Welcome to Atlanta
HIV Testing Terminology and Technology

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The findings and conclusions are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Alphabet Soup

• **Immunoassay (IA)** — a biochemical test that detects the presence of a substance in a biological specimen using the binding of an antibody to its antigen

• **Enzyme immunoassay (EIA or ELISA)** — an immunoassay that uses the catalyzing properties of an enzyme for detection of an immunological reaction

• **Chemiluminescent assay (CIA or CMIA)** — an immunoassay in which the signal is generated by a compound that emits light as the result of a chemical reaction
Alphabet Soup

- **Nucleic acid test (NAT) (qualitative)** – molecular assays for detection of the presence of viral nucleic acids (DNA or RNA); NOTE: Sometimes referred to as nucleic acid amplification test (NAAT)

- **Nucleic acid test (NAT) (quantitative)** – molecular assays for quantification of viral nucleic acids (DNA or RNA); NOTE: Sometimes referred to as viral load assays.
More Soup

- **Clinical Laboratory Improvement Amendments (CLIA)** - Establishes quality standards for all laboratory testing to ensure the accuracy
  - **Waived tests** - “simple laboratory examinations and procedures that have an insignificant risk of an erroneous result.”
    - Point of Care (POC) rapid tests
  - **Moderate Complexity** - most automated tests that do not meet the waiver criteria or provider performed microscopy procedures (PPMP)
    - Automated IAs
  - **High Complexity** - non-automated tests
    - Western blot

Serologic Assay Generations

• 1:\textsuperscript{st} generation immunoassays (IA)
  – Detects HIV antibody (Ab) (IgG) using viral lysates as the antigens (Ag)
    • WB - 1:\textsuperscript{st} generation assay

• 2:\textsuperscript{nd} generation IA
  – Detects HIV Ab (IgG) using recombinant protein and peptide Ags

• 3:\textsuperscript{rd} generation IA
  – Detects HIV Ab (IgG and IgM) using recombinant protein or peptide Ags- “antigen sandwich”

• 4:\textsuperscript{th} generation IA
  – Detects HIV Ab (IgG and IgM) and viral p24 Ag
1st and 2nd Generation IA

plasma/serum/oral fluid

HIV Ag coated matrix
1st - Viral lysate
2nd - rproteins/peptides
+-/- viral lysate

Anti-HIV Abs

Enzyme labeled anti-human IgG

Substrate

Colorometric readout
3rd Generation IA

HIV antigen (Ag) coated matrix

plasma/serum

Anti-HIV IgG or IgM

Labeled HIV Ags

Colorometric or chemiluminescent readout

Substrate or Trigger
4th Generation IA

Plasma/serum

HIV Ags
HIV p24 Ab

Labeled HIV Ags

Labeled Anti p24 Ab

HIV IgG or IgM

HIV p24 Ag

Substrate or Trigger

Colorometric or chemiluminescent readout

OR 2 independent labels that allows independent detection of Ag/Ab
Rapid Test Soup

• Characteristics
  – Individual sample
  – Produces result in 30 min or less
  – Currently 3 general platforms
    • Lateral Flow
      – Sample flows “up” test strip over antigen lines
      – Often CLIA waved
    • Dual Path Platform (DPP)
      – Sample and reagents flow from different directions
    • Flow Thru (Immuno-concentration)
      – Sample flows thru membrane containing antigens
      – Generally moderate complex
  – Most are 2nd generation assays
Immunochromatography
Lateral Flow

Side View
- Wicking Pad
- IgG control antigen
- HIV Ag or Ab
- Conjugate pad with Protein A colloidal gold
- Sample addition port

Top View

Sample addition

Time

Key
- = Anti-HIV IgG
- = IgG (non-HIV)
- = Protein A colloidal gold
- = Antigen
Dual Path Platform (DPP)

After sample addition
Test  Control

Sample 1st

After reagent addition
Test  Control

Reagents 2nd

Designed to increase sensitivity

= HIV Ab
= Protein A colloidal gold
Immunocentrification
Flow Thru
2 Detection Methods

Key
- Green = Anti-HIV IgG
- Purple = IgG (non-HIV) control
- Orange = Enzyme labeled anti-human IgG
- Red = Enzyme substrate
- Brown = Protein A colloidal gold
- Black = HIV antigen
- Beige = non-HIV antigen

Top View
Membrane with HIV and control antigens

Side View
Absorbent Material

Sample
1. Wash
2. Anti-IgG enzyme conjugate

Sample
1. Wash
2. Enzyme substrate

Sample
1. Wash
2. Protein A colloidal gold conjugate
3. Wash

Color reaction produced by enzyme substrate reaction

Color reaction produced by protein A colloidal gold binding to Ab
Supplemental Tests

• Western Blot
  – 1\textsuperscript{st} generation
  – Highly specific when all bands present

• IFA
  – 1\textsuperscript{st} generation
  – Highly specific, but requires training
Western Blot

**LIA**

- Viral proteins transferred to paper
- Plasma/serum/OF
- Enzyme labeled anti-human IgG
- Substrate
IFA

HIV Infected cells

Uninfected cells

plasma/serum

Fluorochrome labeled anti-human IgG + UV light

HIV Infected cells

Uninfected cells
More Acronyms and Terminology 😊

- **PCR** – Polymerase chain reaction
  - *Cyclic* biochemical reaction that requires *multiple temperatures*
  - **RT-PCR**
    - Reverse Transcriptase PCR
      - RNA to cDNA (Viral Load)
    - Real-Time PCR
      - Allows real-time monitoring of product
- **TMA** – Transcription mediated amplification
  - Uses *gene transcription* as the basis for amplification-*isothermal*
- **bDNA** – branched DNA
  - Signal/probe amplification method
Both methods can be multiplexed
Qualitative or Quantitative

Karan et al. Journal of the American Academy of Dermatology, 2005
Nucleic Acid Methods

bDNA

Multiple probes can be used to target multiple sequences

Quantitative

Karan et al. Journal of the American Academy of Dermatology, 2005
Platforms

- Manual
  - More hands on - labor intensive
  - Utilize common lab equipment
  - Less space
  - Longer time to results
  - Lower testing volumes

Bio-Rad GS HIV Combo Ag/Ab EIA

Microwell plate EIA
3rd generation format
p24 antigen
Platforms

- Automated
  - Less labor intensive
  - Dedicated equipment
    - Large space
  - Multiple analytes
  - Higher testing volume
  - Faster time to results
ADVIA® Centaur™ HIV 1/O/2

- 3rd generation format
- Time to result: 1 hour

Ortho VITROS ECi

- 3rd generation format
- Repeat only borderline results
- Time to result: 48 minutes

Abbott Architect Ag/Ab Combo Assay

- Detects p24 antigen and HIV antibody
- Time to result: 29 minutes
Thanks!

Questions?

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Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

**Sensitivity**
20 copies/mL

**Target regions**
gag and LTR regions

Abbott Molecular RealTime HIV-1 m2000 RealTime System

**Sensitivity**
- 40 copies/mL for 1.0 mL
- 40 copies/mL for 0.6 mL
- 75 copies/mL for 0.5 mL
- 150 copies/mL for 0.2 mL

**Target region**
Integrase region of polymerase gene

**Currently not FDA approved for diagnostic use**