NEW HIV IMMUNOASSAY RESOLVES VACCINE INDUCED SEROPOSITIVITY IN HIV VACCINE RV144 AND HVTN204 TRIAL PARTICIPANTS

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HIV Vaccine Trials: How to distinguish between antibodies due to vaccination and HIV infections

- HIV/AIDS Vaccine Trials (HIV affects ~ 35 million people worldwide):
  - More than 40 HIV vaccine candidates have been tested in human studies
  - Some HIV vaccine candidates show some progress in prevention of infection and for reducing disease after infection
  - Thousands of individuals need to be recruited into major efficacy trials to support vaccine licensure

- Vaccinees may show positive results in licensed HIV-1 detection assays due to VISP (antibody responses due to vaccination). According to recent data\(^1\), the rate of VISP demonstrated by FDA licensed EIA tests was 41.7% overall for uninfected subjects who were recipients of various HIV vaccines.

\(^1\)Vaccine-Induced HIV seropositivity/reactivity in noninfected HIV Vaccine Recipients, Cooper et al. JAMA, 2010; 304(3): 275-283
VISP – Vaccine Induced Sero-Positivity

- Vaccine recipients that are falsely diagnosed as ‘HIV INFECTED’ suffer a range of social and economic harms (employment, insurance, recruitment to the armed forces, travel, immigration, blood donations).

- VISP deters people from enlisting in HIV vaccine trials: e.g., 8% of Seattle MSM at a Step study screening appointment did not enroll in the HIV vaccine trial because of concerns about VISP.

- **HIV-Selectest** is a novel HIV Diagnostic test that is being developed to differentiate antibody responses due to vaccination from antibodies produced due to true HIV infection; it measures antibody responses to virus components not included in HIV vaccines.

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4 Vaccine Induced Seropositivity: Seattle HVTU VISP Pilot Project, Mark et al. [www.hvtn.org/meeting/ppt/may09/3/Mark.ppt](http://www.hvtn.org/meeting/ppt/may09/3/Mark.ppt)
HIV-Selectest assay concept and design

- Initially developed by Khurana & Golding (J. Virol 80;2092-99, 2006) as combinatorial approach with two-well format.

- Gene-Fragment Phage Display Library was constructed from entire HIV-1 cDNA genome and screened with sera from seroconverting HIV-infected individuals.

- Three novel early immunodominant epitopes from Gag and envelope gp41 cytoplasmic tail regions not present in most vaccine candidates were selected.

- Consensus multiclade sequences were designed for epitopes gp41-1, gp41-2 and p6-1; in addition, p6-2 was designed as a clade specific sequence for clade C.
Selectest Assay Evolution

• Immunetics was engaged as contractor under NHLBI REDS-II program to convert original HIV Selectest to commercial, validated kit for use in vaccine trials.

• Expanded evaluation led to changes in peptide composition to improve sensitivity and specificity

• Additional antigenic peptides targeted to improve detection of individual clades (clade-specific peptides)

• Modified antigen presentation to increase assay’s capacity for additional peptides and reactivity with serum antibodies; all peptides combined in one well

• Assay chemistry optimization to improve signal/noise ratio and reduce false positives
The assay is configured in a standard indirect ELISA format, in which HIV-specific antibodies in the serum sample are captured by binding to peptide antigens immobilized in the microplate well.
HIV-Selectest current version uses 5 synthetic HIV peptides

• Clade specific peptides were designed using most frequent mutations observed in Clade B or Clade C sequences from Los Alamos HIV database (http://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html) identified using QuickAlign tool. Best performing clade-specific peptides were selected experimentally from a pool of 2-5 peptides with similar mutation frequencies.

• The peptides were from non-optimal epitopes, not usually included in HIV vaccine candidates.

• Current peptide composition of HIV Selectest:
  - Consensus peptides from gp41 envelope sequence
  - Clade specific peptides from gp41 for clades B and C
  - Peptide sequences from non-envelope genes
HIV-Selectest clearly distinguishes HIV positive vs. vaccinee or control sera

648 HIV-1 positives, 400 normal donors and 544 HIV-negative vaccine trial participants from HVTN204 and RV144 trials tested with HIV-Selectest

- **HIV-1 Positive (False-negative - in red)**
- **Normal blood donors**
- **HIV-1 Negative Vaccine trial participants (False-positive - in red)**
## Selectest assay Performance: Vaccine recipients and controls

<table>
<thead>
<tr>
<th>Immunization status</th>
<th>Uninfected vaccine trial participants (# Pos/Total)</th>
<th>Normal donors (# Pos/Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RV 144</td>
<td>HVTN 204</td>
</tr>
<tr>
<td>Pre-immune</td>
<td>1 /120</td>
<td>n/a</td>
</tr>
<tr>
<td>Placebo (from peak response date)</td>
<td>0 /70</td>
<td>0 /92</td>
</tr>
<tr>
<td>Vaccine recipients (peak response date)</td>
<td>1 /170</td>
<td>0 /92</td>
</tr>
<tr>
<td>Vaccine recipients (non-peak)</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Total</td>
<td>2 /360</td>
<td>0 /184</td>
</tr>
<tr>
<td>Specificity* based on combined samples (* point estimate)</td>
<td>99.4%</td>
<td>100%</td>
</tr>
</tbody>
</table>
## Selectest assay Performance: HIV positive serum panels

### Sensitivity of Selectest for HIV sera by clade

(serum panels from HIV infected individuals)

<table>
<thead>
<tr>
<th>Presumed Clade of infection</th>
<th>Clade A (Rwanda)</th>
<th>Clade B (U.S.A.)</th>
<th>Clade C (S. Africa)</th>
<th>Global panels (clades A, B, C, D, E, F, F, G, J, O, ut)</th>
<th>HIV positive panels total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected Positive</td>
<td>100 of 100</td>
<td>192 of 200*</td>
<td>265 of 267</td>
<td>80 of 81</td>
<td>637 of 648</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>&gt;96.0%</td>
<td>99.3%</td>
<td>98.8%</td>
<td>98.3%</td>
</tr>
</tbody>
</table>

*Two of the 200 clade B samples tested HIV negative by the licensed Bio-Rad assay and by Selectest, and may have been incorrectly categorized as HIV positive in the original panel.
Selectest assay Performance: Infected vaccine/placebo recipients

<table>
<thead>
<tr>
<th>Patient status</th>
<th>Sensitivity of Selectest for HIV positive sera from infected VAX003 and VAX004 vaccine trial participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV infected VAX003 &amp; VAX004 trial participants (clades B and B/E)</td>
<td>97.0%</td>
</tr>
<tr>
<td>Placebo recipients</td>
<td>101 of 105</td>
</tr>
<tr>
<td>Vaccine recipients</td>
<td>156 of 160</td>
</tr>
<tr>
<td>Sensitivity based on combined samples</td>
<td>97.0%</td>
</tr>
</tbody>
</table>
Detection of Early Infections

HIV-Selectest vs. Vitros-LS test of early infections

Extremely early infection $S/CO < 4$

Very early infection $S/CO 4-10$

Early infection 105-153 days $S/CO 10-15$

Early infection 153-215 days $S/CO 15-20$
Detection of Samples with Low Viral Load

HIV Selectest detects samples with very low viral load in established HIV patients, including 83% of samples with undetectable viral load*

*Roche Amplicor HIV-1 Monitor Test, Roche Molecular Systems, Inc. Pleasanton, CA or Abbott RealTime HIV-1 Amplification Kit, ABBOTT Molecular, Inc Des Plaines, IL or COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, Roche Molecular Systems, Inc Pleasanton, CA

HIV Selectest detects samples with low viral load in asymptomatic blood donors diagnosed with HIV**

**Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 quantitative assay.
## PPV/NPV: HIV Selectest vs. Licensed HIV ELISA

### Performance of Test in Vaccine Recipient Population *

<table>
<thead>
<tr>
<th>Test</th>
<th>HIV % infected</th>
<th>Sensitivity in Vaccine Recipients</th>
<th>Specificity in Vaccine Recipients</th>
<th>Positive Predictive Value (PPV)†</th>
<th>Negative Predictive Value (NPV)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbot HIV 1/2 (rDNA) EIA</td>
<td>0.7%</td>
<td>99.9%</td>
<td>59.1%</td>
<td>1.69%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Bio-Rad rLAV EIA</td>
<td>0.7%</td>
<td>100.0%</td>
<td>78.6%</td>
<td>3.19%</td>
<td>100.00%</td>
</tr>
<tr>
<td>BioMerieux HIV-1 Plus O Microelisa</td>
<td>0.7%</td>
<td>100.0%</td>
<td>85.3%</td>
<td>4.58%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Bio-Rad HIV-1/2 peptide &amp; HIV-1/2 Plus O</td>
<td>0.7%</td>
<td>100.0%</td>
<td>91.2%</td>
<td>7.42%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Current HIV Selectest EIA</td>
<td>0.7%</td>
<td>98.3%</td>
<td>99.5%</td>
<td>58.09%</td>
<td>99.99%</td>
</tr>
</tbody>
</table>

*Vaccine-Induced HIV seropositivity/reactivity in noninfected HIV Vaccine Recipients, Cooper et al. JAMA, 2010; 304(3): 275-283
† PPV = TP/(TP+FP), NPV = TN/(TN+FN)
10,000 vaccine recipients
0.7% incidence in test population
13% very early infections (< day 53)

**FDA-licensed HIV-1/2 screening test**
- 41.7% FP due to VISP
- 99.9% sensitivity, 99.9% specificity
- NPV > 99.98%
- PPV only 1.6%

**HIV Selectest**
- 0% VISP
- 99% sensitivity, 99.5% specificity
- (est. 40% sensitivity for very early infections)
- NPV = 99.9%
- PPV = 75.6%

**HIV Western Blot**
- 99% sensitivity, >99% specificity
- NPV = 99.99%
- PPV > 98.5%

4250 POS (70 TP, 4180 FP)
5750 NEG (5750 TN, <1 FN)

86 POS (65 TP, 21 FP)
4164 NEG (4159 TN, 5 FN)

65 POS (65 TP, <1 FP)
21 NEG (21 TN, <1 FN)
Acknowledgments

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