Results of a Study to Evaluate the Accuracy and Ease of Use of a Rapid, 60 second HIV Antibody Test Performed by Untrained Operators in POC Test Centers in the United States

Cornerstone of the application for CLIA waiver of the INSTI HIV-1 Antibody Test.

R. Galli,
### CLIA Guidelines Have Changed the CLIA Study Landscape

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Number of Operators</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Contrived plasma or whole blood</td>
<td>Sample matrix same as intended use patient samples</td>
</tr>
<tr>
<td>Sample Particulars</td>
<td>panel of 6 blind coded specimens</td>
<td>Prospectively collected fingerstick and venous blood</td>
</tr>
<tr>
<td>Operator-collected specimens</td>
<td>None</td>
<td>N=1388, HIV unknown and known positives</td>
</tr>
<tr>
<td>Comparator Method</td>
<td>Expected Results</td>
<td>Matching venous blood tested by FDA approved laboratory methods.</td>
</tr>
<tr>
<td>Separate Limit of Detection Study</td>
<td>Not required</td>
<td>Required, by intended use operators and laboratory operators</td>
</tr>
</tbody>
</table>
DEMONSTRATING INSIGNIFICANT RISK OF AN ERRONEOUS RESULT – “ACCURACY”

• “To demonstrate that your device is “accurate” in the hands of the intended operator, we recommend that you perform prospective clinical studies of the device proposed for waiver using patient samples collected in the intended testing environment. In this way, the studies will demonstrate, as closely as possible, how the device performs in the hands of intended operators under the conditions of intended use.”
Clinical Study Purpose

• 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.

• Consultative process between the sponsor and CDRH for final study design.
Clinical Study Design...

Controllable Study Elements:

- Site selection: n=3; varied demographics;
- Operator selection: n=11; varied demographics, experience (none with INSTI)
- Statistically relevant sample size
- Subject recruitment, CRF, inclusion/exclusion criteria
- Blinding algorithm
- Comparator methods; Central Lab
- IRB approved study protocol, ICF.
Clinical Study Design

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- Site selection: n=3; varied demographics;
- Operator selection: n=11; varied demographics, experience (none with INSTI);
- Statistically relevant sample size;
- Subject recruitment, ICF, inclusion/exclusion criteria;
- Blinding algorithm;
- Comparator methods; Central Lab;
- IRB approved study protocol, CRF.

Sponsor Dyspepsia-Inducing Elements:

- **NO** operator training or prior INSTI experience, including FS sample collection;
- **NO** use of INSTI competency assessment panels;
- **NO** on site monitoring for correct INSTI procedure;
- **NO** assurances on how long it would take to find the required minimum of 30 de novo HIV positive subjects (assumed prevalence=2%).
PERFORMING THE INSTI HIV-1 ANTIBODY TEST

INSTI HIV-1 Antibody Test Preparation/Setup

For preparation/setup, follow the instructions as described in the Quality Control section (turn over) of this Guide.

Fingerstick Blood Sample Collection

Read these instructions completely before starting fingerstick

1. Clean finger with alcohol swab, allow for alcohol to dry
2. Twist and pull the groove tip of the lancet
3. Place the lancet on the finger gently off center, press firmly until you hear a click to puncture the skin
4. Squeeze the finger to create a bead of blood
5. Place the pipette tip noncontaminantly into the blood bead. Do not squeeze the pipette bulb; press the finger to keep a level of blood forming.
6. To fill the pipette, capillary action will automatically draw the blood to the black fill line
7. Be sure not to cover the air hole between the black line with your fingers
8. Depending on how the subject bleeds, you may need to continuously pulse the finger.

TEST PROCEDURE to follow after sample collection

NOTE: It is important that the following steps be performed immediately after collection and in the sequence as shown:

1. When the pipette is filled, transfer the specimen/plasma into Solution 1 by squeezing the pipette bulb
2. If does not release, cover the air hole on the black line with your finger and squeeze again
3. Recap Solution 1, and insert a new lancet
4. Pour Solution 1 with specimen into the center of the membrane unit well. Allow to absorb completely, then immediately proceed to the next step
5. Pour Solution 2 into the center of the membrane unit well. Allow to absorb completely, then immediately proceed to the next step
6. Pour Solution 3 into the center of the membrane unit well. Allow to absorb completely, then interpret the results

INTERPRETATION OF THE RESULTS: Read results immediately

Results must be interpreted within five minutes of completing the procedure. Make sure the patient identifier tab on the membrane unit is facing towards you.

NON-REACTIVE

Only one blue control spot appears on top

REACTIVE

Two blue spots, upper one control and one test spot
NOTHE: one spot may be slightly thicker than the other

INVALID

Antibody control spot is an INVALID result

A NON-REACTIVE Test Result means that HIV-1 antibodies were not detected in the specimen. The test result is interpreted as negative for HIV antibodies. However, this does not exclude possible infection with HIV.

A REACTIVE Test Result means that HIV-1 antibodies have been detected in the specimen. The test result is interpreted as Preliminary POSITIVE for HIV-1 antibodies. An INVALID Test Result means that the test was run incorrectly or insufficient specimen was added. Repeat the test once with a new sample collection and INSTI test. If the second test result is also invalid, contact technical assistance at 1-888-614-6994

Follow CDC guidelines to inform the test subject of the test result and its interpretation.
## Operator Demographics

<table>
<thead>
<tr>
<th>Operator ID</th>
<th>Site Description</th>
<th>Experience</th>
<th>Education</th>
<th>Use Rapid Tests?</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1-001</td>
<td>Outreach</td>
<td>3 months</td>
<td>BA/BS</td>
<td>Yes</td>
<td>47</td>
</tr>
<tr>
<td>Site 1-002</td>
<td>Outreach</td>
<td>3.5 years</td>
<td>Some college</td>
<td>Yes</td>
<td>30</td>
</tr>
<tr>
<td>Site 1-005</td>
<td>Outreach</td>
<td>1 year</td>
<td>Some college</td>
<td>Yes</td>
<td>33</td>
</tr>
<tr>
<td>Site 1-006</td>
<td>Outreach</td>
<td>10 years</td>
<td>High school</td>
<td>Yes</td>
<td>44</td>
</tr>
<tr>
<td>Site 2-003</td>
<td>Dr. Office</td>
<td>1 yr, 7 mo.</td>
<td>Tech school</td>
<td>Yes</td>
<td>47</td>
</tr>
<tr>
<td>Site 2-005</td>
<td>Dr. Office</td>
<td>7 years</td>
<td>Ph.D</td>
<td>Yes</td>
<td>63</td>
</tr>
<tr>
<td>Site 2-006</td>
<td>DR. Office</td>
<td>1 yr, 9 mo.</td>
<td>Some college</td>
<td>Yes</td>
<td>27</td>
</tr>
<tr>
<td>Site 3-001</td>
<td>CSO</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Site 3-004</td>
<td>CSO</td>
<td>Unknown</td>
<td>BA/BS</td>
<td>Yes</td>
<td>28</td>
</tr>
<tr>
<td>Site 3-008</td>
<td>CSO</td>
<td>12 years</td>
<td>Tech school</td>
<td>Yes</td>
<td>66</td>
</tr>
<tr>
<td>Site 3-009</td>
<td>CSO</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Yes</td>
<td>44</td>
</tr>
</tbody>
</table>
Clinical Study Details

• 3 intended use study sites selected:
  – Medical office, Ft. Lauderdale, FLA
  – HIV/AIDS Community Service Organization, Phoenix, AZ.
  – HIV testing outreach program, Philadelphia, PA.
• First subject enrolled and tested: 2011-09-14
• Last Subject enrolled and tested: 2012-01-04
• Total subjects in per protocol analysis: 1388:
  – 905 high risk with unknown HIV status attending the study site for standard HIV testing
  – 483 known HIV positives
Clinical Study Methods

- Operators (n=11) blinded to subject identity and HIV status
- Each operator required to test minimum of 5 negative and 5 positive subjects.
- INSTI considered an investigational device: results not provided to subjects or used to determine the subject’s HIV status.
- Untrained operators conducted INSTI testing on fingerstick blood from all subjects according to procedure from the PI and QRG.
- Matching venous blood sent to central lab for CM (Siemens ADVIA Centaur HIV-1/2/O, WB).
- Results of INSTI performed by untrained intended use operators were compared to results of CM performed by trained laboratory operators (positive and negative percent agreement).
- Separate Limit of Detection (LoD) Study performed by operators on panels of prepared plasma samples that were at, slightly above and slightly below the positive cut-off.
Clinical Study Results

Measures of Agreement of INSTI performed by Untrained Users with CM performed by Laboratory Professionals, 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>% Positive Agreement</td>
<td>517/517 (100%)</td>
<td>99.48-100%</td>
<td>99.26-100%</td>
</tr>
<tr>
<td>% Negative Agreement</td>
<td>869/871 (99.8%)</td>
<td>99.31-99.92%</td>
<td>99.17-99.94%</td>
</tr>
</tbody>
</table>
Clinical Study Results

Summary of Results of INSTI Compared to Subject HIV Status as Determined by the CM for Prospective, High Risk, HIV Status Unknown Subjects in the Per Protocol Analysis, n=905

<table>
<thead>
<tr>
<th>INSTI RESULT</th>
<th>Subject HIV Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>34 (3.7%)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>36</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>869</td>
</tr>
<tr>
<td>Invalid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>871</td>
</tr>
</tbody>
</table>
## Limit of Detection Study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Percent Reactive</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weakly Reactive 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1:600</td>
<td>88.3% (53/60)</td>
<td>77.8%- 94.2%</td>
</tr>
<tr>
<td>Weakly Reactive 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1:800</td>
<td>80.0% (48/60)</td>
<td>68.2%- 88.2%</td>
</tr>
<tr>
<td>Weakly Reactive 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1:1200</td>
<td>66.1% (39/59)</td>
<td>53.4%- 76.9%</td>
</tr>
<tr>
<td>Weakly Reactive 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1:1600</td>
<td>34.5% (20/58)</td>
<td>23.6%- 47.3%</td>
</tr>
</tbody>
</table>

- **Expected results**<sup>a</sup>: There should be a greater number of INSTI HIV-1 Antibody Test reactive results than non-reactive results.
- **Expected results**<sup>b</sup>: There should be an equal distribution of reactive and non-reactive INSTI HIV-1 Antibody Test results.
- **Expected results**<sup>c</sup>: There should be a greater number of INSTI HIV-1 Antibody Test non-reactive results than reactive results.
Study Outcomes

• Untrained, intended use operators with varied background were able to collect the FS blood and perform the INSTI test correctly by following only the PI and QRG.
• Their results were highly concordant with CM results obtained by laboratory professionals.
• No INSTI false negative results obtained by untrained intended use operators
• No INSTI invalid results obtained by untrained intended use operators
• Two INSTI false positive results from 871 subjects with HIV negative status.
Study Outcomes

- Data from this untrained user study was highly concordant with the 2007/08 clinical study performed by trained operators, for the PMA submission which used the same CM:

<table>
<thead>
<tr>
<th></th>
<th>Fingerstick Blood</th>
<th>CLIA</th>
<th>PMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSTI Sensitivity</td>
<td>100% (517/517)</td>
<td>99.8% (1095/1097)</td>
<td></td>
</tr>
<tr>
<td>INSTI Specificity</td>
<td>99.8% (869/871)</td>
<td>99.5% (1375/1382)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

• 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 POC test settings negligible.

• CLIA waived status was granted for INSTI by FDA-CDRH on July 20, 2012.
Acknowledgements

• **Anthony La Marca MD**, Therafirst Medical Center, Ft. Lauderdale, FLA

• **Lawrence Waldman MD**, Southwest Center for HIV/AIDS, Phoenix, AZ

• **Ron Powers**, Mazzoni Center, Philadelphia, PA.

And..

_Special thanks to all the intended use operators who participated, and the subjects who volunteered for enrollment._