Which of the Two Make the Best Soul Mate: An Autoimmune or Infectious Antibody?

Maria Crisostomo - September 17, 2017
Disclosure

• No disclosures
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)
Objectives

- Upon completion of this autoimmune module, the learner will have a basic understanding of the following:
  - 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

Gender Bias*

Knowledge Check

• What is an autoantibody?
Knowledge Check

• What is an autoantibody?
• A blood protein produced by our immune system but mistakes our own tissues & cells as foreign invaders
Knowledge Check

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

- What is an autoimmune (AI) disease?
  - An autoimmune disease is a disorder whereby the body mistakes its own tissue and cells for a foreign invader
Knowledge Check

- What triggers an autoimmune disease?
Knowledge Check

• What triggers an autoimmune disease?
• An environmental factor and/or a person’s genetic make-up can trigger an immune response resulting in an autoimmune disease.
Examples of Autoimmune Diseases

**Systemic**
- Thyroid
  - Hashimoto's thyroiditis
  - Grave's disease
- Stomach/Intestinal
  - Atrophic gastritis
  - Autoimm. Hepatitis
  - Celiac Disease
- Adrenal
  - Addison's Disease
- Circulatory
  - Wegener's Granulomatosis
- Kidney
  - SLE
- Skin
  - Scleroderma
- Muscle
  - Dermatomyositis
  - Polymyositis
- Joints
  - Rheumatoid arthritis
  - Mixed Connective Tissue Disease

**Organ Specific**
- Eyes & Mouth
  - Sjogren's Syndrome
- Pancreas
  - Type 1 Diabetes
Autoimmune Testing Disease Categories

- **Typical Autoimmune Test Menu Offering**

  ![Venn Diagram](diagram.png)

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

  **Bio-Rad Autoimmune**
Knowledge Check

• Q: What are the five typical autoimmune disease categories used by IVD manufacturers?
Knowledge Check

• What are the five typical autoimmune disease categories used by IVD manufacturers?
  • Systemic, Organ-specific, Anti-Phospholipid Syndrome, Vasculitis, and Gastrointestinal
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 1\textsuperscript{st} Tier Testing**

(-) No further testing required

(+) Perform 2\textsuperscript{nd} Tier Testing

- *dsDNA*
- *Chromatin*
- *SS-A*
- SS-B
- *Sm*
- Sm/RNP
- RNP
- Jo-1
- Scl-70
- Ribosomal P
- Centro-mere

* = Lupus biomarker
Knowledge Check

• What is a testing algorithm?
Knowledge Check

- What is a testing algorithm
- It is a sequence of test used in combination to improve the accuracy of the laboratory diagnosis of the targeted disease
Knowledge Check

• What are the 3 most commonly test methods used for autoimmune diagnostic testing?
Knowledge Check

• What are the 3 most commonly test methods used for autoimmune diagnostic testing?
  • Immunofluorescence (IFA), Enzyme Immunoassay (EIA) and Multiplex Bead Immunoassay (MBIA)
## 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 positive</td>
<td>Proportion of patients without the disease who test negative</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>True Positive</td>
<td>True Negative</td>
</tr>
<tr>
<td><strong>Ideal Test Result</strong></td>
<td>Negative Test Result</td>
<td>Positive Test Result</td>
</tr>
<tr>
<td><strong>Test Interpretation</strong></td>
<td>They are definitely <strong>not positive</strong> → They <strong>DON’T</strong> have it</td>
<td>They are definitely <strong>not negative</strong> → They <strong>DO</strong> have it</td>
</tr>
<tr>
<td><strong>The Rule</strong></td>
<td>Rule Out (SnOut)</td>
<td>Rule In (SpIn)</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
Test Performance: Sensitivity vs. Specificity
Knowledge Check

• What is assay sensitivity?
Knowledge Check

• What is assay sensitivity?
• It is the ability of the test to detect a disease in a patient with the targeted disease
Knowledge Check

• What is assay specificity?
Knowledge Check

• What is assay specificity?
• It is the ability of the test to not detect a disease in a patient without the targeted disease.
Syphilis Testing
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
Upon completion of this Syphilis module, the learner will have a basic understanding of the following:

- **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 *Treponema pallidum* (TP), subspecies *pallidum, Nichols strain*
- Thin (0.2 μm) spirochete 6-20 μm in length with 10-13 coils
Primary and Secondary Syphilis: Distribution of Cases by Sex & Sexual Behavior, 2015

- Men who have sex with men only (n = 12891)
- Men who have sex with men and women (n = 1338)
- Men who have sex with women only (n = 3178)
- Men without data on sex of sex partners (n = 4140)
- Cases with unknown sex (n = 27)
- Women (n = 2298)
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 and cardiolipin from Treponemes
- Not specific for *T. pallidum* infection
  - May be positive in anti-phospholipid antibody syndrome (APS)
- 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 & 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
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
Manual vs. Automated
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

- What species causes Syphilis?
- *Treponema pallidum* or *T. pallidum*
Knowledge Check

• Which assay is used to monitor syphilis treatment?
Knowledge Check

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

Insert Here
Classic Syphilis Testing Algorithm

Nontreponemal test (RPR or VDRL)*

Reactive

- Treponemal test (EIA, TP-PA, TPHA, or FTA-ABS)
  - Reactive: Syphilis (new case or previously treated case)
  - Nonreactive: Biologic false positive**

- No syphilis or very recent infection; testing concludes

Nonreactive

* If the nontreponemal test is reactive qualitatively, a titer is then quantitated.

** Biologic false positive (BFP) results of nontreponemal tests can occur in the setting of older age, autoimmune disease, intravenous drug use, recent vaccination, or certain infections.
Reverse Sequence Syphilis Algorithm

EIA, enzyme immunoassay; RPR, rapid plasma reagin; TP-PA, Treponema pallidum particle agglutination; FTA-ABS, fluorescent treponemal antibody-absorption.

Source: Infect Med © 2004 Cliggott Publishing, Division of SCP Communications
FIGURE. Composite results of syphilis testing algorithms using treponemal tests for initial screening and likely interpretations* — four laboratories, New York City, October 1, 2005–December 1, 2006†

Initial EIA§ treponemal test (N = 116,822)

- 6,587 (6%)
- 110,235 (94%)

Rapid plasma reagin (RPR) test (n = 6,548)

- 2,884 (44%)
- 3,664 (56%)

Syphilis, old or new. Treatment usually indicated unless previously treated. Retreatment indicated if titer has increased four fold or more.

- Probably old treated syphilis. Treatment might be indicated if not previously treated.
- If false-positive screening treponemal test result suspected, or if not previously treated, retest with a different treponemal test.

Second treponemal test**
(n = 2,512)

- 2,079 (83%)
- 433 (17%)

Treatment indicated, unless a history of treatment exists.

- No treatment, or a third treponemal test can be used to resolve the discrepancy between the two treponemal test results.

* One laboratory provided limited interpretation of the test results; the other three summarized the results without interpretation. No formal recommendations exist regarding the interpretation of results derived from testing algorithms using treponemal tests as the initial test.
† Using a convenience sample of 116,822 specimens. The four laboratories used different testing algorithms. Data shown are a composite of results from all four laboratories.
§ Enzyme immunoassay.
¶ Reactive with EIA treponemal test but nonreactive with RPR test.
** Using Treponema pallidum particle agglutination or fluorescent treponemal antibody 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)
• 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
Nontreponemal test, e.g., RPR

- Treponemal test, e.g., TPPA, EIA, CIA

  + Serodiagnosis
  - BFP

II. Reverse
Treponemal test, e.g., TPPA, EIA

- Quantitative nontreponemal test

  + Serodiagnosis
  - Syphilis unlikely

III. ECDC
Treponemal test, e.g., TPPA, EIA

- A second and different treponemal test

  + Serodiagnosis
  - Syphilis unlikely
• 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?
Knowledge Check

• What are the 2 US Syphilis Testing Algorithms?
• Traditional and Reverse
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
BioPlex Syphilis Total and RPR 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
## 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
• Use of Medication
  • Prevention
  • Antiretroviral Therapy
• FDA and HIV Tests
• Government/National Input
Early Partner

Louis Pasteur
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**

What is HIV/AIDS?

- Acquired Immune Deficiency Syndrome

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

RNA = ribonucleic acid
Typical Course of HIV Infection

CD4+ T Lymphocyte Count (cells/mm³) vs. Weeks vs. Years

- Primary infection
- ± Acute HIV syndrome
- Wide dissemination of virus
- Seeding of lymphoid organs
- Clinical latency
- Opportunistic diseases
- Constitutional symptoms
- Death

HIV RNA Copies per ml Plasma

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
- and rarely, 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>

Source - CDC
Early Stage of HIV: Symptoms

The only way to know is with a test

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

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

- More than 1 million people in the U.S. are living with HIV.
- 1 in 6 people living with HIV are unaware of their infection.
- Gay and bisexual men of all races are the most severely affected by HIV.
- 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.
Diagnoses of HIV Infection among Adults and Adolescents, by Sex, 2009–2013—United States and 6 Dependent Areas

Note: Data include persons with a diagnosis of HIV infection regardless of stage of disease at diagnosis. All displayed data have been statistically adjusted to account for reporting delays, but not for incomplete reporting.

Source - CDC
HIV Status
Diagnoses of HIV Infection among Adults and Adolescents by category

Diagnoses of HIV Infection among Adults and Adolescents, by Transmission Category, 2009–2013 — United States and 6 Dependent Areas

Note: Data include persons with a diagnosis of HIV infection regardless of stage of disease at diagnosis. All displayed data have been statistically adjusted to account for reporting delays and missing transmission category, but not for incomplete reporting.

* Heterosexual contact with a person known to have, or to be at high risk for, HIV infection.

b Includes hemophilia, blood transfusion, perinatal exposure, and risk factor not reported or not identified.

Source - CDC
50,000

New annual HIV infections in US (2013)

Source - CDC
History of HIV
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
HIV History

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
HIV History

1985

– Ryan White is barred from his elementary school because he acquired HIV from a blood transfusion
• Charity single "We Are the World" is recorded by supergroup USA for Africa (Michael Jackson, Lionel Richie and other pop stars)
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
1990

- "The Simpsons " is aired on Fox for the first time
Importance of HIV Testing
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
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
HIV Testing

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)

1985
Abbott
HIV-1 EIA

1987
Vironostika
EIA

1992
Abbott
HIV-1/HIV-2
EIA

2000
Genetic Systems
HIV-1/HIV-2
Peptide EIA

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)

1985
Abbott
HIV-1 EIA

1987
Vironostika
EIA

1992
Abbott
HIV-1/HIV-2 EIA

2000
Genetic Systems
HIV-1/HIV-2
Peptide EIA

2003
GS HIV-1
HIV-2 PLUS O
EIA

2006
Advia
Centaur
HIV 1/0/2 CIA

2008
Ortho VITROS
HIV ½ CIA

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
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
Testing Technology

(Rapid Tests)

Source: Dr Michelle Owen, CDC
Testing Technology

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)
4th Generation Antigen/Antibody (Ag/Ab)

Alere: Determine HIV-1/2 Ag/Ab RT
Testing Technology

(*4th Generation)

1987 Vironostika
EIA

1992 Abbott
HIV-1/HIV-2
EIA

1985 Abbott
HIV-1 EIA

1992 Murex
SUDS

2000 Genetic Systems
HIV-1/HIV-2 Peptide EIA

2002 Procleix
HIV-1/HCV NAT

2003 GS HIV-1
HIV-2 PLUS O
EIA

2006 Procleix Ultrio
HIV/HCV/HBV
NAT

2006 Aptima Qualitative
NAT

2006 Advia Centaur
HIV 1/0/2 CIA

2006 Ortho VITROS
HIV-1/2 CIA

2006 Siemens ADVIA
Centaur HIV Ag/Ab Combo

2008 Procleix HIV/HCV/HBV
NAT

2002 OraQuick
HIV-1/HIV-2 RT

2003 Unigold Recomb
HIV-1 RT

2004 Multispot
HIV-1/HIV-2 RT

2003 GS HIV-1
HIV-2 PLUS O
EIA

2004 Multispot
HIV-1/HIV-2 RT

2002 Abbott
HIV-1/HIV-2 EIA

2002 Murex
SUDS

1999 Roche
Amplicor HIV-1 Monitor

2004 Multispot
HIV-1/HIV-2 RT

2002 OraQuick
HIV-1/HIV-2 RT

2003 Unigold Recomb
HIV-1 RT

2008 Ortho VITROS
HIV-1/2 CIA

2006 Siemens ADVIA
Centaur HIV Ag/Ab Combo

2006 Procleix Ultrio
HIV/HCV/HBV
NAT

2003 GS HIV-1
HIV-2 PLUS O
EIA

2002 Abbott
HIV-1/HIV-2 EIA

1985 Abbott
HIV-1 EIA

1992 Abbott
HIV-1/HIV-2
EIA

1987 Vironostika
EIA

Source: Dr Michelle Owen, CDC and Greg Stewart, Bio-Rad
A 4th Generation Test detects what?

A. IgG antibodies only  
B. p24 Ag  
C. Antibodies and p24 antigen simultaneously  
D. Viral load
A 4th Generation Test detects what?

A. IgG antibodies only  
B. p24 Ag  
C. **Antibodies and p24 antigen simultaneously**  
D. Viral load
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 Bentsen, Lisa McLaughlin, Elizabeth Mitchell, Carol Ferrera, Sally Liska, Robert Myers, Sheila Peel, Paul Swenson, Stephane Gadelle, 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.
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.
Evaluation of the performance of the Abbott ARCHITECT HIV Ag/Ab Combo Assay

Pollyanna Chavez, Laura Wesolowski, 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.
5th Generation Antigen/Antibody (Ag/Ab)

Bio-Rad: BioPlex 2200 HIV Ag-Ab Assay
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) with
  - 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.
Testing Technology

(5th Generation)

1987 Vironostika EIA
1992 Abbott HIV-1/HIV-2 EIA

1985 Abbott HIV-1 EIA
1992 Murex SUDS

2000 Genetic Systems HIV-1/HIV-2 Peptide EIA
2002 Procleix HIV-1/HCV NAT
2003 GS HIV-1 HIV-2 PLUS O EIA
2006 Procleix Ultrio HIV/HCV/HBV NAT

1999 Roche Amplicor HIV-1 Monitor
2002 OraQuick HIV-1/HIV-2 RT
2003 Unigold Recomb HIV-1 RT
2004 Multispot HIV-1/HIV-2 RT

2006 Advia Centaur HIV 1/0/2 CIA
2008 Ortho VITROS HIV-1/2 CIA

2006 Murex SUDS
2000 Abbott HIV-1/HIV-2 EIA

2006 GS HIV-1/2 CIA
2008 Ortho VITROS HIV-1/2 CIA

2006 Procleix Ultrio HIV/HCV/HBV NAT

2006 Genentech HIV-1/2 PLUS O EIA

2006 BioPlex 2200 HIV Ag-Ab

*2011 Abbott ARCHITECT HIV Ag/Ab Combo

*2013 Determine Ag/Ab RT

*2015 Siemens ADVIA Centaur HIV Ag/Ab Combo

Source: Dr Michelle Owen, CDC and Greg Stewart, Bio-Rad
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</th>
<th>Bio-Rad</th>
<th>Siemens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Name</td>
<td>Architect HIV Ag/Ab Combo</td>
<td>GS HIV Combo Ag/Ab</td>
<td>Advia Centaur HIV Ag/Ab Combo</td>
</tr>
<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 Zyptometrix 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

Days before WB positive

- Modified from Owen et al J Clin Micro 2008
HIV-1 Western Blot

- gp160 (env precursor)
- gp120 (outer env or "surface" glycoprotein)
- p65 (reverse transcriptase)
- p55 (core precursor)/p51 (RT)
- gp41 (transmembrane glycoprotein)
- p40 (core)
- p31 (endonuclease)
- p24 (core shell or "capsid")
- 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 (30 µL) specimen or control. Mix well.

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

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

HIV-1 Reactive:
Diagnostic Algorithm

**HIV-1 Positive**
Purple color development for both HIV-1 spots

HIV-2 Reactive:
**Diagnostic Testing Algorithm** - Positive
**Rapid Testing** - Preliminary Positive
Why Develop the Geenius?
### HIV-2
- GP36 peptide
- GP140 peptides
- gp36
- gp140

### HIV-1
- P31 peptide
- GP160 recombinant
- gp160
- gp41 (group M & O)
- p24
- gp41 peptides
- p31*

### Ctl Band
- gp41
- Protein A
- Inside the nucleocapsid
Geenius HIV 1/2 Supplemental

Dispense 15 μl of whole blood or 5 μl of serum/plasma into Well 1

Whole Blood 15 μl or Serum/Plasma 5 μl

2 Drops of buffer into Well 1

Wait 5-7 minutes

5 Drops of buffer into Well 2

Wait 15 to 20 minutes

Read, interpret and report results

Notebook (validated for Geenius® Software)
### 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 INDETERMINATEa</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATEb</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATEc</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 (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1.*  
*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).*  
*Note: Differentiation features managed by proprietary algorithm. |
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

Negative

Pos/Neg Cutoff

Positive
Real Life
Where to Place the Cutoff?

Negative

Positive

High
Sensitivity
Cutoff

High
Specificity
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
“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
1st Generation “confirmatory” tests (WB, IFA) are following 3rd Generation screening tests
New HIV Testing Algorithm
June 26th, 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](http://www.cdc.gov/hiv/testing/lab/guidelines).

Sincerely,

[Signature]

Bernard M. Branson, 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-1 (-)  HIV-1 (+)  HIV-1 (-) or indeterminate
HIV-2 (-)  HIV-2 (+)  HIV-2 (+)  HIV-2 (-)

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

HIV-1 NAT  HIV-1 NAT (+)  HIV-1 NAT (-)

Acute HIV-1 infection  Negative for HIV-1

(+) indicates reactive test result
(-) indicates nonreactive test result
NAT: nucleic acid test
Proposed HIV Testing Algorithm

CLSI published in Nov 2011

Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline

This document provides guidance for laboratorians performing human immunodeficiency virus testing and for the interpretation of results by health care providers in advanced diagnostic laboratories.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.
Proposed HIV Testing Algorithm

JCV Supplement published in Dec 2011
Proposed HIV Testing Algorithm

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

This 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 indications that 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

Imunoassay generations:
- 1st — viral lysate antigens, designed for IgG detection (includes Western blot, IFA)
- 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, p24 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
Proposed HIV Testing Algorithm

Journal of Clinical Virology

Introduction to 2013 Journal of Clinical Virology Supplement on HIV Testing Algorithms

Bernard M. Branson (MD, Special) (Guest Editor)\textsuperscript{a,,*}, Christine C. Ginocchio (PhD, Co-Editor-in-Chief)\textsuperscript{b,c}

\textsuperscript{a} Associate Director for Laboratory Diagnostics, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, Atlanta, GA, United States

\textsuperscript{b} North Shore-LIJ Laboratories, Lake Success, NY, United States

\textsuperscript{c} Hofstra-North Shore School of Medicine, Hempstead, NY, United States
1. Initiate testing for HIV with a 4\textsuperscript{th} 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
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.
Who’s Still Running Western Blot?
5. The HIV-1 Western Blot is no longer part of the recommended algorithm for HIV testing.
Knowledge Check

New Algorithm Recommendations:

Initiate screening with a ____________________
Initiate screening with a 4th generation HIV-1/HIV-2 Ag/Ab combination immunoassay (IA) or 5th generation Ag-Ab assay
Reactive (repeatedly reactive) specimens should be tested with an assay that differentiate HIV-1 from HIV-2 antibodies.
Reactive (repeatedly reactive) specimens should be tested with an assay that differentiates HIV-1 from HIV-2 antibodies.
What changes are to come? How long will it take?
HIV Prevention
Abstinence

- Avoid sexual contact if there is a risk of the partner being HIV positive
Pre-exposure Prophylaxis (PrEP)

- Take a pill every day
- The pill contains two medicines that are also used, in combination with other medicines, to treat HIV. The combined pill is called Truvada
Pre-exposure Prophylaxis (PrEP)

• When someone is exposed to HIV through sex or injection drug use, PrEP can help stop the virus from establishing a permanent infection.
Pre-exposure Prophylaxis (PrEP)

- 2/29/16
- 43 yr old man infected with HIV after taking Truvada for 24 months
- It’s a rare strain of virus that’s resistant to the 2 medicines that make up Truvada
- Scientists believe that PrEP can sometimes be ineffective to resistance as is the case sometimes with ART

Poz conference on Retrovirus
Antiretroviral Therapy
Antiretroviral Therapy (ART)

- Antiretroviral medications are a group of drugs that inhibit different steps in the HIV replication process.
- In this way, they can suppress HIV infection but never entirely eliminate it from the body.
- There are four categories of ARV medications:
Antiretroviral Therapy (ART)

- Nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs)
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
- Protease inhibitors (PIs) (often ritonavir-boosted); and
- Drugs that interfere with viral entry, such as fusion inhibitors and CCR5 antagonists.
The HIV Life Cycle

HIV medicines in six drug classes stop HIV at different stages in the HIV life cycle.

1. **Binding (also called Attachment):** HIV binds (attaches itself) to receptors on the surface of a CD4 cell.

2. **Fusion:** The HIV envelope and the CD4 cell membrane fuse (join together), which allows HIV to enter the CD4 cell.

3. **Reverse Transcription:** Inside the CD4 cell, HIV releases and uses reverse transcriptase (an HIV enzyme) to convert its genetic material—HIV RNA—into HIV DNA. The conversion of HIV RNA to HIV DNA allows HIV to enter the CD4 cell nucleus and combine with the cell’s genetic material—cell DNA.

4. **Integration:** Inside the CD4 cell nucleus, HIV releases integrase (an HIV enzyme). HIV uses integrase to insert (integrate) its viral DNA into the DNA of the CD4 cell.

5. **Replication:** Once integrated into the CD4 cell DNA, HIV begins to use the machinery of the CD4 cell to make long chains of HIV proteins. The protein chains are the building blocks for more HIV.

6. **Assembly:** New HIV proteins and HIV RNA move to the surface of the cell and assemble into immature (noninfectious) HIV.

7. **Budding:** Newly formed immature (noninfectious) HIV pushes itself out of the host CD4 cell. The new HIV releases protease (an HIV enzyme). Protease acts to break up the long protein chains that form the immature virus. The smaller HIV proteins combine to form mature (infectious) HIV.

**Drug Classes:****
- CCR5 Antagonist
- Fusion inhibitors
- Reverse transcriptase inhibitors
- Integrase inhibitors
- Nucleoside reverse transcriptase inhibitors (NRTIs)
- Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
- Protease inhibitors (PIs)
1. **Binding (also called Attachment):** HIV binds (attaches itself) to receptors on the surface of a CD4 cell.

   ![CCR5 Antagonist](image)

2. **Fusion:** The HIV envelope and the CD4 cell membrane fuse (join together), which allows HIV to enter the CD4 cell.

   ![Fusion inhibitors](image)

3. **Reverse Transcription:** Inside the CD4 cell, HIV releases and uses reverse transcriptase (an HIV enzyme) to convert its genetic material—HIV RNA—into HIV DNA. The conversion of HIV RNA to HIV DNA allows HIV to enter the CD4 cell nucleus and combine with the cell’s genetic material—cell DNA.

   - Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
   - Nucleoside reverse transcriptase inhibitors (NRTIs)
**Integration**: Inside the CD4 cell nucleus, HIV releases integrase (an HIV enzyme). HIV uses integrase to insert (integrate) its viral DNA into the DNA of the CD4 cell. Stop integrase inhibitors.

**Replication**: Once integrated into the CD4 cell DNA, HIV begins to use the machinery of the CD4 cell to make long chains of HIV proteins. The protein chains are the building blocks for more HIV.

**Assembly**: New HIV proteins and HIV RNA move to the surface of the cell and assemble into immature (noninfectious) HIV.

**Budding**: Newly formed immature (noninfectious) HIV pushes itself out of the host CD4 cell. The new HIV releases protease (an HIV enzyme). Protease acts to break up the long protein chains that form the immature virus. The smaller HIV proteins combine to form mature (infectious) HIV. Stop protease inhibitors (Pis).
Living with HIV
Living with HIV
Role of FDA in HIV Diagnosis

Pradip N. Akolkar, Ph.D.

Division of Emerging and Transfusion Transmitted Diseases
Office of Blood Research and Review
Center for Biologics Evaluation and Research
HHS agencies in HIV Diagnosis

**CDC:**
- Develops and recommends testing algorithms
- Develops and distributes educational material
- Tracks and maintains epidemiological data

**HRSA**
- Develops and distributes educational material
- Provides some of the funding for testing
HHS agencies in HIV Diagnosis

CMS
• Oversight of the testing labs

FDA
• Evaluates the performance and clinical utility of HIV diagnostic assays
• Approves HIV diagnostic assays for use in the US
What is an In Vitro Diagnostic (IVD) Test?

• IVD = Test Kit = Assay = Test = Device

• **IVD Medical Device:**
  A device, whether used alone or in combination, intended by the manufacturer for the in vitro examination of specimens collected from the human body…
Why Do We Regulate IVDs?

FDA regulates IVDs to ensure they are safe and effective for:

- Their intended use
- By intended user
- In intended environment.
Why Do We Regulate IVDs?

Benefits to public health of safe and effective IVDs:

- Ensuring that the test kits are what they claim to be (traceability, version control)
- Ensuring that labeling language is suitable and that text is clear and unambiguous
Types of HIV Tests

- Blood donor screening tests
- Aid in diagnosis tests
- Monitoring tests
Types of HIV Diagnostic Tests

Preliminary diagnostic tests
- Lab based tests
- Point of care tests
- In-home tests/collection device

Confirmatory Tests

Disease Progression/Monitoring
- Viral load assays
- Drug resistance assays
Summary

- HIV diagnosis is important to contain the spread of HIV infection
- HIV diagnosis is important for timely medical treatment
- Several DHHS agencies have roles in the diagnosis of HIV
- FDA regulates and approves the HIV tests for use in the US
- FDA evaluates the performance of the assay based on the intended use and population
- FDA approves different types of HIV tests
Government / National Input
Ending the HIV/AIDS Pandemic: An Achievable Goal

Speaker: Tony Fauci, MD – NIH

• Prevention is a combination of elements. Once size does not fit all
• Implement the science that we have
  – Care Continuum
    • 69% of those diagnosed with infection are not in care
  – Prevention Continuum
    • Counseling and guidance are needed
    • PrEP is needed – it works anywhere and with anyone
• HIV Vaccine
  – Challenge is that we can’t mimic the way natural infection occurs so have to figure out how to identify the right kind of binding
High Impact Prevention: Science, Practice and the Future of HIV

• **Speaker:** Jonathan Mermin, MD – CDC

• **Strategy Needed**
  – Needs to be cost effective
  – Need to prioritize activity
  – Need to align available funding (CDC)

• **Implementation**
  – Care and Prevention
  – Funding
    • CDC, HHS, HRSA in partnership
    • $45M provided over 3 years to 8 states
10 Ways to Maximize HIV Prevention

• Speaker: Murray Penner – NASTAD

  – Envision world free from HIV
  – Address structural inequalities
  – Prioritize key populations
  – Use Data to improve outcomes
  – Scale up PrEP and treatment
  – Integrate health systems
  – Focus on right people, right places, right practices
  – Cultivate meaningful community engagement
  – Reform broken policies
  – Engage in social action
Acknowledgements

- Special thanks to Bio-Rad Colleagues
  - Greg Stewart
    - Senior Product Manager
      - US Sales & Service Division
      - Hercules, CA
  - Alfredo Villarreal
    - Senior Product Manager
      - Clinical Immunology Division
      - Benicia, 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!