Assessing the sensitivities of laboratory-based and point-of-care HIV antigen-antibody combination tests using a panel of specimens from recently and acutely infected individuals

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Objectives

• Assess the sensitivity of an automated 4\textsuperscript{th} generation IA assay using a panel of specimens from recent and acute HIV infection

• Assess the sensitivity of an antigen/antibody rapid test using panel of specimens from recent and acute HIV infection
RNA Testing of Pooled Specimens

250 ul of each of 10 specimens combined into one tube

test pooled specimen by branched DNA (bDNA) or RT-PCR

RNA Detected

RNA Not Detected

*positive pool*

*negative pool*

Antibody-negative specimens (EIA, or rapid test)
Could be in Window Period (infected but no detectable antibody response yet)

return to + pool constituents test each by RT-PCR or bDNA
The Panel:

- At the time of this study:
  - 64 have enough specimen for panel testing:
    - 29 were Ab neg / RNA pos. on screening, but were found to be pos. on at least one antibody test and negative or indeterminate on a WB (“recent”)
    - 35 are RNA positive / Antibody neg. on 3rd gen, & western blot, all other approved rapids (“acute”)
35 panel members: RNA+, Antibody negative on ALL Ab tests: (“acute” infection)

- Presumed to be the most recently infected individuals (within 1-3 weeks of infection)
- These 35 specimens have RNA levels ranging from 1,177 to $\geq 10,000,000$ RNA copies/ ml
Using the panel to assess antigen-antibody combo tests

- Analyzing a lab-based, automated 4th generation IA
HIV IA: Fourth Generation

- Similar to third generation tests; sandwich capture-detect
- Includes direct HIV antigen detection (p24) in addition to IgM and IgG detection
- Not in use in U.S. (not FDA approved)
4th Generation HIV Ag/Ab Assay

Solid phase:
- Anti-p24 Mab
- HIV-1 and HIV-2 recombinant proteins

Sample:
p24 Ag
- Anti-HIV Ab

Conjugates:
- Anti-p24 Mab
- HIV-1 and HIV-2 proteins

Detection
Direct detection of virion using IA:

- p24 antigen detection (core of virus; most numerous protein—1200 protein copies per virion)
How did the 4th Generation IA (automated) perform with the recent / acute infection panel?

- Detects 57/64 positively (89%)
  - (3rd gen detected 42%)

- Of the 29 “recently infected” specimens: 29/29 (100%)
  - (3rd gen detected 93%)

- Of the 35 “acute” specimens (RNA pos, completely Ab negative: 28/35 (80%)
### Sensitivity of the automated 4\textsuperscript{th} generation immunoassay for antigen: RNA equivalents

- **Viral loads of acute specimens NOT detected by the automated 4\textsuperscript{th} gen combo assay:**

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**Automated 4th generation immunoassay considerations / conclusions**

- Can detect infection in antibody-negative individuals

- Viral load cutoff may be about 30,000 RNA copies / ml

- If used as a replacement for RNA testing, would catch 89% of all specimens currently caught only by RNA pooling

- Is much faster than RNA pooling
Antibody-antigen Rapid test

Performance with the recent/acute panel
A rapid test that determines whether antibody and/or antigen is present

- Requires 50 μl of plasma/test
- Is read like any other rapid test
- Shows one band for antigen detection and another band for antibody detection
Assessing the Rapid Ag/Ab test

- For this study: 58 specimens:
  - 22 RNA+ specimens that were negative by the first antibody screen test; and were indeterminate or negative by WB but were positive at least one other ab test (“recent”)
  - 36 of these 58 were negative by 3rd gen, WB and all FDA-approved rapid tests (“acute”)
Rapid Ag/Ab Performance relative to other rapid tests, using plasma:

- 2/58 positive by OraQuick (3.4%) 

-2/58 positive by Stat-Pak (3.4%) 

-15/58 were positive by Uni-Gold (26%) 

-31/58 positive (for either antigen or antibody) by the Ag/Ab Rapid test (53%) 

(NOTE: in 3 of those 31 specimens– this test was Ab+ where the lab based 3rd gen EIA was negative) 

(there were 0 cases where the lab based 3rd gen EIA was pos and the rapid test was negative)
The Rapid Ag/Ab Performance relative to laboratory based tests:

Using the panel of 58 specimens, as described:

-0/58 positive by 1st or 2nd generation EIA (0%)
-0/58 positive by Western Blot (0%)

-20/58 positive by 3rd generation (IgG / IgM sensitive EIA) (35%)

-46/53 positive by 4th generation (IgG / IgM / p24 sensitive IA) (87%) (for either antigen or antibody)

-31/58 were positive by the Ag/Ab Rapid test (53%) (for either antigen or antibody)
Comparing the Rapid Ab/Ag test to the laboratory-based 4\(^{th}\) gen on specimens that were positive only for RNA (neg. on all FDA-approved Ab tests)

Rapid Ab/Ag:
- Detected antibody or antigen in 13/36 RNA+ specimens that were negative by all antibody tests (36%)
  (for 3 of these: it was Ab+)

Labaratory-based, 4\(^{th}\) gen IA:
- Detected antibody or antigen in 29/36 RNA+ specimens that were negative by all antibody tests gen assay (81%)
Sensitivity of Rapid Ag/Ab test for Virus in RNA copies/ml:

10 specimens pos. for Antigen on the rapid combo:

6 were “off scale” (greater the viral load method could read)

4 had measurable values:

2,915,309
4,571,787
4,589,912
9,289,006

RNA+/ab neg specimens NEGATIVE for antigen by the Rapid Ag/Ab test:

3,427,483
1,531,891
327,333
102,288
446,770
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650,629

…and all others in the 10e5 or less range
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Conclusions for the Rapid Ag/Ab test

• If implemented now, it would be the most sensitive rapid test for detecting recent or acute HIV infection

• It appears better than a lab-based 3rd EIA for antibody detection

• Sensitivity for antigen is quite low relative to lab based IA tests or RNA: may be ~ 3 million RNA copies/ml
Thank You for your Attention

"Cherish the planet": picture drawn by a child at the HIV Prevention Department in St. Petersburg