HIV and Syphilis Diagnosis at DSHS Laboratory

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Current HIV Testing Algorithm - DSHS

Specimen Type

- Serum
  - 3rd gen
  - HIV-1,2+ 0
    - Repeat Reactive in Duplicate
      - ++ / +-
      - Western Blot (Bio-Rad)
        - HIV-1 Ab +
        - HIV-1 Ab Ind
        - Beginning Jan 2010
          - NAAT - Dallas
            - Serum Only

- Discontinue
  - Feb 2011

- DBS
  - rLAV EIA
    - ++ / +-
    - Western Blot
      - (OraSure)
      - HIV – 1 Ab +
      - HIV – 1 Ab Ind
      - HIV – 1 Ab NR

- Oral Fluid WB
  - Confirmation Only

- HIV–1 Ab +
  - HIV–1 Ab NR
## Change in Availability of EIAs

<table>
<thead>
<tr>
<th>Test</th>
<th>FDA Approval Date</th>
<th>% Used by PHL Labs, 2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vironostika HIV-1 Microelisa</td>
<td>1987</td>
<td>58%</td>
</tr>
<tr>
<td>Abbott HIVAB HIV-1/2</td>
<td>1992</td>
<td>11%</td>
</tr>
<tr>
<td>Genetic Systems rLAV</td>
<td>1998</td>
<td>11%</td>
</tr>
<tr>
<td>Gen Sys HIV-1/HIV-2</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>Gen Sys HIV-1/2 Plus O</td>
<td>2003</td>
<td>20%</td>
</tr>
<tr>
<td>Siemens 1/O/2 eHIV</td>
<td>2006</td>
<td></td>
</tr>
<tr>
<td>Ortho Vitros Anti-HIV 1+2</td>
<td>2008</td>
<td></td>
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</tbody>
</table>

- **viral lysate EIA**
- **2nd generation EIA**
- **3rd generation, IgM-sensitive EIA**

Stolen from presentation by Dr Branson, CDC
Current Assays with 15 Seroconverter Panels

Days before Western blot positive when 50% of Specimens Reactive

185 specimens from 15 seroconverters
- Owen et al, J Clin Microbiol 2008

Stolen from presentation by Dr Branson, CDC
Detection of HIV by Diagnostic Tests

- Symptoms
- HIV RNA
- p24 antigen
- HIV EIA*
- Western blot

Weeks Since Infection

0 1 2 3 4 5 6 7 8 9 10

*3rd generation, IgM-sensitive EIA
*2nd generation EIA
*viral lysate EIA

After Fiebig et al, AIDS 2003; 17(13):1871-9

Stolen from presentation by Dr Branson, CDC
Why changing technologies?

~10% of infected persons at the highest risk of transmission are not detected by Antibody tests
Stolen from presentation by Dr Branson, CDC
Window Period & Acute HIV Infection

HIV RNA (plasma)

HIV p24 Ag

HIV Antibody

Acute HIV Infection

Stolen from presentation by Dr Branson, CDC
• 4th Generation Immunoassays – Ag/Ab combo
  – Abbott (Architect)
  – Bio-Rad (Evolis?)
  – Ortho

When? End of 2010 or early 2011?

(Abbott (AxSym-Canada) approved test)
A1: 4th generation HIV-1/2 immunoassay - Proposed

A1+

- A1+
  - Negative for HIV-1 and HIV-2 Antibodies and p24 Ag
  - Initiate care

A1(-)

HIV-1 / HIV-2 discriminatory immunoassay

HIV-1 +
- HIV-1 antibodies detected
  - Initiate care (and viral load)

HIV-2 +
- HIV-2 antibodies detected
  - Initiate care

HIV-1 & 2 (-)
- NAAT
  - NAAT+
    - Acute HIV-1 infection
      - Initiated care
  - NAAT (-)
    - Negative for HIV-1
A1: 4th generation HIV-1/2 immunoassay - Suggested

- **A1+**
  - A2

- **A1(-)**
  - Negative for HIV-1 and HIV-2 Antibodies and p24 Ag

**Western Blot**

- **HIV-1 +**
  - HIV-1 antibodies detected
  - Initiate care (and viral load)

- **HIV-1 -**
  - HIV-1 Ab Ind/NR
  - HIV-2? MS for HIV-2

- **HIV-1 & 2 (-)**
  - NAAT
    - **NAAT+**
      - Acute HIV-1 infection
      - Initiated care
    - **NAAT (-)**
      - Negative for HIV-1
By March 2011

**Avioq EIA HIV-1**

流向图：

- **Oral Fluid**
  - Repeat Reactive in Duplicate
    - **++ / + -**
      - Western Blot (OraSure)
        - **HIV-1 Ab + Initiate care & Viral Load**
        - **HIV-1 Ab Ind**
        - **HIV-1 Ab Neg**
    - **- -**

- **DBS**
  - Repeat Reactive in Duplicate
    - **++ / + -**
      - Western Blot (Bio-Rad)
        - **HIV-1 Ab + Initiate care & Viral Load**
        - **HIV-1 Ab Ind**
        - **HIV-1 Ab Neg**
    - **- -**

**Validate for Serum** (Back-up for testing)
Rapid Testing Algorithm Suggested

Rapid Testing (POC at sites)

- **HIV-1+**
  - Western Blot
  - Ab+ Report HIV-1
  - Initiate care & Viral Load

- **HIV-1-**
  - HIV-1 Ab Ind/Neg
  - (Ag+ or HIV-2? MS 2?)
  - NAAT
  - FP ?
    - NAAT- Neg for HIV-1 or HIV-2/FP+ Very rare
  - NAAT+
    - Acute (Initiate care)
Diagnosis of Syphilis

• **Direct method**
  – Darkfield microscopy
  – DFA
  – PCR

• **Culture (rabbit testes inoculation)**

• **Serology**
  – Non-treponemal tests
    • RPR, VDRL, TRUST
  – Treponemal tests
    • TPPA, FTA-ABS, TPHA, IgG/M EIA, CLIA, Luminex Technology
Current Syphilis Serology Algorithm

Non-treponemal
RPR

Pos / Titer

Neg

Treponemal test - Confirmation

TP-PA +

TP-PA -

FTA-ABS

If TP-PA +/- Inc, RPR>16/TP-PA- or special request
New Serological Testing Algorithm

**Implementation of New Testing Algorithm**

- Initial EIA treponemal test (N = 116,822)
  - POSITIVE: 6,587 (6%)
  - NEGATIVE: 110,235 (94%)
- RPR
  - POSITIVE: 2,884 (2%)
  - NEGATIVE: 3,664 (3%)
  - (false+, past treated, very early/late)
- Second treponemal test (2,512)
  - POSITIVE: 2,079 (83%)
  - NEGATIVE: 433 (17%)

*MMWR 2008;57:872*
Proposed Syphilis Serology Algorithm

Treponemal Test
Bioplex-2200 / EIA

Pos

Neg

Non-treponemal test - titer

RPR +

RPR -

TP-PA +

TP-PA -
Future of HIV Diagnostic Testing

- 4th Generation Ag/Ab Combo EIA

- SAMBA-Simple amplification-based nucleic acid test for HIV-1, Dipstick-based visual detection

- MICT-Magnetic Immuno-Chromatography Testing for rapid HIV-1 p24 Ag

- Rapid confirmation for HIV-1/HIV-2 gp105 band-Brazil

- Next generation POC-handheld microfluidic based

- Battery powered instrumentation for reading
Diagnostics for the Real World:
SAMBA Device and Point of Care Machine

Simple technology
- Sample preparation module in development
- Cartridge with breakable seals
- Isothermal NA amplification ~1 hr
- Dipstick-based visual detection

Front view

SAMBA (Simple AMplification BAased nucleic acid test) machine

Rear view

Lee et al. J. Infect. Dis., April supplement 2010
Advanced Liquid Logic

Digital microfluidics
- Cartridge is fabricated using low-cost printed-circuit-board technology
- No pipes, pumps or valves
- Discrete droplets are manipulated electrically (electrowetting) within an oil-filled cartridge
- Use whole blood with a magnetic bead capture protocol
WAVE 80 Biosciences

L. Mazzola: Poster #40

- Continuous-flow microfluidics
- Onboard lyophilized reagents
- No fluid exchange
- Disposable
- Finger-stick sampling
- Licensed microchip and assay technology
- Wave 80 proprietary IP

Branched DNA-like Nucleic Acid Signal Amplification

- Fully sensitive signal amplification
- No risk of amplifying non-targeted RNA
- No temperature or stability issues

- Flexible instrument design
- Luminescent readout
- Robust operation
- Low maintenance
POC Technology Pipeline

**Near POC**
- Tabletop assays using finger-stick blood will be evaluated in clinical trials over the next year (DAIDS can assist in evaluation)

**Next generation POC**
- Handheld microfluidic-based battery powered assays require an additional year of development before clinical trials

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**Other Potential Products**
- Inverness
- iQuum
- Northwestern
- CDC
- Thermal Gradient
- BioHelix
Flow-Through Real-Time PCR

60 °C  

MRSA Titration

Hua et al., Analytical Chemistry, 2010
Multiplex POC Test for Confirmation of HIV 1&2

Chembio Diagnostic Systems, INC.-The Dual Path Platform (DPP®) Technology
DPP–Syphilis Screen & Confirmation
Multiplex features

- **Multiplexing is**
  - The ability to perform and record multiple reactions occurring simultaneously in a single reaction vessel

- **Multiplex features include:**
  - Improve workflow efficiencies
    - Generate up to 22 simultaneous results from a single sample
  - Incorporate real-time assay integrity checks
  - Warehouse and retrieve unordered results
    - Reduces need to collect and run second patient sample
8 micron beads
  Electron micrograph of pre-dyed beads

3 coating layers
  Each bead is coated with three distinct layers, internal latex core, iron crystal coating and external reactive surface

Dying process
  Each bead is dyed with a ratio of yellow and red dye to create a distinct bead color. Each colored bead is unique to its respective analyte
Addition of patient sample
If antibodies are present in patient sample, they bind to their respective bead antigen in the reaction vessel.

Washing step
Two wash steps are employed to eliminate unbound material. First after addition and incubation with primary sample and second after addition and incubation of conjugate.

Addition of conjugate
Conjugate is added after first wash step and incubated. Second wash step removes unbound conjugate material.
Multiplex detection

Beads aspirated into probe
Aliquot of bead solution from reaction vessel is aspirated into the flow cell

Beads lined up in single file
Beads move single file through a flow cytometer

Each bead subjected to two lasers
Red or classification laser determines which bead is present
Green or reporter laser determines if bead is negative or positive and level of intensity for positive beads

Characterizes 150 beads
The BioPlex 2200 classifies a minimum of 150 beads from each analyte prior to making a determination
Multiplex IQ (internal quality) beads

Employ 3 IQ beads with each specimen
To ensure assay integrity for each patient result

Serum Verification Bead
SVB confirms that the sample is plasma or serum, flags for short sample and sample dilution

Internal Standard Bead
Real time measurement to standardize detector voltage fluctuation

Reagent Blank Bead
Identifies samples with non-specific binding antibodies
BioPlex-2200

- **Advantages**
  - CDC recommendation of new syphilis algorithm
  - Advanced technology with interfacing to LIS
  - Random access platform
  - Avoid transcription error thru automation
  - Limited biohazard exposure
  - Reduced TAT
  - Workflow advantage and savings?
  - BioPlex expandable serology menu
  - Space saving – no need of old EIA equipments

- **Disadvantages**
  - Cost
  - New algorithm interpretation?
BioPlex 2200 Current Menu

510(k) cleared

- **Syphilis IgG**
  - r15, r17, r47
- **ToRC IgG/IgM**
  - Toxoplasma
  - Rubella
  - CMV
- **MMRV IgG**
  - Measles
  - Mumps
  - Rubella
  - Varizella zoster

**HSV 1 and 2**
- HSV 1
- HSV 2
BioPlex 2200 Future Menu

• Other Panels –2011 +
  – HIV Combo (Ag/Ab)
  – Hepatitis A, B, C Panels
  – Lyme Panel

  – Cardiac Damage
  – Cardiac Risk Assessment
  – Diabetes (Hemoglobin A1c)
  – Urine Toxicology
  – Gastrointestinal
  – Phospholipids
# Future Workflow at DSHS Lab

<table>
<thead>
<tr>
<th>Current</th>
<th>Serology</th>
</tr>
</thead>
<tbody>
<tr>
<td>STD</td>
<td>VPD</td>
</tr>
<tr>
<td>Syphilis, HIV, Hep C</td>
<td>MMRV, Hep B &amp; A</td>
</tr>
<tr>
<td>RPR, TP-PA, IFA, and EIA</td>
<td>EIA, IFA</td>
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| 2010-2011 BioPlex | Syphilis IgG | MMRV - IgG |
| 2011-2012 BioPlex | Hep C, Syphilis IgM | R - IgM, MM - IgM? | Toxo/CMV IgG, IgM |
| 2012-2013 BioPlex | HIV-1/2 Ag/Ab | Hep B & Hep A | Lyme, Arbo? as needed? |

<table>
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<tbody>
<tr>
<td>Syphilis</td>
</tr>
<tr>
<td>HIV</td>
</tr>
<tr>
<td>Hep C</td>
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HSV 1 and 2 IgG ???