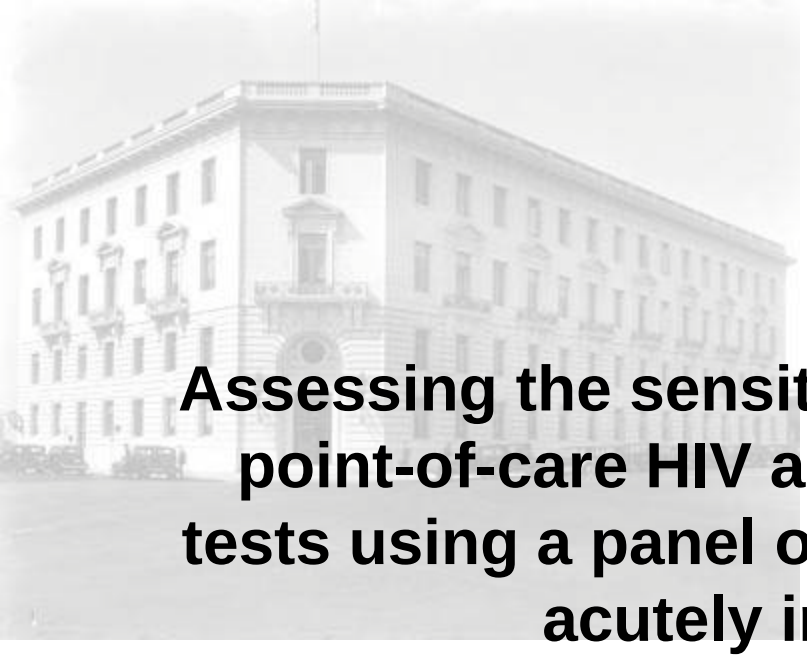


Assessing the sensitivities of laboratory-based and point-of-care HIV antigen-antibody combination tests using a panel of specimens from recently and acutely infected individuals

Pandori, M.W., Louie, B., Pilcher, C., Klausner, J.D., Vallari, A. , Harris, B., Holzmayer, V., Hackett, J.Jr., Liska, S.

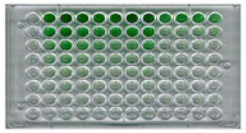
San Francisco Department of Public Health, San Francisco, CA
University of California, San Francisco, San Francisco, CA
Abbott Diagnostics, Abbott Park, IL

Objectives

- **Assess the sensitivity of an automated 4th 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



Antibody-negative specimens (EIA, or rapid test)

Could be in Window Period (infected but no detectable antibody response yet)

250 ul of each of 10 specimens combined into one tube

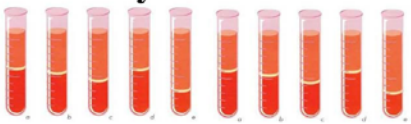
test pooled specimen by branched DNA (bDNA) or RT-PCR

RNA Detected } ***positive pool***

RNA Not Detected

negative pool

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



The Panel:

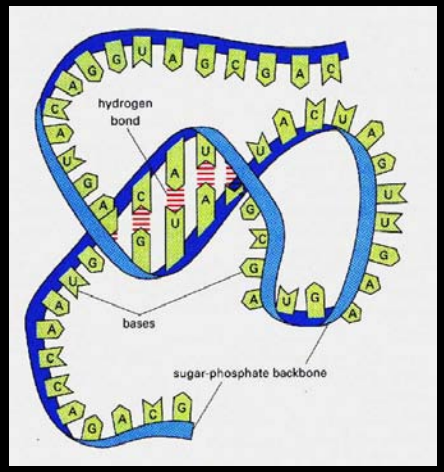
- At the time of this study:

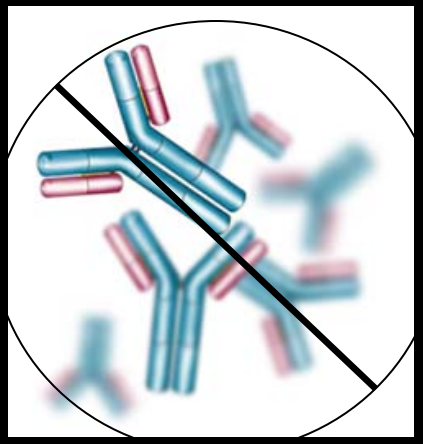
- 64 have enough specimen for panel testing:

  -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 ≥10,000,000 RNA copies/ ml

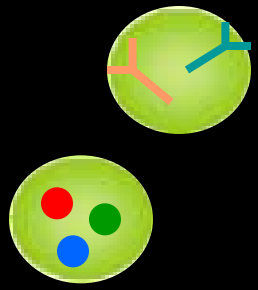
Using the panel to assess antigen-antibody combo tests

- 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

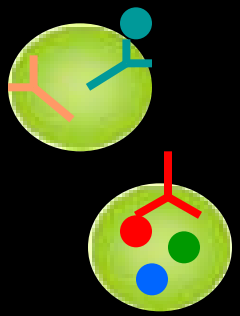


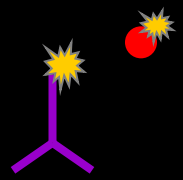
Solid phase:
Anti-p24 Mab
+
HIV-1 and HIV-2
recombinant
proteins

Sample

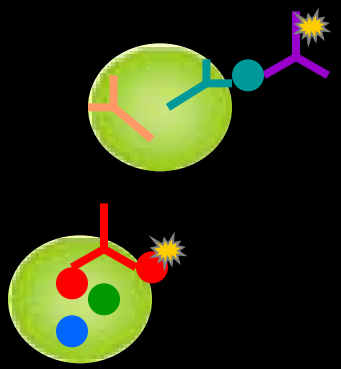
p24 Ag

Anti-HIV Ab





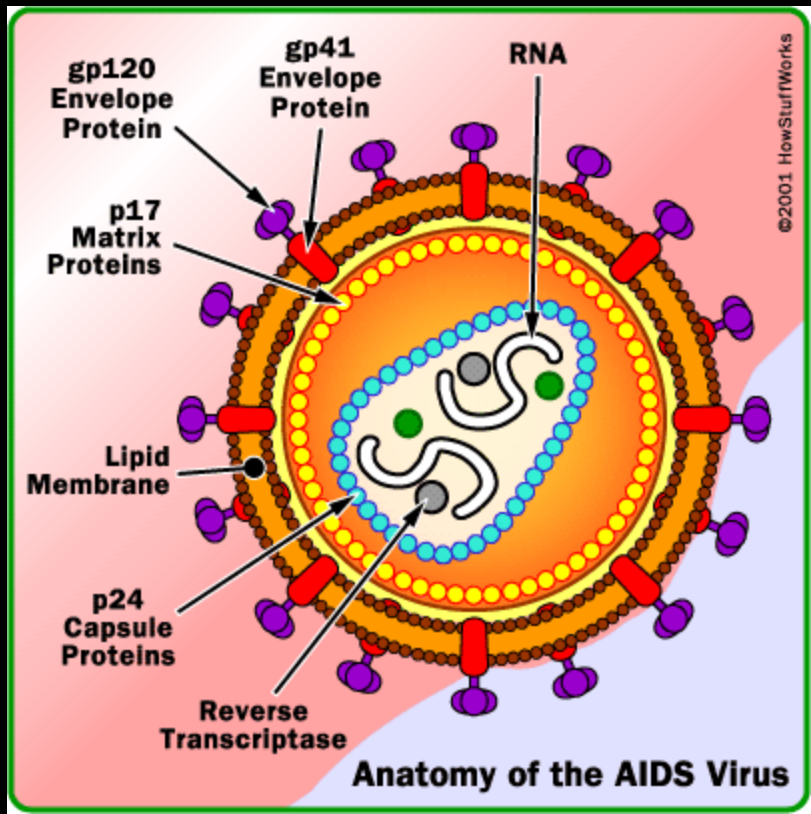
Conjugates:
Anti-p24 Mab
+
HIV-1 and HIV-2
proteins



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 4th generation immunoassay for antigen: RNA equivalents

Viral loads of acute specimens NOT detected by the automated 4th gen combo assay:

RNA copies / ml:

1,177
3,921
5,770
6,373
9,855
12,852
14,062

Viral loads of acute specimens ***detected*** by automated 4th gen combo assay:

RNA copies / ml:

30,734
43,173
69,599

And higher

Sensitivity of the automated 4th generation immunoassay for antigen: RNA equivalents

Viral loads of specimens NOT detected by the automated 4th gen combo assay:

RNA copies / ml:

1,177
3,921
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Viral loads of specimens detected by automated 4th gen combo assay:

RNA copies / ml:

30,734
43,173
69,599

And higher

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 pos on 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 4th 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+)

Laboratory-based, 4th 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
427,490
650,629

…and all others in the10e5 or less range

Sensitivity of Rapid Ag/Ab test for Virus in RNA copies/ml:

10 specimens pos. for Antigen on the rapid combo:

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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*