

HIV: Update on Rapid Diagnostics and the CDC Testing Algorithm

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Objectives

- Understand the history of HIV in the United States and the World
- Understand the evolution of HIV testing
- Understand the goals of international bodies to combat the spread of HIV/AIDS, especially the 90-90-90 project of UNAIDS
- Review the new CDC guidelines and algorithm and the important updates since its release, including the use of molecular testing in HIV1 and HIV2
- Understand the Point of Care testing options for HIV detection, the challenges early in its release, and current updates on performance and CDC recommendations.

HIV- What is it?

Human Immunodeficiency Virus- Virus that attacks the body's immune system, specifically the CD4 cells (T cells), which help the immune system fight off infections.

Untreated, HIV reduces the number of CD4 cells in the body, making the person more likely to get infections, especially "opportunistic" infections or cancers.

The presentation of opportunistic diseases take advantage of a very weak immune system, and signal the person has AIDS, the last stage of HIV infection

What are the stages of HIV?

Stage 1: Acute HIV infection (AHI)

- Within 2 to 4 weeks after infection with HIV, people experience a flu like illness, which may last for a few weeks. When people have acute HIV infection, they have a large amount of virus in their blood and are very contagious
 - To diagnose AHI, molecular testing (NAT) or a Combo Ag/Ab test is needed
- Stage 2: Clinical Latency (HIV Inactivity)
 - HIV active but reproduces slowly. Can last a decade or longer, asymptomatic.
 - People on treatment can stay here indefinitely
- Stage 3: Acquired immunodeficiency syndrome (AIDS)
 - Very damaged immune system with opportunistic infections. CD4 <200.

History of HIV

- Pre-1980:
 - Widely believed HIV originated in the Democratic Republic of Congo around 1920 with HIV crossed species from chimpanzees to humans. The current epidemic started in the 1970's, with HIV in 1980 spread to five continents. (North America, South America, Europe, Africa, and Australia, by which time 100,000 to 300, 000 people may have already been infected
- 1980's
 - 1981: case of rare lung disease called Pneumocystis Carnii (PCP) were found in five young healthy gay men in Los Angeles, and increases of a cancer called Kaposi's Sarcoma. 270 Total Reported cases of "severe immune deficiency" in gay men

History of HIV

- 1980s:
 - 1982: sexually transmitted nature of disease was discovered, syndrome initially called “gay related immune deficiency”. CDC later renames as “AIDS”.
 - 1983:
 - AIDS found in women, demonstrating passage via heterosexual sex. Doctors at the Pasteur Institute in France discover Lymphadenopathy Associated Virus (LAV), possible cause of AIDS
 - Transmission by casual contact, food, water, air ruled out by CDC.
 - 1984: LAV (also called HTLV III) confirmed as cause of HIV and test created for the purpose of screening the national blood supply. An ELISA for IgG was officially released in 1985.
 - 1986: Virus officially renamed HIV. 38,401 AIDS cases in world in 85 countries.

History of HIV

- 1980's:
 - 1987: First Antiretroviral drug approved by FDA, zidovidine (AZT). The Western Blot is also approved for Diagnosis for more specificity
 - 1989: Reported AIDS cases in USA reached 100,000
- 1990's
 - 1992: FDA licensed a 10 minute testing kit for HIV to be used by healthcare professionals. AIDS is the leading cause of death for US men 25-44 y.o.
 - 1993: estimated 2.5 million AIDS cases globally. CD4 <200 definition for AIDS.
 - 1994: FDA approves an oral HIV test. AIDS leading cause of death for all Americans age 25-44.
 - 1995: FDA approves first protease inhibitor, beginning era of highly active antiretroviral treatment (HAART), immediate decline of 60-80% in AIDS deaths and hospitalization where can be afforded.

History of HIV

- 1990's
 - 1996: FDA approves first home testing kit, a urine testing kit, HIV viral load testing, and non-nucleoside transcriptase inhibitor.
 - 1999: AIDS is forth biggest cause of death worldwide, number one in Africa, 83 million people with HIV, 14 millions deaths to AIDS.
- 2000's:
 - 2000: United nations announces the Millennium Development Goals, which include goals to reverse spread of HIV, malaria, and TB
 - 2002: FDA approves first rapid HIV test with 99.6% accuracy in 20 minutes.
 - 2003: WHO announces 3 by 5 initiative to bring HIV treatment to 3 million people by 2005

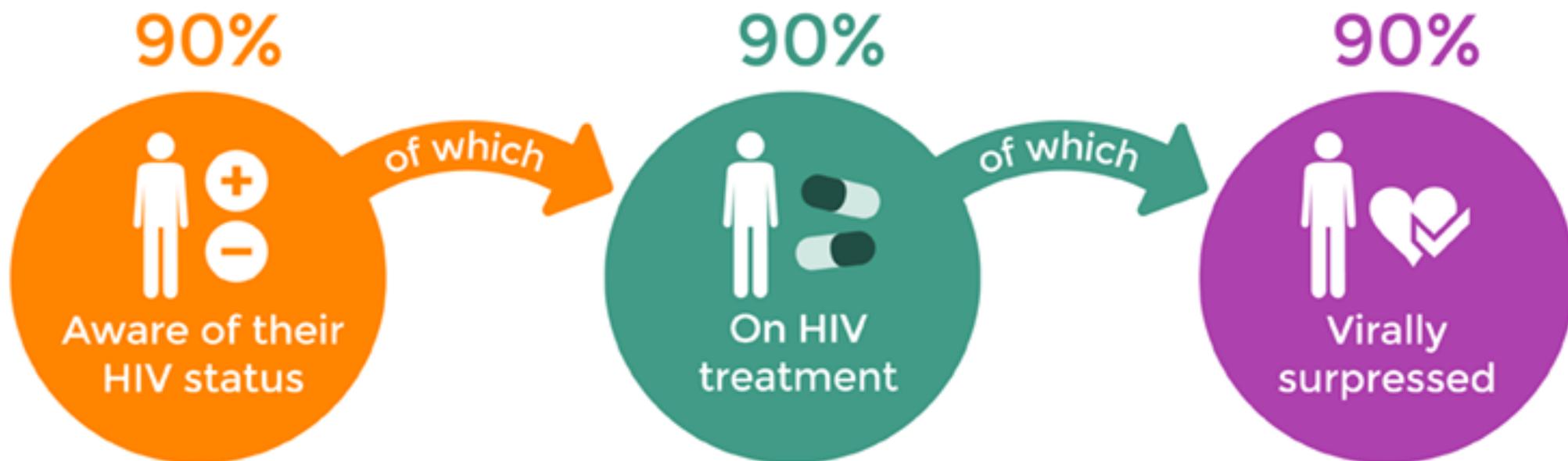
History of HIV

- 2010's:
 - 2011: Trials show early initiation of antiretroviral treatment reduced the risk of HIV transmission by 96%
 - 2013: 35 million people living with HIV
 - 2014: UNAIDS launches the ambitious 90-90-90 targets to dramatically increase diagnosis and treatment by 2020
 - 2015: UNAIDS announces that the Millennium Development Goal relating to HIV and AIDS has been reached six months ahead of schedule, 15 million people received treatment for HIV. CDC announces more than 90% of new HIV infections could be prevented by diagnosis of those living with HIV.
 - CDC reports 50% of young Americans living with HIV don't know it.
 - 2017: For the first time ever, more than half of the global population with HIV are receiving ART, a record of 19.5 million people.

90-90-90 by 2020

- Initiative launched by UNAIDS in 2014 as a “Fast Track” strategy.
- Outlined plans to step up the HIV response in low and middle income countries to meet a UNAIDS Strategic Development goal (SDG 3) to end AIDS by 2030.
- Outlines the need to reduce new HIV infections and AIDS related death by 90%.
- Goals by 2020:
 - 90% of people with HIV know their status
 - 90% of HIV diagnosed receiving ART therapy
 - 90% of those receiving ART will have viral suppression

KEY 2020 FAST TRACK TARGETS

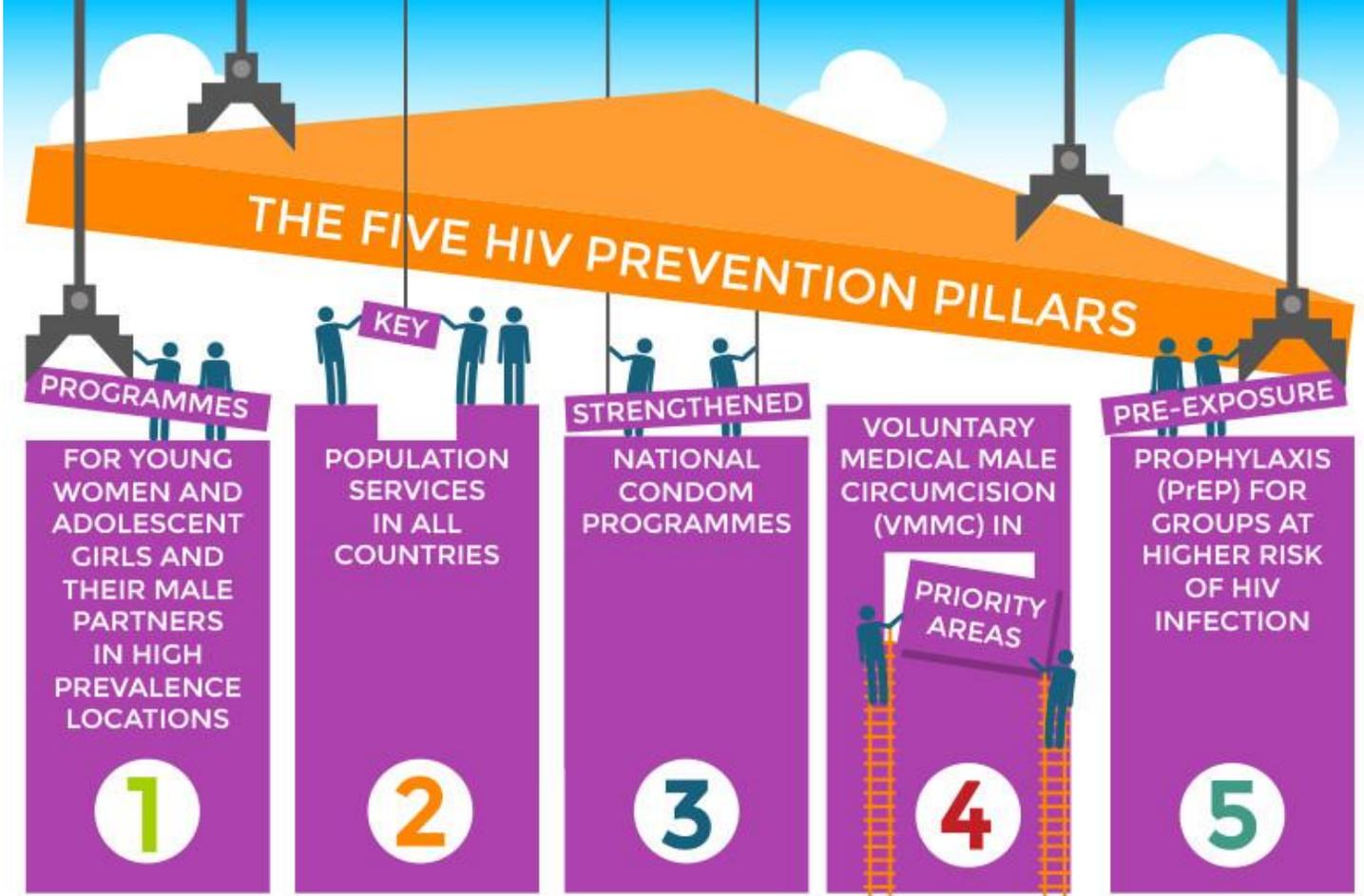


30 million
people on
treatment



Fewer than
500,000
new HIV
infections
annually





The Five HIV prevention Pillar outline approach to achieve 90-90-90.

The following targets have been set for 2020.

- 1) Fewer than 500,000 people newly infected with HIV (75% reduction from 2010)
- 2) Fewer than 500,000 people dying from AIDS related illness
- 3) Elimination of HIV related discrimination.



- 1** Reduce new HIV infections among women and girls to under 100,000.
- 2** Ensure that 90% of people at risk of HIV can access prevention services.
- 3** Make 20 billion condoms available annually in low- and middle-income countries.
- 4** Provide 25 million more men with VMMC.
- 5** Provide PrEP to 3 million people at high risk of HIV.

Evolution of HIV Testing

Early (IgG) antibody testing ("First Generation Testing")

Following 1983 isolation of virus. Developed using separate samples of virus from HTLV III (Abbot and Electronucleonics) and LAV (Genetic Systems) isolates.

Used ELISA and chemiluminescence methods, targeted to proteins isolated from virus infected cultures.

Sensitive to IgG only, had a window between infection to positivity of up to 12 weeks.

Primarily used to screen blood supply, so very sensitive but non-specific

Early false positives

- Early false positives:
 - Other infections
 - Autoimmune disease
 - Pregnancy
- Therefore two confirmatory tests FDA approved
 - Western Blot Assay, and HTLV immunofluorescence assay
 - Each of these still:
 - Only detected IgG
 - Antibody negative window of 6-12 weeks

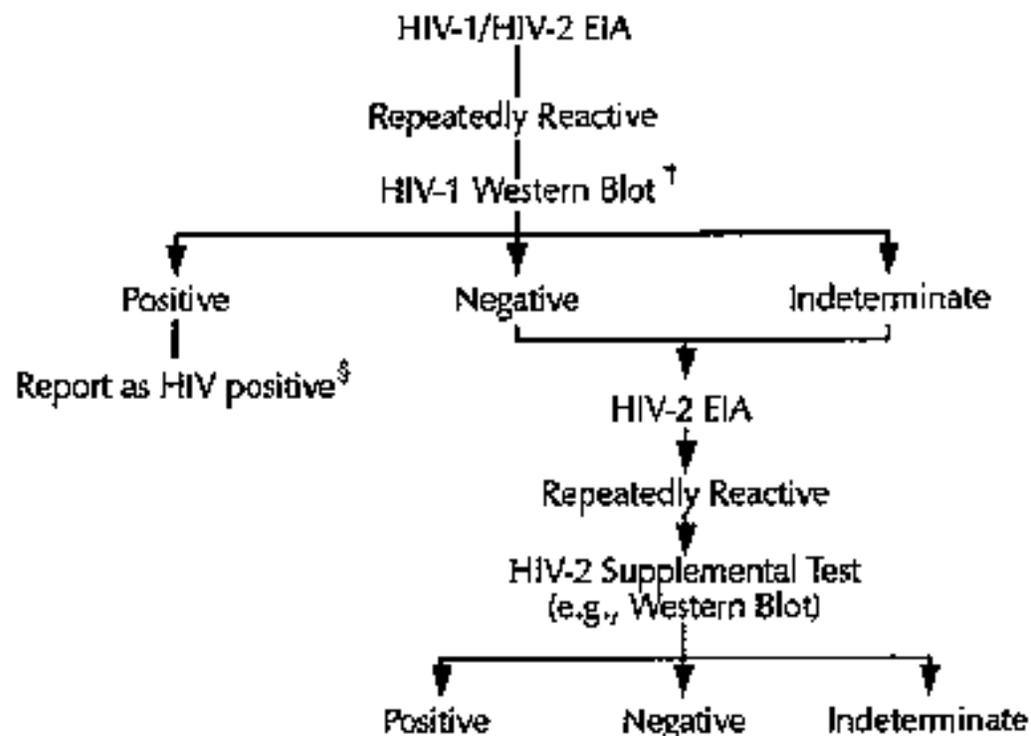
Evolution of HIV Testing

- IgG sensitive testing, with recombinant antigens (Second Generation)
 - Recombinant antigens, especially p24, greatly improved the specificity of the assays
 - Also added HIV-2 proteins and HIV-1 Group O proteins
 - Also reduced the antibody negative window to 4-6 weeks
 - (HIV-2 detection meant HIV-2 confirmation was added to testing algorithm)
- IgG and IgM sensitive Antibody Testing (Third Generation)
 - Addition of IgM detection, while not useful by itself, decreased the antibody negative window to 3 weeks, in combination with IgG.
- Note: a p24 detection ELISA was available at this time too.

Third Generation Testing Notes:

- Confirmation of Screens were by either Western Blot or IFA
- Since the confirmation screens were insensitive to HIV-2, Screen POS and confirm neg could be tested for HIV-2
- Quantitative and Qualitative molecular Assays could reduce the time from infection to detection compared to these screening assays, however due to cost are not effective for generalized screening
- HIV PCR's are recommended for neonatal diagnosis, however, because maternal HIV IgG antibodies may cross the placenta and result in false positives

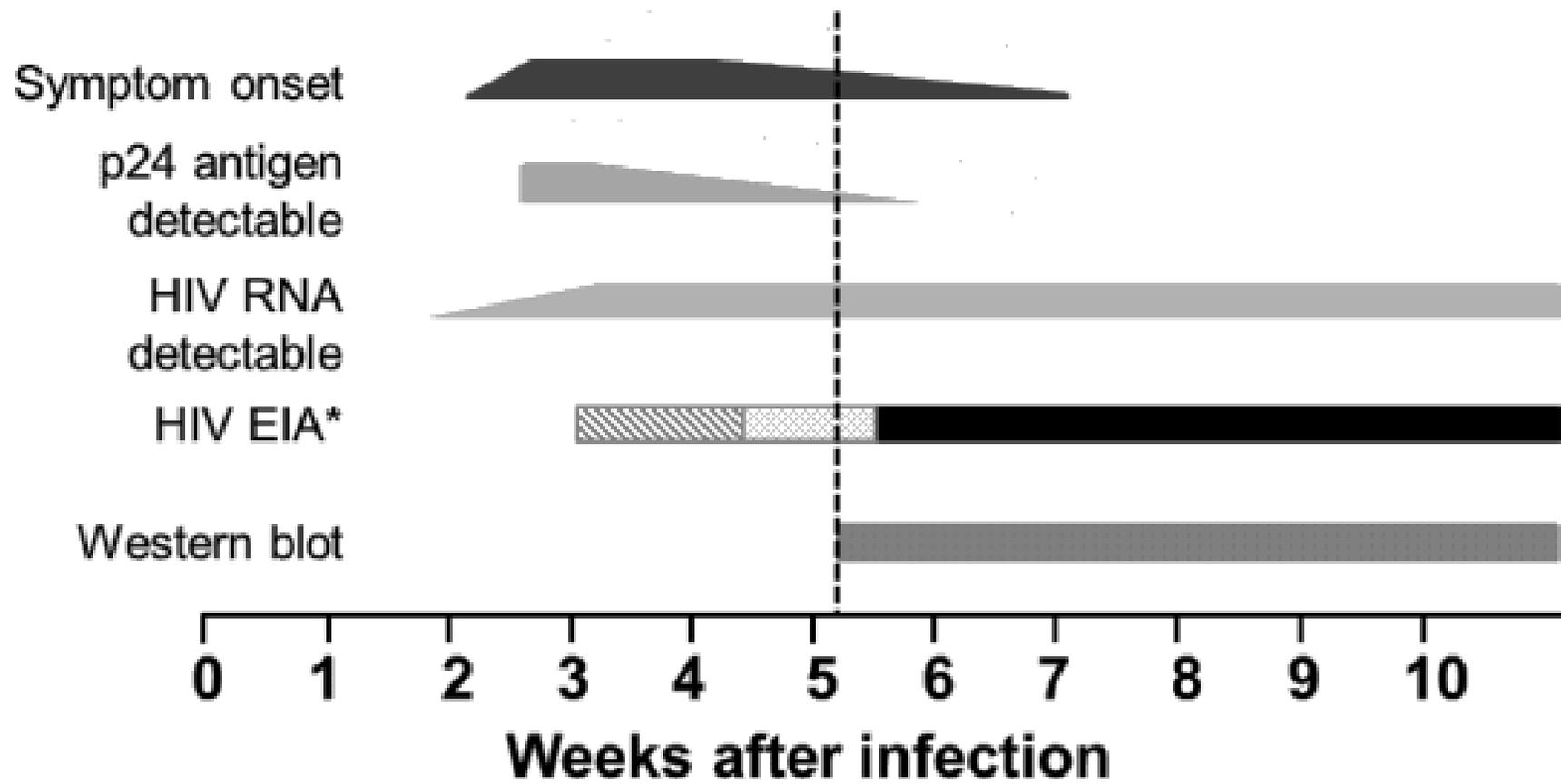
FIGURE 1. Centers for Disease Control/Food and Drug Administration testing algorithm for use with combination HIV-1/HIV-2* enzyme immunoassays (EIAs)



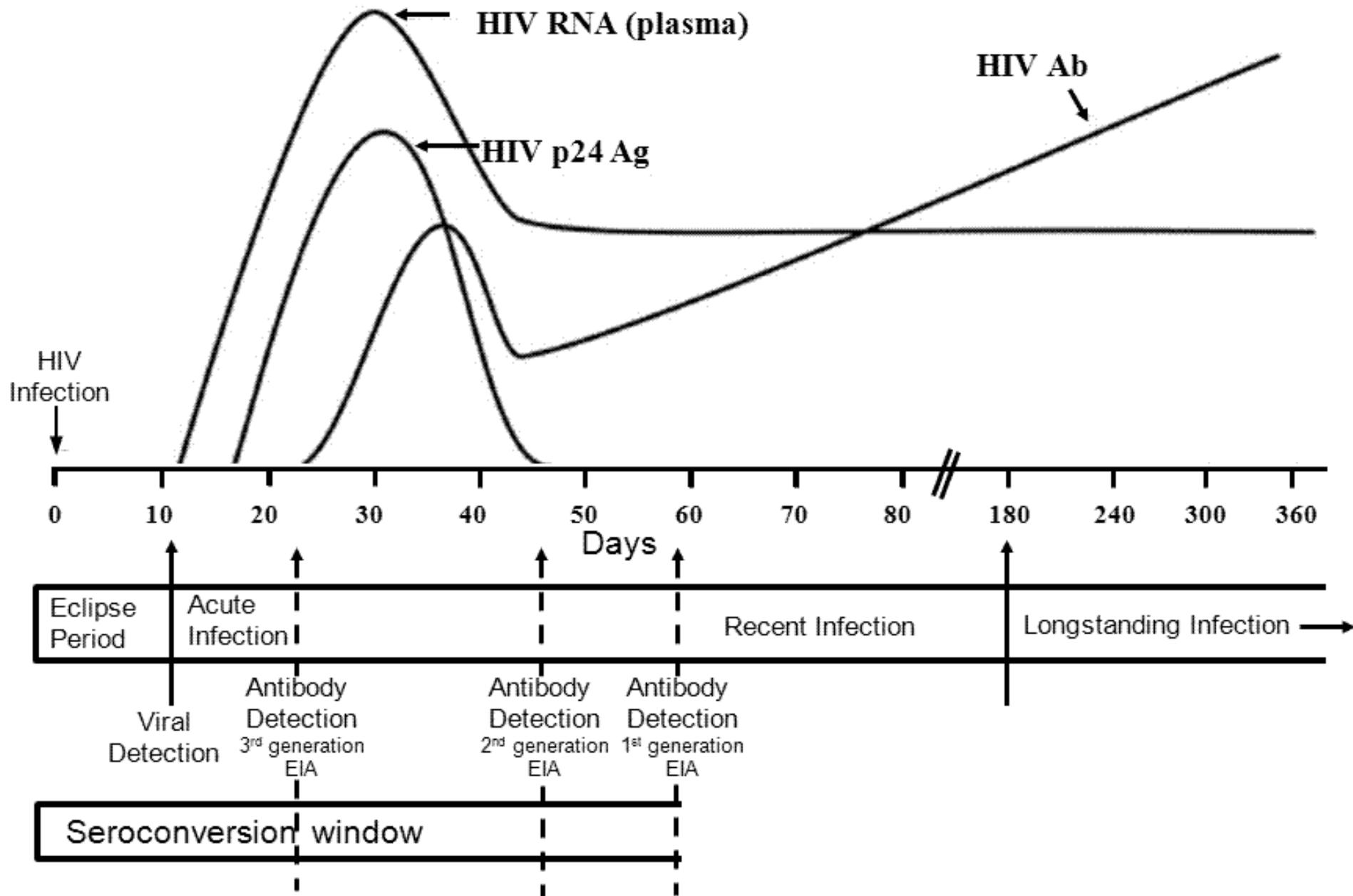
*HIV = Human immunodeficiency virus.

†An immunofluorescence assay (IFA) for HIV-1 antibodies has recently been licensed by the Food and Drug Administration and can be used instead of Western blot. Positive and negative IFA results should be interpreted in the same manner as similar results from Western blot tests. An indeterminate IFA should first be tested by HIV-1 Western blot and then as indicated by the Western blot results.

§Perform HIV-2 EIA only if there is an identified risk factor for HIV-2 infection.

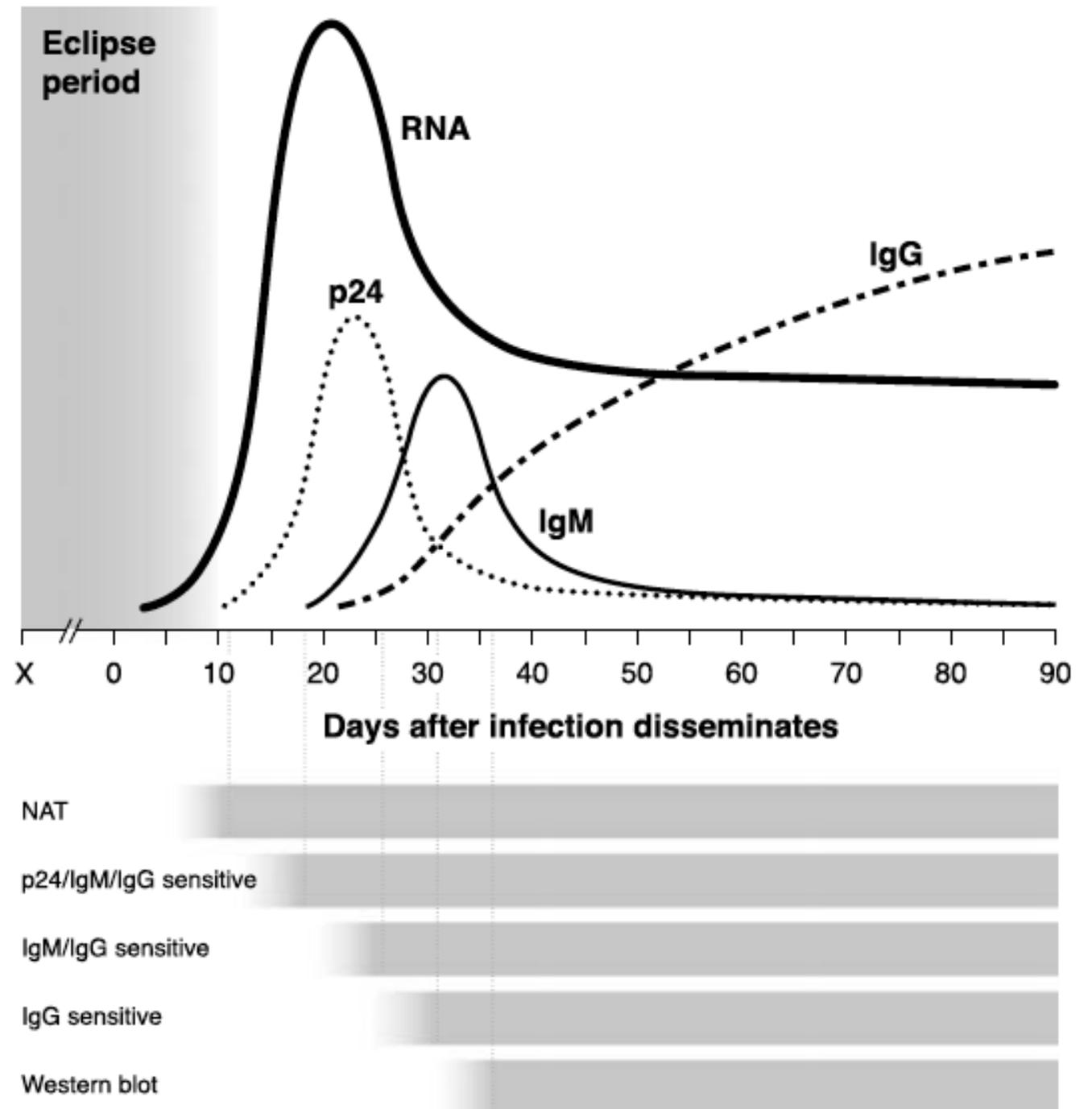


-  *3rd-generation, IgM-sensitive EIA
-  *2nd-generation EIA
-  *Viral lysate EIA



Comparison of serology versus technology

Note on p24:
free p24 is detected by assays. Once antibodies rise, they bind to p24 (and clear from blood stream). This decreases detectability. However, by then Antibody testing is Reactive.



Newest Generation Assays

- Combination Antigen Antibody assays (Fourth Generation Assays)
 - In Late 1990's, HIV assay that detected antibodies to HIV-1 and HIV-1 IgG and IgM plus an antigen (p24) were developed.
 - Are still either ELISA or chemiluminescence
 - Decrease Test Window to 2 weeks.
 - First fourth generation procedure cleared by the FDA was the Abbott Architect method, approved in 2010.
 - Architect had a sensitivity of 99.94% and repeat testing specificity of 99.5% in a cohort of 3,386 HIV pos, 7551 HIV negative, and 58 patients with acute HIV patients.

Consequent Approvals

- Bio-Rad's GS ELISA FDA cleared in 2011
 - 100% sensitivity and 99 to 100% specificity
- Siemen's Advia HIV Combo assay
 - To be used on the Centaur
 - Approved in 2015
 - Sensitivity of 100% for antibody, antigen sensitivity of 97.4%, and specificity of 99.69

“Fifth Generation”

Bio-Rad BioPlex 2200 HIV ag/Ab assay, FDA approved in 2015

Provides separate results for HIV antibody results and for Antigen positivity

100% sensitivity and 99.5% specificity

Will need an update to the algorithm:

- Provides separate HIV1 and HIV2 results for no need for differentiation test

- Specimens reactive for p24 only do not need antibody confirming procedure, and antibody only do not need Ag confirm

Change to nomenclature of testing by FDA

- Since the first HIV diagnostic test FDA approved in 1985, four “generations” of testing have been developed, each improving and shortening the window period.
- The official nomenclature for HIV is changing
- New Categorizations are based on analytic targets (i.e. HIV-1 IgG, p24 antigen) rather than a generation numbers.
- Each category is then subcategorized into point of care or laboratory based
- This is because, with improvement in technology, windows and accuracy vary within ‘generations’, more descriptive of performance to name by target.

HIV Assay Diagnostic Testing Evolution

| Assay progression | Indirect ELISA (HIV-1,2) | | Sandwich ELISA HIV1,2 IgG & IgM | Sandwich ELISA HIV1,2 IgG & IgM + p24 Ag | |
|------------------------------|---|--|--|--|--|
| | HIV-1 lysate | HIV-2 lysate | HIV peptide | p24 | |
| Year | 1985 | 1987 | 1991 | 1997 | 2015 |
| Generation | 1 st | 2 nd | 3 rd | 4 th | 5 th |
| Antigen (Ag) Source | Virus Infected Cell Lysate | Lysate & Recombinant | Recombinant & Synthetic peptides | Recombinant & Synthetic peptides | Recombinant & Synthetic peptides |
| Specificity | 95-98% | >99% | >99.5% | 99.5% | 99.5% |
| Sensitivity | 99% | >99.5% | >99.5% | >99.8% | 100% |
| Negative Window | 8-10 weeks | 4-6 weeks | 2-3 weeks | 2 weeks | 2 weeks |
| Detects Antibody (Ab) and Ag | IgG Anti HIV-1 | IgG anti HIV-1 and IgG anti HIV-2 | IgG and IgM anti HIV-1, HIV-2 and Group O | IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag | IgG and IgM anti HIV-1, HIV-2 and Group O. Also detects HIV-1 p24 Ag |
| Results | Single result | Single result | Single result | Single result; does not differentiate Ab from Ag positivity | Separate HIV-1 and HIV 2 Ab and Ag results |
| Confirming Tests | HIV-1 western blot (WB) or immunofluorescence (IFA) | HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative | HIV-1 WB or IFA, HIV-2 ELISA and WB if HIV-1 confirm is negative | HIV-1.2 differentiation Assay followed by qualitative HIV-1 RNA PCR if differentiation assay is negative | Not determined at the time of this writing |

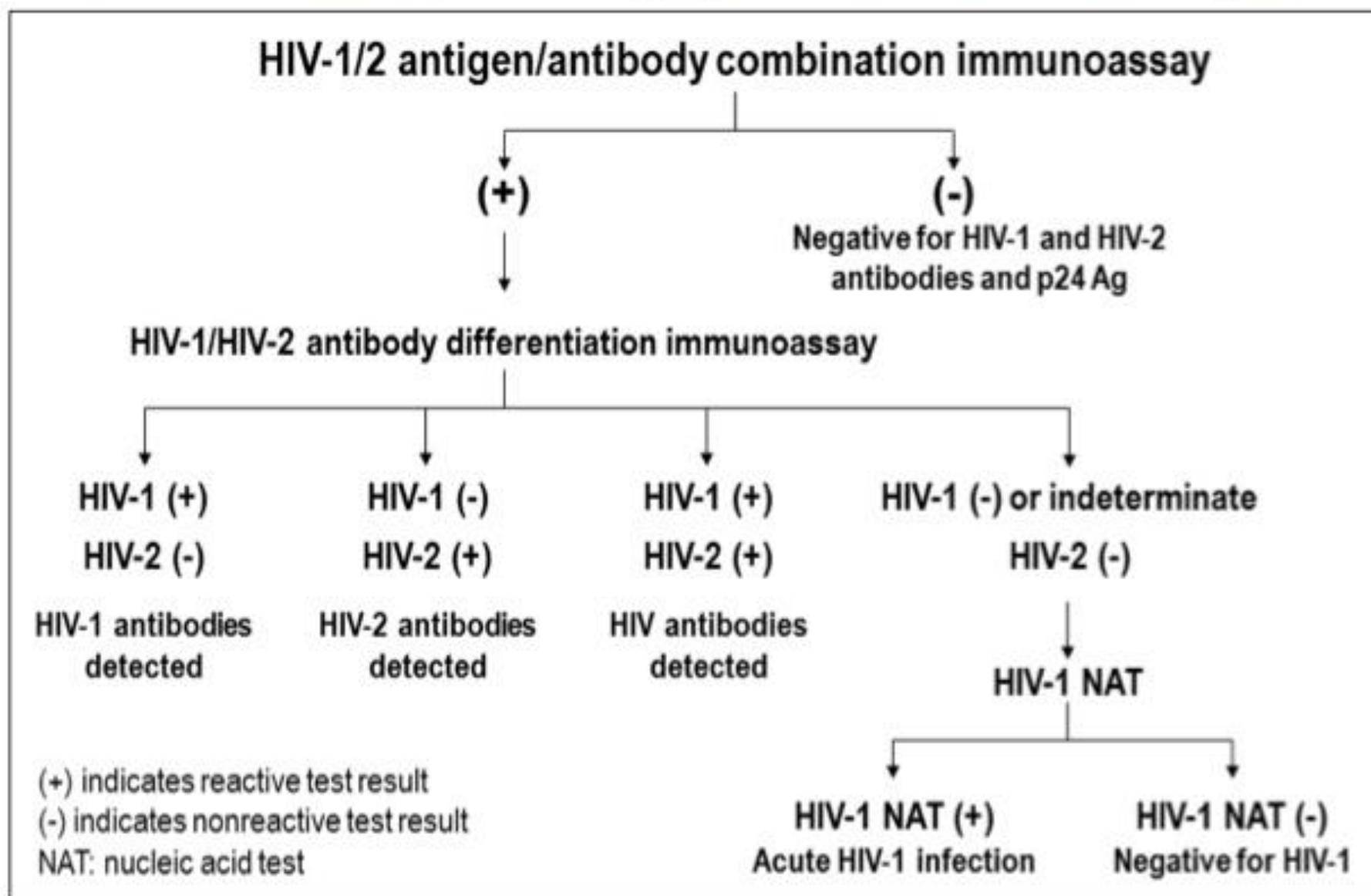
New CDC guidelines

- Centers for Disease Control published guidelines for serodiagnosis of HIV type 1 infections in 1989, centered around antibody detection
- Updated in 1992 for HIV-2, and 2004 to add protocols for confirmation of reactive rapid antibody test results.
- The CDC did a major update in 2014 on their recommendations for HIV testing, based on evidence collected from 2007 to December 2013. This guidance was updated in August 2016 and October 2017 for new evidence for Point of Care Combo Ag/Ab test and a change to the confirmation instrument.

Why updates to the HIV algorithm needed?

- FDA approval of Improved HIV assays that allow detection of HIV sooner than previous immunoassays
- Evidence that relying on Western Blot and IFE for confirmation can produce false negative or indeterminate results early in infection
- Recognition that risk of HIV transmission from persons with acute and early infection is much higher than that from person with chronic infection
- Evidence of significant benefit from ART in all patients including those with acute infection
- Demonstration that most HIV-2 infections detected by screening assays are misclassified as HIV-1 by Western Blot.

Recommended Laboratory HIV Testing Algorithm for Serum or Plasma Specimens



What are the advantages of the Algorithm

- More accurate lab diagnosis of acute HIV-1 infection
- More accurate laboratory diagnosis of established HIV-1 infection
- More accurate laboratory diagnosis of HIV-2 diagnosis
- Fewer indeterminate results
- Faster turn around time for most test results

Advantages of the new algorithm

- Previous testing algorithm for HIV-1 fails to identify acute HIV-1 infections
 - Specimens with nonreactive antibody immunoassay results and reactive NAT results (representing acute HIV infection), have been described in 4% to 32% of all new HIV diagnosis at the time of testing in some populations, especially men who have sex with men
 - Retrospective testing of specimens from high risk persons demonstrated that 3rd generation immunoassays were reactive in 20-37% of specimens that were HIV-1 Western Blot negative but NAT reactive, but 4th generation immunoassays were reactive in 62% to 83% of specimens that were NAT reactive but nonreactive with earlier generation immunoassays

Advantages of New Algorithm

- Assays that detect HIV-1 infection earlier are now widely available
 - new generations of immunoassays with improved sensitivity for detecting early HIV-1 infection can narrow the interval between the time of infection and initial reactivity
 - In 2006, 74% of labs use 1-2 gen testing, in 2012 92% of labs use 3rd or 4th gen immunoassay.
 - However, these immunoassays become reactive days to weeks before the HIV-1 Western Blot does.
 - Therefore using Western Blot for confirmation of these sensitive assays can produce false negative results during seroconversion

Advantages of New Algorithm

- The risk of HIV-1 transmission from persons with acute and early infection is much higher than that from persons with established infection
 - Extremely high levels of infectious virus are present in blood in serum and genital secretions during “acute” HIV-1 infection and persist 10-12 weeks.
 - Rate of sexual transmission during acute infection is 26 times as high as that during established HIV infection
 - Acute HIV infection, because of the high HIV viral load, can account for 10-50% of all new HIV-1 transmission, despite it’s short duration.

Advantage of New algorithm

- Initiation of Antiretroviral therapy (ART), during the early stage of HIV-1 infection can benefit patients and reduce HIV transmission.
 - Treatment of acute and early HIV-1 infection with combination ART improves laboratory markers of disease progression.
 - Since very high levels of virus in blood and genital secretions increase infectiousness during and immediately after acute HIV infection, initiating treatment during acute infection can also reduce the risk of HIV-1 transmission substantially

Advantages of New Algorithm

- The use of HIV-1 Western blot in the previous algorithm misclassifies the majority of HIV-2 infections
 - Correct identification of HIV-2 infections is challenging, but distinguishing it is important because some ART agents effective against HIV-1 are not effective against HIV-2.
 - Screening with test designed for HIV-1 miss 15% to 53% of HIV-2 infections
 - When HIV-1/HIV-2 immunoassays are reactive, but the western blot was negative or indeterminate, the previous recommendation advised specific testing for HIV-2
 - In addition, HIV- Western Blot was incorrectly positive in 46% to 85% of specimens from persons found to be infected with HIV-2, resulting in inappropriate treatment.

Advantages of New algorithm

- Change of confirmation step from Western Blot to a HIV-1/HIV-2 Differentiation Assay reduces change of false negative confirmation
 - The HIV1/HIV2 differentiation assay detects HIV-1 antibodies earlier than the HIV-1 Western Blot, reduces indeterminate results, and identifies HIV-2 infections.
 - Turnaround time is shorter
 - Costs are lower

Advantages of New Algorithm

- Specimens that are reactive on a combo Antigen/Antibody test but negative on the HIV1/2 differentiation tests should be tested by a FDA approved HIV-1 NAT.
 - HIV-1 NAT results can distinguish acute HIV-1 infection from false positive initial immunoassay result in specimens with a reactive Antigen/antibody immunoassay and non reactive HIV1/2 differentiation assay.
 - Note: HIV-1 NAT will not detect HIV-2 and no HIV-2 NAT is FDA approved.

Currently available HIV screening Tests, lab use only, moderate to high complexity

- Architect HIV Ag/Ab Combo, Moderate Complexity, Plasma/Serum
- Siemens Centaur HIV Ag/Ab Combo, Moderate Complexity, Serum/Plasma
- Bioplex 2200 HIV Ag/Ab, Mod Complexity, Plasma, Li and Na Heparin, Serum
- BioRad GS HIV combo Ag/Ab EIA, High complexity, TAT 3 hours, Plasma/Serum

Currently Available HIV screening test, lab use only, moderate to high complexity

- Roche Elecsys HIV combi PT, Ab and Ag, plasma/serum
- Antibody only assays
- Advia Centaur HIV 1/O/2, Moderate Complexity, Plasma/Serum
- Bio-Rad GS HIV 1-2 Plus O, High Complexity, 3 hour TAT, Plasma/Serum
- Ortho/Vitros ECL/ECiQ HIV 1+2, High Complexity, Plasma/Serum
- HIV-1 Microelisa system, High Complexity, IgG only, 3 hours TAT, Plasma, serum, dried blood spots, oral fluid

What about Oral Fluid Tests

- Specimen type makes a difference.
- Oral fluid immunoglobulin levels are >300 times lower than plasma
- Test using oral fluid transudates are significantly less sensitive than whole blood
- Tests using whole blood are less sensitive than those using serum or plasma
- Oral antibodies also rise later to detectable than in serum, so the window period is prolonged by roughly 29 days
- Whole blood roughly 55% cells, so less target per ml specimen

How does technology affect window period comparatively across platforms

TABLE 1. Window Periods of HIV Tests, by Category

| Category (No. Tests Included) | 25%* Will Have a Reactive Result by Day | 50%* Will Have a Reactive Result by Day | 75%* Will Have a Reactive Result by Day | 99%* Will Have a Reactive Result by Day |
|---|---|---|---|---|
| p24/IgM/IgG-sensitive laboratory tests (4)† | 13.0 | 17.8 | 23.6 | 44.3 |
| p24/IgM/IgG-sensitive POC test (1)‡ | 14.8 | 19.2 | 24.6 | 43.1 |
| IgM/IgG-sensitive laboratory tests (3)§ | 18.4 | 23.1 | 28.8 | 49.5 |
| IgM/IgG-sensitive POC tests (2) | 24.2 | 29.3 | 35.3 | 57.4 |
| IgG-sensitive laboratory test (1)¶ | 26.5 | 30.6 | 35.9 | 54.1 |
| IgG-sensitive POC tests (5)** | 26.7 | 31.8 | 37.8 | 57.8 |
| IgG-sensitive supplemental tests (1)†† | 28.2 | 32.9 | 38.6 | 57.7 |
| Western blot (1)‡‡ | 31.0 | 36.5 | 43.2 | 64.8 |

*Columns correspond to the 25th, 50th, 75th, and 99th percentiles of window period distributions, averaged across all tests in a given category, as calculated by Delaney et al.⁴

†ADVIA Centaur HIV Ag/Ab Combo, ARCHITECT HIV Ag/Ab Combo, BioPlex 2200 HIV Ag-Ab, and GS HIV Combo Ag/Ab EIA.

‡Determine HIV-1/2 Ag/Ab Combo.

Testing Methodology

IgG sensitive Tests

- Anti HIV antibodies from patient specimen bind to recombinant or synthetic HIV antigen immobilized on the solid phase of the assay (usually gp41 for HIV1, gp36 for HIV2), and then a detection agent is applied

IgM plus IgG sensitive tests

- As with IgG only tests, solid phase antigens bind patient antibodies, but instead of labeled protein A, a second HIV antigen which is labeled or enzyme linked. (Antigen Sandwich)

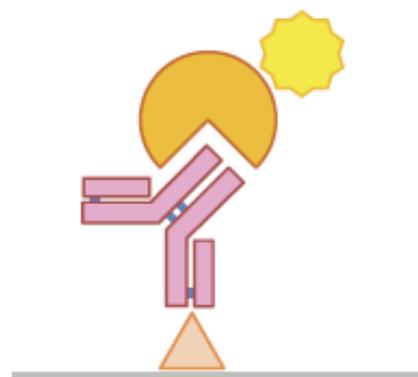
Testing methodology

Antigen Antibody Combo Tests

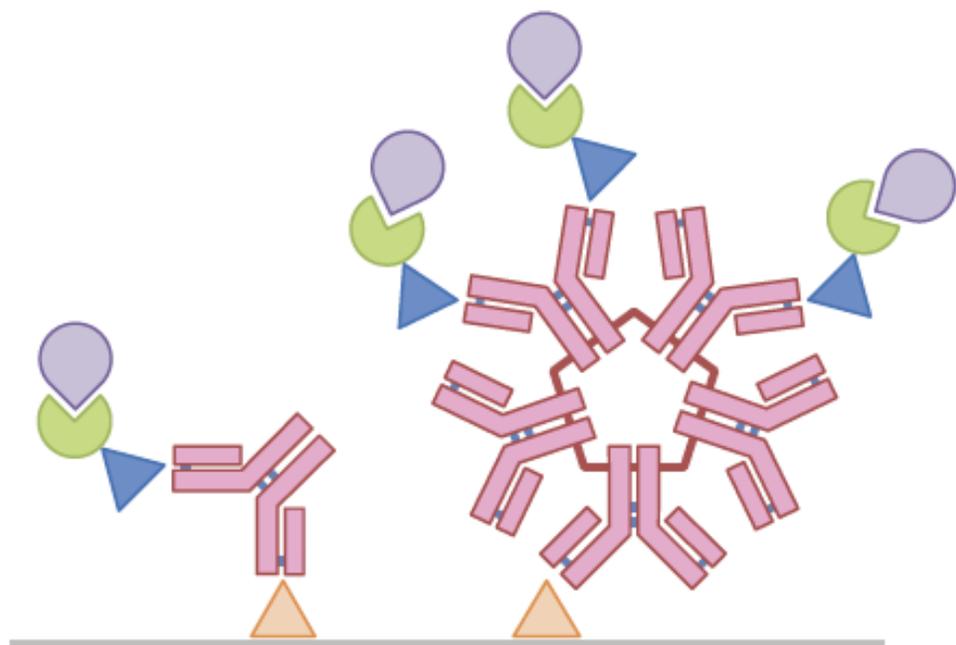
- Pair a IgG/IgM sensitive test with a separate p24 antigen detection test. Antigen from patient is first captured by immobilized anti p24 antibody on solid phase, then a separate, labeled antigen is applied, forming a detectable complex.
- Some report 'reactive' if either is POS, some report each separately.

Point of Care Tests

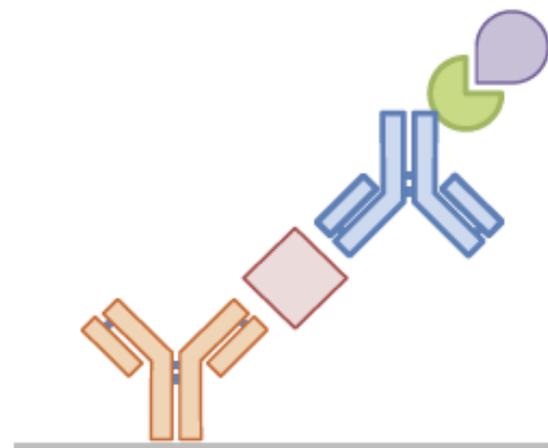
- Same as antibody tests, but detection reagent is often a conjugate of Protein A with colloidal gold or selenium.
- Use one 1 of 2 methods:
 - Lateral Flow: specimen is drawn through an antigen impregnated strip by capillary action
 - Flow through: Patient's specimen and reagents are sequentially applied to a membrane embedded with HIV antigens



IgG sensitive



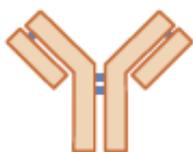
IgM / IgG sensitive



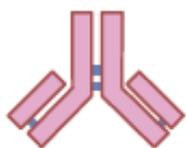
**p24 Ag detection
(in p24 / IgM / IgG
sensitive tests)**



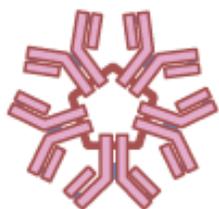
Synthetic or recombinant HIV antigens



Monoclonal anti-p24 antibody



Anti-HIV IgG (from patient)



Anti-HIV IgM (from patient)



Protein A-linked colloidal gold



p24 antigen (from patient)

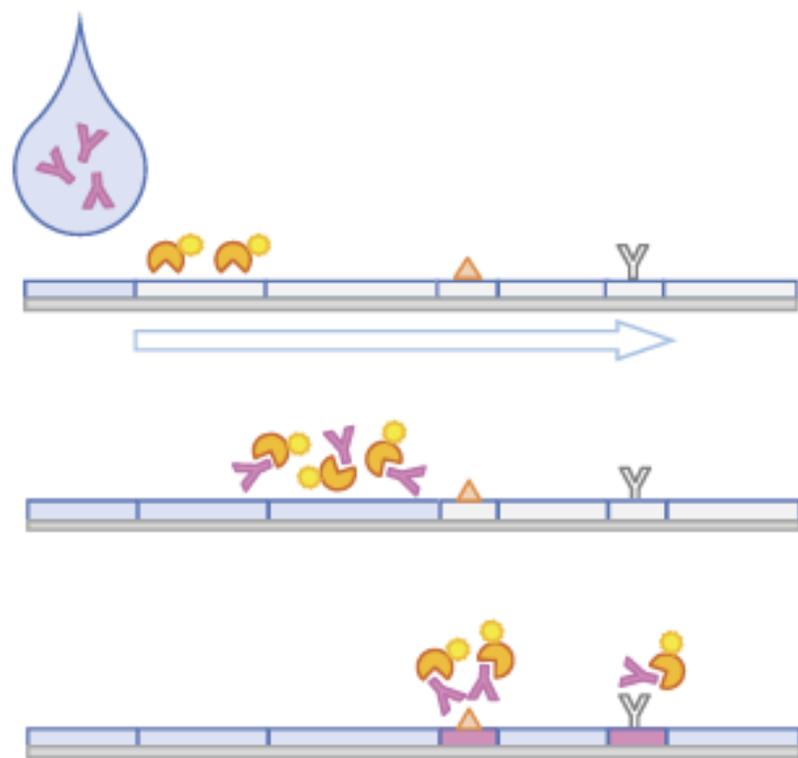


Enzyme-linked anti-p24 antibody

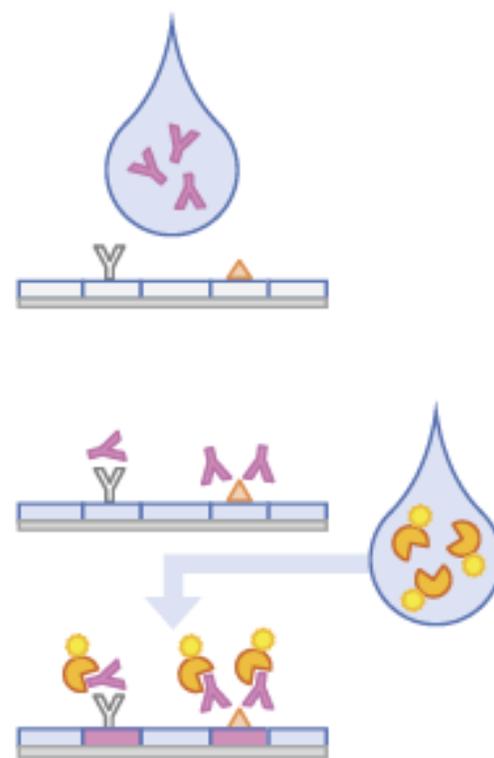


Detection reagent

Lateral flow



Flow-through



Synthetic or recombinant HIV antigens



Anti-HIV antibody (from patient)



Immobilized anti-human antibody



Protein A-linked colloidal gold

HIV 1-2 Differentiation Test

- Has replaced the Western Blot completely in the algorithm
- Single use immunochromatographic test, used in whole blood serum, or plasma
- Antibodies for HIV1 p31, gp160, p14, gp41, and HIV2 gp36 and gp140
 - Banding patterns and intensities on a Geenius Cassette are read by an automated reader connected to a personal computer and interpreted by software.
- All data in the original 2014 CDC guidelines were from the BioRad Multispot
- The manufacturer (Bio-Rad) recently ceased manufacture of the MultiSpot, replaced with the Geenius HIV 1-2 Confirmatory Assay
 - Now has a reader and additional reportable results
 - The Geenius approved as a supplementary test for the HIV algorithm and not intended as a primary screening device

Updated Comparison results for Geenius

- Kato, Kondo, et al. (Oct 2018) most recently Directly compared Modern Western Blot to the current “Geenius” assay
 - Western Blot still used more commonly in Japan
- Comparators:
 - Bio-Rad Geenius HIV1-2 Differentiation Assay
 - Bio-Rad NEW LAV BLOT 1 and 2 (kits for HIV1 and HIV2, respectively)
 - The only WB kits approved by the Pharmaceuticals and Medical Devices Agency of Japan
- Geenius had 99.3% sensitivity for established HIV vs 98.6% for WB (n=146)
- In a population of 20 acute HIV patients, Geenius reclassified seven indeterminate HIV 1 WB's to positive and three of those as negative

Summary of comparison

- Sensitivity for established HIV similar.
- Geenius gave seven positive results in 20 NLB1 negative or indeterminate samples from Acute HIV patients. (Decreases need for HIV NAT)
- Provided positive results earlier than NLB in two of five seroconversion panels, showing increased sensitivity
- For 140 HIV-1 negative samples including 10 false positives, Geenius have 136 negative and NLB1 gave 112 negative results, showing better specificity
- Crossreactivity for HIV-1 in HIV-2 pos specimens was improved over WB. 18 samples that were only HIV1 that were dual positive on WB were resolved as HIV-1 only on Geenius. Overall discrimination rates were 97.7% for Geenius and 87.5% for NLB1 + NLB2

CDC guidelines were updated August 2016

- This update was driven by the Replacement of Multispot by the Geenius:
- Possible Results from Genius:
 - HIV negative, HIV-2 indeterminate, HIV-1 indeterminate, HIV indeterminate, HIV-1 positive, HIV-2 Positive, HIV-2 Positive (with HIV1 cross reactivity), and HIV positive untypeable
- Three results were added to the Geenius versus MultiSpot, the update addressed these

New results and interpretation for Geenius

- HIV2 Positive with HIV-1 cross reactivity:
 - Result should be considered HIV-2 positive. This result is distinct from HIV positive untypeable, which indicates possible infection with HIV-1 and HIV-2 (rare). Either this or the untypeable result need medical care
- HIV-2 indeterminate results:
 - REQUIRE ADDITIONAL TESTING. Repeat Geenius on same sample.
 - If repeat NEGATIVE, negative is final result and HIV-1 NAT needed.
 - If repeat indeterminate: Perform HIV-1 NAT as repeat HIV2 indeterminates may be HIV1. If NAT positive treat as HIV1. If the HIV1 NAT is negative, refer the specimen for testing with a different HIV2 assay or repeat testing in 2-4 weeks (note the package insert states for repeat HIV2 indeterminate repeat the testing in 2-4 weeks, but CDC issued additional recommendation above.

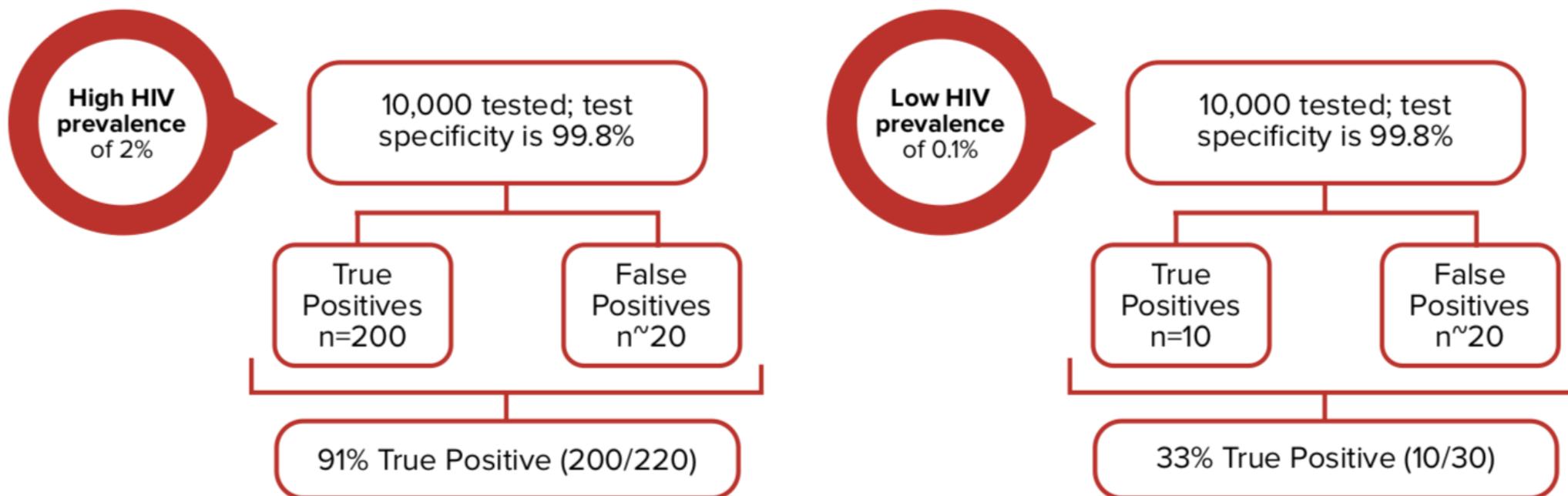
CDC update for Geenius

- HIV Indeterminate:
 - Should prompt the same testing sequence as described for HIV2 indeterminate.
 - HIV1 NAT conducted
 - If POS call specimen HIV1 POS
 - If NEG HIV 1 NAT, refer for another HIV2 assay or repeat testing in 2-4 weeks.

False Positives

- The impact of false positives (decreased Positive Predictive value) is greatest in areas that have low prevalence of HIV infection
- What are the common causes of False Positives:
 - Specimen mix up
 - Technical factors (interferents, heterophile antibodies, etc)
 - Mislabeling
 - Improper handling
 - Misinterpretation of visually read results
 - Participation in a vaccine study
 - Autoimmune disorders

Impact of Prevalence of Utility of “Positives”



Point of Care Testing

- Point of Care Testing for HIV has changed and improved over the years to meet needs
 - Rapid testing for Health Care Professionals
 - Waived test available for home use
 - IgM and IgG antibody testing
 - One FDA approved Combo Ag/Ab test
 - Alere Determine HIV 1-2 Ag/Ab Combo
 - *This will focus on USA available tests, two other test are available outside of US

What is available- Rapid Tests in Clinical Settings

- Chembio DPP HIV 1/2, HIV Ab only, Serum Plasma Whole Blood
- Chembio SURE CHECK HIV 1/2, HIV Ab, Serum plasma Whole Blood
- Clearview HIV 1-2 STAT PAK, HIV Ab, Serum plasma Whole Blood.
- INSTI HIV1/HIV2 Rapid Antibody Test, Ab Only, Plasma, Whole Blood
- MedMira Reveal G3 Rapid HIV1 Antibody Test, Serum Plasma
- OraQuick ADVANCE Rapid HIV 1-2, Ab Only, Plasma Whole Blood
- UniGold Recombigen HIV, Ab HIV-1 only, Serum Plasma Whole Blood.

- Combo Test:
 - Determine HIV 1-2 Ag/Ab Combo Test, Ag and Ab, Serum Plasma Whole Blood

What is available - Waived

- Chembio DPP HIV 1/2, HIV Ab only, Finger Stick Whole Blood, oral fluid, venous whole blood
- Chembio SURE CHECK HIV 1/2, HIV Ab, Finger stick, venous Whole Blood
- Clearview HIV 1-2 STAT PAK, HIV Ab, Finger stick, venous Whole Blood
- INSTI HIV1/HIV2 Rapid Antibody Test, Ab Only, Finger stick Whole Blood
- OraQuick ADVANCE Rapid HIV 1-2, Ab Only, Finger Stick Whole Blood, oral fluid, venous whole blood
- UniGold Recombigen HIV, Ab HIV-1 only, Finger stick, venous Whole Blood

- Combo Test:
 - Determine HIV 1-2 Ag/Ab Combo Test, Ag and Ab, Finger stick Whole Blood

Early concerns- Point of Care HIV Screening

- When the initial Guidelines were released in 2014, there were insufficient data to recommend the use of the Rapid, Point of Care Combo Antigen/Antibody test available at the time.
 - Alerre Determine Combo Ag/Ab
- At the time, there were concerns of sensitivity and specificity of the Antigen (p24) portion of the Assay
- Multiple studies since then have been released, providing more evidence
 - While the results vary, there are some conclusions that can be drawn

Select Literature review

- Duong, Parekh, et al (2014) conducted Home testing and lab testing on whole blood and serum on 18,172 individuals in Swaziland.
 - Performance of the antibody component was comparable to laboratory based testing
 - 12 samples that were Ag+ and Ab- were evaluated for viral load and were negative. (False positive)
 - 12 double nonreactive patients had Positive NAAT results, considered a False negative.
 - Other Studies in UK and Australia found similar results, with no acute infections detected with false positives present (many non Type B's present)
 - This was attributed to multiple factors:
 - Much worse performance in Whole Blood
 - Lack of p24 amplification step
 - Possible decreased reaction to non-Type B HIV infections (not found in US)

Evaluation at UCLA

- Stafylis and Klausner (August 2017) compared the Standard Bioline HIV Ag/Ab Combo to Alere Determine Combo, compared to RNA and Lab based Combo Ag/Ab test in 133 frozen serum samples
- The sensitivity on serum was high but not as high as package insert (95%) and not as high as laboratory based combo testing (Consistent with other studies) and specificity was 100%.
 - Alere yielded five false negative results (SD Bioline nine)
- For 13 antibody negative/RNA positive samples, Lab testing identified 10/13 and Alere identified 12/13
- P24 component had a sensitivity of 76.9%, other studies ranged from 2.9% to 88%

Evaluating Performance in Serum

- Masciotra and Owen, et al, (2017) tested 508 plasma samples from New York, North Carolina, and San Francisco
- Determine Combo detected 337 of 396 (85.1%) of architect reactive, confirmed HIV-1 infections
 - Detected 50% (55/110) of Architect reactive, HIV RNA POS, MS negative samples (Acute infection samples)
 - Detected 11/14 (78.8%) of Architect reactive, HIV RNA POS, MS indeterminant sample
 - Detected 271/272 (99.6%) of architect reactive, Multispot positive samples
- Conclusion: Antigen performance was better than other studies, but only in plasma (whole blood much worse), and was not as good as lab based tests. Antibody portion was not as good as lab-based tests but as good as other Antibody only Rapid Tests.

CDC supplement to Guidance- Oct 2017

- With updated data, CDC issued a technical update on the use of the Alere Determine Combo Rapid Test
 - FDA recommendation is still to use laboratory based tests since they are more sensitive to ANY point of care tests for early infection.
 - However, Alere Combo can detect infection earlier than antibody only tests.
 - For laboratories in which instrumented Combo testing is not available, Determine can be used with serum/plasma (whole blood not mentioned) as the first step in laboratory algorithm. This approach will not detect infection as early as the instrumented tests, and labs are advised to acknowledge the limitations of the testing procedure when reporting results.
 - There are limited data on the performance of the Antigen portion of the test. When antigen only is detected, supplemental testing may be offered. Further data on antigen in this test is pending

CDC supplement to Guidance- Determine

- Data is not available to advise on testing with whole blood
- When a preliminary positive is received in a lab from a waived application (whole blood), start with antigen/antibody test as beginning of algorithm
- In summary, when instrumented combo tests are available, those are preferred. However, in smaller labs, Determine may be used as a screening test.

What to do with POC combo tests?

Always start with instrumented Ag/Ab testing where possible as it is superior to all point of care (and antibody only instrumented tests)

If need POC testing, the performance of the antigen portion has not been well characterized, but it is less sensitive than instrumented tests. Specificity in the US needs to be better characterized. Antibody portion is at least equal to the other rapid tests and may perform between the POC and the instrumented antibody only tests.

For rapid combo tests, may be advisable to send presumptive positives for confirmation to a lab with instrumented Ag/Ab test. If negative, can stop and report negative. (Only MANDATORY for waived/whole blood samples, NOT for serum/plasma. This option is at the discretion of the lab at that point)



Thank You for your Time!

Questions