HIV Testing in 2016:
- Where we stand.
- Where we’re headed.

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Disclosures

Dr Branson has served as a consultant to

- Gilead Sciences, Inc.
- Caldwell-Everson
- Daktari Diagnostics
- Siemens Healthcare
Objectives

- Compare the relative merits of different FDA-approved HIV tests for different circumstances.
- Describe how new HIV rapid and lab tests fit in with current testing recommendations.
- Recognize the increasing role that RNA tests play in HIV diagnosis
Outline

- The basics
- New tests: rapid, lab, & supplemental
- How differences between tests relate to accuracy
- What’s on the horizon
What is your role with HIV testing?

1. I order HIV lab tests and give results to patients.
2. I perform rapid HIV tests and interpret the results.
3. Patients are referred to me after they have tested HIV-positive.
4. I manage an HIV testing program.
5. I work in a lab and perform all kinds of tests, including HIV.
HIV Infection and Laboratory Markers

- HIV RNA (plasma)
- HIV p24 Ag
- HIV Antibody
- IgM
- IgG

Screening for HIV: Can We Afford the False Positive Rate?

Klemens B. Meyer, M.D., and Stephen G. Pauker, M.D.


We are a testing culture: we test our urine for drugs; we test our sweat for lies. It is not surprising that we should also test our blood for the acquired immunodeficiency syndrome (AIDS). But before we screen low-risk groups for antibody to the human immunodeficiency virus (HIV), we should consider what the results will mean.
Use of Two Tests in Sequence

- After a **positive** screening test, conduct a second test on persons who test positive.
- Decision rule: Must be positive on - both tests - either test

- Positive on **both** tests = reduces false positives
  - Increases specificity, but reduces sensitivity
Net Specificity, Sequential Tests (Both Tests Positive)

- Net Specificity = Spec1 + Spec2 – (Spec1 x Spec2)

- HIV Example: Spec1 = .996, Spec2 = .998
  - Spec1 + Spec2 = 1.994
  - Spec1 x Spec2 = .994
  - Net Specificity = 1.00
HIV Diagnostic Algorithm: 1989

“The Public Health Service recommends that no positive test results be given to clients/patients until a screening test has been repeatedly reactive (i.e., greater than or equal to two tests) on the same specimen and a supplemental, more specific test such as the Western blot has been used to validate those results.”
1989: State of the Art

EIA

Western blot

- gp 160
- gp 120
- p55
- gp41
- p31
- p24
1989 Almanac

- Berlin Wall comes down
- Tiananmen Square
- Exxon Valdez oil spill
- U.S. invades Panama
Treatment Era

- 1989 – PCP prophylaxis
- 1993 – PCP and MAC prophylaxis
- 1996 – Potent ART with protease inhibitors
Percentage of persons at testing sites who do not return for their HIV test results

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<thead>
<tr>
<th></th>
<th>HIV Positive</th>
<th>HIV Negative</th>
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<tr>
<td>1995</td>
<td>25%</td>
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<td>42%</td>
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<td>1998</td>
<td>38%</td>
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<td>43%</td>
<td>48%</td>
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<td>2000</td>
<td>42%</td>
<td>47%</td>
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Source: CDC Client Record Database, Publicly-funded HIV testing
Health-care providers should provide preliminary positive test results before confirmatory results are available in situations where tested persons benefit.
The Roll-out of Rapid HIV Testing in The United States
CLIA-waived
The Roll-out of Rapid HIV Testing in The United States
Rapid HIV test a weather-vane for waived tests

Karen Lusky

The Food and Drug Administration’s waiver of the OraQuick rapid HIV-1 antibody fingerstick test last year sent shock waves through the clinical laboratory community. Many feared the agency’s move signaled a crack in the regulatory dam that would bring a flood of other waived tests that could hardly be
Rapid HIV tests can be performed only by:

1. A doctor or nurse.
2. A clinical laboratory.
3. An HIV counselor who has received training.
4. Any of the above.
Rapid HIV Test Restrictions

- Sale is restricted to clinical laboratories that:
  - Have an adequate quality assurance program, and
  - Where there is insurance that operators will receive and use instructional materials.

- Test is approved for use only by an agent of a clinical laboratory.

- Test subjects must receive the “Subject Information Notice” prior to specimen collection.
CLIA-waived rapid HIV-antibody tests

- Oraquick Advance
- DPP HIV 1/2
- Chembio Sure Check
- INSTI HIV 1/2
- Chembio Stat Pak
- Uni-Gold Recombigen
2003 Almanac

- Department of Homeland Security Established
- Euro adopted by the EU
- Enron collapses
- U.S. invades Iraq
HIV-1 antigens and RNA

- gp120
- gp 41
- p24
- RNA

Membrane Associated (Matrix) Protein MA p17
Protease PR p9
Polymersase RT & RNase H RNH p66
Integrase IN p32
Which antibodies do rapid tests detect?

1. p24 antibody
2. gp41 antibody
3. gp120 antibody
4. gp41 and gp120 antibody
5. All three: p24, gp41, and gp120
How large a specimen?

Oraquick Advance: 5 µL

DPP HIV 1/2: 10 µL

Chembio Sure Check: 3 µL

INSTI HIV 1/2: 50 µL

Chembio Stat Pak: 5 µL

Uni-Gold Recombigen: 50 µL
How long does it take?

Oraquick Advance: 20-40 min

DPP HIV 1/2: 5 + 10-15 min

Chembio Sure Check: 15-20 min

INSTI HIV 1/2: 1 min

Chembio Stat Pak: 15-20 min

Uni-Gold Recombigen: 10-12 min
What's new?

Chembio SureCheck

DPP HIV 1/2

INSTI HIV 1/2

Determine HIV-1/2 Ag/Ab Combo
Product name change: Clearview Complete is now Sure Check
DPP HIV-1/2

- Finger-stick, oral fluid
- Swab gums 4 times (15 seconds) or 10 µL whole blood
- Read time 10-25 min blood
  40 min oral fluid
Dual Path Platform Technology

Conjugate

Specimen

Strip 1

Strip 2
INSTI HIV-1/2

- CLIA-waived for whole blood, finger-stick
- 50 µL specimen volume
- Results <1 minute
- Detects IgM antibodies

Moshgabadi et al, J Clin Virol 2015
Abbott Architect Ag/Ab Combo
2010 p24 Antigen
Control

Antibody gp41, gp120

Siemens Advia Centaur® CHIV
2015

“4th Generation”

- CLIA-waived
- Whole blood (50µL)
What’s new in the lab?
Abbott Architect Ag/Ab Combo 2010

“4th Generation”

Bio-Rad Ag/Ab Combo 2011

“Ag/Ab Combo”

Siemens Advia Centaur® CHIV 2015
On-board Refrigeration of Multiple Different Assays
Random Access Multiplatform analyzers for HIV testing

STAT sample requests without pausing
Results in <60 minutes
“5th generation” HIV Ag/Ab Combo Assay

- Beads conjugated to HIV-1 Group M (gp160) and O antigens, HIV-2 gp36 antigen, and p24 antibody

- Distinguishes between
  - p24 antigen
  - HIV-1 antibodies
  - HIV-2 antibodies

BioPlex 2200 HIV Ag-Ab
July 2015
Which factor is least important for the accuracy of a rapid HIV antibody test?

1. The type of specimen (serum, whole blood, oral fluid).
2. The volume of specimen the test requires.
3. The way the test is designed.
4. The time it takes to run the test.
5. The prevalence of HIV in the population tested.

0% 0% 0% 0% 0%
Accuracy: Performance Characteristics

- **Sensitivity**
  - The ability of the test to identify correctly those who **have** the disease
    - HIV: ≥99.8%

- **Specificity**
  - The ability of the test to identify correctly those who **do not have** the disease
    - HIV: ≥99.8%
Interpreting HIV Test Results

For a laboratory test:

**Sensitivity:** Probability test=positive if patient=positive

**Specificity:** Probability test=negative if patient=negative

For a person:

**Predictive value:**

  Positive: Probability patient=positive if test=positive
  Negative: Probability patient=negative if test=negative
Patients without disease:

100% negative predictive value

Patients with disease:

100% positive predictive value

100% sensitivity

100% specificity

Test Results

Frequency

Cutoff

Positive or negative?
Where to Set the Cutoff?

- If the penalty for missing a case is high (e.g., the disease is fatal and treatment exists, or disease spreads easily):
  - Use a cutoff with high sensitivity
  - Maximize true positives

- Higher sensitivity = lower specificity
  - Increases false-positives
Test Results

Cutoff

100% Sensitivity

100% Specificity

100% negative predictive value

PATIENTS WITHOUT DISEASE

100% positive predictive value

PATIENTS WITH DISEASE

Frequency
Example: Test 10,000 persons

HIV prevalence = 3%

Test Specificity = 99.8% \(\frac{2}{1000}\)

True positive: 300
False positive: 20

Positive predictive value: \(\frac{300}{320} = 94\%\)
Example: Test 10,000 persons

Test Specificity = 99.8% (2/1000)

HIV prevalence = 3%
True positive: 300  False positive: 20
Positive predictive value: 300/320 = 94%

HIV prevalence = 0.1%
True positive: 10  False positive: 20
Positive predictive value: 10/30 = 33%
What is the Window Period?

1. The period after infection when HIV is undetectable.
2. The interval when HIV RNA is detectable but antibodies have not yet developed.
3. The time after infection before antibodies appear.
4. The time you must wait before reading rapid test results.
Evolution of HIV Tests: Four Generations
Evolution of HIV Tests

- **1st generation:** whole viral lysate, detects IgG antibody
- **2nd generation:** synthetic peptides, detects IgG antibody
- **3rd generation:** detect IgM and IgG antibody
- **4th generation:** detects IgM, IgG antibodies, p24 antigen
26 seroconverters were analyzed with 14 tests
17 seroconverters with WB positive used for cumulative frequency analysis
Sequence of Test Positivity Relative to WB (plasma)

166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency

Bangkok Tenofovir Study:
Delayed HIV detection by oral fluid in patients on PReP

Participants receiving tenofovir (who became HIV-infected) took longer to develop a reactive OraQuick (191.8 days) than participants receiving placebo (16.8 days)


1985 Abbott HIV-1 EIA

1987 Vironostika EIA

1989 Abbott HIV-1/HIV-2 EIA

1990 Genetic Systems HIV-1/HIV-2 Peptide EIA

1991 Cambridge Western blot

1992 Fluorognost IFA

1992 Genetic Systems rLAV (HIV-1)

1998 Genetic Systems HIV-1/HIV-2 Rapid Test

1998 Cambridge Western blot

2000 Abbott Architect Ag/Ab Combo CIA

2000 Genetic Systems HIV-1/HIV-2 Plus O EIA

2003 Ortho Vitros HIV 1+2 CIA

2003 GS HIV-1 HIV-2 Peptide EIA

2006 Advia Centaur 1/O/2 CIA

2006 Abbott Architect Ag/Ab Combo CIA

2008 OraQuick HIV-1/HIV-2 Rapid Test

2009 Avioq HIV-1 EIA

2009 Unigold Reveal HIV-1 Rapid Tests

2009 Bioplex Ag/Ab Combo

2010 INSTI HIV-1 Rapid Test

2010 Abbott Architect Ag/Ab Combo CIA

2011 Bio-Rad Ag/Ab Combo EIA

2013 Determine Combo Ag/Ab Rapid test

2015 Siemens Ag/Ab Combo CIA

2015 Bioplex Ag/Ab Combo

3rd & 4th gen lab screening tests

1st gen confirmatory tests

2nd gen rapid tests
Limitations of the 1989 HIV Algorithm

- Antibody tests do not detect infection in ~10% of infected persons at highest risk of transmission

- Western blot confirmation is less sensitive during early infection than many widely used screening tests
Pooled RNA Screening for Acute HIV Infection
CDC Acute HIV Infection Study
1-Stage RNA Pooling, antibody-negative specimens

16 Specimens

- 80 HIV testing clinics in Florida
- 14 STD clinics and 1 MSM clinic in Los Angeles
- 3 STD clinics in New York

- Patel et al, Archives Int Med 2010
## Acute HIV Screening: 99,111 tested

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<tr>
<th>Test Combination</th>
<th>Positive (%)</th>
<th>False (%)</th>
</tr>
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<tbody>
<tr>
<td>EIA-RR/WB+ RNA+</td>
<td>1,136 (1.1%)</td>
<td>1,094 (96.3%)</td>
</tr>
<tr>
<td>EIA-RR/WB+ RNA-</td>
<td>42 (3.7%)</td>
<td>30 (0.03%)</td>
</tr>
<tr>
<td>EIA-RR/WB-ind RNA+</td>
<td>3 (10.0%)</td>
<td>27 (90.0%)</td>
</tr>
<tr>
<td>EIA-neg/RNA+ Acute HIV</td>
<td>52 (0.05%)</td>
<td>48 (92%)</td>
</tr>
<tr>
<td>False-pos RNA</td>
<td>4 (8%)</td>
<td></td>
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*Patel et al, CDC, Archives Int Med 2010*
Why Is Acute HIV Important?
Increased Risk of Sexual Transmission of HIV


Virus 75-750 times more infectious

1/30 - 1/200

1/1000 - 1/10,000

1/500 - 1/2000

1/100 - 1/1000

HIV RNA in Semen (Log_{10} copies/ml)

Acute Infection 3 wks

Asymptomatic Infection

HIV Progression

AIDS
Acute HIV: Partner Notification

- Persons with acute HIV infection
  - 2.5 times as many sex partners
  - 1.9 times as many partners newly diagnosed with HIV

...as did persons with new diagnosis of established HIV infection

Moore et al, JAIDS 2009
RNA vs. Laboratory Ag/Ab Assay

- RNA+/ 3rd gen-negative specimens detected by Ag/Ab assay:
  - 38 of 46 (83%) – Australia*
  - 10 of 14 (71%) – CDC AHI study**
  - 51 of 61 (84%) – CDC panel***

- 4 days after RNA – 9 seroconversion panels***

* Cunningham P, HIV Diagnostics Conf 2007
** Patel P, CROI 2009
*** Owen M, CROI 2009
HIV-2 Infection

- Remains uncommon in U.S., but
  - Does not respond to NNRTIs, some PIs (first line therapy)
  - Undetectable by HIV-1 viral load tests

- Misclassification by HIV-1 Western blot:
  - 54/58 (93%) HIV-2 patients tested had positive HIV-1 WB (NYC)\(^1\)
  - 97/163 (60%) HIV-2 cases reported had positive HIV-1 WB (CDC)\(^2\)

- HIV-2 often diagnosed after immunologic deterioration in patient with negative viral load

\(^1\) Torian et al, Clinical Infectious Disease 2010
\(^2\) MMWR July 2011
Goals for 2010 HIV Diagnostics Conference

- Review the available evidence
- Identify the needs of different stakeholders
- Develop a menu for specific applications
- **Begin consensus process** for updating testing recommendations
Goals for 2010 HIV Diagnostics Conference

If you don't recommend new algorithms, we'll kill this dog.
Proposed HIV Diagnostic Algorithm

A1: 4th generation HIV-1/2 immunoassay

A1+ → A1(-)

A1(-)
Negative for HIV-1 and HIV-2 antibodies and p24 Ag

A1+ → A2

HIV-1/HIV-2 differentiation immunoassay

HIV-1 +
HIV-1 antibodies detected
Initiate care (and viral load)

HIV-2 +
HIV-2 antibodies detected
Initiate care

HIV-1&2 (-)

NAAT

NAAT+ Acute HIV-1 infection
Initiate care

NAAT (-) Negative for HIV-1
2013 Almanac

- U.S. Government shutdown
- Pope Benedict resigns
- Kim Jung Un threatens the US (after a bad haircut)
- U.S. doesn’t invade anyone
Laboratory Testing for the Diagnosis of HIV Infection

Updated Recommendations

Published June 27, 2014

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4th generation HIV-1/2 immunoassay

HIV-1/HIV-2 antibody differentiation immunoassay

- HIV-1 (+) or indeterminate
- HIV-2 (-)

HIV-1 antibodies detected

HIV-2 antibodies detected

Acute HIV-1 infection: RNA (+)

Negative for HIV-1: RNA (-)
Determine Combo Ag/Ab with Whole Blood

- In 6 studies involving >26,000 persons, Determine Combo failed to detect p24 antigen in whole blood from any of the 26 acute infections.

- CDC study: Nine seroconverters showed a median delay of 6 days between DC reactivity with plasma and reactivity with whole blood

- 2016 CDC HIV Diagnostics Conference: http://hivtestingconference.org
Evaluation of Newly Approved HIV Tests

- > 6000 specimens were collected from mostly MSM attending STD clinics in Los Angeles, CA from 2003-2005
- Tested with 6 rapid tests, (including Multispot) BioRad 1/2/O (3rd gen), WB and Aptima
- In 2010-2011, tested with Vitros HIV-1/2/0, Siemens Advia HIV-1/2/0, Abbott 3a77 (all 3rd Gen) and Abbott Ag/Ab Combo (4th gen)
- 644 EIA/WB Positive
- 15 WB neg/IND, Aptima positive (Acute)
- 988 WB and Aptima negative;
  - 211 False-positive on at least one test

-Delaney et al, CDC: 2016 HIV Diagnostics Conference
## Summary of screening test performance

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<td>53% (27-79)</td>
<td>100.0% (99.0-100)</td>
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<td>98.3% (97.3-99.0)</td>
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<td>40% (16-68)</td>
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-Delaney et al, CDC: 2016 HIV Diagnostics Conference
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-Delaney et al, CDC: 2016 HIV Diagnostics Conference
4th generation HIV-1/2 immunoassay

(+)

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)  HIV-1 (-)  HIV-1 (+)
HIV-2 (-)  HIV-2 (+)  HIV-2 (+)

HIV-1 antibodies detected  HIV-2 antibodies detected  HIV antibodies detected

HIV-1 (-) or indeterminate HIV-2 (-)

RNA

RNA (+)  RNA (-)

Acute HIV-1 infection  Negative for HIV-1

Negative for HIV-1 and HIV-2 antibodies and p24 Ag
A reactive Ag/Ab test result followed by a negative antibody test result most often means:

1. The patient has acute HIV infection
2. The first (Ag/Ab) test is false-positive
3. The second (antibody) test is false-negative
4. The patient has HIV-2 infection.
**Example:** Test 10,000 persons

**Ag/Ab Immunoassay**

Test Specificity = 99.8% \( (2/1000) \)

HIV prevalence = 3%

True positive: 300  False positive: 20
Positive predictive value: \( \frac{300}{320} = 94\% \)

Established Infection

Acute HIV prevalence = 0.02%

True positive: 2  False positive: 20
Positive predictive value: \( \frac{2}{22} = 9\% \)

Acute Infection
Positive Predictive Value: Newborn Screening

- 3.7 million infants each year, screened, twice

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<tr>
<th></th>
<th>Cases</th>
<th>Incidence</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PKU</td>
<td>289</td>
<td>1:13,050</td>
<td>2.65%</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>54</td>
<td>1:62,800</td>
<td>0.57%</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1203</td>
<td>1:3,300</td>
<td>1.77%</td>
</tr>
<tr>
<td>Adrenal Hyperplasia</td>
<td>51</td>
<td>1:25,100</td>
<td>0.53%</td>
</tr>
</tbody>
</table>

- Arch Pediatr Adolesc Med, 2000
FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay
HIV-1/HIV-2 Differentiation Assays

FDA approved, March 2013
- Serum Control
- HIV-1 Recombinant gp41
- HIV-1 Peptide gp41
- HIV-2 Peptide gp36
- Multispot HIV-1/HIV-2

FDA approved, Oct. 2014
- Geenius HIV-1/HIV-2

Product Withdrawal
July 29, 2016
Where’s My Western Blot?
What HIV Specialists Need to Know about Updated HIV Testing Recommendations
Geenius™ HIV-1/2 Lines

HIV-1 & HIV-2 Associated Lines

- gp36
- gp140
- gp160
- gp41 (group M & O)
- p24
- p31*

HIV-2

HIV-1

Control Band

* inside the nucleocapsid
Dual Path Platform: 
Add 
5 µL serum/plasma 
Or 
15 µL whole blood 
to specimen well
Add 5 drops buffer
To buffer well.
Wait min 15-20 min (max 30 min) for results
Insert test cassette in reader for automated interpretation
## Geenius Results: New Interpretations

<table>
<thead>
<tr>
<th>HIV-1 RESULT</th>
<th>HIV-2 RESULT</th>
<th>ASSAY INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>HIV NEGATIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Negative</td>
<td>HIV-1 INDETERMINATE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATE&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATE&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
</tbody>
</table>
| Indeterminate| Positive     | HIV-2 POSITIVE with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1<sup>*</sup>.  
<sup>*</sup>Note: Differentiation features managed by proprietary algorithm. |
| Positive     | Positive     | HIV POSITIVE Untypable (undifferentiated): Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare) <sup>*</sup>.  
<sup>*</sup>Note: Differentiation features managed by proprietary algorithm. |

<sup>a</sup> HIV-1 band(s) detected but did not meet the criteria for HIV-1 Positive  
<sup>b</sup> HIV-2 band(s) detected but did not meet the criteria for HIV-2 Positive  
<sup>c</sup> HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive
Arizona Experience 3/16 - 9/16

- 179 specimens submitted for Geenius testing
  - 111 (62%) HIV-1 positive
  - 56 (31%) HIV-negative
  - 7 (4%) HIV-1 indeterminate
  - 2 (1%) HIV-2 indeterminate
  - 1 (0.5%) HIV-2 positive
Persons with HIV and Awareness of HIV Status, United States - 2012

Number HIV infected: 1,201,100

Number unaware of their HIV infection: 168,300 (14 %)

Estimated new infections annually: 47,500

CDC HIV Surveillance Supplemental Report, 2014
PrEP

ANOTHER BLUE PILL FOR SEX
**PrEP: PrEP works... if you take it**

### iPrEx Open Label Extension Study (MSM)

<table>
<thead>
<tr>
<th>Adherence by Drug Concentration</th>
<th>HIV Incidence per 100 PY</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 pills/week</td>
<td>4.7</td>
</tr>
<tr>
<td>&lt;2 pills/week</td>
<td>2.3</td>
</tr>
<tr>
<td>2-3 pills/week</td>
<td>0.6</td>
</tr>
<tr>
<td>≥4 pills/week</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Women might need to be more adherent than MSM

Percent of Women Achieving Effective Drug Concentrations in CD4+ Cells

**Pharmacokinetics in 49 healthy female volunteers**

<table>
<thead>
<tr>
<th>Rectal Tissue</th>
<th>Female Genital Tract Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-7 doses/week (28% adherence) protects colorectal tissue</td>
<td>6-7 doses/week (85% adherence) to protect female genital tract tissue</td>
</tr>
</tbody>
</table>
Quick Case Study

- 25 year old MSM in an HIV-discordant relationship, on PrEP since 12/2015. Perfect adherence by history. Also had 2 other sex partners.
- 4th generation testing repeatedly negative until May 2016:
  - Positive 4\textsuperscript{th} gen, negative Multispot, HIV RNA<20, signal detected
- Repeat 2 and 4 weeks later: same results.
What would you do next?

1. Tell the patient he is not infected
2. Repeat tests in 6 weeks
3. Order a Western blot
4. Order a genotype

2003 study: false-negative Oraquick and waning or absent gp41 titers in patients on early, effective therapy
Bangkok tenofovir study...

Genotype: Multiple drug resistance mutations (including TDF/FTC), virus not related to that of his virally suppressed partner.

- O’Connell et al, J Clin Micro 2003
Follow-up Testing: Persons on PrEP

- Every 3 months:
  - HIV Ag/Ab Combo test
  - STI testing

- Test positivity while on PrEP (resistance or poor adherence) might be delayed, especially with rapid tests
On the Horizon...
4th generation HIV-1/2 immunoassay

HIV-1/HIV-2 antibody differentiation immunoassay

- HIV-1 (+) and HIV-2 (-): HIV-1 antibodies detected
- HIV-1 (-) and HIV-2 (+): HIV-2 antibodies detected
- HIV-1 (+) and HIV-2 (+): HIV antibodies detected
- HIV-1 (-) or indeterminate
- HIV-2 (-)

HIV-1 RNA viral load

- RNA (+): Acute HIV-1 infection
- RNA (-): Negative for HIV-1

June 27, 2014
HIV Nucleic Acid Test (NAT) for Diagnosis: Qualitative vs Viral Load

- APTIMA HIV-1 qualitative RNA assay is the only NAT FDA-approved for diagnosis

- Under FDA and CLIA regulations, clinicians can order HIV-1 RNA viral load tests, but labs cannot use them as a reflex part of the algorithm
How about...

4th generation HIV-1/2 immunoassay

(+)

HIV-1 RNA viral load

(-) Negative for HIV-1 and HIV-2 antibodies and p24 Ag

VL detectable HIV-1 infection

VL (-) HIV-1/HIV-2 antibody differentiation assay

HIV-1+ (Viral suppression)

HIV-2+ HIV-2 infection

Negative

Useful clinical information
“Point-of-Care” Nucleic Acid Tests

- Xpert HIV-1 viral load
  - 1 ml plasma
  - Results in 90 minutes
  - LOD 32 copies/mL
  - CE-marked December 2014

GeneXpert

Not available in U.S.
“Point-of-Care” Nucleic Acid Tests

- Xpert HCV viral load
  - 1 mL serum or plasma
  - Genotypes 1-6
  - Range 10 – 100,000,000 IU/mL
  - Results in 105 minutes
  - CE-marked April 2015

Not available in U.S.
“Point-of-Care” Nucleic Acid Tests

- 25 µL whole blood specimen
- HIV-1 or HIV-2 viral load in 60 minutes
- CE-marked March 2015

*Not available in U.S.*
“Point-of-Care” Nucleic Acid Tests

- “Lab in a Tube”
- Influenza A/B – FDA cleared
- Strep A – FDA cleared
  - Results in 15 minutes
  - CLIA-waived May 2015
- HIV under development
What’s Next: 2017 Almanac?
Summary

- HIV tests keep getting better
- Only labs can perform HIV tests
  - If you conduct HIV tests, you are a lab
  - Meet requirements to maintain CLIA certificate of waiver
- RNA viral load will play an increasingly important role in HIV diagnosis
- You never know what’s next