Which of the Two Make the Best Soul Mate: An Autoimmune or Infectious Antibody?
Maria Crisostomo, September 20, 2019

Disclosure

- Employee and stock holder of Bio-Rad Laboratories
Outline

At the completion of the session, the participant will be able to:

- Describe the evolution of diagnostic tests utilized in the areas of Autoimmune, Syphilis, and HIV diagnostic testing
- Evaluate alternative testing algorithms in these three areas of diagnostic testing based on the clinical utility of the respective diagnostic tests
- Analyze the immunological reactivity between antigen and antibody and how their "ideal" pairing contribute to the performance of an assay (sensitivity, specificity, PPV, & NPV)

Autoimmune - Outline

- Antibody, autoantibody & antigen
- Autoimmunity & autoimmune diseases
- 5 primary categories of autoimmune diseases
- Test methods used for autoimmune diagnostic testing
- Autoimmune testing algorithm
- Test performance
Autoimmune Testing

Antibody vs. Autoantibody vs. Antigen

- **Auto:** Self
- **Antibody:** a blood protein produced by our immune system to protect against foreign invaders
- **Autoantibody:** a blood protein produced by our immune system but mistakes our own tissues & cells as foreign invaders
What is an Autoimmune Disease?

• Immune means resistant to a foreign invader

• An autoimmune disease is a disorder whereby the body mistakes its own tissue and cells for a foreign invader

Why do people get autoimmune disease?

Environmental Trigger
Genetics Predisposition

Immune System

Autoimmune Response
Normal immune response

Variable clinical presentation
Autoimmune Diseases*

- ~80 autoimmune diseases have been described; most of these diseases are rare (low prevalence)
- Common diseases include autoimmune thyroid disease, rheumatoid arthritis and celiac disease
- Every patient is unique and presents with different clinical symptoms and autoantibody profile
- Systemic Lupus Erythematosus (SLE) or Lupus is the prototypical systemic rheumatic disease known as “The Disease of a Thousand Faces”

Autoimmune Disease Burden*

- Affect up to 8% of the US population
- Is responsible for $100B in annual direct healthcare costs in the US

*Source: American Autoimmune Related Diseases Association, Inc.

Gender Bias*

Knowledge Check

• What is an autoantibody?

Knowledge Check

• What is an autoimmune (AI) disease?
Knowledge Check

• What triggers an autoimmune disease?

Examples of Autoimmune Diseases

- Thyroid: Hashimoto’s thyroiditis, Grave’s disease
- Stomach/Intestinal: Atrophic gastritis, Autoimmune Hepatitis, Celiac Disease
- Adrenal: Addison’s Disease
- Pancreas: Type 1 Diabetes

Autoimmune Diseases

- Circulatory: Wegener’s Granulomatosis
- Muscles: Dermatomyositis, Polymyositis
- Kidneys: SLE
- Skin: Scleroderma
- Joints: Rheumatoid arthritis
- Mixed Connective Tissue Disease

Systemic

Organ Specific
Autoimmune Testing Disease Categories

- **Typical Autoimmune Test Menu Offering**

  - Systemic
  - Gastrointestinal
  - Vasculitis
  - Organ-specific
  - Anti-Phospholipid Syndrome

Knowledge Check

- Q: What are the five typical autoimmune disease categories used by IVD manufacturers?
Common Test Methods

- **Solid Phase:** Glass Side
- **Detection:** Epifluorescent Microscope
- **Result:** Subjective

- **Solid Phase:** Microwell
- **Detection:** Spectrophotometer
- **Result:** Objective

- **Solid Phase:** 8 µm magnetic Bead
- **Detection:** Flow Cytometer
- **Result:** Objective

What is a Testing Algorithm?

- A **testing algorithm** is a sequence of tests used in combination to improve the accuracy of the laboratory diagnosis of the targeted disease based on testing of serum or plasma specimens.

- A **testing algorithm** can also be described as tiered testing (i.e. first tier, second tier, etc.), whereby the result of the initial test (first tier or screening test) will determine if a subsequent test (second tier or confirmatory test) will be used to confirm the initial test result.
Let’s take a look at typical ANA testing algorithm

**ANA Screen or 1st Tier Testing**

- **(-)**: No further testing required
- **(+)**, Perform 2nd Tier Testing


* = Lupus biomarker

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**Knowledge Check**

- What is a testing algorithm?
Knowledge Check

- What are the 3 most commonly test methods used for autoimmune diagnostic testing?

Test Performance: Sensitivity vs. Specificity

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>Proportion of patients with a disease who test <strong>positive</strong></td>
<td>Proportion of patients without the disease who test <strong>negative</strong></td>
</tr>
<tr>
<td><strong>100% (1.0) Means</strong></td>
<td>The test correctly identify every person who <strong>has</strong> the target disorder</td>
<td>The test correctly identify every person who <strong>does not have</strong> the target disorder</td>
</tr>
<tr>
<td><strong>Statistical Outcome</strong></td>
<td><strong>True Positive</strong></td>
<td><strong>True Negative</strong></td>
</tr>
<tr>
<td><strong>Ideal Test Result</strong></td>
<td><strong>Negative Test Result</strong></td>
<td><strong>Positive Test Result</strong></td>
</tr>
<tr>
<td><strong>Test Interpretation</strong></td>
<td>They are <strong>definitely not positive</strong> → They <strong>DON'T have it</strong></td>
<td>They are <strong>definitely not negative</strong> → They <strong>DO have it</strong></td>
</tr>
<tr>
<td><strong>The Rule</strong></td>
<td><strong>Rule Out (ScoOut)</strong></td>
<td><strong>Rule In (Spla)</strong></td>
</tr>
</tbody>
</table>
Test Performance: Sensitivity vs. Specificity

- Sensitivity = If you got it, we detect it

- Specificity = If you don’t got it, we won’t detect it
Knowledge Check

- What is assay sensitivity?

Knowledge Check

- What is assay specificity?
Syphilis Testing

Outline

Upon completion of this Syphilis module, the learner will be able to:

• Describe the common test methods used for Syphilis testing

• Describe the common test algorithms used for Syphilis screening and confirmation

• Describe the multiplexing method used for Syphilis testing
Outline

• Syphilis Incidence
• Serologic Tests
  – Manual
  – Automated
• Testing algorithms
  – Classic or “Traditional”
  – Reverse
  – European
• BioPlex® 2200 Syphilis Test
  – A new, combined approach

Manual Semi-Automated
1984 – Teaching “Aerobics”

Etiology of Syphilis

- Venereal syphilis is caused by spirochete bacterium, *Treponema pallidum* (TP), subspecies *pallidum*, Nichols strain
- Thin (0.2 μm) spirochete 6-20 μm in length with 10-13 coils
Syphilis - Infectious Stages

Primary Syphilis
- Painless Chancre
  - Spontaneously resolves in 9-90 days

Secondary Syphilis
- Diffuse Rash, Swollen Glands
  - Spontaneously resolves in 6-8 weeks

Latent Syphilis
- Asymptomatic
  - Spontaneously resolves in 2-4 years

Tertiary Syphilis
- Cardiovascular, Cutaneous Diseases, Neurosyphilis
  - 3-20 years

CDC - US Syphilis Prevalence (rates per 100K people)

Average Prevalence (Positivity Rate): 5%

Rate per 100,000 population:
- <=2.9 (n=13)
- 3.0-5.0 (n=14)
- 5.1-7.3 (n=13)
- >=7.4 (n=14)

Guam 1.2
Virgin Islands 7.7
Puerto Rico 15.0
Syphilis Incidence, CDC Statistics

Primary and Secondary Syphilis: Distribution of Cases by Sex & Sexual Behavior, 2015

Laboratory Testing
Cassette Players Don’t Rewind Themselves

Direct Organism Detection: Fluid from Lesion

- Dark Field Microscopy
- DFA-TP
  - Direct Fluorescent Antibody – Treponemal pallidum
- DFAT-TP
  - Direct Fluorescent Antibody Tissue – Treponemal pallidum
Serologic Assays

Non-Treponemal Assays

• Detects antibodies to lipoprotein material from damaged cells, cardiolipin from Treponemes and other bacteria

• Not specific for *T. pallidum* infection
  – May be positive in anti-phospholipid antibody syndrome (APS); not directed against causative agent of syphilis

• Classically used for screening test and monitoring disease response to treatment
  – In reverse sequence algorithm; used as a second line test
Non-Treponemal Assays

- **RPR- Rapid Plasma Reagin**
  - Anti-phospholipid (cardiolipin) antibody
  - Phospholipids with charcoal beads
  - Screening test
  - May become negative in late stage disease
  - Will decline (slowly) with successful treatment
  - Macroscopic test
  - Not used on CSF
  - Can be semi-automated (ASI) or fully automated (Gold Standard AX100 & Bio-Rad BioPlex 2200)

- **VDRL- Venereal Disease Research Laboratory Test**
  - Similar to RPR
  - Flocculation, test with freshly prepared liposomes of cardiolipin, lecithin and cholesterol
  - Microscopic test
  - Used for CSF

Syphilis Serology, 2

- Both non-treponemal tests are titered following a positive screening test
- Prozone effect may occur
  - Common in flocculation tests
  - May result in false negative test results in high antibody titer, due to interference in forming of antigen-antibody lattice
RPR

- Non-reactive
- Reactive

VDRL Slide
Syphilis: Treponemal Tests

- FTA-ABS
  - Immunofluorescent procedure using a non syphilitic (Reiter) strain of *T. pallidum* to absorb out non specific treponemal antibodies
  - Not titered
  - Remains positive in >85% of individuals throughout life
  - False positives (beaded pattern?) associated with rheumatic diseases and pregnancy
Treponemal Assays, 2

• TP Agglutination Assays
  – Agglutination assay with Treponemal antigens coupled to red blood cells (HA-TP, MHA-TP) or latex (TP-PA) particles
  – May be more specific than FTA; fewer false positives, especially in pregnant women
• ELISA/chemiluminescent (CIA) assays
• Multiplex bead immunoassays (MBIA)

TP-PA

[Image of TP-PA test with reactive and non-reactive samples]

Reactive

Non-reactive
ELISA / Chemiluminescent Assays

- Treponemal specific assays
- Automated/Semi Automated platforms
- High volume testing
- Objective interpretation
- Screening test
  - Blood Supply
  - Reverse Sequence Algorithm
Save the Manuals!!!

Knowledge Check

- What species causes Syphilis?
Knowledge Check

- Which assay is used to monitor syphilis treatment?
Manual “Methods”

Classic Syphilis Testing Algorithm

- Nonprenomal test (RPR or VDRL)*
  - Reactive
  - Nonreactive

- Treponemal test (EIA, TP-PA, TPHA, or FTA-ABS)
  - Reactive
  - Nonreactive

  - Syphilis (new case or previously treated case)
  - Biologic false positive**

* If the nonprenomal test is reactive qualitatively, a titer is then quantitated.
** Biologic false positive (BFP) results of nonprenomal tests can occur in the setting of other age, autoimmune diseases, intravenous drug use, recent vaccination, or certain infections.
Reverse Sequence Syphilis Algorithm

Diagram showing the algorithm for diagnosing Syphilis, with steps for nonreactive and reactive results, including EIA, RPR test, TP-PA, FTA-ABS, and other diagnostic tests.
Performance of Reverse Sequence Algorithm (MMWR, Feb 11, 2011)

- 5 Laboratories using reverse sequence algorithm from 2006-2010
- 56.7% of specimens reactive on the EIA/CIA were non reactive on an RPR/VDRL
- 31.6% of these discordant specimens were nonreactive using a second treponemal assay (TP-PA/FTA)
CDC Recommendations (MMWR, Feb 11, 2011)

- Traditional screening algorithm is recommended
- Reverse sequence may be used if a laboratory has appropriate equipment
  - Discordant specimens should be tested using the TP-PA instead of the FTA-ABS as the third level
  - Studies have been published since 2011 supporting the use of the automated Treponemal Specific Assays as the first line of testing

Three Algorithms

I. Traditional
   Non-treponemal test, eg, RPR
   Treponemal test, eg, TPPA, EIA, CIA
   + Serodiagnosis - BFP
   Treponemal test, eg, TPPA, EIA
   + Quantitative non-treponemal test
   + Serodiagnosis
   + Syphilis unlikely
   A second and different treponemal test
   + Serodiagnosis
   + Syphilis unlikely

II. Reverse
   Treponemal test, eg, TPPA, EIA
   Quantitative non-treponemal test
   - Serodiagnosis
   + Syphilis unlikely
   A second and different treponemal test
   + Serodiagnosis
   + Syphilis unlikely

III. ECDC
    Treponemal test, eg, TPPA, EIA
    + Syphilis unlikely
Tong, et al., Clin Infect Dis. 2014 Apr;58(8):1116-24

• Compared classical, reverse and European algorithms
• Classical algorithm: 76% accuracy
• Reverse algorithm: 99.9% accuracy
• European algorithm: 99.6% accuracy

Performance of Automated Treponemal Specific Assays

• Park, et al., 2016. JCM, 54:163-167
• Compared 6 different automated syphilis IgG assays to FTA-ABS, n= 615
  – Architect, Centaur, Cobas, HISCL, Immunoticles, and Mediace.
  – Agreement: 98-99.8%
  – Sensitivity: 96.8-99.4%
  – Specificity: 98-100%
• Discrepant specimens were false positives or from past, treated individuals
Performance of Automated Treponemal Specific Assays

- Compared Mediace RPR vs Mediace TPLA
- N= 24,681
- Reverse algorithm found 190 screen positive, traditional algorithm found 30 screen positive
- 140/190 were confirmed by RPR and/or TPPA
- Reverse algorithm detected 110 more true positives than traditional at a cost of more false positives

Knowledge Check

- What are the 2 US Syphilis Testing Algorithms?
But, Is There a Way to Have Both Treponemal and Non-Treponemal Results Simultaneously?

Can Replaying a Song Be as Easy as Selecting Repeat Option?
Finally…Auto Playing…Bluetooth

But, Is There a Way to Have Both Treponemal and Non-Treponemal Results Simultaneously?

YES!!!
BioPlex 2200 Syphilis Total and RPR Assay

Multiplex Treponemal and RPR Dual Assay

- Random Access Multiplex Analyzer
- Beads
  - Treponemal fusion protein rTP47/rTP17
  - Cardiolipin
  - Internal Standard
  - Serum Verification
- Conjugate
  - Monoclonal anti IgG and Monoclonal anti IgM
- Results
  - Total Anti Treponemal
  - Total RPR (Cardiolipin)
  - RPR Titer
Intended use

- An initial qualitative test for syphilis diagnosis
  - Not intended for screening blood or plasma donors
- Second qualitative step in either the standard or reverse algorithm
- Dilutions can be run to determine an RPR (Cardiolipin) end point titer up to 1:64 (1:2048)

BioPlex Test Result Interpretation

<table>
<thead>
<tr>
<th>BioPlex Syphilis Total</th>
<th>BioPlex RPR Total</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>Reactive</td>
<td>Presumptive evidence of infection</td>
</tr>
<tr>
<td>Reactive</td>
<td>Non reactive</td>
<td>Primary or latent infection or previously treated or untreated syphilis. Recommend additional testing consistent with clinical findings</td>
</tr>
<tr>
<td>Non reactive</td>
<td>Reactive</td>
<td>Presumptive evidence of early and/or incubating infection. Possible cross reactivity with other spirochetes/related antigens. Recommend additional testing consistent with clinical findings</td>
</tr>
<tr>
<td>Non reactive</td>
<td>Non reactive</td>
<td>No serological evidence of infection. Early or incubating infection cannot be excluded.</td>
</tr>
</tbody>
</table>
Bio-Rad In-House Data

  - BioPlex RPR Clinical sensitivity
    - 92.7% (n=41) for untreated syphilis
    - 82.7% (n=81) for treated syphilis
  - BioPlex Treponemal assay clinical sensitivity
    - 97.6% (n=41) for untreated syphilis
    - 95.1% (n=81) for treated syphilis

Bio-Rad In-House Data

- Zheng, et al., 2017 APHL Conference
  - Compared BioPlex results to a comparator result consisting of Diasorin LIASON treponemal assay, BD Macro-Vue RPR and Fujirebo SERODIA-TP-PA
  - Positive agreement: 98.7% (n=541)
  - Negative agreement: 98.5% (n=675)
OK, so I can have both results simultaneously. Which Algorithm should I use?

“The Lady or the Tiger” Ending. You decide!!!

• You can follow the classical algorithm and use the RPR results as the primary
• Or, follow the reverse algorithm and use the treponemal result as the primary
• If the reverse is chosen, a 3rd level of testing (TP-PA) will be needed for discordant specimens
Syphilis Testing Summary

- CDC has recommended the traditional algorithm
- Automated treponemal specific tests offer increased sensitivity, possibly at a cost of reduced specificity
  - The CDC and ECDC reverse algorithms use these assays as their initial test
- The BioPlex System provides both treponemal and non treponemal results in a single test with rapid turnaround time. Each lab can choose which algorithm they should follow

Acknowledgements

- Special thanks to
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    - Immunologist
      - Summa Health, Akron, OH
    - Professor of Pathology
      - Northeast Ohio Medical University
      - Rootstown, OH
Teaching “Zumba”

Wireless Music

HIV Testing – Historical Review and Preparing for the Future
Objectives

- Describe the disease progression of HIV History of HIV
- Explain how HIV diagnostic tests have increased in sensitivity FDA and HIV Tests
- Describe the importance of the current HIV testing algorithm related to public health

Outline

- Introduction to HIV
- History of HIV
- Importance of HIV Testing
- Evolution of HIV Tests
- HIV Testing Algorithm
Early Partner

• French biologist, microbiologist and chemist

• Renowned for his discoveries of the principles of vaccination, microbial fermentation and pasteurization

• Remembered for his remarkable breakthroughs in the causes and prevention of diseases

Louis Pasteur
Born: December 27, 1822; Dole, France
Died: September 28, 1895 (aged 72)
Marnes-la-Coquette, France

Bio-Rad Facility in Marnes-la-Coquette, France
Luc Montagnier, MD

Discovers virus for HIV in 1983 while working at Pasteur Institute
Awarded 2008 Nobel prize in Medicine for identifying virus that causes AIDS

Introduction to HIV
What is HIV/AIDS?

- **Human Immunodeficiency Virus**

  ![Image 1](http://aids.gov/)

  Images, charts, and statistics gathered from http://aids.gov/

What is HIV/AIDS?

- **Acquired Immune Deficiency Syndrome**

  ![Image 2](http://aids.gov/)

  Images, charts, and statistics gathered from http://aids.gov/
Anatomy of the Virus

RNA = ribonucleic acid

Typical Course of HIV Infection

RNA = ribonucleic acid
HIV Disease Progression

The first 6 months after infection, virus levels are higher and the risk of transmission is greatest. Evidence that starting HIV treatment early lowers the risk of developing AIDS or other serious illness. Early treatment of these patients and outreach to those they may have infected reduces the risk of further transmission.

Early Detection of HIV is Key

The first 6 months after infection, virus levels are higher and the risk of transmission is greatest. Evidence that starting HIV treatment early lowers the risk of developing AIDS or other serious illness. Early treatment of these patients and outreach to those they may have infected reduces the risk of further transmission.
The Many Flavors of HIV

- Two major types of HIV:
  - HIV-1 and HIV-2
- HIV-2 infection is most common in West Africa
- HIV-1 infection is more frequent than HIV-2 infection in most of the world
- HIV-2+ patients require different antiretroviral therapy than HIV-1 patients

How Does One Get HIV?

HIV CAN BE TRANSMITTED THROUGH...

- Sexual Contact
- Injection Drug Use
- Pregnancy, Childbirth & Breast Feeding
- Occupational Exposure
- Blood Transfusion/Organ Transplant

Images, charts, and statistics gathered from http://aids.gov/
### HIV Transmission

<table>
<thead>
<tr>
<th>Infection Stage</th>
<th>Transmission Hazard per Person-year</th>
<th>Mean Duration, Years (%)</th>
<th>No. (%) New Transmissions, by Sexual Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serial Monogamy</td>
</tr>
<tr>
<td>Acute</td>
<td>2.76</td>
<td>0.24 (2%)</td>
<td>0.10 (9%)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0.106</td>
<td>8.38 (82%)</td>
<td>0.77 (71%)</td>
</tr>
<tr>
<td>AIDS</td>
<td>0.760</td>
<td>0.75 (16%)</td>
<td>0.21 (20%)</td>
</tr>
</tbody>
</table>

### Early Stage of HIV: Symptoms

- **Within 2-4 weeks after exposure to HIV:** many, but not all, people who are infected experience flu-like symptoms, often described as the “worst flu ever.”
- **Many HIV+ people do not have symptoms:** they may not look or feel sick, often people only begin to feel sick when they progress toward AIDS.
- **If you think you may have been exposed to HIV:** regardless of whether you have experienced any symptoms, it is important to get tested as soon as possible.

The only way to know is with a test.

US HIV Statistics

1 in 6 living with HIV are unaware of their infection.

About 1 in 4 new HIV infections is among youth ages 13-24.

Most of them do not know they are infected, are not getting treated, and can unknowingly pass the virus on to others.

CDC - US HIV Prevalence (rates per 100K people)

Rates of HIV diagnoses among adults and adolescents in the US in 2015, by State.

Avg. Prevalence (Positivity Rate): 12%

Source - CDC
US HIV Statistics

Diagnoses of HIV Infection among Adults and Adolescents by gender

HIV Status

Diagnoses of HIV Infection among Adults and Adolescents by category
HIV Status

~40,000
New annual HIV infections in US (2017)


Knowledge Check

- Who discovered HIV?
Knowledge Check

- Can one be HIV positive and not have AIDS?

History of HIV
HIV History

1981

- Outbreak in NY and CA of rare form of cancer among gay population
  - Kaposi’s Sarcoma

- ERs in NY began seeing multiple cases of healthy people with flu-like symptoms and rare form of pneumonia
  - Pneumocystis
1983

- 33 countries around the world had confirmed cases

- Dr. Montagnier at Pasteur Institute in France isolated a retrovirus related to the outbreak of HIV and then AIDS

1983

- The final episode of M.A.S.H. aired with more than 125 million viewers tuned in to watch
### HIV History

**1984**
- Dr. Robert Gallo from NIH isolated a retrovirus that is reported to also be responsible for AIDS
  - Same as that found by Dr. Montagnier (who received Nobel Prize in 2008)
- Canadian flight attendant nicknamed “patient zero” - believed to be responsible for introducing the virus to the general population

---

**1984**

- The first Apple Macintosh goes on sale
1985

- Ryan White is barred from his elementary school because he acquired HIV from a blood transfusion

1985

Charity single "We Are the World" is recorded by supergroup USA for Africa (Michael Jackson, Lionel Richie and other pop stars)
### HIV History

**1987**

- First treatment available for people with HIV
  - Retrovir (AZT) is FDA approved

---

**1987**

Richard Branson and Per Lindstrand make the first transatlantic hot-air balloon flight. 2,790 miles from Sugarloaf Mountain, Maine, to Ireland.

*Virgin Atlantic Flyer*
HIV History

1990
- Ryan White Care Act is enacted by Congress to provide government sponsored funds for the care of people with HIV and AIDS

"The Simpsons " is aired on Fox for the first time

Extra Credit: How many episodes?
Still on air with >630 episodes to date
Importance of HIV Testing

Recommended Testing: CDC

Revised Recommendations for HIV Testing of Adults, Adolescents, and Pregnant Women in Health-Care Settings
Recommended Testing: CDC

Adults and Adolescents
- Routine, voluntary HIV screening for all persons 13-64 in health care settings, not based on risk
- HIV testing of people at high risk for HIV infection at 1x/yr
- Intended for all health care settings: in patient services, emergency rooms, urgent care clinics, STD clinics, primary care settings

Recommended Testing: USPSTF

Screening for HIV

U.S. Preventive Services Task Force Recommendation Statement

Release Date: April 2013
**Recommended Testing: USPSTF**

Adults and Adolescents – expanded in 2013
- Routine HIV screening for all persons 15–65 years old who are not known to be at high risk
- Younger adolescents and older adults who are at increased risk should also be screened
- All pregnant women for HIV, including those in labor who are untested and whose HIV status is unknown

**HIV Testing**

The proportion of adults who have ever been tested for HIV increased from 37 percent in 2000 to 45 percent in 2010

HIV Testing

Laboratory

Point of Care
Knowledge Check

What type of test(s) does your lab perform?

A. Screening  
B. Supplemental/Confirmatory  
C. POC

Evolution of HIV Tests

Screening
1st Generation Antibody (Ab)

- HIV is viral lysate as antigen
- Detects IgG antibodies
- Specific for HIV-1 Group M, subtype B

Testing Technology

(1st Generation)

1987 Vironostika EIA

1985 Abbott HIV-1 EIA

Source: Dr. Michelle Owen, CDC
2nd Generation Antibody (Ab)

- Synthetic peptides or recombinant proteins as capture antigen
- Detects IgG antibodies
- Specific for HIV-1 Group M, subtype B and HIV-2

Testing Technology

(2nd Generation)

Collectively – the CDC now refers to both of these generations as IgG tests

Source: Dr Michelle Owen, CDC
3rd Generation Antibody (Ab)

- Detects IgM and IgG antibodies
- Specific for HIV-1 (Groups M and O) and HIV-2 antibodies

Testing Technology

(3rd Generation)

Source: Dr. Michelle Owen, CDC
A 3rd Generation Test detects what?

A. HIV-1 only
B. IgM and IgG antibodies
C. IgG antibodies only
D. p24 Ag

Knowledge Check

A 3rd Generation Test detects what?

A. HIV-1 only
B. IgM and IgG antibodies
C. IgG antibodies only
D. p24 Ag
HIV Rapid Test

- OraQuick HIV-1/HIV-2 RT
- Unigold Recomb HIV-1 RT
- Multispot HIV-1/HIV-2 RT
- Ortho VITROS HIV 1/2 CIA

Testing Technology

- (Rapid Tests)

- Abbott HIV-1 EIA (1987)
- Abbott HIV-1 EIA (1992)
- Murex SUDS (1992)
- Abbott HIV-1 EIA (1987)
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Source: Dr. Michelle Owen, CDC
4th Generation Antigen/Antibody (Ag/Ab)

- Detects HIV-1 \textbf{p24} antigen
- Detects IgM and IgG antibodies
- Simultaneous detection, not differentiation of antigen and antibodies
- Ability to detect acute infection
4th Generation Antigen/Antibody (Ag/Ab)

Abbott: ARCHITECT HIV Ag/Ab Combo

4th Generation Antigen/Antibody (Ag/Ab)

Bio-Rad: GS HIV Combo Ag/Ab EIA
4th Generation Antigen/Antibody (Ag/Ab)

Siemens: ADVIA Centaur® HIV Ag/Ab Combo (CHIV)

Note: Can differentiate between Ag and Ab, but not within Abs (HIV 1 / HIV 2)
A 4th Generation Test detects what?

A. IgG antibodies only  
B. p24 Ag  
C. Antibodies and p24 antigen simultaneously  
D. Viral load
4th Gen vs 3rd Gen : Bio-Rad

Performance evaluation of the Bio-Rad Laboratories GS HIV Combo Ag/Ab EIA, a 4th generation HIV assay for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma

Christopher Panther, Lisa McLaughlin, Elizabeth Mitchell, Carol Ferrera, Sally Liska, Robert Myers, Sheila Pool, Paul Swenson, Stephanie Gadele, M. Kathleen Shriver

Results
GS HIV Combo Ag/Ab EIA detection in samples from individuals in two separate populations with acute HIV infection was 95.2% (20/21) and 86.4% (38/44).

Sensitivity was 100% (1603/1603) in known antibody positive [HIV-1 Groups M and O, and HIV-2] samples. HIV p24 antigen detection was 100% (53/53) in HIV-1 culture supernatants.

HIV-1 seroconversion panel detection improved by a range of 0–20 days compared to a 3rd generation HIV test. Specificity was 99.9% (5989/5996) in low risk, 99.9% (959/960) in high risk and 100% (100/100) in pediatric populations.
4th Gen vs 3rd Gen : Bio-Rad

Conclusion

• The GS HIV Combo Ag/Ab EIA significantly reduced the diagnostic window when compared to the 3rd generation screening assay, enabling earlier diagnosis of HIV infection.

• The performance parameters of the Bio-Rad GS HIV Combo Ag/Ab EIA are well suited for use in HIV diagnostic settings.

4th Gen vs 3rd Gen : Abbott

Evaluation of the performance of the Abbott ARCHITECT HIV Ag/Ab Combo Assay

Pollyanna Chaves, Laura Wiesolowski, Pragna Patel, Kevin Delaney, S. Michele Owen
Results

- Based on results from the initial ARCHITECT test, sensitivity was 99.94% (95% confidence interval [CI]: 99.79, 99.99) and specificity was 98.78% (95% CI: 98.51–99.01).
- Repeat testing resulted in corrected specificity of 99.50% (95% CI: 99.31, 99.64).
- Also, 48 AHI specimens (83%) were detected by this screening assay.

Conclusion

- The sensitivity and specificity of the ARCHITECT combination assay are very high and most AHIs were detected by the assay.
- Use of Ag/Ab combination assays may improve the number of AHIs identified relative to existing FDA-approved HIV-antibody only based serologic assays, particularly in high incidence populations.
The BioPlex 2200 HIV Ag-Ab multiplexed assay is Bio-Rad’s next (5th) generation HIV diagnostic test system.

“5th Generation” BioPlex 2200 HIV Ag-Ab assay design

Simultaneously detects and reports:

- HIV Ag-Ab (overall result)
- HIV-1 p24 Ag
- HIV-1 Ab (groups M & O)
- HIV-2 Ab

*Separate* reporting of HIV-1 p24 helps identify acute infections. Includes HIV-1 and HIV-2 Ab *Differentiation*.
HIV Ag or Ab Dyed Bead Mix

- The 5th generation assay design allows for the simultaneous detection and identification of multiple HIV analytes for each sample processed.
- The bead reagent consists of a mixture of four distinct populations of dyed microparticle beads, in addition to three internal quality beads that assure quality results.

Beads are combined into single “Bead Reagent” for multiplex analysis

Testing Technology

(5th Generation)

Source: Dr Michelle Owen, CDC and Greg Stewart, Bio-Rad
HIV Testing

Analytical Sensitivity

- Used CLSI protocol to determine Limit of Detection (LOD) at cutoff for reference HIV-1 p24 Ag
  - WHO Standard: **0.33 IU/mL**
  - (range: 0.29-0.35 IU/mL)
  - French National Standard (ANSM): **5.2 pg/mL**
  - (range: 5.0-5.4 pg/mL)

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Abbott Assay Name</th>
<th>Bio-Rad Assay Name</th>
<th>Siemens Assay Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO Reference</td>
<td>1.032 IU/mL</td>
<td>0.65 IU/mL</td>
<td>1.05 IU/mL</td>
</tr>
<tr>
<td>French ANSM</td>
<td>18.39 pg/mL</td>
<td>14.78 pg/mL</td>
<td>9.04 pg/mL*</td>
</tr>
</tbody>
</table>

*Used Zypometrix panel instead of French ANSM; thus not equivalent (33.49 pg/mL OUS)
Evolution of HIV Tests
Confirmatory
Sequence of Test Positivity Relative to WB

15 Seroconverter panels - 50 % Positive Cumulative Frequency

- Modified from Owen et al J Clin Micro 2008

HIV-1 Western Blot

- gp160 (env precursor)
- gp120 (outer env or "surface" glycoprotein)
- p55 (reverse transcriptase)
- p55 (core precursor)/p9 (RT)
- gp41 (transmembrane glycoprotein)
- p24 (core or "capsid")
- p31 (endonuclease)
- p18 (core matrix)
HIV-1 Western Blot

APHL/CDC criteria for positive WB: Any two of gp160/120, gp41, p24

Multispot HIV 1/2 Rapid Test
Multispot HIV 1/2 Rapid Test

Remove foil. Label cartridge and specimen or control test tubes.

Add 2 dropperfuls (300 μL) of specimen diluent to each test tube. Add 1 drop (60 μL) specimen or control. Mix well.

Pour specimen into the prefiltor. Wait 2 min. Remove and discard prefiltor.

Fill cartridge with wash solution and let absorb. Add 3 drops conjugate. Wait 2 min.

Fill with wash solution and let absorb. Repeat. Add 3 drops development reagent. Wait 5 min.

Fill with stop solution. Allow to absorb and read results.

Multispot HIV 1/2 Rapid Test

Spot Identity

Procedural Control
Recombinant HIV-1
HIV-1 Peptide
HIV-2 Peptide

HIV-1 Reactive:
Diagnostic Algorithm
HIV-1 Positive
Purple color development for both HIV-1 spots

HIV-1 Reactive:
Rapid Testing
HIV-1 Preliminary Positive
Purple color development for one or both HIV-1 spots

HIV-2 Reactive:
Diagnostic Testing Algorithm - Positive
Rapid Testing - Preliminary Positive

BIO-RAD
Why Develop the Geenius?
Geenius HIV 1/2 Supplemental

Control Band

<table>
<thead>
<tr>
<th>HIV-2</th>
<th>HIV-1</th>
<th>Ctl Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP36 peptide</td>
<td>GP140 peptide</td>
<td>Protein A</td>
</tr>
<tr>
<td>P31 peptide</td>
<td>GP160 recombinant</td>
<td></td>
</tr>
<tr>
<td>P24 recombinant</td>
<td>GP41 peptides</td>
<td></td>
</tr>
<tr>
<td>ENV</td>
<td>ENV</td>
<td>POL</td>
</tr>
</tbody>
</table>

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### Geenius HIV 1/2 Supplemental

<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*</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATE*</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATE</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>
<tr>
<td>Indeterminate</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
</tbody>
</table>
| Positive     | Positive     | HIV-2 POSITIVE with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity is due to cross-reacting and precedes confirmation of HIV-1.*  
  *Note: Differentiation features managed by proprietary algorithm. |
| Positive     | Positive     | HIV POSITIVE (differential): 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).*  
  *Note: Differentiation features managed by proprietary algorithm. |

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### Assay Performance
Assay Performance Characteristics

- Sensitivity: Positive in disease
- Specificity: Negative in absence of disease
- Positive Predictive Value (PPV): Odds that a positive represents a true positive
- Negative Predictive Value (NPV): Odds that a negative represents a true negative
- So, what is a true positive and a true negative?

Ideal Situation

![Graph showing ideal situation with positive and negative distributions and pos/neg cutoff](image-url)
Real Life
Where to Place the Cutoff?

In low-risk populations, immunoassay false positives and prevalence effect positive predictive values.
**Moral of Story**

- Screening tests (high sensitivity) performed in low risk individuals have poor positive predictive value.
- Screening tests performed in individuals selected based on clinical symptoms have high positive predictive value.
  - Shotgun approach to testing leads to poor PPV
- Using a confirmatory test will reduce false positives and increase the overall PPV

**HIV Testing Algorithm**
Old HIV Testing Algorithm

1989 Guideline: Confirmatory Testing

“The Public Health Service recommends that no positive test results be given until a screening test has been repeatedly reactive on the same specimen and a supplemental, more specific test such as the Western blot has been used to validate those results.”

Source: Dr Michelle Owen, CDC
Old HIV Testing Algorithm

**Laboratory Algorithm 2**

HIV-1/HIV-2 Immunoassay, With Supplemental NAAT Option

A1

- HIV-1/HIV-2 Immunoassay
  - A1+
    - Repeat A1 in duplicate
  - A1
  - A1(± or +−)

A1(± or +−)

- OR
  - B1
    - HIV-1 WB or HIV-1 IFA
      - Positive
      - Negative
      - Indeterminate
  - B2
    - Individual HIV-1 NAAT
      - Positive
      - Negative
      - Positive

**BIO-RAD**
Old HIV Testing Algorithm

The Bottom Line Is…

1\textsuperscript{st} Generation “confirmatory” tests (WB, IFA) are following 3\textsuperscript{rd} Generation screening tests

New HIV Testing Algorithm
June 20th, 2014

Dear Colleague:

The Centers for Disease Control and Prevention (CDC) has issued updated recommendations for HIV testing by laboratories in the United States. The new recommended algorithm has several advantages over the previous testing algorithm: more accurate laboratory diagnosis of acute and established HIV-1 infection, more accurate laboratory diagnosis of HIV-2 infection, fewer indeterminate test results, and faster turnaround times for most test results. The complete recommendations titled Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations and a quick reference guide can be downloaded from the Division of HIV/AIDS Prevention’s website at http://www.cdc.gov/hiv/testing/lab/guidelines.

Sincerely,

[Signature]

Bernard J. Benson, M.D.
Associate Director for Laboratory Diagnostics
Division of HIV/AIDS Prevention
National Center for HIV, Viral Hepatitis, STD and TB Prevention

US HIV Testing Algorithm

Laboratory Testing for the Diagnosis of HIV Infection:
CDC June 2014 recommendations

HIV-1/2 antigen/antibody combination immunoassay

(+)

HIV-1/2 antibody differentiation immunoassay

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

HIV-1 antibodies detected

HIV-2 antibodies detected

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

HIV antibodies detected

HIV-1 (+) or indeterminate

HIV-2 (-)

HIV-1 NAT

(-) indicates reactive test result

(-) indicates nonreactive test result

NAT: nucleic acid test

HIV-1 NAT (+)

Acute HIV-1 infection

HIV-1 NAT (-)

Negative for HIV-1
Proposed HIV Testing Algorithm

CLSI published in Nov 2011

JCV Supplement published in Dec 2011
Proposed HIV Testing Algorithm

DRAFT Recommendations:
Diagnostic Laboratory Testing for HIV Infection in the United States

The draft information is distributed solely for the purpose of pre-dissemination peer review and public comment under applicable information quality guidelines. This draft information has not been formally disseminated by the Centers for Disease Control and Prevention, the Health Resources and Services Administration, or the U.S. Department of Health and Human Services. This draft information does not represent and should not be construed to represent any agency determination or policy. The draft report describes use of tests for some indicationsthat do not reflect labeling approved by the U.S. Food and Drug Administration (FDA) at the time of publication.

Presented at the 2012 HIV Diagnostics Conference Feedback Session held on December 14, 2012

Definitions
Immunocassette generations:

- 1st: viral lysate antigen, designed for IgG detection (includes Western blot, RIA)
- 2nd: synthetic peptide or recombinant protein antigens, designed for IgG antibody detection
- 3rd: synthetic peptide or recombinant protein antigens, designed for IgM and IgG antibody detection
- 4th: synthetic peptide or recombinant protein antigens, IgM antibody, designed to detect IgM and IgG antibodies and p24 antigen

Acute HIV infection, for the purpose of these recommendations, is defined as the interval between the appearance of detectable HIV RNA and development of detectable IgG antibody.
Proposed HIV Testing Algorithm

Recommended Key Changes for HIV testing on Serum or Plasma

1. Initiate testing for HIV with a 4th generation antigen/antibody combination immunoassay.

http://www.cdc.gov/hiv/testing/lab/guidelines
Recommended Key Changes for HIV testing on Serum or Plasma

1. Initiate testing for HIV with a 4th generation antigen/antibody combination immunoassay.

2. Test specimens with a repeatedly reactive antigen/antibody immunoassay results with an antibody immunoassay that differentiates HIV-1 antibodies from HIV-2 antibodies. As of June 27, 2014, the Multispot HIV-1/2 Rapid Test is the only assay approved by the FDA for this indication. Note that the criteria for interpretation of Multispot test results, when it is used as a differentiation assay in the diagnostic algorithm, require the presence of both HIV-1 indicators for a positive interpretation.

http://www.cdc.gov/hiv/testing/lab/guidelines

3. Specimens that are reactive on the initial 4th generation immunoassay and nonreactive or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with and HIV-1 nucleic acid test (NAT).

http://www.cdc.gov/hiv/testing/lab/guidelines
Recommended Key Changes for HIV testing on Serum or Plasma

3. Specimens that are reactive on the initial 4th generation immunoassay and nonreactive or indeterminate on the HIV-1/HIV-2 antibody differentiation immunoassay should be tested with and HIV-1 nucleic acid test (NAT).

4. Laboratories should use this same testing algorithm, beginning with a 4th generation immunoassay, with serum or plasma specimens submitted for testing after a reactive (preliminary positive) result from any rapid HIV test (including the HIV-1/HIV-2 antibody differentiation assay, when it is used as an initial rapid test, and the HIV-1/HIV-2 antigen/antibody combination rapid test). No further testing is required if the result of the laboratory’s initial 4th generation immunoassay is nonreactive.

http://www.cdc.gov/hiv/testing/lab/guidelines

New Algorithm

Who’s Still Running Western Blot?
5. The HIV-1 Western Blot is no longer part of the recommended algorithm for HIV testing.

http://www.cdc.gov/hiv/testing/ab/guidelines

Knowledge Check

New Algorithm Recommendations:

Initiate screening with a ________________
New Algorithm Recommendations

Reactive (repeatedly reactive) specimens should be tested with an assay that
___________ HIV-1 from HIV-2 antibodies.

What changes are to come? How long will it take?

Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens

- HIV-1/2 antigen/antibody combination immunoassay
  - (+) HIV-1 (+) or HIV-2 (+)
  - (-) Negative for HIV-1 and HIV-2 antibodies and p24 Ag

  HIV-1/2 antibody differentiation immunoassay
  - (+) HIV-1 antibodies detected
  - (-) HIV-2 antibodies detected
  - (-) HIV antibodies detected
  - HIV-1 (+) HIV-1 NAT (+) Acute HIV-1 infection
  - HIV-1 (-) or Indeterminate HIV-1 NAT (-) Negative for HIV-1
2019 CDC Proposing Alternative Algorithm

An alternative laboratory testing algorithm

- HIV-1/2 Ag/Ab combination assay
  - (+) HIV-1 viral load quantification
  - (-) Target not detected considered negative

- HIV-1 RNA detected at any level considered positive

- HIV-1/2 Ab differentiation assay
  - (+) Indeterminate; may need further testing
  - (-)
**Summary**

- HIV diagnosis is important to contain the spread of HIV infection
- HIV diagnosis is important for timely medical treatment

**Acknowledgements**

- Special thanks to Bio-Rad Colleagues
  - Alfredo Villarreal
    - Senior Product Manager
    - Clinical Immunology Division, Benicia, CA
  - Greg Stewart
    - Senior Product Manager
    - US Sales & Service Division, Hercules, CA
Can Replaying a Song Be as Easy as Selecting Repeat Option?

Which option do you prefer?

Question to Ponder

• Which of the Two Make the Best Soul Mate: An Autoimmune or Infectious Antibody?
Thank You!