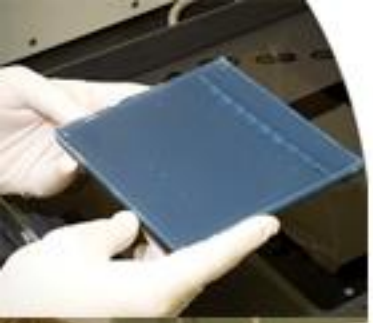
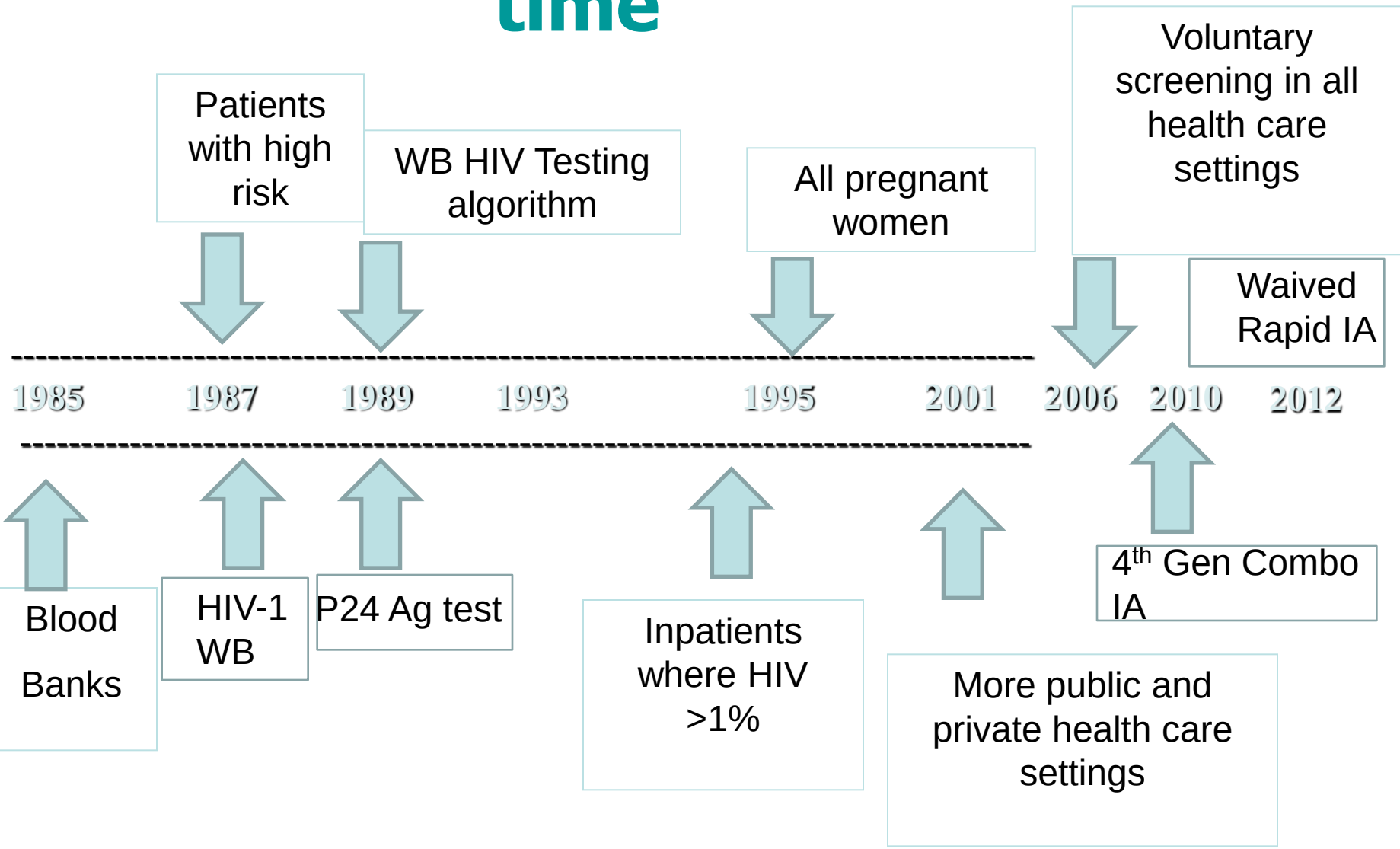


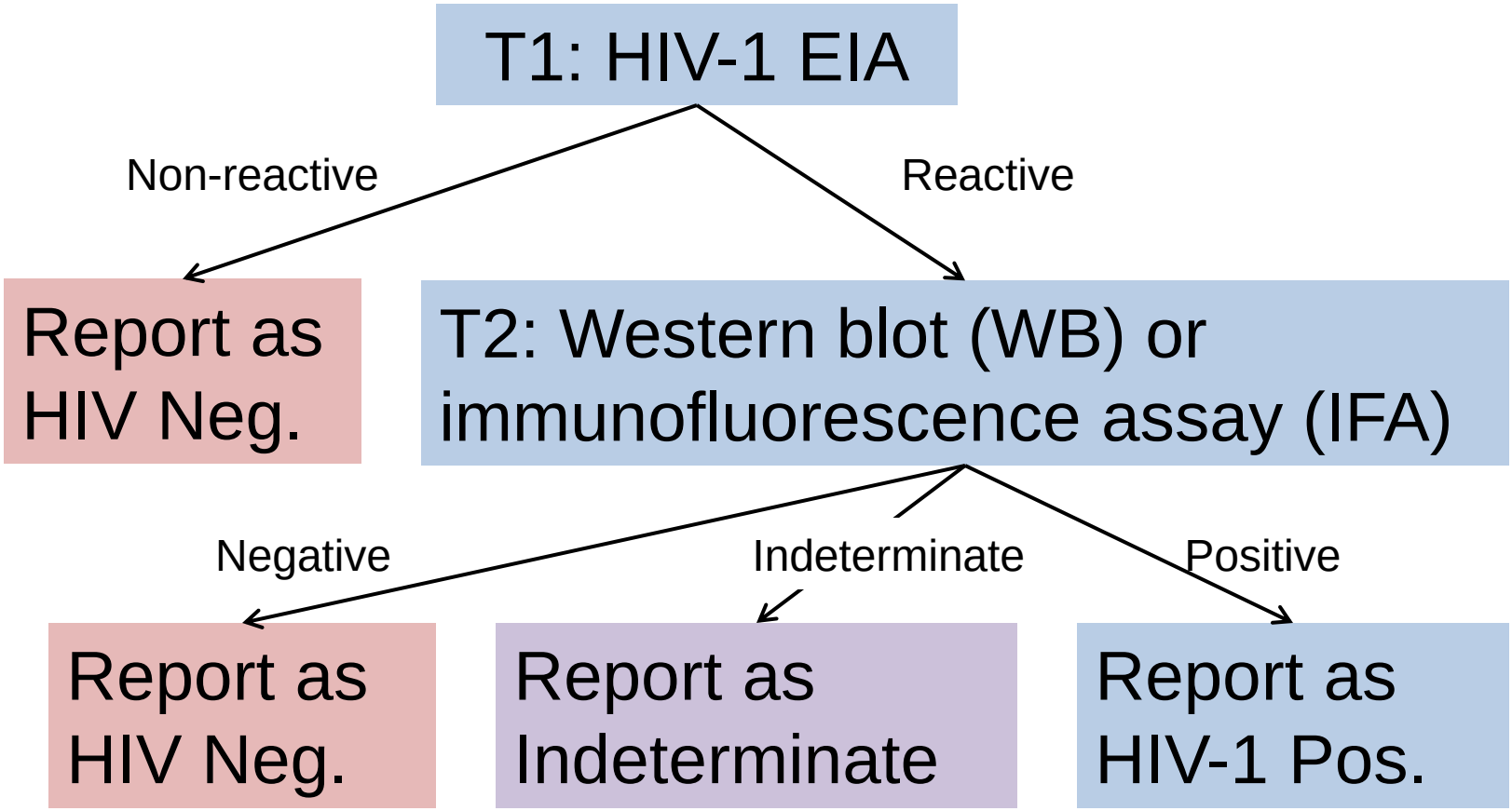

HIV Testing Technology and the Latest Algorithm

David Warshauer, PhD, D(ABMM)
Deputy Director, Communicable Diseases
Wisconsin State Laboratory of Hygiene

HIV Testing has changed over time

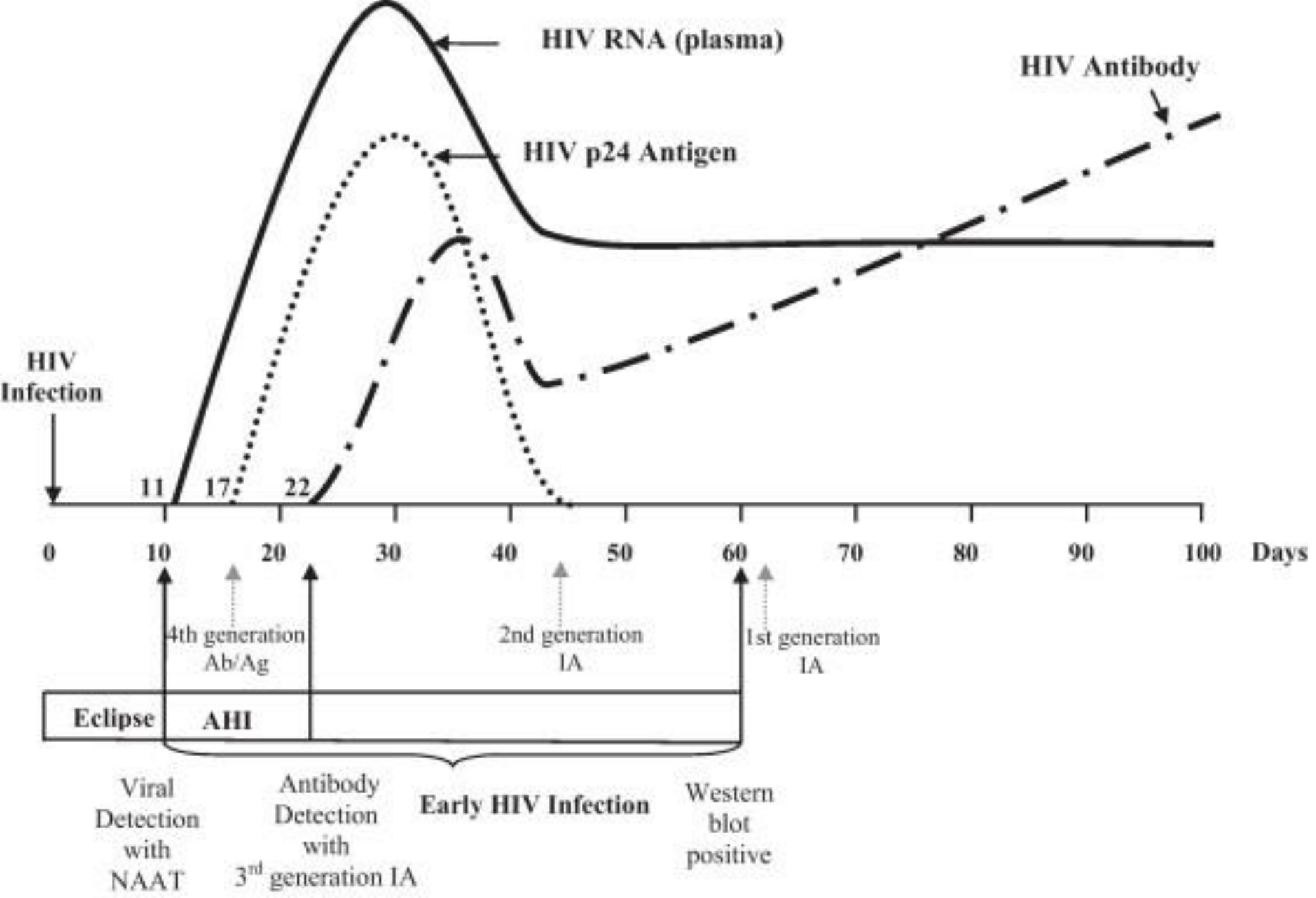


1989 HIV Algorithm



New HIV testing strategies/algorithms

- 1989 algorithm remained largely unchanged for over 30 years
- Learned more about HIV-1 and HIV-2 disease
  - Disease progression
  - Virus dynamics
  - Window Period
- Advancements in technology and molecular tests



sciencedirect.com

Evolution of the HIV Immunoassays

- Designed for *sensitivity* and use as screening tests
- Reactive result considered “preliminary positive”
  - Supplemental testing needed to confirm
- IAs have become more sensitive with each new generation
  - 1st gen EIA: HIV-1 viral lysates; indirect IA using labeled antihuman IgG
    - Required significant dilution to overcome non-specific reactions with cellular protein contaminants

Evolution of the HIV Immunoassays

- 1991---2nd gen IA: Improved sens & spec
  - HIV-1/HIV-2
  - Synthetic peptide or recomb Ag w/wo viral lysates
  - IgG
- 1992---3rd gen IA: HIV-1/HIV-2
  - synthetic peptide or recomb Ag
  - IgM + IgG Ab
  - Ag sandwich format; lower specimen dilution; increased sensitivity

Evolution of the HIV Immunoassays

- 2010---4th gen IA
  - Similar to 3rd gen w/ addition of p24 Ag detection
- 5th gen multiplex flow IA:
  - differentiates HIV-1 Ag, HIV-1 and HIV-2 Ab (Bio-Rad)

4th Generation HIV Ag/Ab Combo Assays

Test Name (Manufacturer)	Instrument	Specimen Types	Result Output
Abbott ARCHITECT HIV Ag/Ab Combo (Abbott Diagnostics)	Fully automated, random access (i2000SR)	Serum Plasma	Nonreactive Reactive
GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories)	Manual or semi-automated instrument (Evolis)	Serum Plasma	Nonreactive Reactive
ADVIA Centaur HIV Ag/Ab Combo (Siemens Healthcare Diag.)	Fully automated, random access (Centaur/Centaur XP)	Serum	Nonreactive Reactive

4th Generation Ag/Ab Test

- 3 FDA-approved kits available
  - ARCHITECT HIV Ag/Ab Combo (Abbott)
  - GS HIV Ag/Ab Combo EIA (Bio-Rad)
  - ADVIA Centaur Ag/Ab CIA
- Detect HIV-1 p24 Ag, HIV-1 and HIV-2 antibodies
- Reactive result:
  - Doesn't distinguish between Ag and Ab
  - Preliminary positive
  - Supplemental testing required

5th Generation Ag/Ab Test

- BioPlex 2200 HIV Ag/Ab Test
  - Detects and differentiates HIV-1 Ag, HIV-1 Ab, and HIV-2 Ab in serum or plasma
  - Early infection detection similar to other laboratory Ag/Ab tests
  - High sensitivity and specificity
  - Further data needed on performance in the algorithm

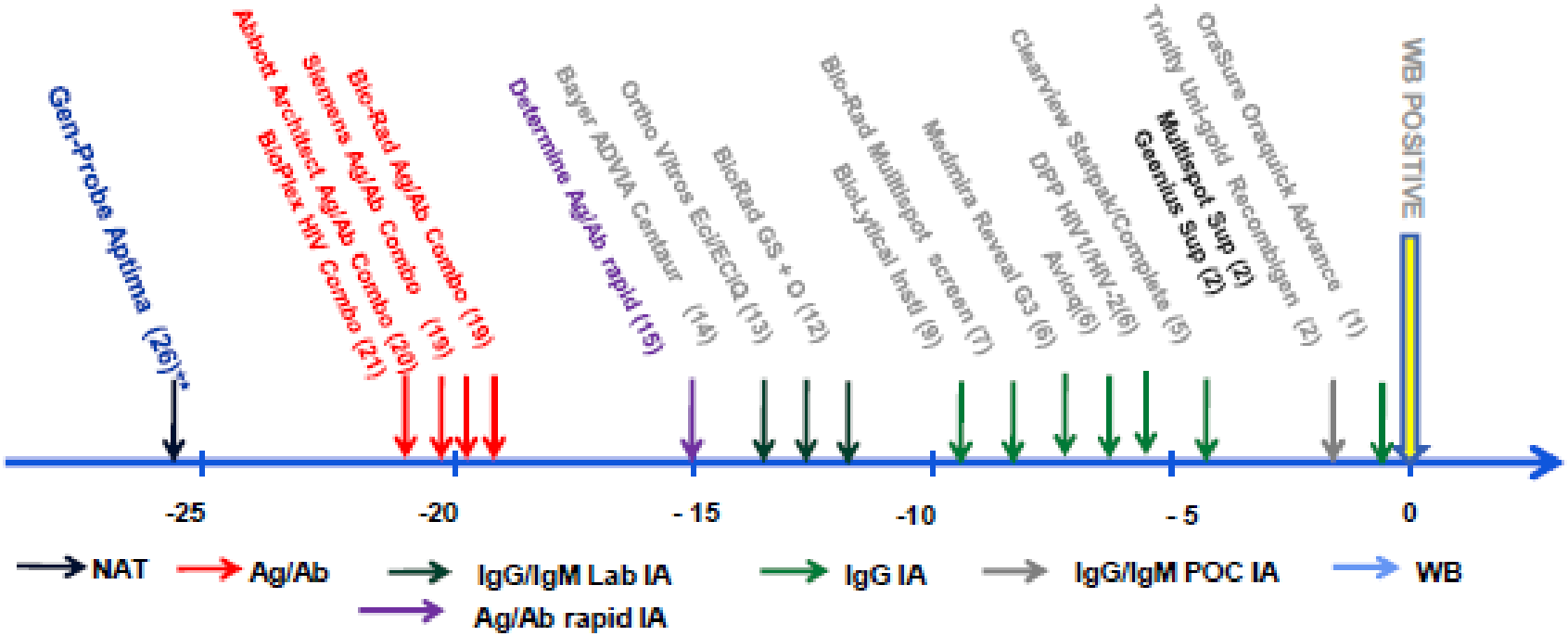
Changes that impact HIV testing strategies/algorithms

- Evolving technology
  - Availability of rapid tests
  - Increased sensitivity of screening assays
    - Western blot and IFA now much less sensitive than screening assays which they are intended to “confirm”

Western blot (WB)

- Designed for *specificity*
- Must meet specific criteria for a positive interpretation
- Indeterminate results occur for a variety of reasons
  - Early infection, late infection, HIV-2 infection, other
- Western blot technology has not advanced
  - Newer immunoassays are more sensitive than WB
- Lack of sensitivity can lead to false negative and inconclusive results

Sequence of HIV Assay Reactivity During Early HIV Infection Relative to Western Blot*



*Assay sensitivity above is based on frozen plasma only. Whole-blood and oral fluid has not been characterized for early infection.
**Current data suggests that the Gen-Probe Aptima can detect HIV-1 RNA ~5-28 days after infection.

Adapted from Owen et al J Clin Micro 2008 and Masciotra et al J Clin Virol 2011

HIV Test Strategy Workgroups

- Two workgroups were formed in October 2006
  - Focused on strategies for both POC and Laboratory settings
- HIV Diagnostics Conference in December 2007
  - Data was presented to substantiate various algorithms
- Status Report started fall 2008
  - APHL/CDC Steering Committee produced a report of the current status of the proposed algorithms

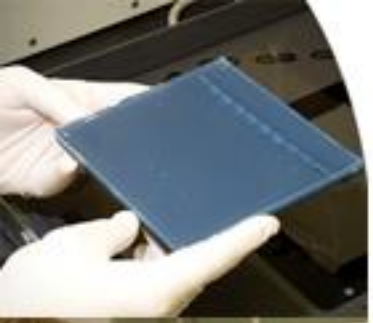
- 2010 HIV Diagnostics Conf.
  - Announced consideration of Multispot as supplelmental assay
- 2012 HIV Diagnostics Conf.
  - Draft recommendations for a new HIV testing algorithm
- 2014 HIV Diagnostics Conf.
  - CDC publishes new HIV testing algorithm

Representation on Laboratory and POC Workgroups

- Association of Public Health Laboratories
- American Clinical Laboratory Association
- American Society of Microbiology
- Blood Banks
- College of American Pathologists
- US Centers for Disease Control and Prevention
- Commercial Laboratories
- US Department of Defense
- US Food and Drug Administration
- HIV Program Staff from NASTAD and Public health departments

What are we looking for from these new testing strategies?

- Resolution of indeterminates
- Ability to confirm HIV-2 infections
- Increased detection of acute infection
- Assays that can be used as screening or confirmatory/ supplemental tests and as part of multi-test algorithms
- Guidance for laboratory confirmation of POC rapid tests



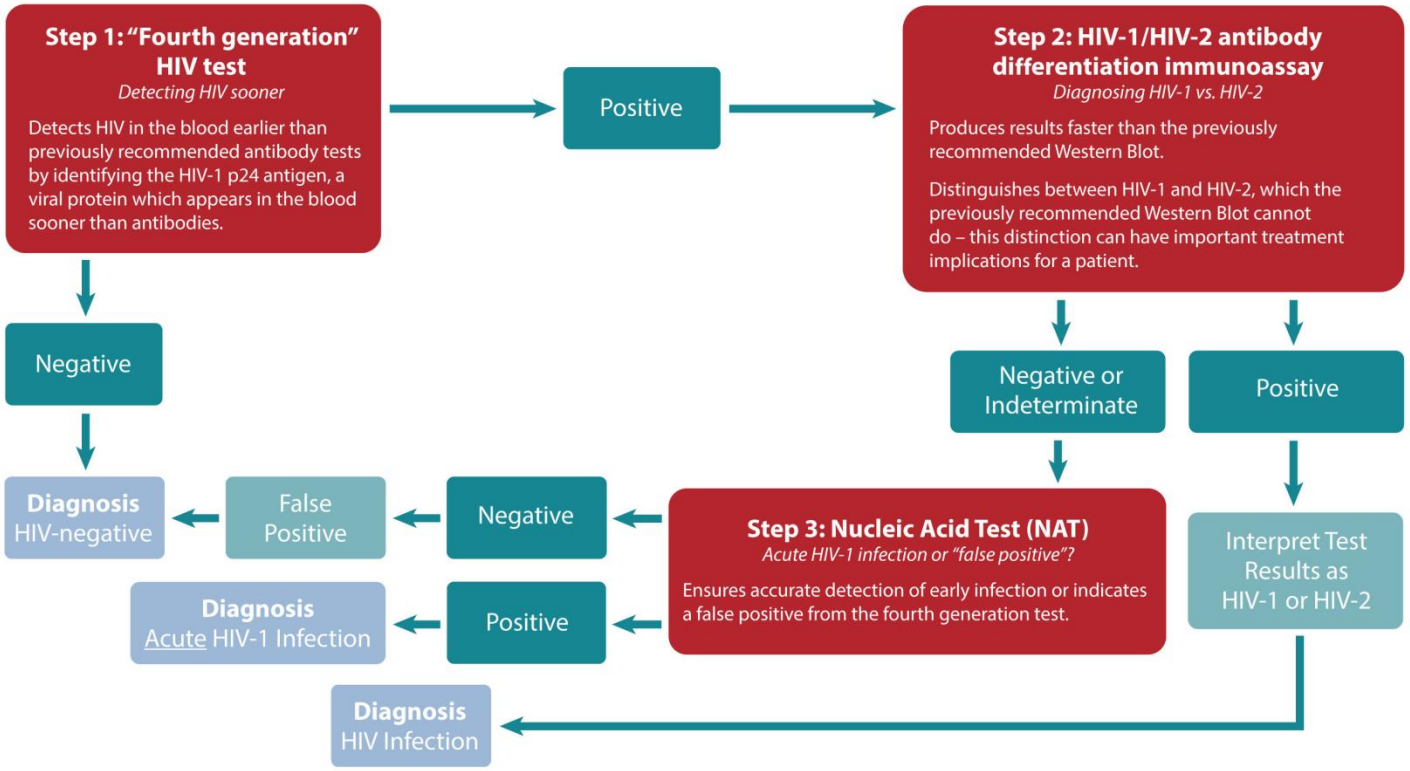
Laboratory Algorithms

New CDC Recommendations for HIV Testing in Laboratories

A step-by-step account of the approach

CDC's new recommendations for HIV testing in laboratories capitalize on the latest available technologies to help diagnose HIV infections earlier – as much as 3-4 weeks sooner than the previous testing approach. Early diagnosis is critical since many new infections are transmitted by people in the earliest ("acute") stage of infection.

By putting the latest testing technology to work in laboratories across the United States, we can help address a critical gap in the nation's HIV prevention efforts.



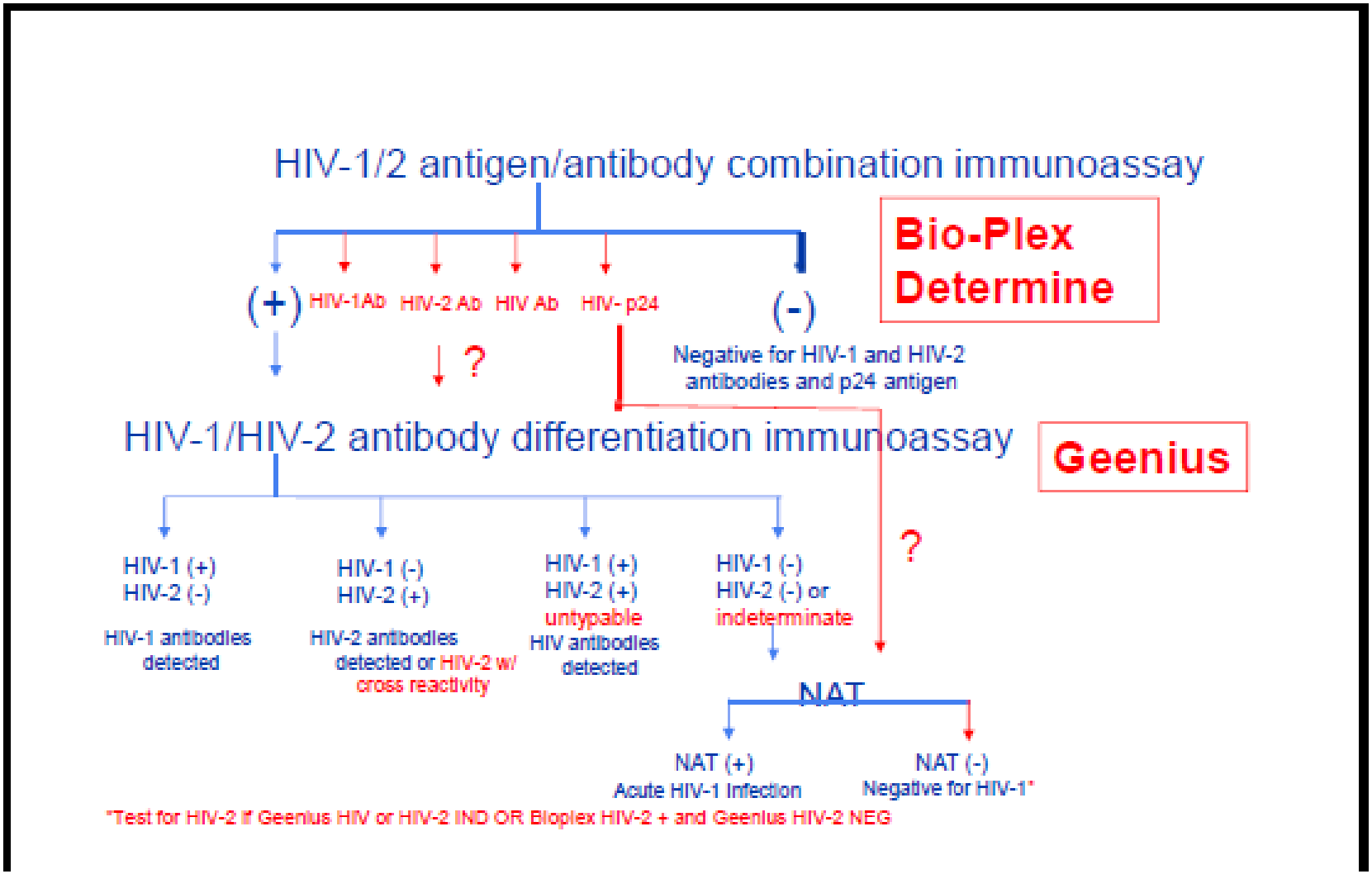
This graphic is designed to illustrate key concepts of the new testing approach in laboratories. For more detail, please see the full guidelines here: http://www.cdc.gov/hiv/pdf/HIVtestingAlgorithmRecommendation-Final.pdf.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

www.cdc.gov/nchhstp/newsroom

JUNE 2014

Algorithm with 5th Generation Test



Advantages of the New Algorithm

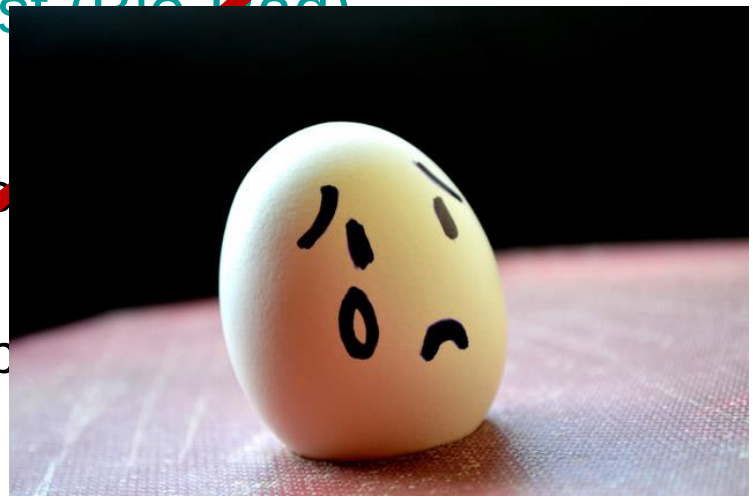
- 4th Gen Ag/Ab more sensitive and specific
- Allows detection of acute HIV infection
- Eliminates expensive, labor intensive, and problematic WB
- Allows detection of HIV-2 infection
- Geenius HIV-1/HIV-2 is simple, rapid, and less expensive than WB

HIV-1 NAT

- Only one HIV-1 RNA test FDA-approved for use as an *aid in the diagnosis* of HIV-1 infection
  - APTIMA® HIV-1 RNA Qualitative Assay (Gen-Probe)
- Several HIV-1 quantitative tests (viral load)
  - FDA approval is for *patient monitoring*, i.e. assess prognosis, monitor effects of therapy
  - Not intended as a diagnostic test to confirm the presence of HIV-1 infection
- WSLH using proviral DNA RT-PCR

HIV-1/HIV-2 Discriminatory Immunoassay

- Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad)
- FDA-approved:
  - To detect & differentiate antibodies
  - Suitable for multi-test algorithms
  - Current package insert does not sp
    supplemental test



- Single-use rapid test
- Results in ≤30 min
- Not CLIA waived
- Serum or plasma

Geenius HIV 1/2 Supplemental Assay

Geenius HIV ½ Supplemental Assay

- FDA approved Oct 2014
- Replaces Multispot
- Differentiates HIV-1 and HIV-2 antibodies
- 30 minutes

Geenius

Test Antigens (lines, left to right)

1. HIV-2 gp36 (env peptide)
2. HIV-2 gp140*
3. HIV-1 p31 (pol peptide)
4. HIV-1 gp160 (env recomb prot)
5. HIV-1 p24 (core recomb prot)
6. HIV-1 gp41(env peptides)
7. Control (protein A)

*Multimer of HIV-2 gp36 env peptide

Protein A colloidal gold detection

Geenius™ Compared to Multispot

Multispot	Geenius
Rapid testing (2004) or Supplemental (2013)	Supplemental use only (confirmation)
Manual reading and interpretation	Geenius Reader and automatic interpretation on Geenius Software
Manual result entry into LIS/LIMS	Bi-directional connection to LIS/LIMS
Manual labeling	Full traceability; barcode identification
Dilution protocol in PI to resolve cross-reactivity	No Equivalent
Serum or plasma	Serum, plasma, fingerstick or venous whole blood

Geenius™ and Multispot Results

Multispot Results	Geenius Results
Nonreactive	Nonreactive
Reactive: HIV-1 positive	Reactive: HIV-1 positive
Reactive: HIV-2 positive	Reactive: HIV-2 positive
No Equivalent	Reactive: HIV-2 positive with HIV-1 cross-reactivity
Reactive: HIV positive (undifferentiated)	Reactive: HIV positive untypable (undifferentiated)
Indeterminate: HIV-1 indeterminate	Indeterminate: HIV-1 indeterminate
No Equivalent	Indeterminate: HIV-2 indeterminate
No Equivalent	Indeterminate: HIV indeterminate

Geenius Results-What They Mean

- HIV-2 positive with HIV-1 cross-reactivity
  - Antibody to HIV-2 confirmed
  - HIV-1 reactivity does not meet criteria to be considered positive
    - only one HIV-1 envelope band detected
    - Gp160 or gp41
    - Indicative of HIV-1 cross-reactivity
  - Final Interpretation Reported
    - HIV-2 Positive
  - Patient should be referred to care for HIV-2 infection

What results are seen with HIV-2 Ab positive specimens?

- Package Insert Data for 200 specimens
  - HIV-2 Positive for 38.5% (108/200)
  - HIV-2 with HIV-1 cross-reactivity for 54% (108/200)
  - HIV Undifferentiated for 6% (12/200)
  - HIV indeterminate for 1.5% (3/200)

Geenius Results-What They Mean

- HIV-2 Indeterminate
  - Repeat before reporting
  - If repeat is “HIV-negative, report as such
  - If repeat is “HIV-1positive” or HIV-2 positive”, report as such
  - If repeat is HIV-2 indeterminate, report as HIV-2 indeterminate
    - Result may indicate acute HIV-1 infection
    - HIV-1 NAT should be performed

Geenius Results-What They Mean

- HIV-2 Indeterminate (con't)
  - If HIV-1 NAT is negative
    - Refer specimen for testing with a different HIV-2 test
    - Or repeat testing in 2-4 weeks

Geenius Results-What They Mean

- HIV Indeterminate
  - Bands present for both HIV-1 and HIV-2, but positive criteria not met for either
  - Repeat before reporting
  - If repeat is “HIV-negative, report as such
  - If repeat is “HIV-1positive” or HIV-2 positive”, report as such
  - If repeat is HIV indeterminate, report as HIV indeterminate
    - Result may indicate acute HIV-1 infection
    - HIV-1 NAT should be performed

Geenius Results-What They Mean

- HIV indeterminate
  - Perform HIV-1 NAT
    - If NAT pos, refer patient to care for HIV-1
    - If NAT neg, refer specimen for HIV-2 testing with NAT or different antibody test
    - or repeat testing in 2-4 weeks

Geenius Results-What They Mean

- HIV-1 indeterminate
  - Same testing sequence as for HIV indeterminate with except additional HIV-2 testing is not necessary

Geenius Results-What They Mean

- HIV Positive Untypable (undifferentiated)
  - Antibodies to HIV-1 and HIV-2 confirmed in the specimen
  - May occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1
  - Or co-infection with both HIV-1 and HIV-2
  - Repeat to confirm before reporting
  - Interpret similar manner as “HIV indeterminate”

Geenius Results-What They Mean

- HIV Positive Untypable (undifferentiated)
  - Perform HIV-1 NAT
    - If NAT pos, refer patient to care for HIV-1
    - If NAT neg, refer specimen for HIV-2 testing with NAT or different antibody test

      repeat testing in 2-4 weeks

Geenius Results Reporting

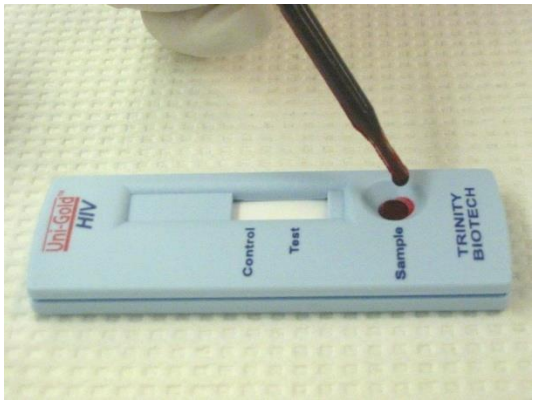
Not Reported

Reported

HIV-1 result	HIV-2 result	Geenius Assay Interpretation
Negative	Negative	**HIV Negative**
Indeterminate	Negative	**HIV-1 Indeterminate**
Negative	Indeterminate	**HIV-2 Indeterminate**
Indeterminate	Indeterminate	**HIV Indeterminate**
Positive	Negative	**HIV-1 Positive**
Positive	Indeterminate	**HIV-1 Positive**
Negative	Positive	**HIV-2 Positive**
Indeterminate	Positive	**HIV-2 Positive**
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

Point of Care Rapid HIV Testing

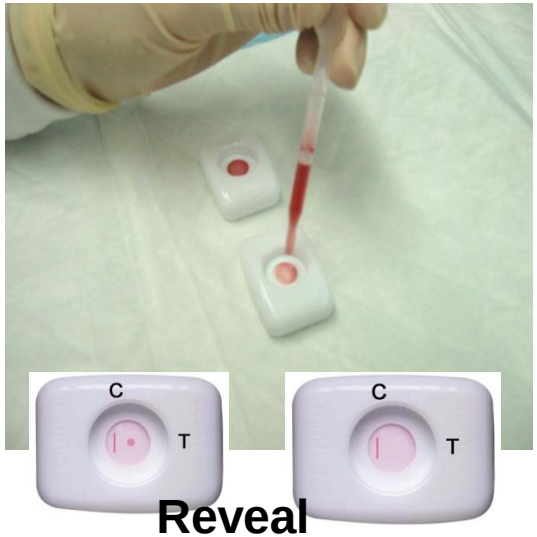
Approved Tests for the Point of Care Algorithms



Uni-Gold Recombigen

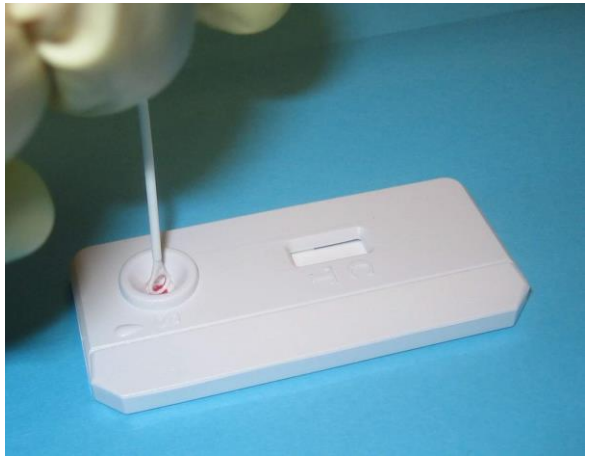
~~**Multispot HIV-1/HIV-2**~~

Clearview Complete HIV 1/2



Reveal G3

OraQuick ADVANCE



Clearview HIV 1/2 Stat Pak

Product	Specimen type		CLIA Category		Exp.
Unigold Recombigen HIV	Whole Blood Serum, Plasma		**Waived** Moderate Complexity		1 yr
OraQuick ADVANCE HIV 1/2	Oral Fluid, Whole Bld Plasma		**Waived** Moderate Complexity		6 mos.
Reveal G3 Rapid HIV-1	Serum, Plasma		Moderate Complexity		1 yr
Clearview STAT-PAK HIV-1/2	Whole Blood Serum, Plasma		**Waived** Moderate Complexity		2 yrs
Clearview Complete HIV-1/2	Whole Blood Serum, Plasma		**Waived** Moderate Complexity		2 yrs
Alere Determine	Whole Blood		**Waived** Moderate Complexity		15 mo

FDA Approved HIV Rapid Tests

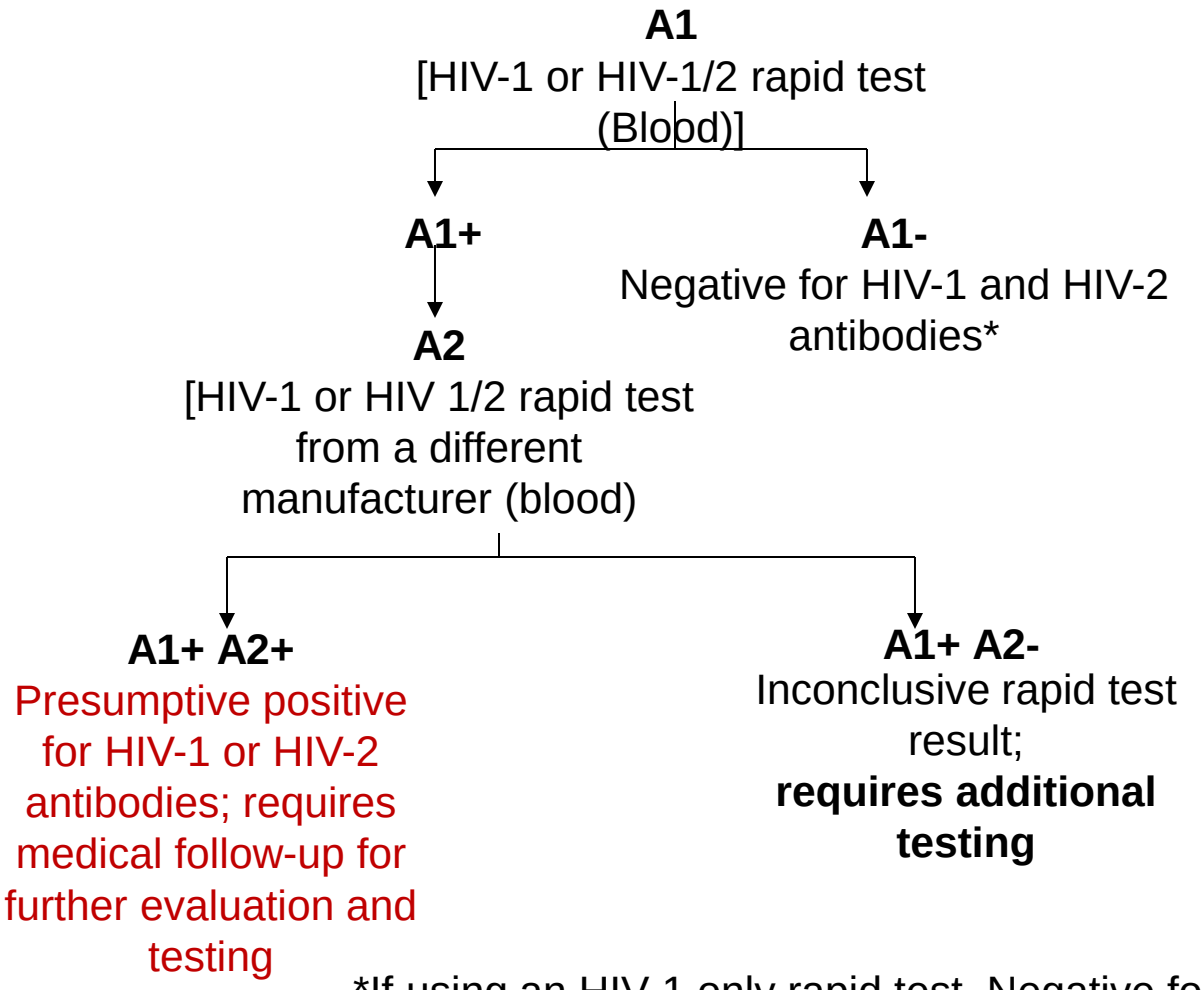
Product	Manufacturer	Analyte	Specimen Type	Sensitivity	Specificity	FDA Approval
Unigold Recombigen HIV	Trinity Biotech www.trinityusa.com	HIV-1	Whole blood, Serum, Plasma	100%	99.7%	Dec. 2003
OraQuick ADVANCE HIV 1/2	Orasure Technologies www.orasure.com	HIV-1 HIV-2	Whole blood, Oral fluid, Plasma	99.6% BL 99.3% OF	100% BL 99.8% OF 99.9% plasma	June 2004
Reveal G3 Rapid HIV-1	MedMira www.reveal-hiv.com	HIV-1	Serum, Plasma	99.8%	99.1% serum 98.6% plasma	Oct. 2006
Clearview STAT-PAK HIV-1/2 **&** Complete HIV-1/2	Inverness Med. www.invernessmedicalpd.com	HIV-1 HIV-2	Whole blood, Serum, Plasma	99.7%	99.9%	May 2006
Determine HIV-1/2 Ag/Ab	Alere www.alerehiv.com	HIV-1 Ab HIV-2 Ab HiV-1 Ag	Whole blood, serum, plasma	99.9	99.7% BL 99.6% serum 99.7% plasma	2013,2014

Non-Clinical Site Algorithms

- Single rapid test with immediate linkage to clinical provider if reactive
- Single rapid test followed by lab-based follow up testing if reactive
  - WDPH algorithm for CTS
- Single rapid test immediately followed up a 2nd rapid test on-site if reactive
  - If both tests reactive, link to HIV care
  - If second test nonreactive, refer to laboratory or clinical provider for follow-up testing

Testing Strategy for Point of Care HIV Rapid Testing Facilities

Two Rapid Tests (A1/A2) Performed in Sequence on Blood
(A1 and A2 must be different rapid tests)



*If using an HIV-1 only rapid test, Negative for **HIV-1 antibodies only**

POC Two Test Algorithm

- Requires that technicians be proficient with 2 tests
  - Limited training, expertise required
- Little chance for false-positive or false-negative algorithm results
- Likely to work well in high prevalence settings
- In low prevalence areas majority of specimens will require lab resolution

POC Testing using Multi-Rapid Test Algorithms

- Addresses low return rate for confirmatory test results experienced in some settings
- Can optimize care and treatment for HIV-infected persons
  - HC settings can aid in definitive diagnosis and therefore inform clinical decisions quickly
  - All settings can facilitate referral to HIV care, especially with mobile and transient populations
- Can optimize prevention
  - Messages about HIV test results can be clearer and more persuasive; optimizes prevention and care engagement
  - Facilitates provision of partner services

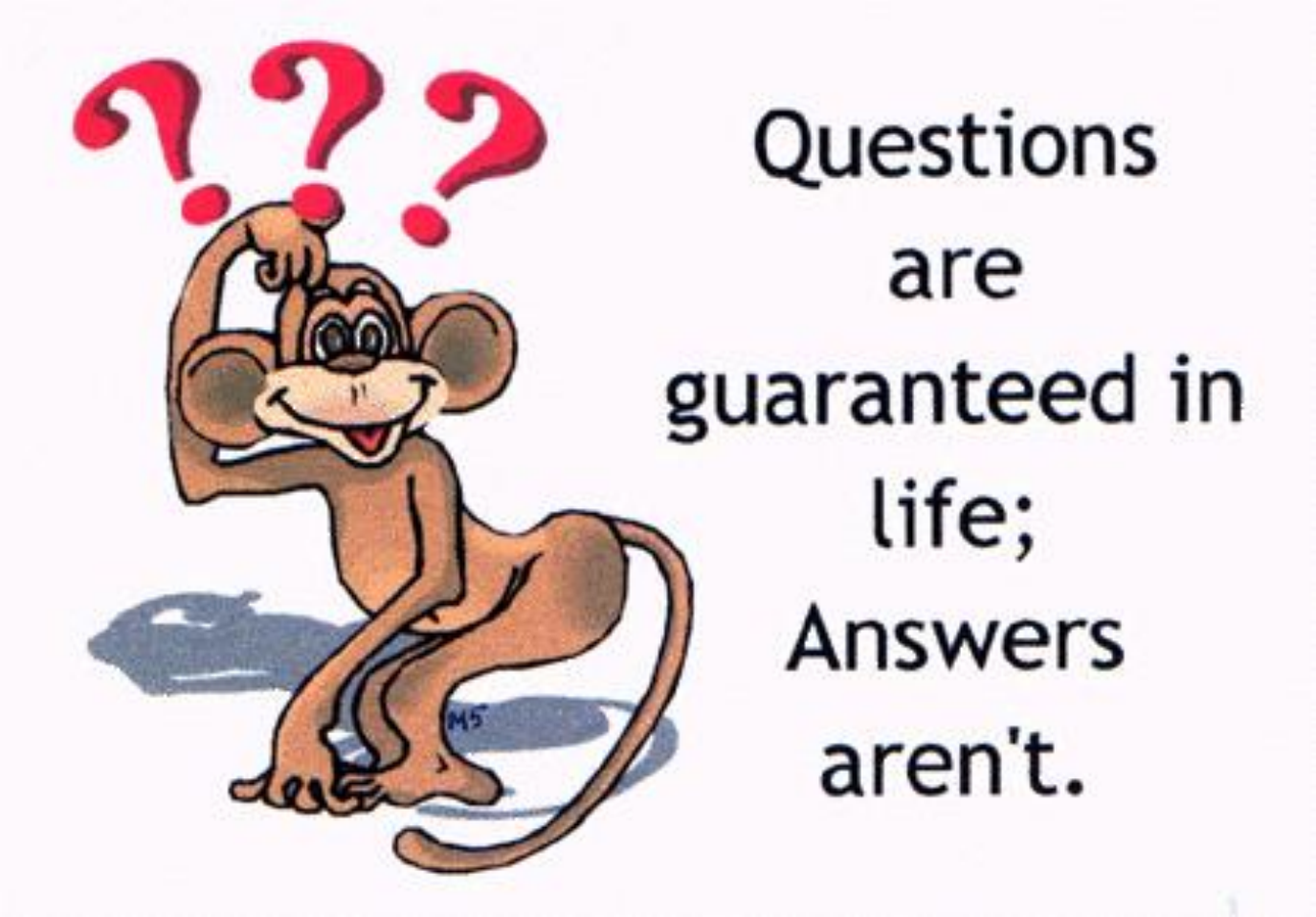
Oral Fluid Testing

- Not part of CDC's recommended algorithm
- Requires a testing algorithm that includes the Western blot
- Does not detect infection as early as blood tests

More Information

- CDC and APHL. Laboratory testing for the diagnosis of HIV infection: updated recommendations. http://stacks.cdc.gov/view/cdc/23447

- APHL.HIV Diagnostic Informational Updates *Modified on March 17, 2016.* https://www.aphl.org/programs/infectious_disease/Documents/2015_Informational%20Update_02_12_16_FINAL.pdf
- Link to 2016 HIV Diagnostic Conference: http://hivtestingconference.org/


Questions
are
guaranteed in
life;
Answers
aren't.