent HIV infections are laboratory-based, which limit their application in resource-poor areas with limited laboratory capability. This study assessed the performance of a rapid, lateral-flow HIV-1 incidence-prevalence (I-P) test for distinguishing recent from long-term infections.

Methods: The rapid I-P test uses an eight-branched gp41-peptide applied at a high concentration for diagnostic detection of HIV antibodies and a multi-subtype recombinant gp41-protein applied at a low concentration to distinguish recent from long-term HIV infections. Long-term infections are identified by antibody reactivity to both antigens, while antibodies present in recent HIV infections are detected only by the high concentration line. Protein A is used as an internal assay control. The evaluation specimen set (Subtype B) included commercial seroconversion panels (n=25, total specimens = 121) (Seracare, Inc.), two long-term HIV antibody-positive longitudinal panels; (Panel 1: n=54, total specimens = 568; Panel 2: n=105, total specimens = 987), and seroconversion panels collected over a 24-month period (n=8, total specimens = 79) and those who were not on antiretroviral therapy. Specificity was assessed with HIV-nonreactive specimens, n = 100, and HIV-2 antibody-positive specimens, n=88. Rapid I-P data were compared to the commercial BED HIV-1 incidence assay and to epidemiologic data.

Results: All 56 HIV-1 positive members of the commercial seroconversion panels collected within 28 days of seroconversion were classified as recent HIV-1 infections by the I-P assay. The rapid I-P test classified 557 of the 568 (98.19%) long-term longitudinal panel 1 specimens as long-term infections using 183 days as the mean duration of recency (MDR). Ten false-recent specimens were in late stage AIDS. Panel 2 had an 89.2% concordance with the BED HIV-1 incidence assay for classifying recent versus long-term infections with discordants skewed slightly to recent by the RIP assay. Specimens collected < 183 days after seroconversion (n=30) were classified as recent infections, and specimens collected = 183 days after seroconversion were classified as long-term infections (n=49) in the 24-month panel. Specificity using the negative and HIV-2 specimens was 100% with no specimens reactive with either antigen line; all tests had valid internal controls.

Conclusion: The rapid I-P test may be applicable in a variety of settings to identify high incidence areas for HIV prevention efforts. More work is needed to compare rapid I-P test results with other tests for recent HIV infection and to establish the MDR.
New LAg-Avidity EIA for HIV Incidence Surveillance

Juehn Shin Maa1, J. Maa1 and M. Su2

1. Maxim Biomedical, Inc.
2. Maxim Biotech, Inc.

Objective: Maxim HIV-1 Limiting Antigen Avidity (LAG-Avidity) EIA Test is a single well in vitro 96-well format enzyme immunoassay that measures the increasing avidity of HIV antibodies after seroconversion. Antibody avidity reflects relative binding strength (Fig. 1a), a functional property, of developing antibodies and is likely to be more robust in detecting recent HIV-1 infections than the assays that detect levels of antibodies, which is a passive parameter. Newly (<6 months) acquired HIV-1 infections are usually seen to have lower avidity HIV IgG in comparison to their long-term counterparts. The Maxim LAg-Avidity EIA Test is to be used for surveillance purposes only. For example, in population incidence estimates to aid in prevention programs, targeting resources, monitoring and evaluation, and identifying high-risk cohorts for prevention research, which include vaccine trials.

Methods: Multiple lots of LAg-Avidity EIA kits were manufactured by Maxim and evaluated by both Maxim and CDC for its performance. A standard of procedure was followed by both sites (Fig. 1b). Controls and samples were diluted 1:101 with sample buffer. 100 µl of controls and samples were incubated with limited quantity of antigen rIDR-M coated EIA plate for 60 minutes at 37 °C. After washing, 100 µl of dissociation buffer was added and further incubated for 15 minutes at 37 °C. After washing, 100 µl of goat anti-human IgG-HRP was added and incubated for 30 minutes at 37 °C. After another washing, 100 µl of TMB substrate was added and incubated for exact 15 minutes at 25 °C and then stop the reaction by adding 100 µl of stop solution. The result was obtained by reading the plate at 450 /630 nm EIA reader.

Results: We have tested multiple panels from CDC (700 samples) and SeraCare (Panel 601- 15 samples) by performing intra-lot comparison (one operator, multiple runs), inter-operator comparison (one lot, multiple operators), and inter-lot comparison (multiple lots, one operator). The statistic analysis of data indicated the new kit with multiple improvements is predictive of recent HIV-1 infection as the original developed assay from CDC.

Conclusion: The new assay kit gives the same predictive result as the original developed method with the following conclusions:

<table>
<thead>
<tr>
<th>Maxim LAg</th>
<th>Recent</th>
<th>Long-Term</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent</td>
<td>79</td>
<td>4</td>
<td>83</td>
</tr>
<tr>
<td>Long-Term</td>
<td>3</td>
<td>614</td>
<td>617</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>618</td>
<td>700</td>
</tr>
</tbody>
</table>

Concordance 99.0%
Kappa 0.952
2. Concordance (R2 values) between operators ranged from 0.97-0.99 for OD and 0.96-0.99 for ODn.
3. Lot to Lot had a R2 = 0.9944.
4. Coating Consistency - A total of 3 plates across lots had an overall %CV of 5.95

The following are unique features modified from original CDC methods:

1. All components are stable at 2-8°C eliminating frozen package.
2. Color code buffers (sample diluent, dissociation buffer) make easy visible identification of sample processing.
3. The specific features comparison between BED and LAg-Avidity EIA are:

<table>
<thead>
<tr>
<th>Feature</th>
<th>BED</th>
<th>LAg-Avidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Technology Developer</td>
<td>CDC approved</td>
<td>Maxim optimized/CDC approved</td>
</tr>
<tr>
<td>Antigen Used</td>
<td>Synthetic BED peptide</td>
<td>Multi-subtype rIDR-M protein</td>
</tr>
<tr>
<td>HIV Subtype Recognized</td>
<td>Major variants of all HIV-1 subtypes</td>
<td>Major variants of all HIV-1 subtypes</td>
</tr>
<tr>
<td>Assay Principle Based</td>
<td>HIV specific IgG/ total IgG</td>
<td>Limiting antigen avidity</td>
</tr>
<tr>
<td>Incidence Window Period</td>
<td>Variable on subtypes</td>
<td>Uniform on subtypes</td>
</tr>
<tr>
<td>Stable of Kit Components</td>
<td>Controls and conjugate stable at -20°C / Other components stable at 2~8°C</td>
<td>All components stable at 2~8°C</td>
</tr>
<tr>
<td>Color Coded Buffers</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Volume Used/ Procedure</td>
<td>100~200 µL dependent on procedure step</td>
<td>All 100 µL throughout procedure steps</td>
</tr>
<tr>
<td>Total Assay Time</td>
<td>~ 4 hours</td>
<td>~ 2 hours</td>
</tr>
<tr>
<td>Misclassification</td>
<td>High, &gt;2-3%</td>
<td>Low, &lt;1%</td>
</tr>
</tbody>
</table>

Fig. 1A Antibody Avidity Principle Fig. 1B One-well LAg-Avidity EIA Assay Principle
CEPHIA: A Specimen and Data Repository for Independent Evaluation of HIV Incidence Assays


Objective: To estimate HIV incidence from cross sectional survey samples, assays have been developed to distinguish recent from longstanding HIV infections. Previous assays have suffered from both lack of standardization of the “mean duration of recency” (window period), and high rates of “false recent” misclassification. The Collaborative for Evaluation of Performance of HIV Incidence Assays (CEPHIA) was charged in 2011 by the WHO Working Group on HIV Incidence Assays and Bill and Melinda Gates Foundation to independently evaluate single assays and algorithms for recent HIV infection.

Methods: Collaborations were developed between CEPHIA and cohort partners to collect substantial numbers of blood plasma specimens with specific characteristics, representing all major HIV subtypes. To facilitate the planned multi-assay comparative evaluation, specimens were only sought that had >10 mL available and all were sub-aliquoted into multiple replicates of 100 uL. Associated data also collected included dates of seroconversion, CD4 count, HIV subtype and viral load.

Results: The repository consists of 1) A “Long Seroconversion Series” set (n=2139, Ab seroconversion date known <90d) to estimate each assay’s “mean duration of recency”; 2) A “False Recent Rate” set (n=1365; seroconversion date known <90 d and infected > 365 d); and 3) “False Recent Challenge” specimens (n=422, including elite controllers, patients with advanced AIDS and ART related sero-reversion). Evaluation panels comprised of blinded specimens from the above sets have been finalized and are being deployed to CEPHIA laboratories (HPA-London; BSRI-San Francisco) for evaluation of existing predicate assays. In addition to the specimen sets described above for the planned multi-assay evaluation, smaller (n=250) “Qualification panels” have been used by CEPHIA laboratories and partner laboratories (ISS-Rome; JHU-Baltimore; CDC/DHAP-Atlanta) for blinded evaluations of candidate assays in order to “qualify” them for inclusion in the larger multi-assay evaluation. Small (n=50) “Developmental panels” are also available to assay developers for blinded or unblinded evaluations of assays still in development.

Conclusion: The CEPHIA repository represents a new resource available to interested researchers, programs and industry, to catalyze more rapid development and deployment of HIV incidence assays, and to accomplish a rigorous, independent evaluation of assays and assay algorithms that are proposed for use in the field.