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

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Disclaimer: The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention

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The STOP Study Methods

The STOP Study: Screening Targeted Populations to Interrupt On-going Chains of HIV Transmission with Enhanced Partner Notification

• On-going prospective study evaluating methods to detect acute HIV infection linked to enhanced partner services in New York City, San Francisco, and North Carolina

• Participants were > 12 years and receiving HIV testing at one of 12 HIV testing venues in sexually transmitted infection clinics and community-based HIV testing programs
The STOP Study Methods

The STOP Study: Screening Targeted Populations to Interrupt On-going Chains of HIV Transmission with Enhanced Partner Notification

- (I guess better than the “STPTIOCHTEPN” Study….)

- On-going prospective study evaluating methods to detect acute HIV infection linked to enhanced partner services in New York City, San Francisco, and North Carolina

- Participants were ≥ 12 years and receiving HIV testing at one of 12 HIV testing venues in sexually transmitted infection clinics and community-based HIV testing programs
The STOP Study Methods

The STOP Study: **S**creening **T**argeted Populations to Interrupt **O**n-going Chains of HIV Transmission with Enhanced **P**artner Notification

- On-going prospective study evaluating methods to detect acute HIV infection linked to enhanced partner services in New York City, San Francisco, and North Carolina
- Participants were > 12 years and receiving HIV testing at one of 12 HIV testing venues in sexually transmitted infection clinics and community-based HIV testing programs
HIV Testing Performed

• Screening with the Architect (Abbott), an HIV-1/HIV-2 combination Antigen/Antibody (Ag/Ab) immunoassay

• Specimens with repeatedly reactive Architect results were tested with Multispot and either an HIV-1 Western blot (Bio-Rad) or an immunofluorescence assay (IFA).

• Specimens with a reactive Architect result but a negative confirmatory result (i.e., negative Multispot, Western blot, or immunofluorescence assay results) were resolved with an HIV-1 nucleic acid amplification test (NAAT)
Objectives

1. To compare the Multispot (Bio-Rad), a rapid HIV-1/HIV-2 antibody differentiation assay, as a confirmatory test with the HIV-1 WB and IFA in a prospective study.

2. To evaluate the yield of the HIV-1 nucleic acid amplification assay (NAAT) for specimens with discordant screening and confirmatory results in a prospective study.
Architect Ag/Ab Combo HIV Test Results - New York City, San Francisco, and North Carolina, September 2011 – 2012

Screening with Architect Ag/Ab Combo (4th generation) HIV Test
N=37,876

Repeatedly Reactive Architect
n=654 (1.7%)

Negative Architect
n= 37,222
Screening with Architect Ag/Ab Combo (4th generation) HIV Test
N=37,876

Repeatedly Reactive Architect
n=654 (1.7%)

Multispot HIV-1/HIV-2 differentiation assay testing

Negative Architect
n=37,222
Multispot HIV-1/HIV-2 Differentiation Assay

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)
- HIV Reactive but Undifferentiated
  n=19 (1.7%)
- HIV-2 Reactive
  n=0 (0%)
- Non- Reactive
  n=90 (13.8%)
Multispot HIV-1 Reactive Results

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)
  - Both HIV-1 Spots
    n=536 (98.3%)
  - Recombinant HIV-1 Spot only
    n=6 (1.1%)
  - HIV-1 Peptide Spot only
    n=3 (0.6%)

- HIV Reactive but Undifferentiated
  n=19 (1.7%)

- HIV-2 Reactive
  n=0 (0%)

- Non-Reactive
  n=90 (13.8%)
Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  - n=545 (83.3%)
  - Both HIV-1 Spots
    - n=536 (98.3%)
    - Western blot (n=388)
      - positive 384 (99.0%)
      - indeterminate 3* (0.8%)
      - negative 1* (0.2%)
      * NAAT reactive

- HIV Reactive but Undifferentiated
  - n=19 (1.7%)
  - Recombinant HIV-1 Spot only
    - n=6 (1.1%)
    - IFA (n=148)
      - positive 142 (95.9%)
      - indeterminate 5* (3.4%)
      - negative 1* (0.7%)
      * NAAT reactive

- HIV-2 Reactive
  - n=0 (0%)

- Non-Reactive
  - n=90 (13.8%)
Multispot HIV-1 Reactive with One Spot

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)
  - Both HIV-1 Spots
    n=536 (98.3%)
    - Western blot (n=3) positive 3
  - Recombinant HIV-1 Spot only
    n=6 (1.1%)
    - IFA (n=3) positive 3
- HIV Reactive but Undifferentiated
  n=19 (1.7%)
- HIV-2 Reactive
  n=0 (0%)
- Non-Reactive
  n=90 (13.8%)
HIV Reactive but Undifferentiated
n=19 (1.7%)

HIV-2 Reactive
n=0 (0%)

Non-Reactive
n=90 (13.8%)

HIV-1 Reactive
n=545 (83.3%)

Both HIV-1 Spots
n=536 (98.3%)

Recombinant HIV-1 Spot only
n=6 (1.1%)

HIV-1 Peptide Spot only
n=3 (0.6%)

Western blot (n=3)
positive 2
negative 1*

* One false positive result (0.18% of all HIV-1 reactives; 95% CI: 0.00% - 0.98%)
Details: Western blot and NAAT negative on same specimen, repeat Architect and NAAT negative on new specimen one week later.
Multispot HIV Reactive but Undifferentiated

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)

- HIV Reactive but Undifferentiated
  n=19 (1.7%)

- HIV-2 Reactive
  n=0 (0%)

- Non- Reactive
  n=90 (13.8%)

Western blot (n=14) positive 14

IFA (n=5) positive 5
Multispot HIV Reactive but Undifferentiated

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)

- HIV Reactive but Undifferentiated
  n=19 (1.7%)

- HIV-2 Reactive
  n=0 (0%)

- Non-Reactive
  n=90 (13.8%)

- HIV-1 Reactive at 1:10 dilution
  n=17

- HIV-1 Reactive at 1:100 dilution
  n=1

- Undifferentiated at 1:100 dilution
  n=1

HIV-1 Western blot: positive
HIV-1 NAAT: reactive
HIV-2 EIA: reactive
HIV-2 Western blot: pending
Multispot Non-Reactive

Architect Reactive tested with Multispot
N=654

HIV-1 Reactive
n=545 (83.3%)

HIV Reactive but Undifferentiated
n=19 (1.7%)

HIV-2 Reactive
n=0 (0%)

Non-Reactive
n=90 (13.8%)

NAAT reactive
n = 47  (52.2%)

NAAT negative
n = 43  (47.8%)
Multispot Non-Reactive with NAAT Reactive

Architect Reactive tested with Multispot
N=654

HIV-1 Reactive
n=545 (83.3%)

HIV Reactive but Undifferentiated
n=19 (1.7%)

HIV-2 Reactive
n=0 (0%)

Non-Reactive
n=90 (13.8%)

NAAT reactive
n = 47  (52.2%)

NAAT negative
n = 43  (47.8%)

Western blot (n=22)
- positive 2  (9%)
- indeterminate 5  (23%)
- negative 15  (68%)

IFA (n=25)
- positive 2  (8%)
- indeterminate 1  (4%)
- negative 22  (88%)
Multispot Non-Reactive; NAAT Non-Reactive

Architect Reactive tested with Multispot
N=654

HIV-1 Reactive
n=545 (83.3%)

HIV Reactive but Undifferentiated
n=19 (1.7%)

HIV-2 Reactive
n=0 (0%)

Non-Reactive
n=90 (13.8%)

NAAT reactive
n = 47  (52.2%)

NAAT negative
n = 43  (47.8%)

Western blot (n=15)
negative 15

IFA (n=16)
negative 16
Multispot Non- Reactive; NAAT Non-Reactive

Architect Reactive tested with Multispot
N=654

- **HIV-1 Reactive**
  - n=545 (83.3%)

- **HIV Reactive but Undifferentiated**
  - n=19 (1.7%)

- **HIV-2 Reactive**
  - n=0 (0%)

- **Non-Reactive**
  - n=90 (13.8%)

**NAAT reactive**
- n = 47  (52.2%)

**NAAT negative**
- n = 43  (47.8%)

**Western blot (n=15)**
- negative  15

**IFA (n=16)**
- negative  16

Remaining 12 were not tested by Western blot
Multispot HIV-1/HIV-2 Differentiation Assay

Architect Reactive tested with Multispot
N=654

- HIV-1 Reactive
  n=545 (83.3%)

- HIV Reactive but Undifferentiated
  n=19 (1.7%)

- HIV-2 Reactive
  n=0 (0%)

- Non-Reactive
  n=90 (13.8%)

- NAAT reactive
  n = 47 (52.2%)

- NAAT negative
  n = 43 (47.8%)

- 610 / 654 Architect-positive specimens confirmed by Western blot, IFA or NAAT
## STOP Study HIV Laboratory Algorithm Substudy

<table>
<thead>
<tr>
<th><strong>Multispot</strong></th>
<th><strong>IFA / Western blot</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>-- Positive for 563 / 610 (92%) confirmed infections</td>
<td>-- Positive for 557 / 610 (91%) confirmed infections</td>
</tr>
<tr>
<td>-- 1 “indeterminate” (undifferentiated HIV-1/2)</td>
<td>-- 14 indeterminate</td>
</tr>
<tr>
<td>-- 1 False Positive HIV-1 Peptide spot</td>
<td>-- 0 False Positives</td>
</tr>
</tbody>
</table>
Multispot vs. HIV-1 Western Blot to confirm true positive HIV infections (n=429)

<table>
<thead>
<tr>
<th></th>
<th>Multispot Reactive</th>
<th>Multispot Non-Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot Positive</td>
<td>403 (93.9%)</td>
<td>2 (0.5%)</td>
</tr>
<tr>
<td>Western Blot Indeterminate</td>
<td>3 (0.7%)</td>
<td>5 (1.2%)</td>
</tr>
<tr>
<td>Western Blot Negative</td>
<td>1 (0.2%)</td>
<td>17 (4.0%)</td>
</tr>
</tbody>
</table>

One false positive Multispot result not included
McNemar’s exact p-value = 0.69
Multispot vs. HIV-1 Immunofluorescence Assay (IFA) to confirm true positive HIV infections (n=181)

<table>
<thead>
<tr>
<th></th>
<th>Multispot Reactive</th>
<th>Multispot Non- Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFA Reactive</td>
<td>150 (82.9%)</td>
<td>2 (1.1%)</td>
</tr>
<tr>
<td>IFA Indeterminate</td>
<td>5 (2.8%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>IFA Non-Reactive</td>
<td>1 (0.6%)</td>
<td>22 (12.2%)</td>
</tr>
</tbody>
</table>

McNemar’s exact p-value = 0.29
• Multispot was comparable to Western blot and IFA for confirming HIV infection

• One of nine Multispot results, however, with only one HIV-1 spot was false positive (one of three peptide-only positive specimens)
### Multispot False Positive: Case Details

- Male, in his 50s, originally from West Africa, living in US for 10 years, self-reported a negative HIV test result on immigration, history of syphilis treated 10 years ago

<table>
<thead>
<tr>
<th>Initial HIV Testing</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid EIA (Oraquick)</td>
<td>negative</td>
</tr>
<tr>
<td>Architect</td>
<td>repeatedly reactive (S/CO: 3.75 / 1.37 / 1.39)</td>
</tr>
<tr>
<td>Multispot</td>
<td>HIV-1 reactive (HIV-1 peptide only)</td>
</tr>
<tr>
<td>3(^{rd}) gen EIA (BioRad)</td>
<td>negative</td>
</tr>
<tr>
<td>Pooled NAAT (Aptima)</td>
<td>negative</td>
</tr>
<tr>
<td>HIV-1 viral load (Abbott m2000)</td>
<td>undetectable</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow-up HIV Testing (2 weeks later)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architect</td>
<td>negative</td>
</tr>
<tr>
<td>HIV-1 viral load (Abbott m2000)</td>
<td>undetectable</td>
</tr>
</tbody>
</table>
One other interesting finding

Are all MS-confirmed infections positive for HIV RNA?
Multispot HIV-1 Reactive Tested with NAAT—
New York City specimens only, September 2011 – 2012

Architect Reactive tested with Multispot
N=346

- HIV-1 Reactive
  - n=301 (87.0%)
  - NAAT reactive
    - n = 296 (98.3%)
  - NAAT negative
    - n = 5 (1.7%)

- HIV Reactive but Undifferentiated
  - n=14 (4.0%)

- HIV-2 Reactive
  - n=0 (0%)

- Non- Reactive
  - n=31 (9.0%)
<table>
<thead>
<tr>
<th></th>
<th>Architect</th>
<th>Multispot</th>
<th>Rapid – Oraquick</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reactive</td>
<td>HIV-1 Reactive</td>
<td>Reactive</td>
<td>New diagnosis, partner of one year known HIV positive, history of drug use, not asked about ART use, did not link to care</td>
</tr>
<tr>
<td>2</td>
<td>Reactive</td>
<td>HIV-1 Reactive</td>
<td>Reactive</td>
<td>New diagnosis, visiting from Caribbean, documented negative test in 2010</td>
</tr>
<tr>
<td>3</td>
<td>Reactive</td>
<td>HIV-1 Reactive</td>
<td>Reactive</td>
<td>Previous positive, diagnosed in 1999, re-establishing care, unclear if on ART, viral load 9 days after HIV testing undetectable</td>
</tr>
<tr>
<td>4</td>
<td>Reactive</td>
<td>HIV-1 Reactive</td>
<td>Reactive</td>
<td>Previous positive, diagnosed in 2008, refused interview, unknown if on ART, linked to care</td>
</tr>
<tr>
<td>5</td>
<td>Reactive</td>
<td>HIV-1 Reactive</td>
<td>Reactive</td>
<td>Previous positive, diagnosed in 2009, refused interview, unknown if on ART</td>
</tr>
</tbody>
</table>
Conclusions

- Multispot had comparable frequency of false negative results as Western blot and IFA
Conclusions

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- Regardless of the confirmatory assay used, NAAT is necessary additional diagnostic test
Conclusions

- Multispot had comparable frequency of false negative results as Western blot and IFA
- Regardless of the confirmatory assay used, NAAT is necessary additional diagnostic test
- **NAAT successfully resolved all of the discordant screening and confirmatory results as either:**
  - false negative confirmatory test (i.e., acute HIV) or
  - false positive Architect result
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