

HIV Update in Laboratory Testing

Patricia Slev, PhD, D(ABCC)

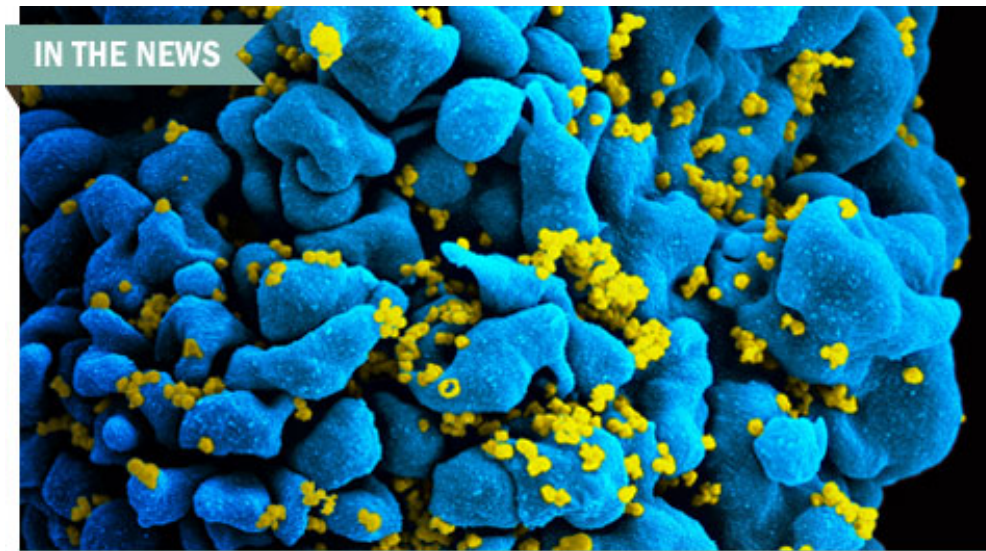
Objectives

- Explain the advances in HIV diagnostics, including fourth generation Ag/Ab combination HIV screening assays
- Describe the new CDC HIV diagnostic algorithm
- Explain appropriate testing algorithm and understand interpretation of laboratory results for HIV

Questions

- What is a fourth generation HIV screening assay (describe)
- Is there a rapid test that detects both HIV Ag& Ab (true or false)
- Preliminary results from a rapid test must proceed to confirmation with the Western blot (true or false)

August 7, 2014

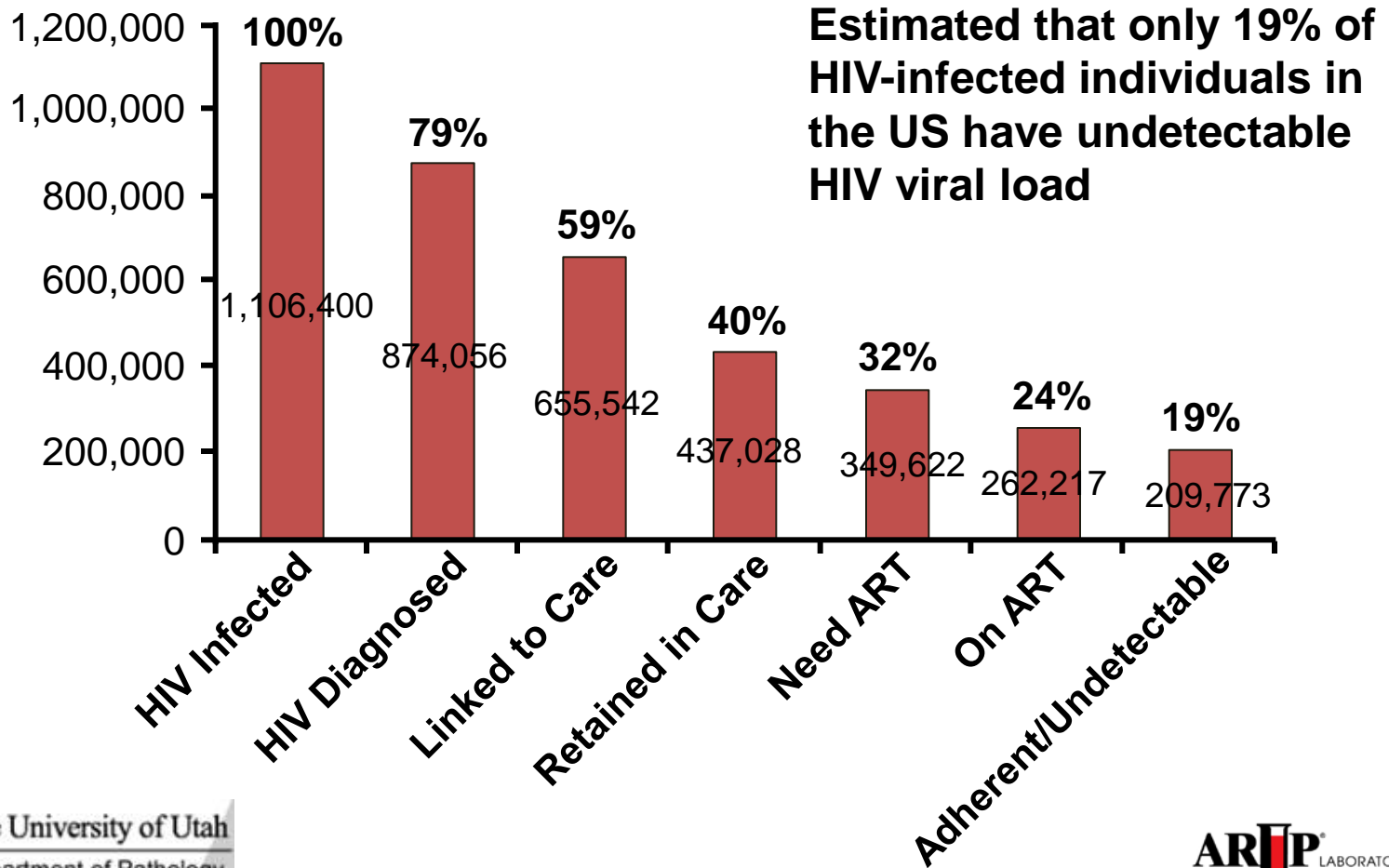


© National Institute of Allergy and Infectious Diseases

Updated HIV Testing Guidelines

CDC, APHL together offer recommendations for HIV testing, based on the best available scientific evidence. [READ MORE](#)

HIV in the US



2006 CDC Guidelines “Universal Testing”

- Routine HIV
voluntary, not based on risk
- Opt-Out
option to decline, general consent for care includes HIV testing
- Population
13 -64 years old
- Venue
inpatient services, ED, urgent care, STD clinics,
substance abuse and correctional facilities

USPSTF – “Universal Screening” (2013)

Annals of Internal Medicine

| **CLINICAL GUIDELINE**

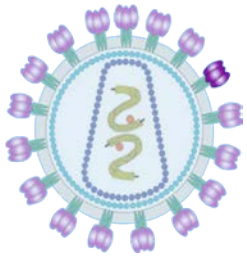
Screening for HIV: U.S. Preventive Services Task Force Recommendation Statement

Virginia A. Moyer, MD, MPH, on behalf of the U.S. Preventive Services Task Force*

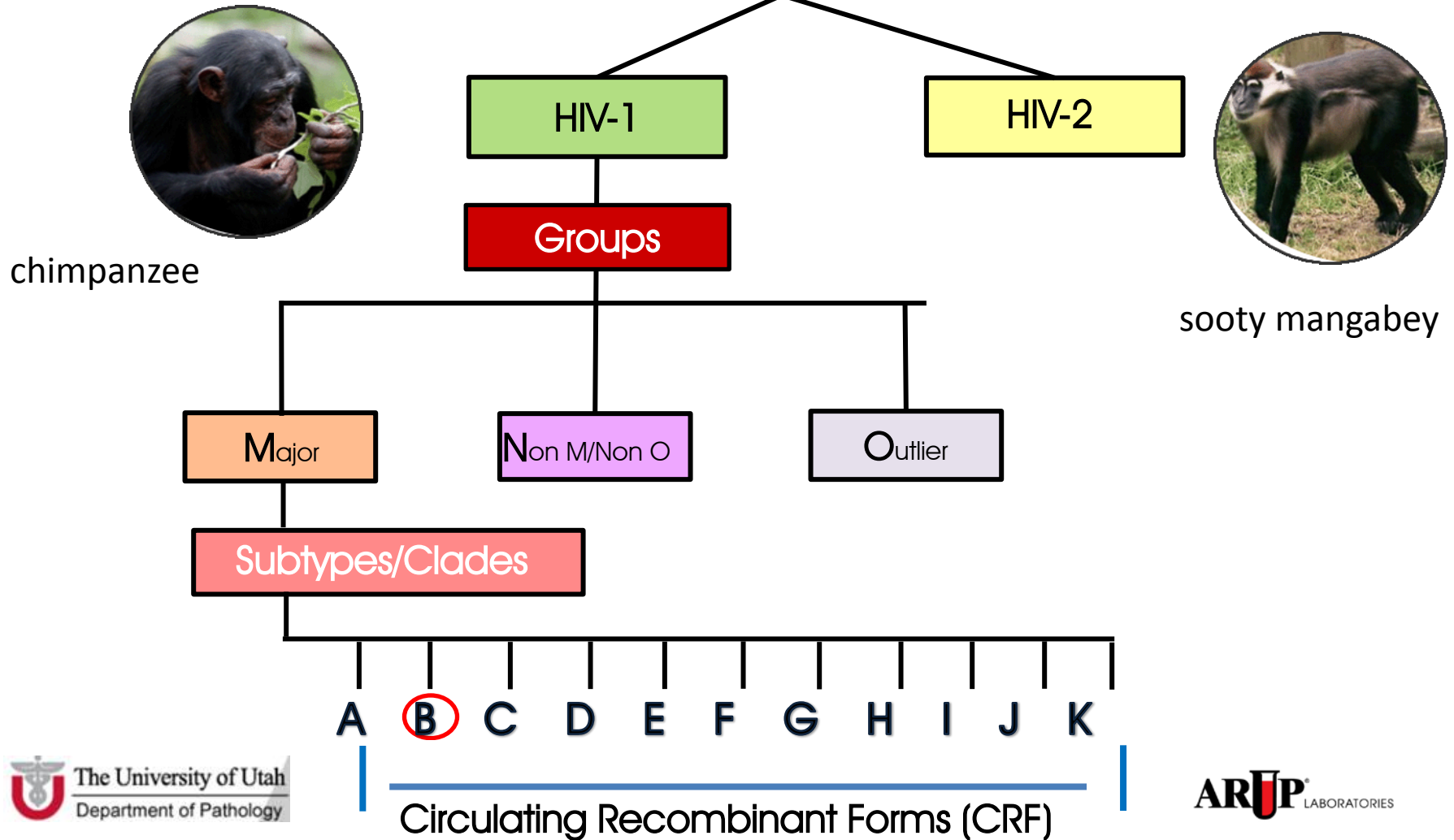
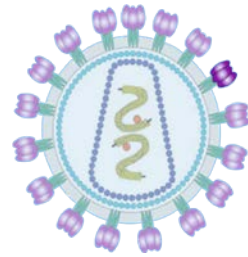
6/13

Grade A Recommendation for Routine HIV Testing
in individuals 15-65 yrs of age

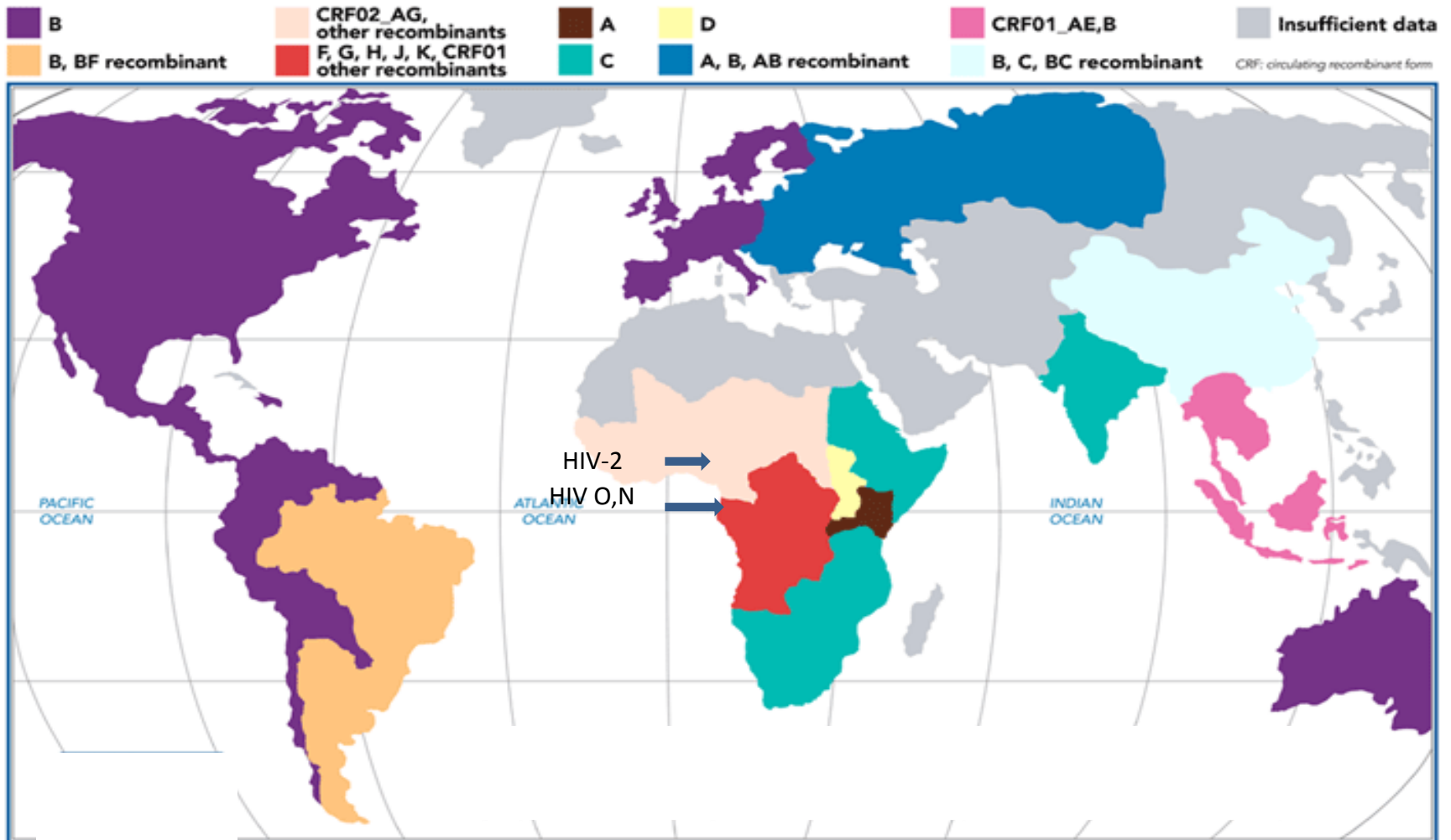
Impact - Reimbursement



Human Immunodeficiency Virus



HIV Distribution



McCutchan, Henry M. Jackson Foundation (Rockville, Maryland). McCutchan and colleagues are indebted to the many international collaborators who helped develop the data used to generate this map.

HIV-2

(prior recommendations)

Persons at risk for HIV-2 infection include

- Sex partners of a person from a country where HIV-2 is endemic
- Sex partners of a person known to be infected with HIV-2
- People who received a blood transfusion or a nonsterile injection in a country where HIV-2 is endemic
- People who shared needles with a person from a country where HIV-2 is endemic or with a person known to be infected with HIV-2
- Children of women who have risk factors for HIV-2 infection or are known to be infected with HIV-2

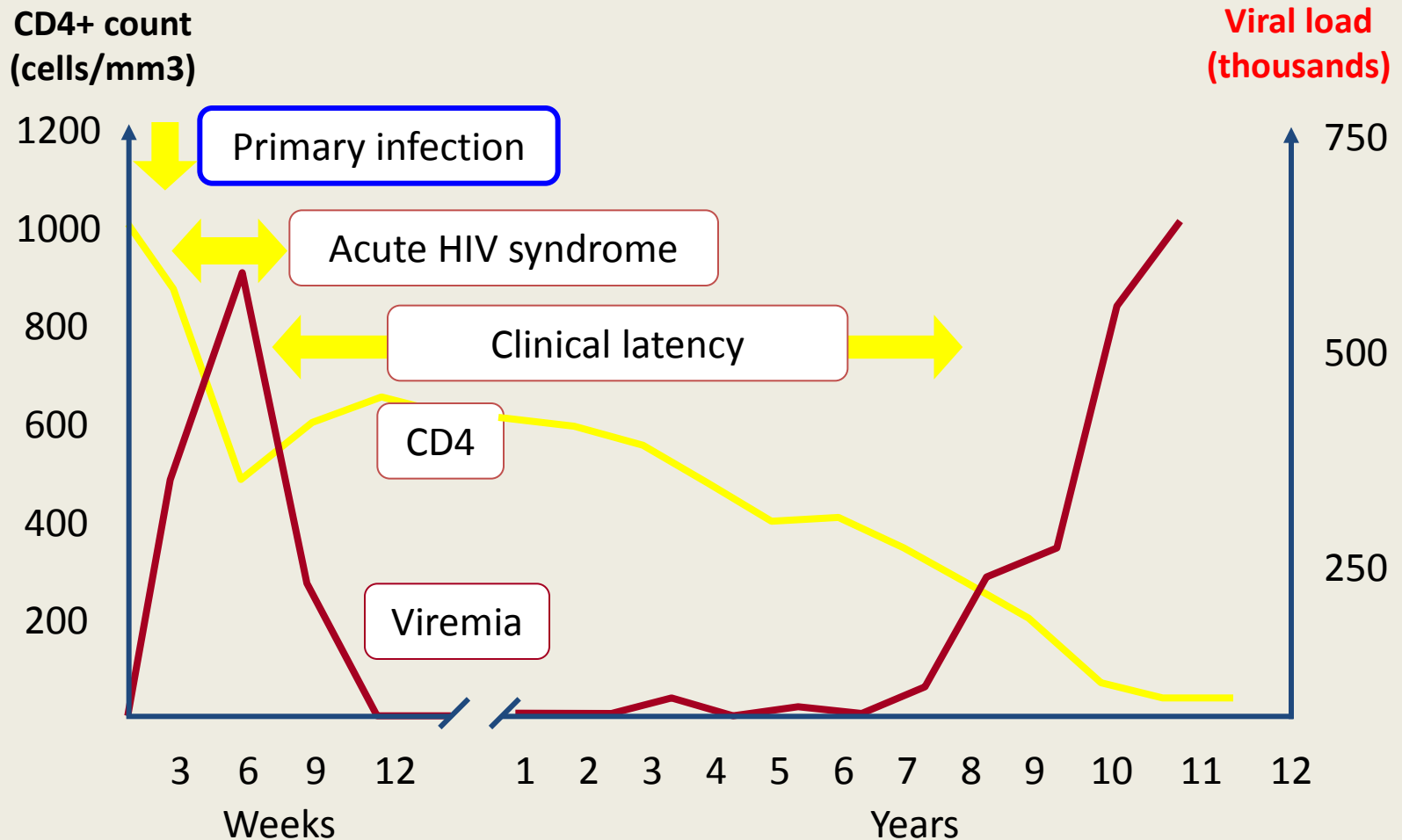
HIV-2 testing is also indicated for

- People with an illness that suggests HIV infection (such as an HIV-associated opportunistic infection) but are not HIV-1 positive
- People for whom HIV-1 Western blot exhibits the unusual indeterminate test band pattern of gag (p55, p24, or p17) plus pol (p66, p51, or p32) in the absence of env (gp160, gp120, or gp41)

- ***HIV Cases ?***

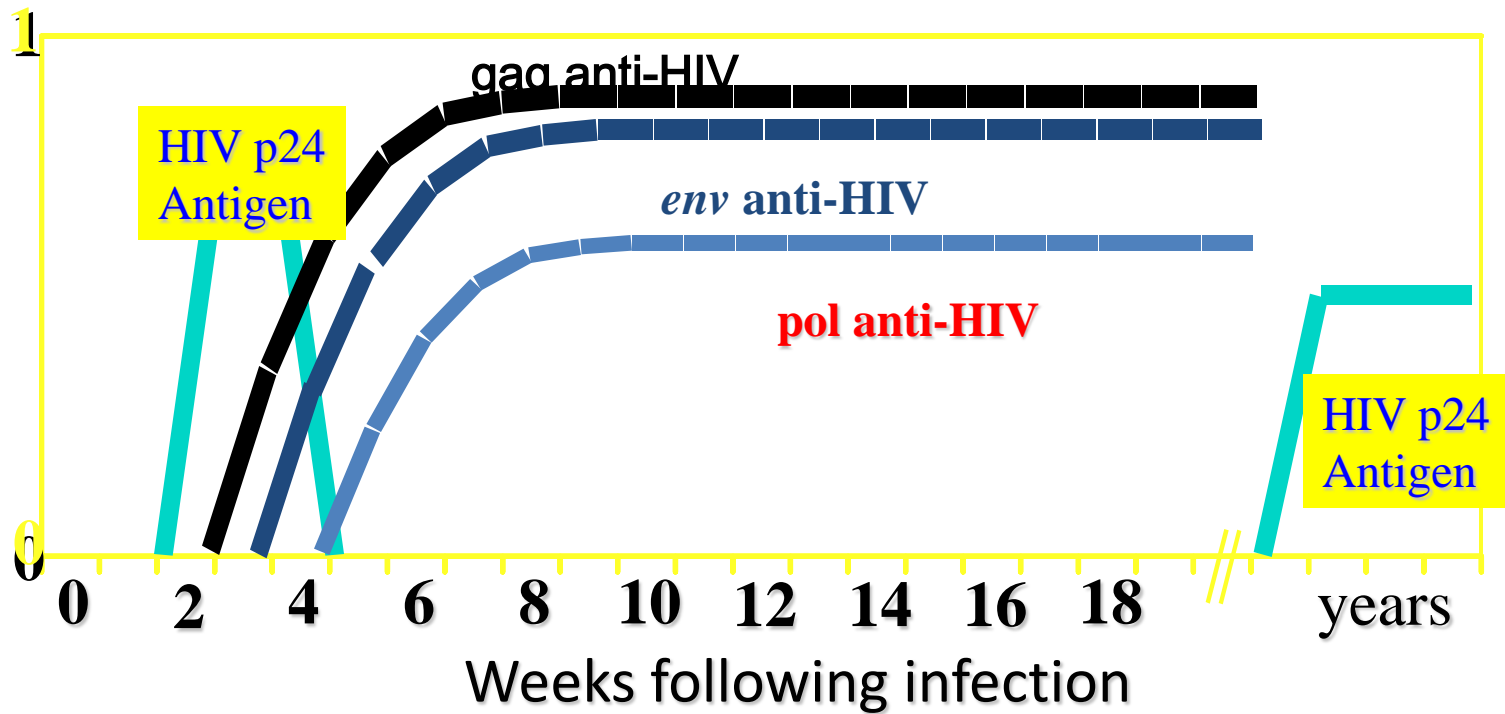
166 confirmed cases between 1988-2010; 0.01% of all HIV cases in the US
81% people born in West Africa; most positive on HIV-1 Western blot

HIV Infection Course



HIV Serological Response

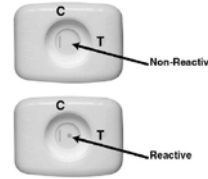
Typical response following infection



“Traditional” HIV Diagnostic Algorithm

1 Screen

immunoassay (EIA/CIA)
rapid tests

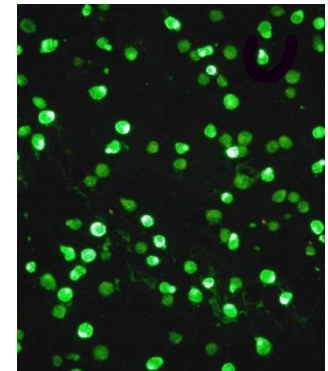
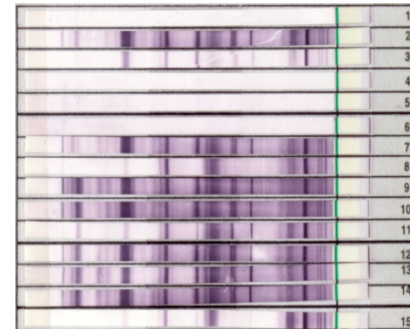


2 Confirmation for HIV-1

Western blot (98%)

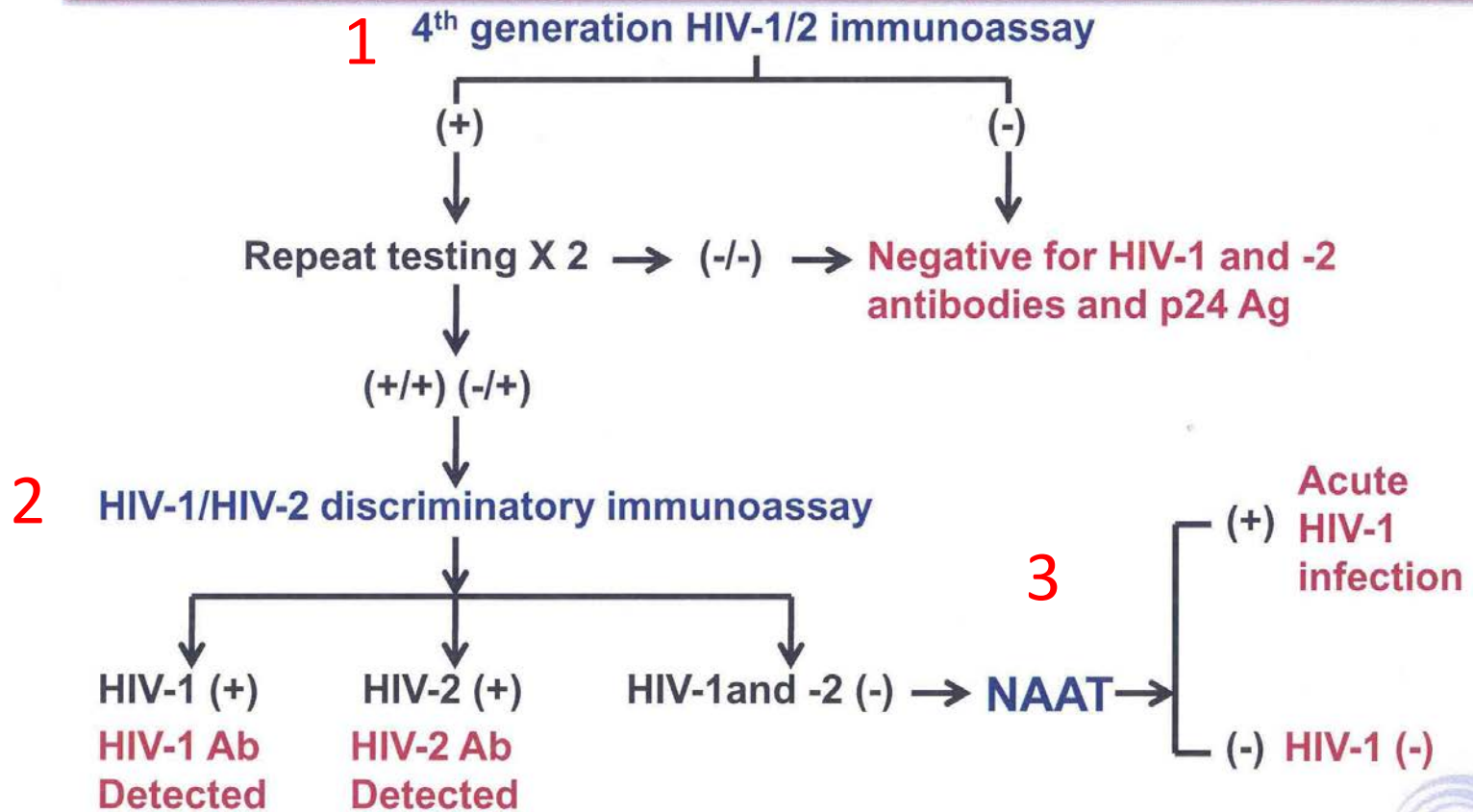
IFA

Nucleic Acid Amplification Test *



*Note: TMA format, qualitative assay only FDA approved nucleic acid amplification test (NAAT) for diagnosis and confirmation. There are no viral load tests approved for diagnosis

CDC Diagnostic Algorithm (2014)



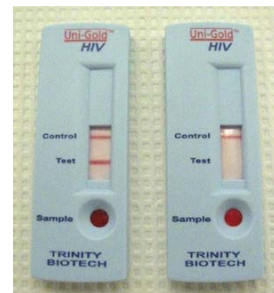
*Could be an IgM sensitive Ab immunoassay if Ag/Ab combination assay is unavailable
AACC. Clinical Laboratory News. 2010

Rapid Test – Point of Care

- 8 FDA approved
- Most are equivalent to 2nd gen assays
- One kit Ag/Ab combo (not incorporated in the algorithm)
- One kit approved for in-home testing
- Only one kit discriminates between HIV-1 and HIV-2
- Sample types

plasma, serum, whole blood, oral fluid

unprocessed sample types (oral fluid & whole blood) are CLIA waived, all others are moderately complex

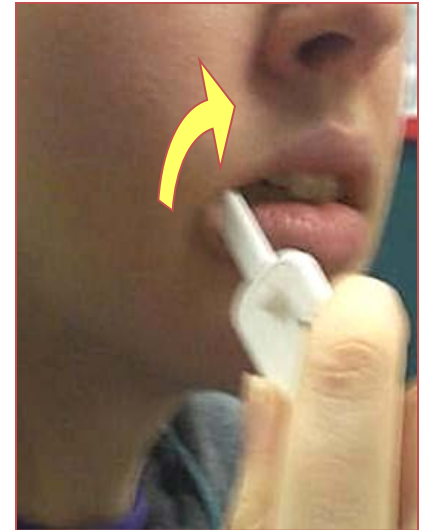


OraQuick[®] Advance

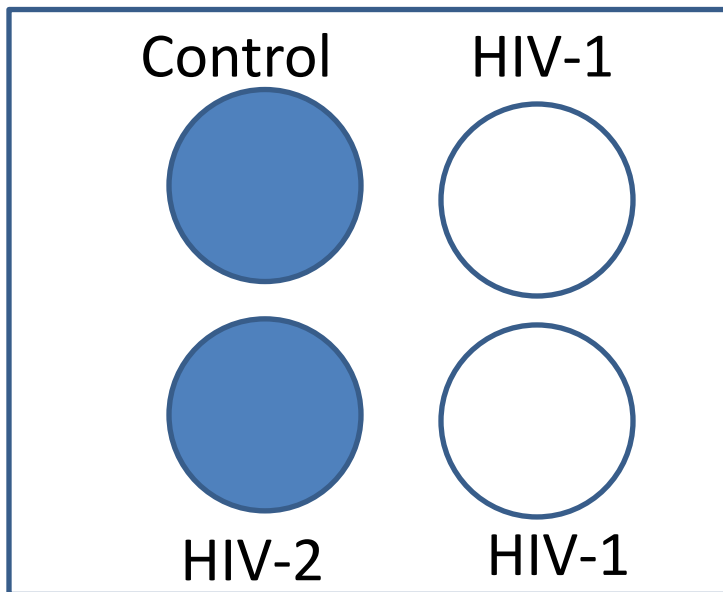


- Synthetic gp-41 (HIV-1)
- Synthetic gp-36 (HIV-2)
- Goat anti-human IgG

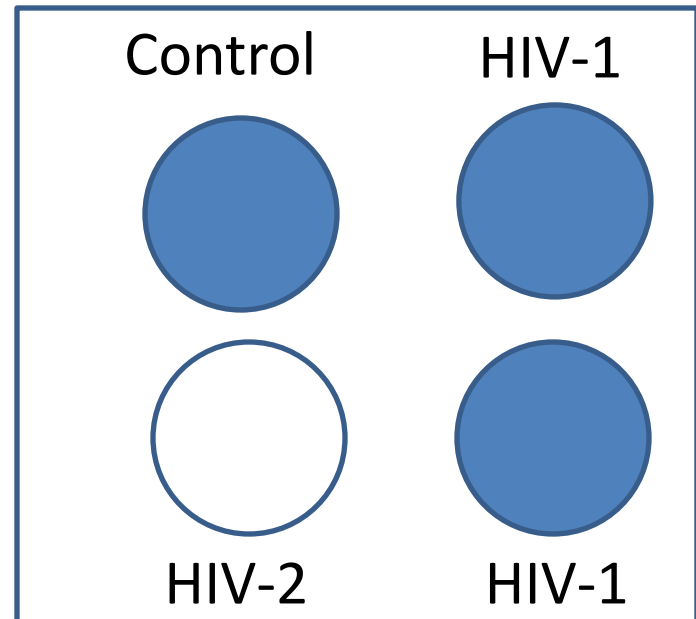
Photograph from CDC: www.cdc.gov/hiv/rapid_testing



HIV-1/HIV-2 Differentiating Assay



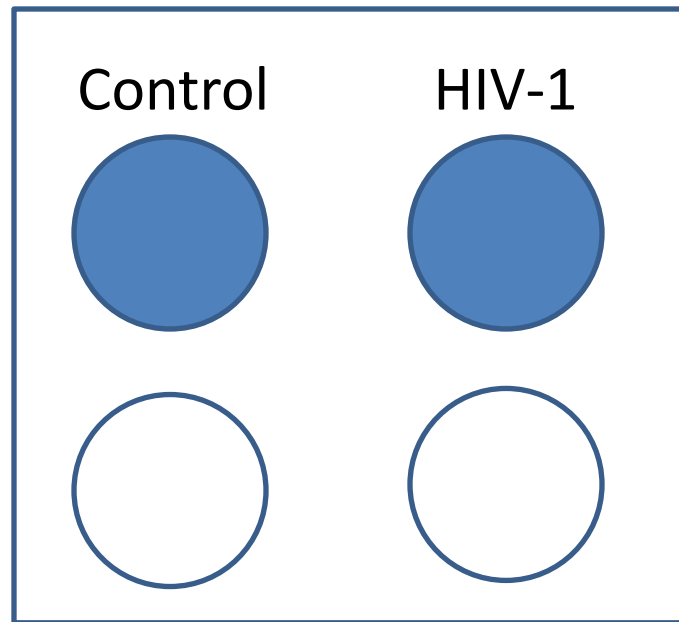
HIV-2 Ab Detected



HIV-1 Ab Detected



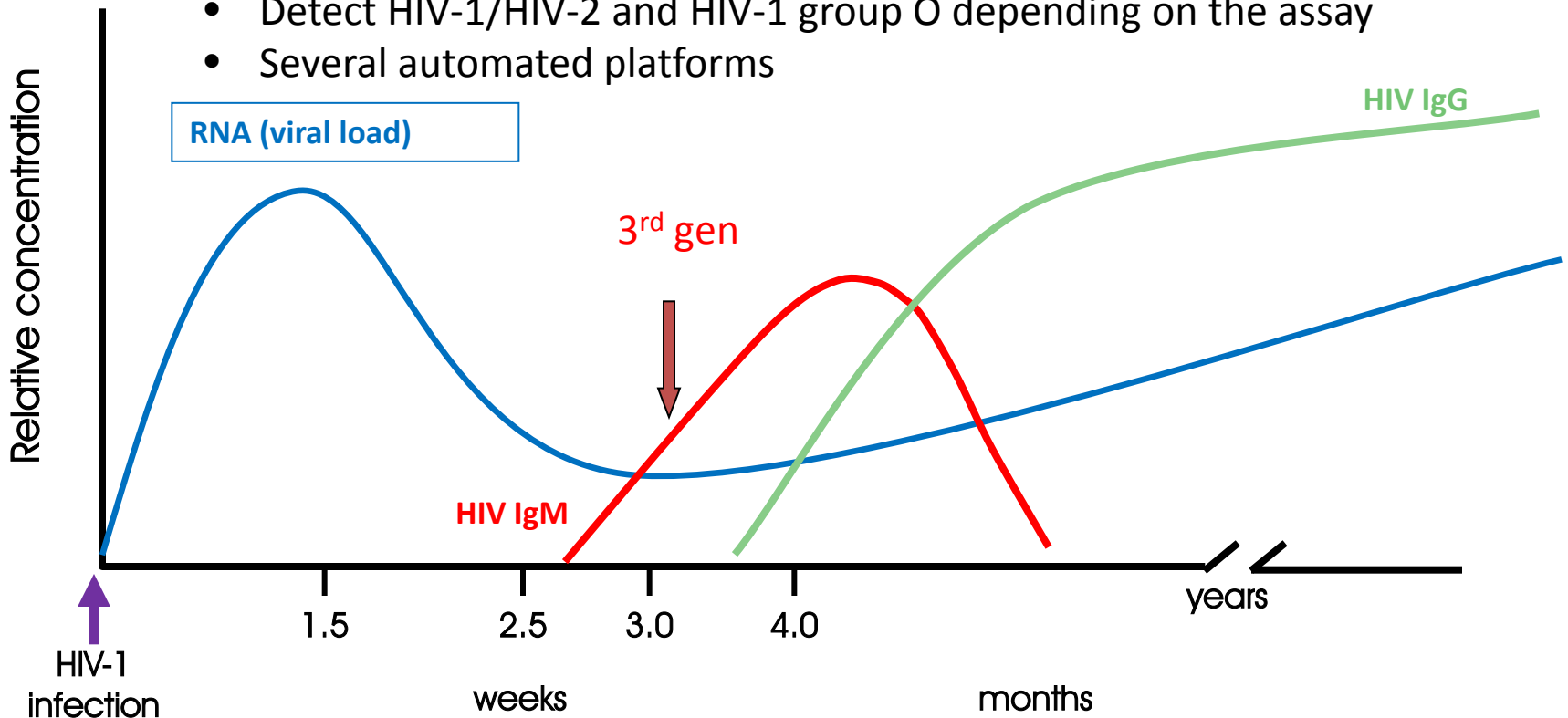
Screen / Supplemental Interpretation



Screen - preliminary positive
Supplemental - indeterminate

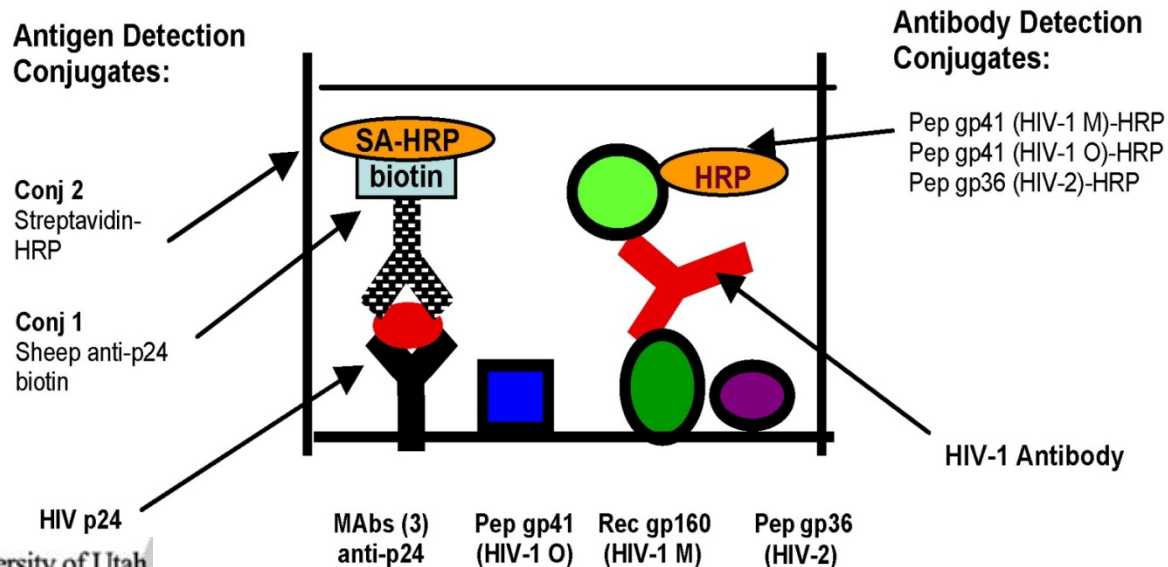
HIV Ab Screening Assays (3rd gen – IgM and IgG)

- Third generation assays (IgG/IgM); antigen sandwich assay
- Detect HIV infection on day 22
- Detect HIV-1/HIV-2 and HIV-1 group O depending on the assay
- Several automated platforms

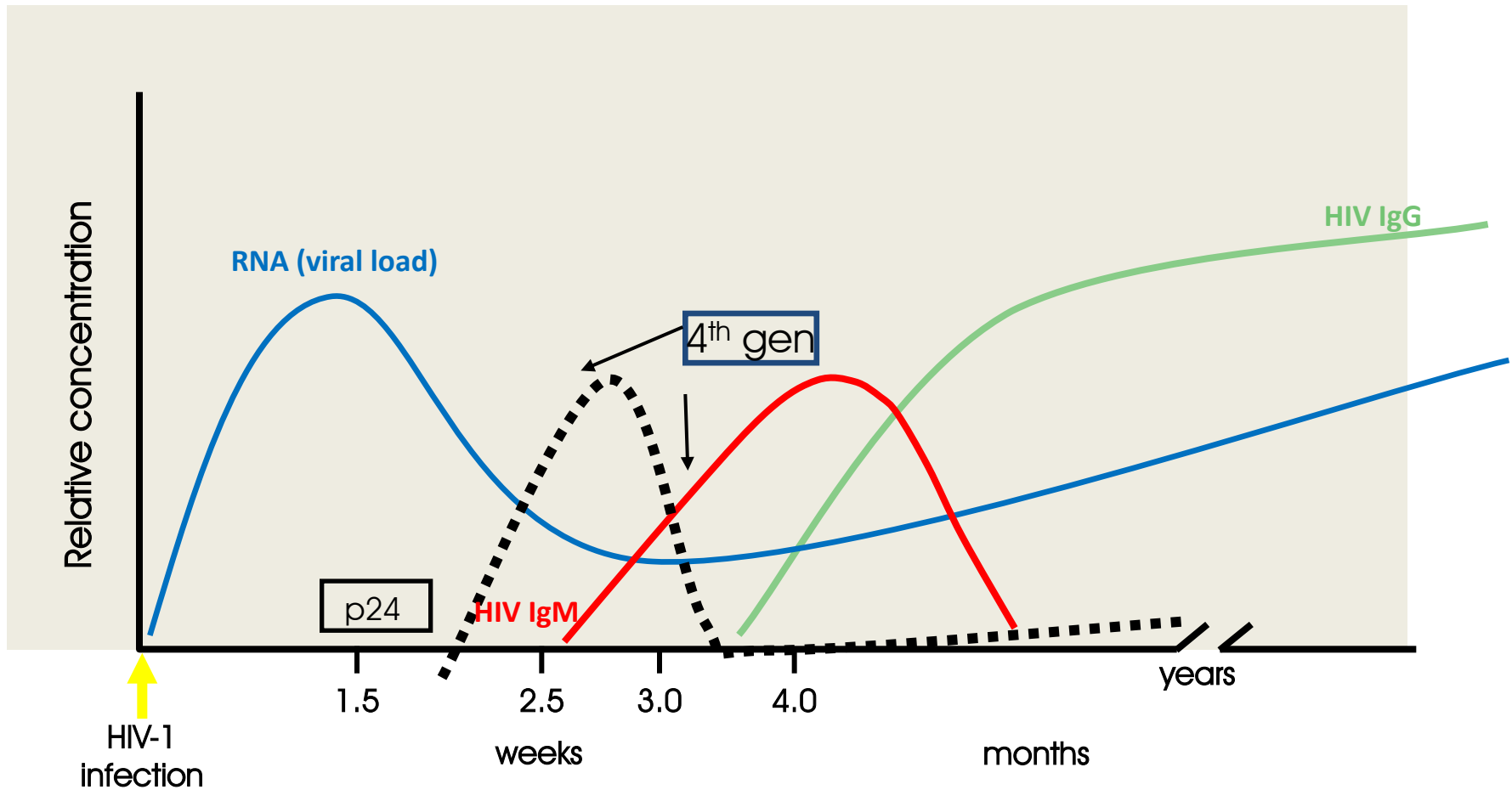


HIV Antigen/Antibody Combination Assays (4th gen – p24 Ag/IgM/IgG)

- Detect both HIV -1 (group O) and HIV-2 antibodies and p24 antigen
- Do not distinguish between Ab+ or Ag+
- Do not differentiate between HIV-1 and HIV-2
- Only two FDA – cleared assays



Earlier Detection of HIV Infection: (4th generation)



Detects infection at 2.5 -3.0 weeks, 5 days earlier than 3rd gen

Combo Ag/Ab & Acute HIV Infection (4th generation)

| Acute HIV patient | Days from 1 st bleed | HIV-1 RNA copies (mL) | GS HIV Combo Ag/Ab | Historical results | | |
|-------------------|---------------------------------|-----------------------|--------------------|--------------------|-----------|-----|
| | | | | HIV-1/HIV-2 EIA | HIV-1 EIA | WB |
| 1 | 0 | >500,000 | R | NR | NR | Neg |
| | 56 | | R | R | R | Pos |
| 2 | 0 | 183,850 | R | NR | NR | Neg |
| | 16 | 10,479 | R | R | R | Pos |
| | 42 | | R | R | R | Pos |
| 3 | 0 | >500,000 | R | R | | Neg |
| | 141 | | R | R | R | Pos |
| 4 | 0 | >500,000 | R | NR | NR | Neg |
| | 19 | | R | R | R | Pos |
| 5 | 0 | >500,000 | R | R | R | Neg |
| | 21 | | R | R | R | Ind |
| | 64 | | RR | R | R | Pos |

Adapted from Bentsen et al. Journal of Clinical Virology 2011.

HIV Combo Ag/Ab Specificity (4th generation)

| Low Risk Population | Number tested | HIV Ag/Ab Combo | Repeatedly reactive Samples | | Specificity (#negative/total) |
|-----------------------------|---------------|----------------------------------|-----------------------------|----------------------------|-------------------------------|
| | | | WB positive (%positive) | HIV-2 positive (%positive) | |
| | | Repeatedly Reactive (% Reactive) | | | |
| Health insurance applicants | 2000 | 6 (0.30%) | 2 | 0 (0.00%) | 99.8% |
| Normal blood donors | 2000 | 0 (0.0%) | NT | NT | 100% |
| Pregnant women | 1000 | 2 (0.20%) | 1 | 0 (0.00%) | 99.9% |
| Military recruits | 1000 | 3 (0.30%) | 1 | 0 (0.0%) | 99.8% |
| Healthy pediatric subjects | 100 | 0(0.0%) | NT | NT | 100% |
| Total | 6100 | 11 (0.18%) | 4 | 0 (0.0%) | 99.89% |

Adapted from Bentsen et al. Journal of Clinical Virology 2011.

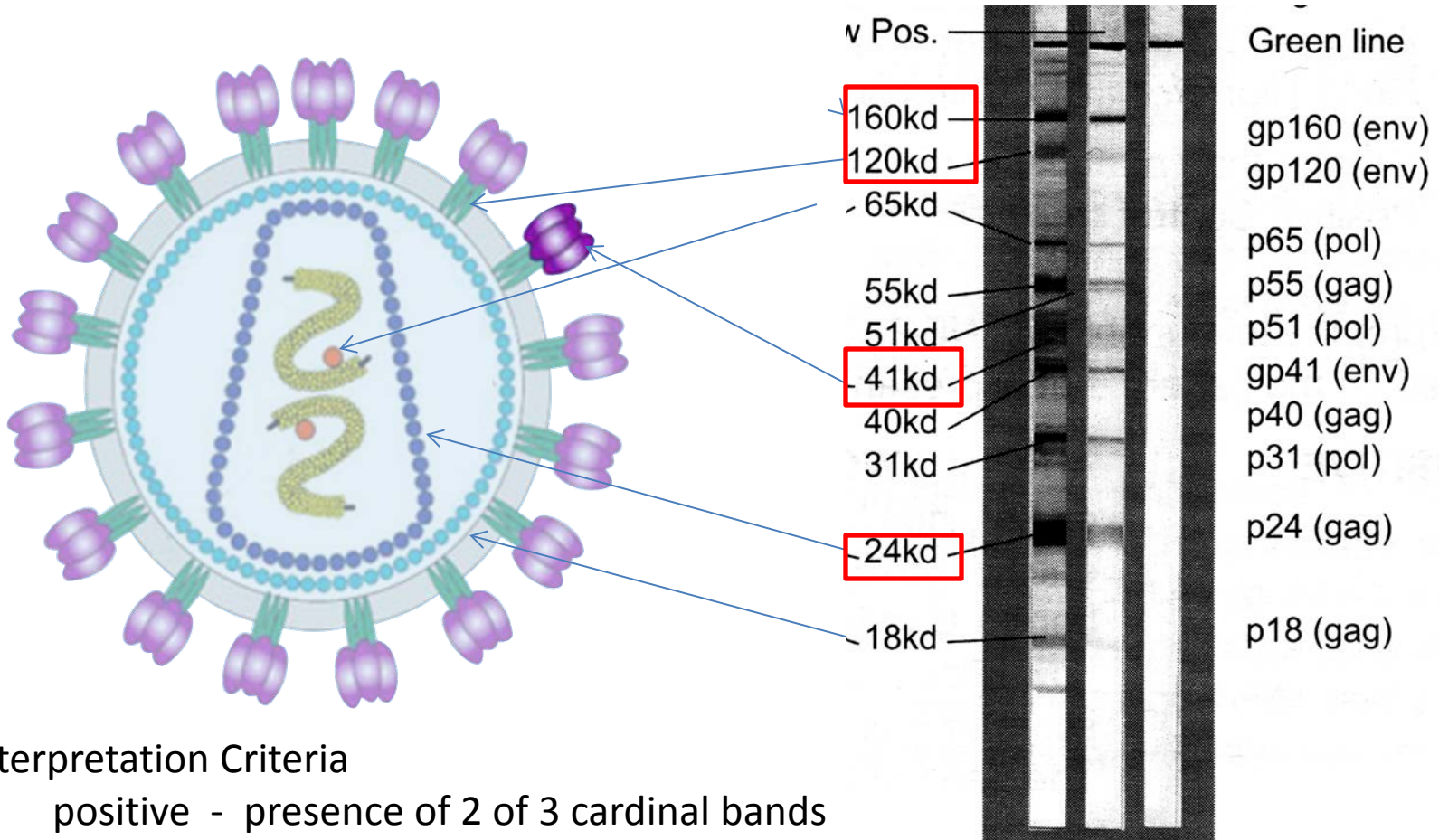
False Positive Immunoassay Results

- Vaccinations
 - flu
 - rabies
- HIV vaccine trials
- Autoimmune disease
- Heterophile Antibodies
- Other viral infections

Supplemental/Confirmatory Testing

- Assume the infection rate is 1 per 500
- Testing 10,000 random subjects will yield
 - 20 false repeatedly reactive
 - 19 true repeatedly reactive
 - 9,960 true nonreactives
 - 1 false nonreactives
- Therefore, $PV^+ = 49\%$, $PV^- = 99.99\%$
- Testing needed to separate repeat reactives

Confirmation by Western Blot



CDC Interpretation Criteria

- positive - presence of 2 of 3 cardinal bands
- negative - absence of all bands
- indeterminate - does not meet + or - criteria

Why Not the Western Blot ?

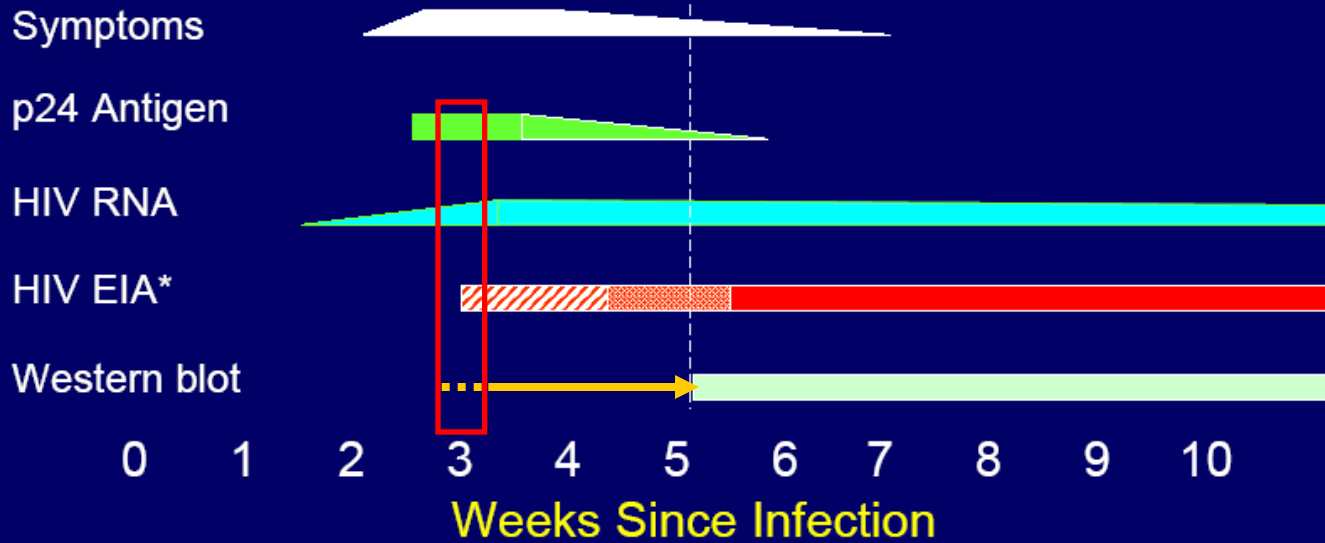
- Diagnostic Limitations
 - indeterminate/inconclusive results, require follow-up
 - insensitive compared to current screening assays
 - HIV-2 misclassification
- Practical Limitations
 - access
 - expense
 - turn around time
- High Specificity for HIV Infection

Western Blot “Indeterminate”

- Indeterminate results may be due to
 - infected but in the “window”
 - advanced disease, AIDS
 - HIV vaccinated
 - infected with HIV-2
 - uninfected, cross reactivity
 - viral or non-viral bands, recent flu and rabies vaccinations, multiple pregnancies, recipients of multiple transfusions, autoimmune disease
 - study followed 99 blood donors – 91 stable indeterminate Western blot patterns over 30 months
- Indeterminate results require follow-up
 - repeat Western blot – 3 indeterminate results spanning 6 months = negative nucleic acid amplification test (NAAT)




Sensitivity of HIV Assays

Detection of HIV by Diagnostic Tests



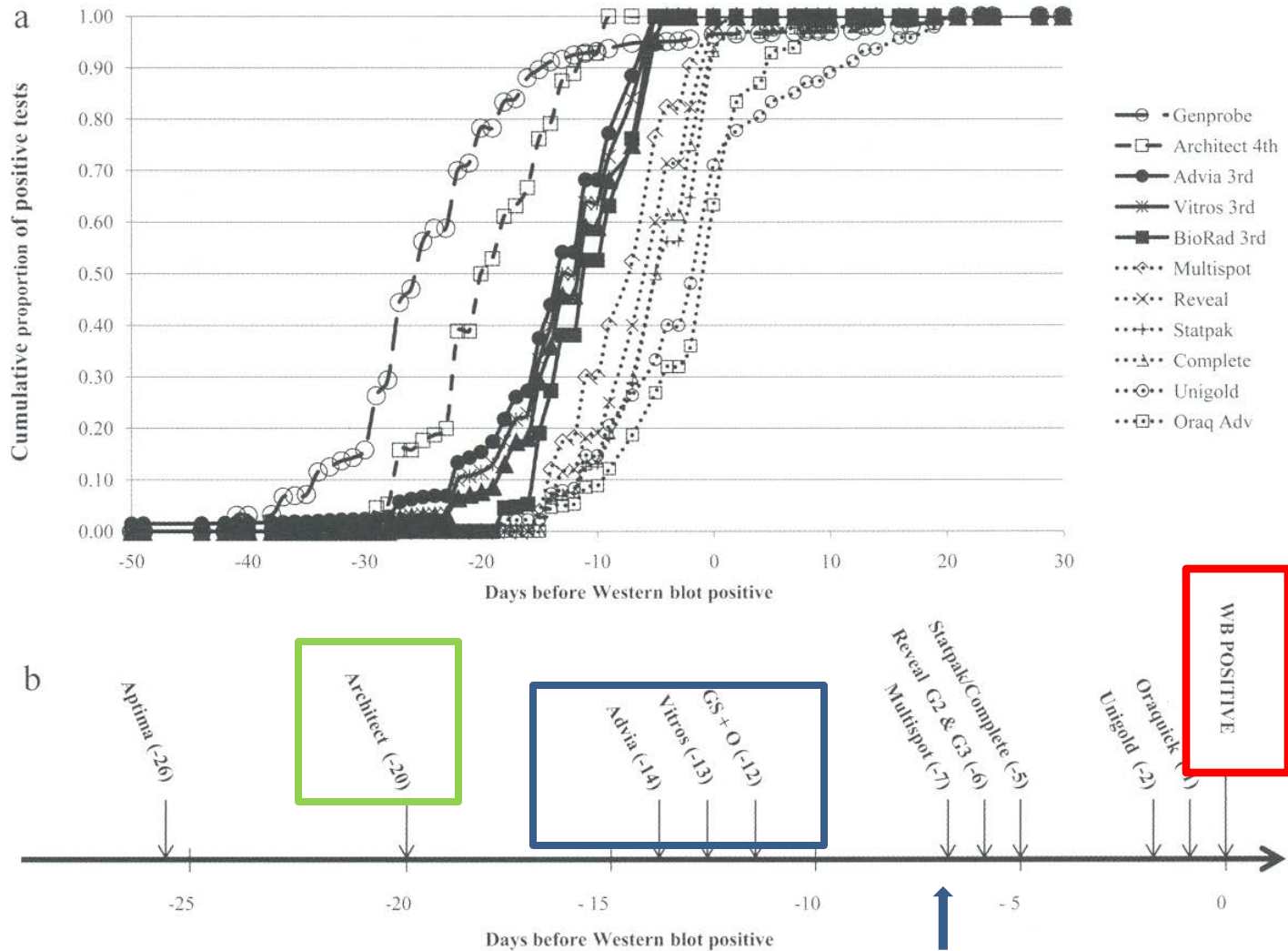
After Fiebig et al, AIDS 2003;
17(13):1871-9



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



Detecting HIV Infection and Current Assays



HIV-1 vs HIV-2 and Western Blot

Percentage of specimens with each HIV-1 Western blot band in 114 specimens collected from persons infected with HIV-2 and 1761 specimens positive for HIV-1 by Western blot and Multispot HIV-1/HIV2 assay.

| | p17 | p24 | p31 | p40 | gp41 | p51 | p55 | p66 | gp120 | gp160 |
|------------------|------|------|------|------|------|------|------|------|-------|-------|
| HIV-2 (n=114) | | | | | | | | | | |
| Present | 18.4 | 93.9 | 83.3 | 88.6 | 1.8 | 74.6 | 73.7 | 29.8 | 10.5 | 48.3 |
| Present but weak | 14.9 | 4.4 | 7.0 | 9.7 | 0.9 | 17.5 | 17.5 | 10.5 | 10.5 | 22.8 |
| Absent | 66.7 | 1.8 | 9.7 | 1.8 | 97.4 | 7.9 | 8.8 | 59.7 | 79.0 | 29.0 |
| HIV-1 (n=1761) | | | | | | | | | | |
| Present | 78.8 | 91.4 | 95.2 | - | 97.4 | 97.2 | 93.3 | 95.0 | 98.6 | 99.9 |
| Present but weak | 6.3 | 7.3 | 2.0 | - | 1.7 | 1.4 | 1.3 | 2.8 | 0.6 | 0.1 |
| Absent | 14.9 | 1.4 | 2.8 | - | 0.9 | 1.4 | 5.4 | 2.2 | 0.8 | 0.0 |

Adapted from Nasrullah et al. Journal of Clinical Virology 2011.

HIV-2 Infection Classification by Western Blot

Comparison of two HIV-1 Western blot interpretive criteria applied to specimens collected from 114 persons known to be infected with HIV-2,^a

| | Alternative HIV-1 WB criteria + , η (%) | | | |
|---------------|---|---------------|-----------|-------------|
| | Negative | Indeterminate | Positive | Total |
| Negative | 1 (0.9) | 0 (0.00) | 0 (0.0) | 1 (0.9) |
| Indeterminate | 0 (0.0) | 60 (52.6) | 0 (0.0) | 60 (52.6) |
| Positive | 0 (0.0) | 40 (35.1) | 13 (11.4) | 53 (46.5) |
| Total | 1 (0.9) | 100 (87.7) | 13 (11.4) | 114 (100.0) |

Adapted from Nasrullah et al. Journal of Clinical Virology 2011 .

HIV-1 /HIV-2 Differentiation Assay VS Western Blot

| | HIV 1/2 Diff Assay Positive | | HIV1/2 Diff Assay Negative | | Total |
|------------------|-----------------------------|-------|----------------------------|-------|-------|
| | N | Row % | N | Row% | N |
| WB positive | 8670 | 99.9% | 8 | 0.1% | 8678 |
| WB negative | 3 | 15.8% | 16 | 84.2% | 19 |
| WB indeterminate | 23 | 36.5% | 40 | 63.5% | 63 |
| Total | 8696 | 99.3% | 64 | 0.7% | 8760 |

Adapted from Torian et al. Journal of Clinical Virology 2011.

NAAT for HIV Diagnosis

- Transcription Mediated Amplification (TMA)
- Screening of high-risk populations
- Known exposure such as needle-stick
- Testing patients with acute HIV-1 symptoms and known exposure
- Screening of newborn babies born to infected mothers
- HIV vaccine studies
- **Resolution arm for new screening algorithms**

TMA vs Real-time PCR Tests

| | TMA | Real Time (1) | Real Time (2) |
|---------------------|-------------------|------------------|--------------------|
| Sensitivity | 30 copies/ml | 40 copies/ml | 20 copies/ml |
| Genotypes | A-O | A-O | A-G |
| Amplicon control | Strand Capture | Closed | UTP/UNG, closed |
| Automation | No (U.S.) | Yes | Yes |
| FDA approval | Diagnosis | Monitor | Monitor |

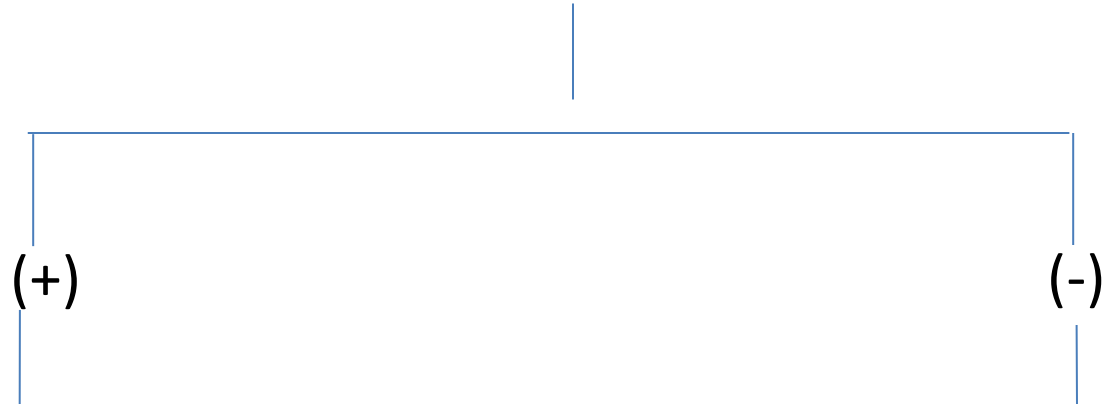
Molecular Take-Home Points

- Only TMA format is approved for HIV diagnosis
Automation may eventually occur
- Viral Load tests may have equivalent “analytic performance” compared to TMA
Guidelines stirred interest in claims for diagnosis
Process will be slow
- Very few LDT HIV-2 RNA assays available

Rapid Tests



1 HIV-1/HIV-2 Ag/Ab combination immunoassay
(recommended, sensitive 3rd gen allowed)



2 HIV-1/HIV-2 Differentiation Assay

Negative for HIV-1
HIV-2 Abs and p24 Ag

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

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

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

HIV-1 (-) or IND, HIV-2 (-)

HIV-1 Ab

HIV-2 Ab

HIV- Abs
undifferentiated

3 HIV-1 NAAT

(+)

(-)

Acute HIV-1

Negative for HIV-1

HIV Summary

- New algorithm encourages use of HIV Ag/Ab combo assay to improve detection of acute HIV infection
 - Only two lab platforms currently available for Ag/Ab Combo assays
 - Sensitive 3rd gen allowed
- New algorithm replaces the Western blot supplemental testing with HIV-1/HIV-2 discriminatory assay to improve detection of HIV-2 infection
 - Only one rapid test platform can discriminate between HIV-1 and HIV-2 infection
 - Interpretation for the differentiation assay depends on use (screen vs supplemental)
 - Indeterminate results are possible

HIV Summary

- NAAT is formally incorporated into the algorithm
 - There is only one qualitative molecular assay approved for HIV diagnosis, TMA format, that is not automated and therefore not readily available
 - NAAT are designed to detect HIV-1
 - NAAT for HIV-2 are not FDA –cleared
- Rapid tests must proceed to 4th gen lab test, the starting point in the algorithm
 - **including preliminary positive samples with Ag/Ab 4th gen rapid test**
 - rapid tests are no longer confirmed with Western blot

Thank you!

