Point of Care
Molecular Diagnostics

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Henry Jackson Foundation

Vaccine Research Program, Division of AIDS, NIAID, NIH
Vaccine Specific Issue

1) Advantages of rapid tests
   a) Quick (loss to follow-up)
   b) Easy (minimal infrastructure)
   c) Cost ↓

2) Antibody-based tests will not be effective
   a) Vaccine-induced seropositivity (VISP)
   b) React positively with licensed diagnostic tests (rapids, EIA, WB)
   c) Potential to unblind
   d) Can be present >10 yrs
Vaccine Diagnostic Needs

**SELECTest**
- Being developed by Westat® for blood donors (NHLBI)
  - Able to differentiate true HIV infection from VISP

- Identify conserved sequences in p6 and gp41
  - Recognized soon after infection (similar sensitivity to 3rd gen)
  - Do not contain protective epitopes
  - Are not part of most current HIV vaccines in development

*Alternatively: directly detect virus*
- Eliminate the chance of a future vaccine containing the same epitopes as the diagnostic assay
- Nucleic acid testing (NAT)
  - Efficacy trials are performed in resource-limited settings
  - NAT requires expensive equipment, reagents and highly skilled technicians with sufficient infrastructure to support testing
- Need for simple, affordable and robust molecular point-of-care diagnostic device
Beyond Diagnostic Needs of Vaccines

**Additional applications for POC NAT**

- Early infant diagnosis (maternal antibodies)
- Identify acutely infected (RNA+/Ab-) individuals (window phase highly viremic)
- Test and treat (limit transmission, reduce TB co-infections)
- Monitor for therapeutic efficacy (compliance and viral DR)
- Monitor for infection in PrEP (low dose treatment may accelerate DR)
- Disaster readiness (blood transfusions in earthquake)
- Genuine desire to do good
- Identified medical need
- Matched technology

"Valley of Death"

FUNDING GAP

- Product launch
- Access to markets

**Venture Capital**
Difficult to convince investing in a product with the intended use in disadvantaged and vulnerable populations in the poorest settings
BAA: Contract mechanism

BAA: Broad Agency Announcement (peer reviewed)
- Government identifies research area and specifications
- Offeror responds with the statement of work (SOW)

Three contracts were awarded September 2009
- Advanced Liquid Logic (5.2M)
- Diagnostics for the Real World (4.7M)
- Wave 80 Biosciences (7.5M)

Scope of work to be funded: All phases of technology development, product development, validation except:
  1. basic research on core POC platform
  2. Phase I, II, or III clinical trials

Anticipated timeline: 3-5 year program
- Technology/product development
- Analytical/pre-clinical studies
- Clinical studies
**BAA: Performance & Operational Characteristics**

<table>
<thead>
<tr>
<th><strong>Sample</strong></th>
<th>type</th>
<th>whole blood (plasma...)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>volume</td>
<td>100-200 uL</td>
</tr>
<tr>
<td></td>
<td>preparation</td>
<td>1-3 steps</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Assay</strong></th>
<th>LOD</th>
<th>200-1000 copies/mL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>sensitivity</td>
<td>90-95%</td>
</tr>
<tr>
<td></td>
<td>specificity</td>
<td>99.5-99.9%</td>
</tr>
<tr>
<td></td>
<td>subtypes</td>
<td>M,N,O</td>
</tr>
</tbody>
</table>

| **Diagnostic** | time-to-result | 90-120 minutes |
|                | shelf life at 37 C| 12-24 months   |
|                | humidity         | 70 %           |
|                | transportation stress | 50 C for 48-72 hours |

| **Controls** | negative            | full process negative |
|             | positive             | internal positive     |

| **Biosafety** | containment         | closed, self-contained system |
|               |                     | no biosafety cabinet required |
|               |                     | unprocessed sample transfer only, |
|               |                     | no open handling of material |

| **Instrument** | handheld            | portable               |
|                | power requirements  | battery powered        |

| **Reporting** | interface           | LED readout, electronic data transfer |
|               |                     | flexible database architecture: capture, store, integrate |

| **Training**   | community health worker | < 1 hr |
|                | high school diploma    | < 8 hrs |

| **Cost**       | per test result       | $12 - $20 USD |

Balancing the Needs

WHO guidelines (2006)
- Do not recommend the routine use of VL testing for diagnosing treatment failure due to the high cost and feasibility (currently being revised) in resource-limited settings
- Affordable molecular diagnostics which reduce loss to follow up will allow health care workers to monitor patient compliance and viral drug resistance
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

SAMBA (Simple AMplification BAased nucleic acid test) machine

Lee et al. J. Infect. Dis., April supplement 2010
Performance of SAMBA detection of HIV-1 in clinical samples (Barts hospital, London)

- SAMBA detected 189 of 191 HIV positive samples

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No. samples (%)</th>
<th>Viral load</th>
<th>No. SAMBA +</th>
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<tbody>
<tr>
<td>A</td>
<td>31 (16.3)</td>
<td>214 - 5x10⁵</td>
<td>31/31</td>
</tr>
<tr>
<td>B</td>
<td>25 (13.2)</td>
<td>78 - 6x10⁶</td>
<td>24/25</td>
</tr>
<tr>
<td>C</td>
<td>30 (15.8)</td>
<td>278 - 6x10⁵</td>
<td>30/30</td>
</tr>
<tr>
<td>D</td>
<td>9  (4.8)</td>
<td>7x10³ - 1.8x10⁵</td>
<td>9/9</td>
</tr>
<tr>
<td>F</td>
<td>7  (3.8)</td>
<td>268 - 6x10⁴</td>
<td>7/7</td>
</tr>
<tr>
<td>G</td>
<td>5  (2.7)</td>
<td>937 - 5.7x10⁴</td>
<td>5/5</td>
</tr>
<tr>
<td>H</td>
<td>1  (0.6)</td>
<td>526</td>
<td>1/1</td>
</tr>
<tr>
<td>J</td>
<td>4  (2.2)</td>
<td>7x10³ - 2x10⁵</td>
<td>4/4</td>
</tr>
<tr>
<td>K</td>
<td>5  (2.7)</td>
<td>1x10³ - 2x10⁴</td>
<td>5/5</td>
</tr>
<tr>
<td>Recombinants</td>
<td>74 (38.8)</td>
<td>54 - 4x10⁷</td>
<td>73/74</td>
</tr>
<tr>
<td>Negatives</td>
<td>225</td>
<td>0/225</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = 98.95% (189/191)  Specificity 100% (0/225)
Highly sensitive signal amplification
No risk of amplifying non-targeted RNA
No temperature or stability issues

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

- Flexible instrument design
- Luminescent readout
- Robust operation
- Low maintenance

Branched DNA-like Nucleic Acid Signal Amplification

- signal amplifying probe complex
- high-specificity label probe
- viral RNA target capture probe

L. Mazzola: Poster #40
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
Flow-Through Real-Time PCR

MRSA Titration

Hua et al., Analytical Chemistry, 2010
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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