New HIV Testing Algorithms

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New HIV Testing Algorithm

- **Prior to 2013, California laboratories only allowed to report HIV indicative results from list outlined in MMWR.**

- **2011: Clinical and Laboratory Standards Institute (CLSI) recommended new Standard Lab Algorithm**
  - **Detection of Acute HIV infection**
  - **Differentiation of HIV1/HIV2**
  - **Fast turnaround time**

- **Effective June 26, 2013: Regulation change allows California labs to use any HIV testing algorithm recommended by Centers for Disease Control and Prevention (CDC), Association of Public Health Labs (APHL) , CLSI and US HHS.**

HIV Diagnosis: New Algorithms and Evolution of HIV Diagnostics

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CDC Division of HIV/AIDS Prevention
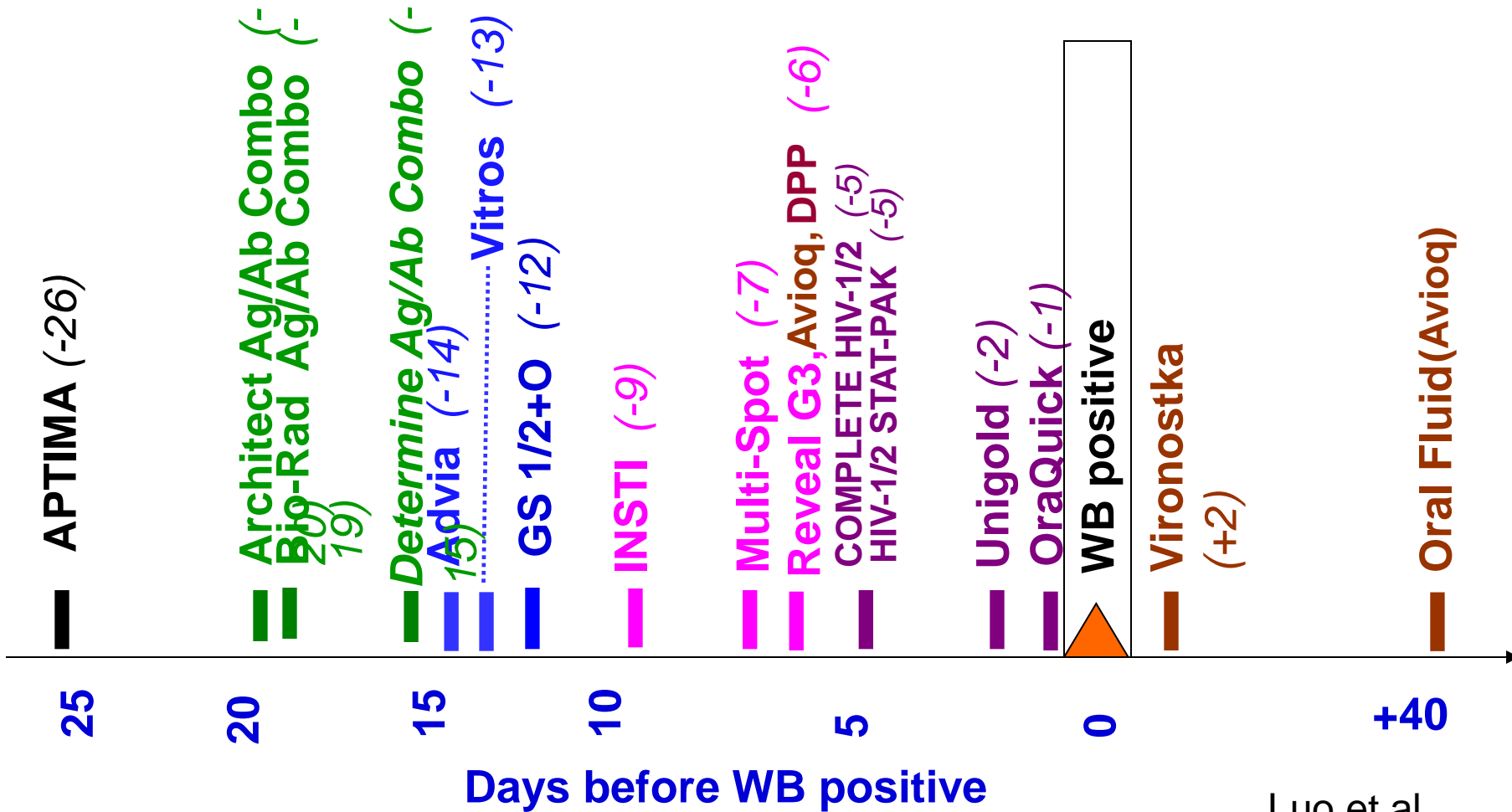
Limitations of Antibody Testing

- **Antibody tests do not detect infection in ~10% of infected persons at highest risk of transmission**

- **Western blot confirmation less sensitive during early infection than many widely used screening tests**

- **Antigen/antibody combo tests now FDA-approved can detect most antibody-negative persons during highly infections acute infection stage**

Evolution of HIV Tests

- 1st generation: whole viral lysate, detects IgG antibody
- 2nd generation: synthetic peptides, detects IgG antibody
- 3rd generation: detect IgM and IgG antibody
- 4th generation: detects IgM, IgG antibodies, p24 antigen
- "Combi" tests: detect both HIV-1 and HIV-2 antibodies
- Nucleic acid tests: detect HIV RNA
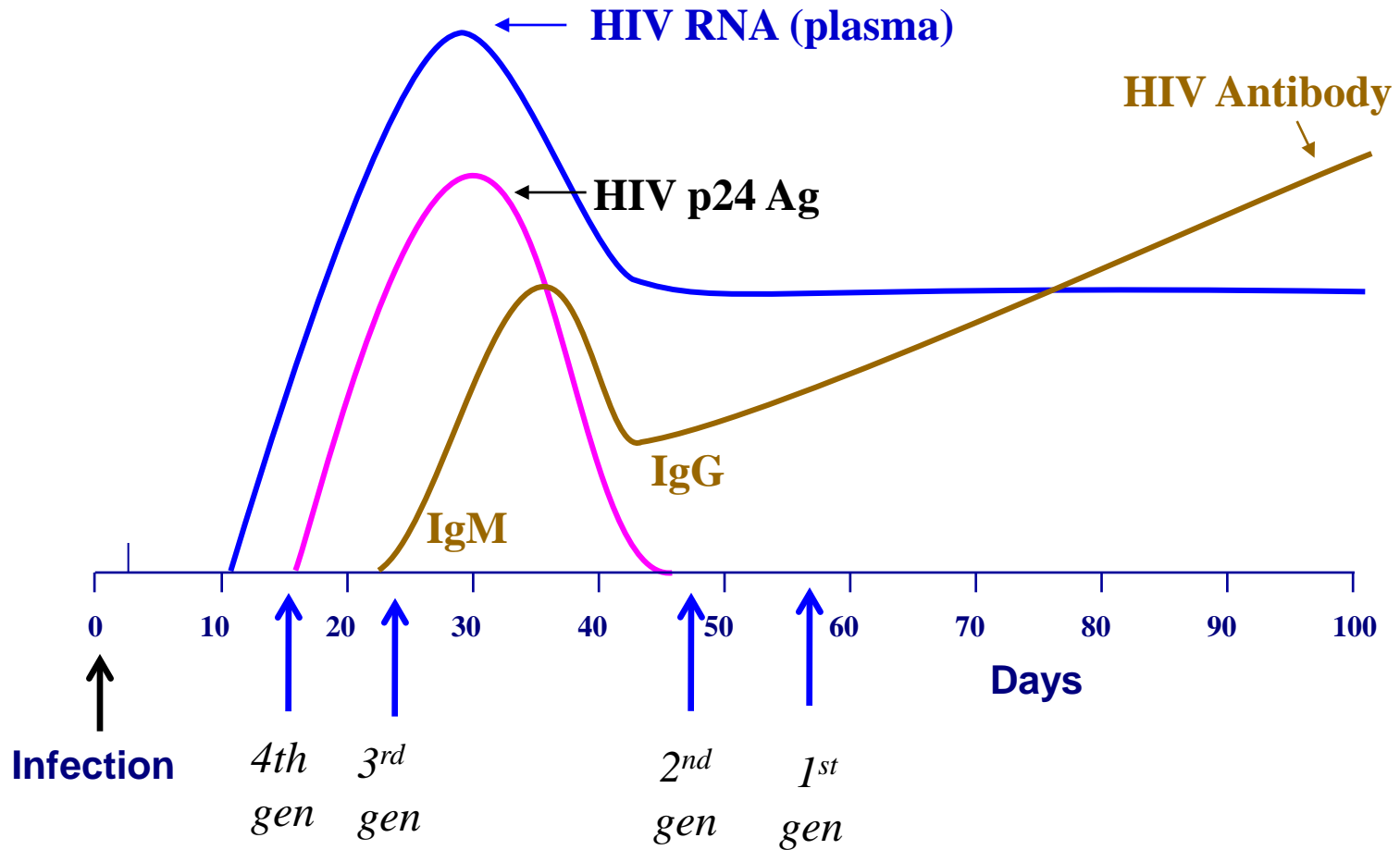
Sequence of Test Positivity Relative to WB (plasma)

166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency

Days before WB positive

APTIMA (-26)

Architect Ag/Ab Combo (-20)
Bio-Rad Ag/Ab Combo (-19)

Determine Ag/Ab Combo (-15)
Advia (-14)
Vitros (-13)

GS 1/2+O (-12)

INSTI (-9)

Multi-Spot (-7)
Reveal G3, Avioq, DPP (-6)
COMPLETE HIV-1/2 (-5)
HIV-1/2 STAT-PAK (-5)

Unigold (-2)
OraQuick (-1)

WB positive

Vironostka (+2)

Oral Fluid (Avioq)

25   20   15   10   5   0   +40

Modified from *Masciotra et al, J Clin Virol 2011, 2013* and *Owen et al, J Clin Micro 2008*

Luo et al,
J Clin Virol
2013

HIV Infection and Laboratory Markers

HIV RNA (plasma)

HIV Antibody

HIV p24 Ag

IgG

IgM

Days

0   10   20   30   40   50   60   70   80   90   100

Infection

4th gen

3rd gen

2nd gen

1st gen

Acute HIV Infection

Recent or Stage "0" HIV Infection → 180 days

Modified after Busch et al. Am J Med. 1997

Acute HIV Infection vs. Stage 0 HIV Infection

- **Acute HIV infection: phase of HIV disease immediately after infection during which the initial burst of viremia in newly infected patients occurs; anti-HIV antibodies are undetectable while HIV RNA or p24 antigen are present**

- **"Recent" HIV infection generally is considered the phase up to 6 months after infection during which anti-HIV antibodies are detectable.**

  - **Stage 0 HIV Infection: up to 180 days after infection**

- **"Early HIV infection": either acute or recent HIV infection.**

CDC: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm

AIDS Info: http://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-treatment-guidelines/20/acute-hiv-infection#

HIV-2 Infection

- **Remains uncommon in U.S., but**
  - **Does not respond to NNRTIs, some PIs (first line therapy)**
  - **Undetectable by HIV-1 viral load tests**

- **Misclassification by HIV-1 Western blot:**
  - **54/58 (93%) HIV-2 patients tested had positive HIV-1 WB (NYC)[*]**
  - **97/163 (60%) HIV-2 cases reported had positive HIV -1 WB (CDC)[**]**

- **HIV-2 often diagnosed after immunologic deterioration in patient with negative viral load**

Torian et al, Clinical Infectious Disease 2010
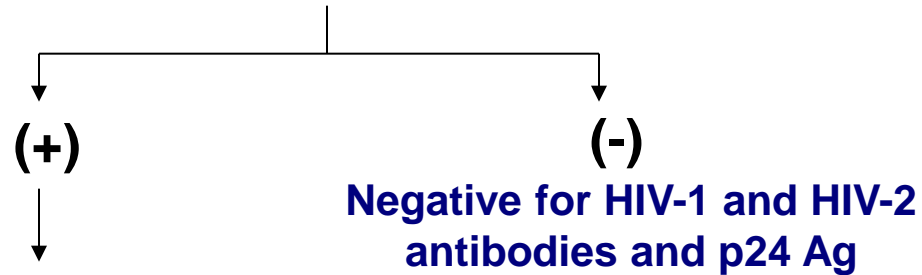
**MMWR July 2011*

Lab Data Changes

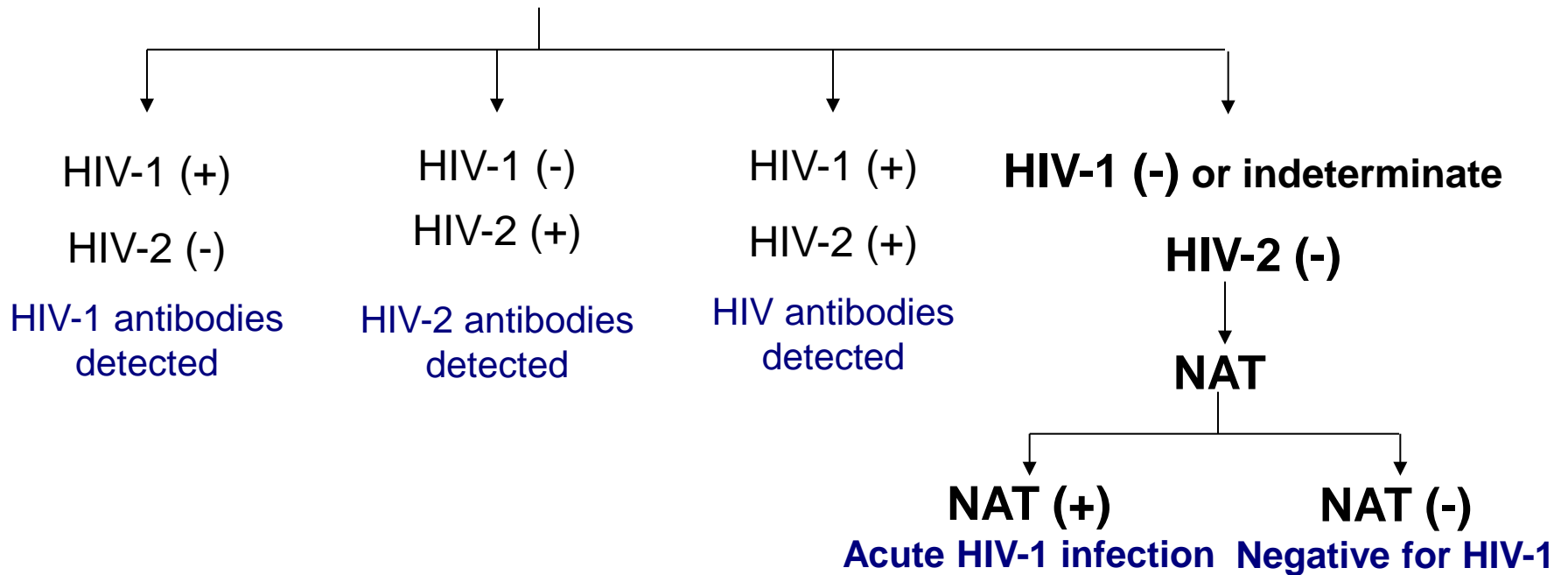| Removed	Added
Detection Tests: **HIV-1 RNA PCR (QUAL)** **HIV-1 PROVIRAL DNA (QUAL)**	**HIV-1 RNA/DNA NAAT* (Qualitative)** **HIV-2 RNA/DNA NAAT* (Qualitative)**
Viral load tests: **HIV-1 RNA NASBA** **HIV-1 RNA bDNA** **HIV-1 RNA RT-PCR** **HIV-1 RNA**	**HIV-1 RNA/DNA NAAT* (Quantitative)** **HIV-2 RNA/DNA NAAT*(Quantitative)**
Antibody tests	**HIV 1/2 Differentiating Test (Multispot)** **HIV-2 Western Blot**

*NAAT (also NAT) = Nucleic Acid Amplification Test

CDC/APHL Proposed New HIV Testing Algorithm

4th generation HIV-1/2 immunoassay

(+)   **(-)**

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

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)

HIV-2 (-)

HIV-1 antibodies detected

HIV-1 (-)

HIV-2 (+)

HIV-2 antibodies detected

HIV-1 (+)

HIV-2 (+)

HIV antibodies detected

HIV-1 (-) or indeterminate

HIV-2 (-)

NAT

NAT (+)   **NAT (-)**

**Acute HIV-1 infection**   **Negative for HIV-1**

NAT: nucleic acid test (e.g., RNA)

FDA-approved 4th Generation Tests

- **Abbott Architect 4th Gen Ag/Ab Combo Assay**
  - **Chemiluminescent immunoassay that detects p24 antigen and HIV antibody**
  - *results in 29 minutes*

- **Bio-Rad GS HIV Combo Ag/Ab EIA**
  - **3rd generation Ab format plus p24 antigen**

- **Determine Combo Rapid HIV 1/2 Ag/Ab Test**
  - **Distinguishes Ag from Ab**

FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay
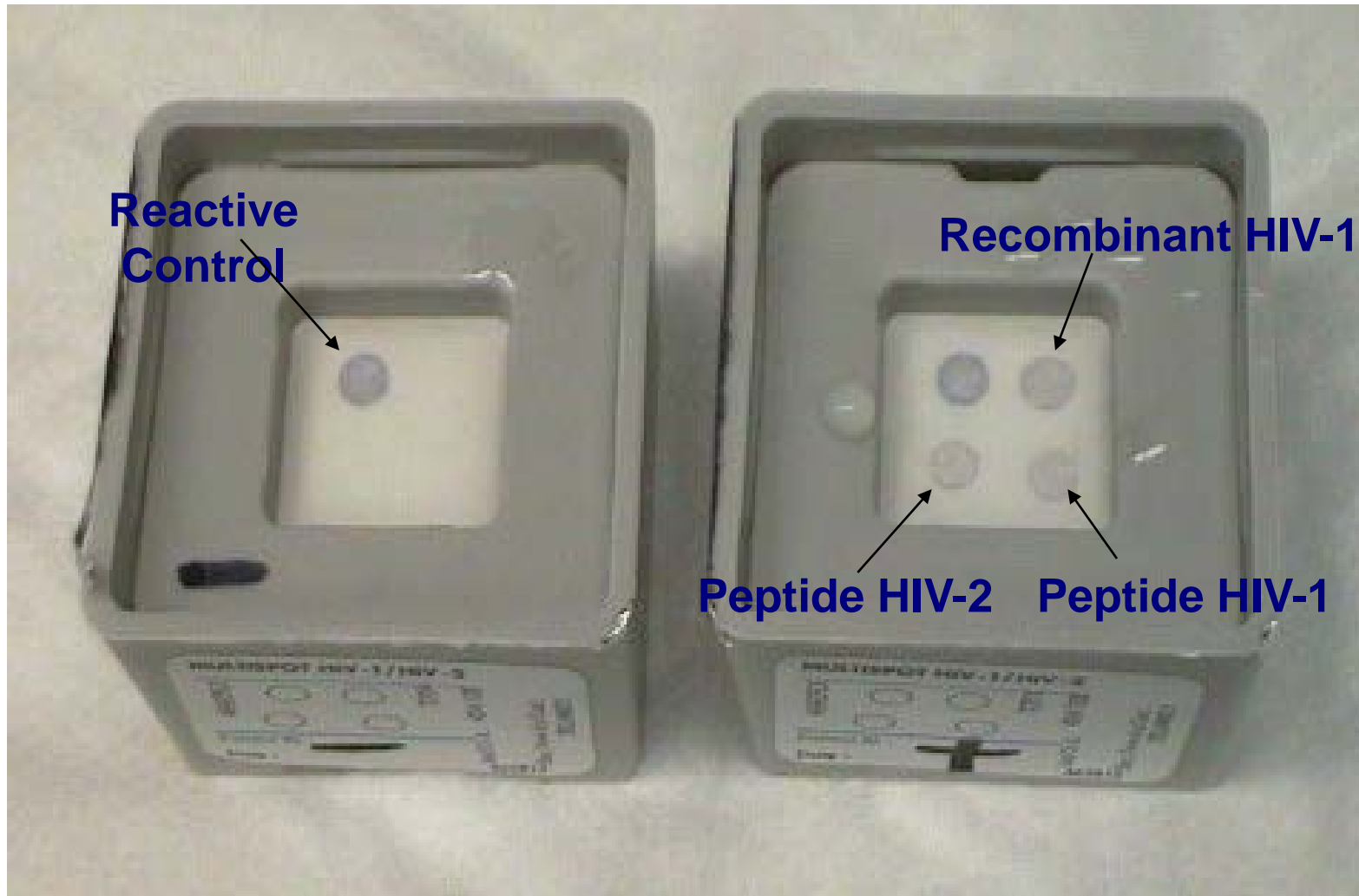
Interpretation for Diagnostic Testing Algorithm that Differentiates HIV-1 and HIV-2 Antibodies:

Nonreactive
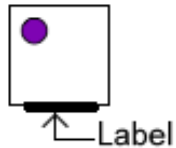
Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies. Additional testing is recommended, including HIV nucleic acid testing (NAT).
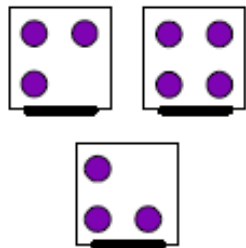
↑ Label

Reactive

HIV-1 POSITIVE:
The Procedural Control Spot shows purple color development and **both** the recombinant HIV-1 Spot and the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Positive for HIV-1 antibodies
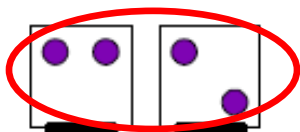
HIV-2 POSITIVE
The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development. Test result is interpreted as Positive for HIV-2 antibodies

HIV POSITIVE (Undifferentiated):
The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development as well as one or both HIV-1 Spots. In this case, the specimen may be tested by additional methods which allow for differentiation between HIV-1 and HIV-2. See diutional procedure which follows.

Indeterminate

HIV-1 INDETERMINATE: The Procedural Control Spot shows purple color development and either the recombinant HIV-1 Spot or the HIV-1 Peptide Spot shows purple color development, but not both HIV-1 Spots. Test result is interpreted as Indeterminate for HIV-1 antibodies and testing for HIV nucleic acid is recommended.

Nucleic Acid Test (NAT) for Diagnosis

- **APTIMA HIV-1 qualitative RNA assay is only NAT FDA-approved for diagnosis**

- **Clinicians can order HIV-1 viral load tests, but labs cannot use them as a reflex part of the algorithm**

- **Any NAT, including Viral Load, or p24 positive result is reportable and sufficient for a reportable case in eHARS**
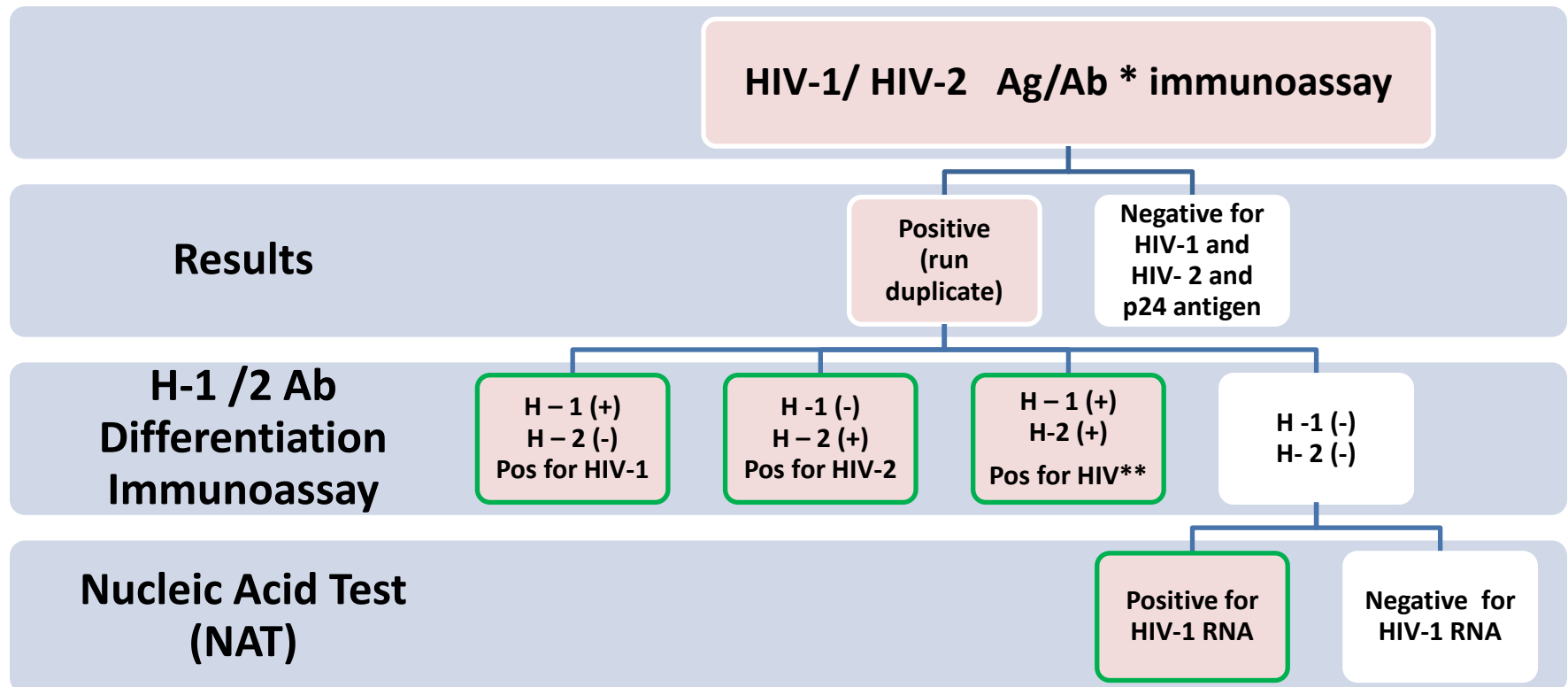
Guidance for Reporting Results from HIV Testing Algorithm

1st test: 4th Gen Ab/Ag	2nd test: HIV-1/2 Ab Different'n IA	3rd test: HIV-1 NAT	Overall Interpretation	Reporting to LAC DPH
Nonreactive	NA	NA	No evidence of HIV infection	NOT required
Reactive	HIV-1 (+), HIV-2 (+) HIV-1 (+), HIV-2 (-) HIV-1 (-), HIV-2 (+) HIV-1/2 (+) undifferentiated	NA	Evidence of HIV-1 and/or HIV-2 infection	Report 1st, 2nd test results
Reactive	HIV-1/2 (-) or indeterminate	Detected	Acute HIV-1 infection	Report 1st, 2nd, 3rd test results
Reactive	HIV-1/2 (-) or indeterminate	Not detected	No evidence of HIV infection	NOT required

Note: Table Adapted from CDC, NY State and City of SF HIV Surveillance information.

CDC Website

- **Below is the link to the CDC HIV testing website, which includes links to several documents pertaining to the new testing algorithm.**

- **http://www.cdc.gov/hiv/testing/lab/guidelines/index.html**

- **http://www.cdc.gov/hiv/pdf/HIVtestingAlgorithmRecommendation-Final.pdf**

Algorithm 1 – Ag/Ab Combination

HIV-1/ HIV-2   Ag/Ab * immunoassay

Results
- Positive (run duplicate)
- Negative for HIV-1 and HIV- 2 and p24 antigen

H-1 /2 Ab Differentiation Immunoassay
- H – 1 (+) H – 2 (-) Pos for HIV-1
- H -1 (-) H – 2 (+) Pos for HIV-2
- H – 1 (+) H-2 (+) Pos for HIV**
- H -1 (-) H- 2 (-)

Nucleic Acid Test (NAT)
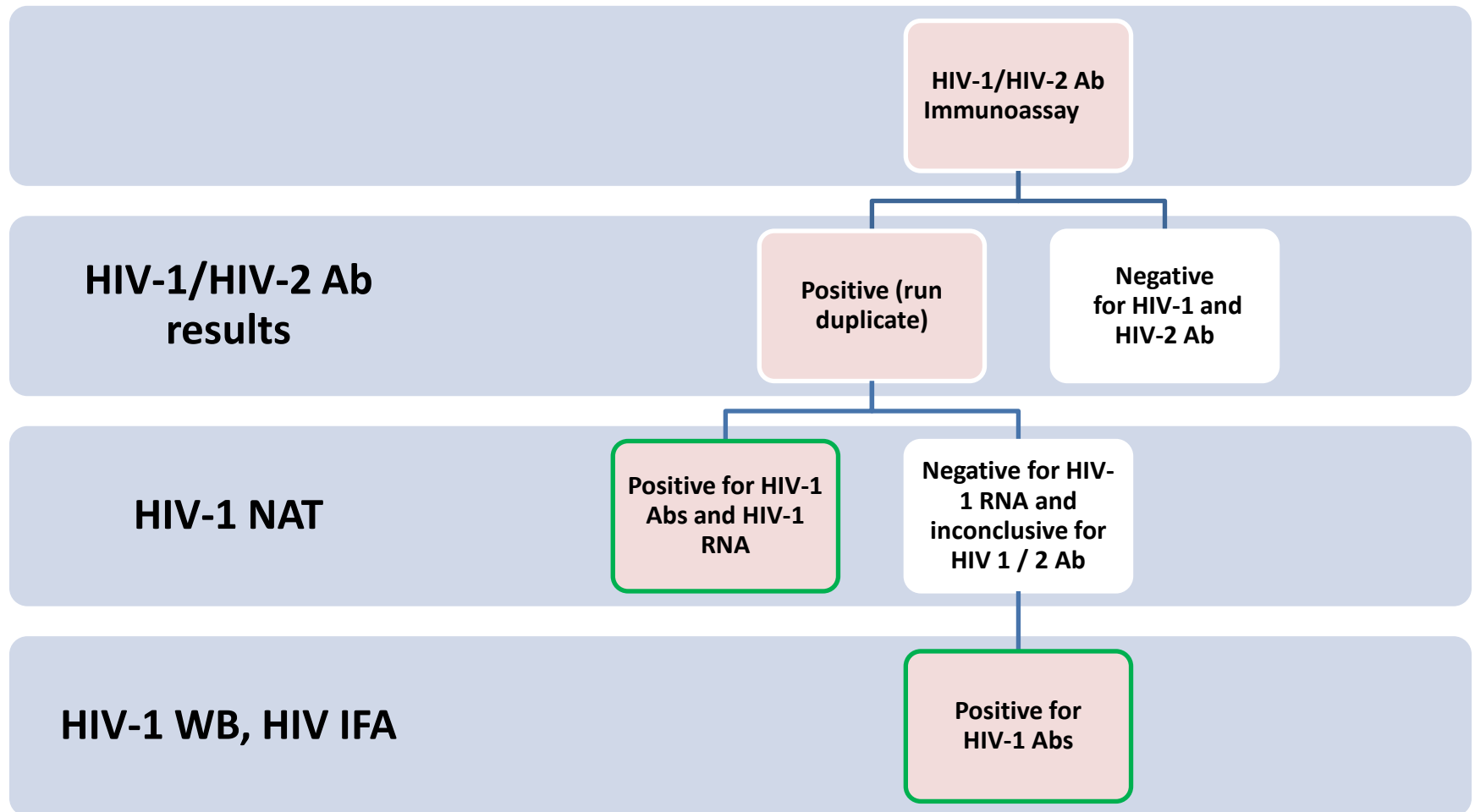- Positive for HIV-1 RNA
- Negative for HIV-1 RNA

*Antigen/antibody immunoassays (4th generation assays):
- ARCHITECT HIV Ag/Ab combo assay (Abbott)
- GS HIV Combo Ag/Ab EIA (Bio-Rad)

** Need further testing to differentiate HIV-1 from dual infection

Algorithm 2: Antibody test with supplemental tests

HIV-1/HIV-2 Ab results

HIV-1 NAT

HIV-1 WB, HIV IFA

HIV-1/HIV-2 Ab Immunoassay

Positive (run duplicate)

Negative for HIV-1 and HIV-2 Ab

Positive for HIV-1 Abs and HIV-1 RNA

Negative for HIV-1 RNA and inconclusive for HIV 1 / 2 Ab

Positive for HIV-1 Abs

Algorithm 2a: Antibody test with supplemental tests

HIV-1/HIV-2 Ab results

HIV-1 WB, HIV IFA, Line IA

HIV-1 NAT

HIV-1/HIV-2 Ab Immunoassay

Positive (run duplicate)

Negative for HIV-1 and HIV-2 Ab

Positive for HIV-1 Abs

Negative for HIV-1 WB and inconclusive for HIV 1 / 2 Ab

Indeterminate

Positive for Abs and HIV-1 RNA

Positive for Abs and HIV-1 RNA

County of Los Angeles Public Health
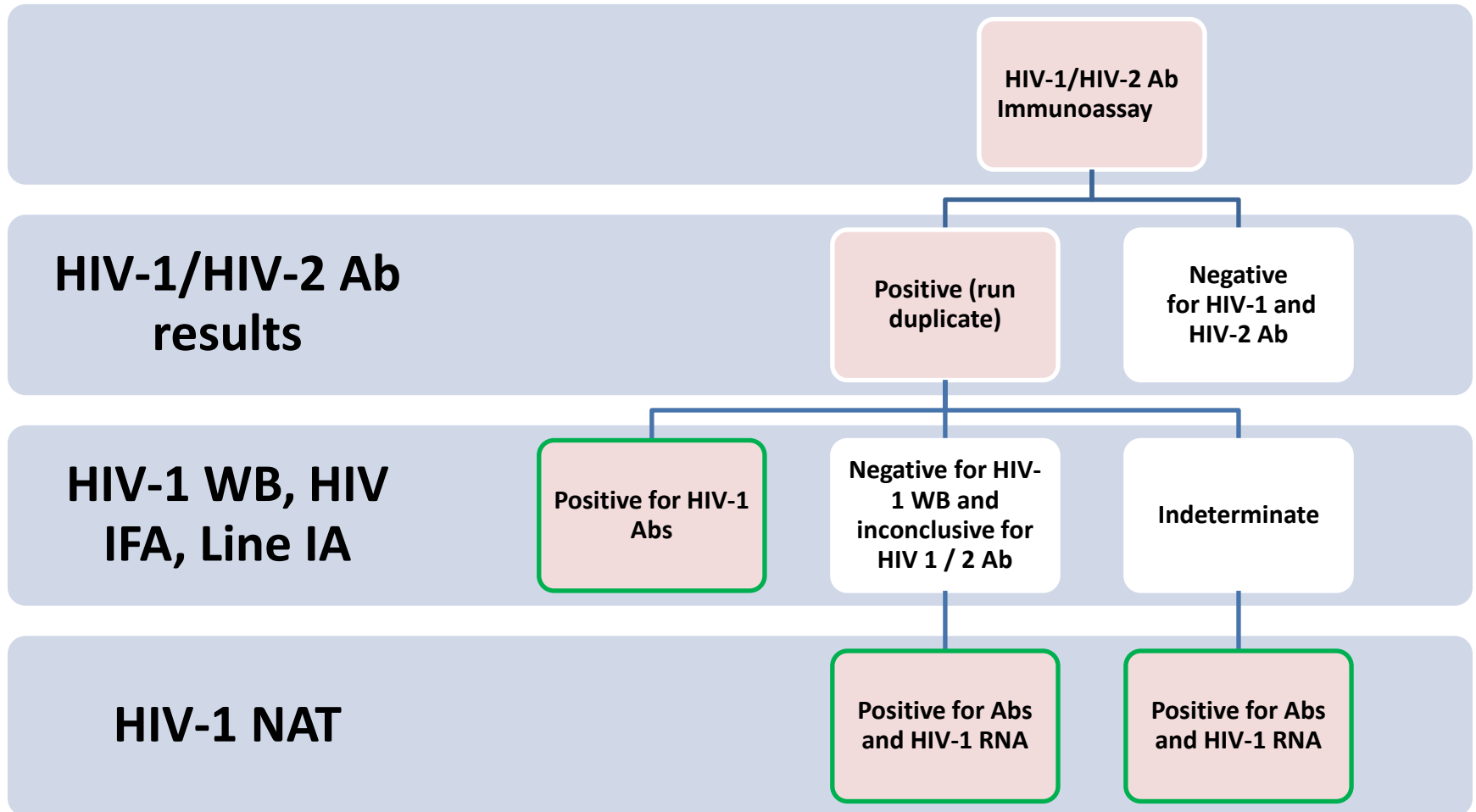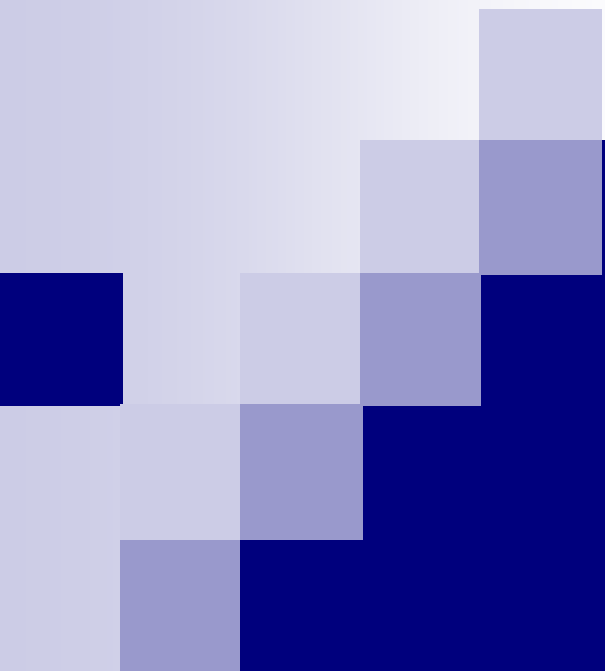
Algorithm 3: Presumptive Diagnosis with sequential Antibody tests

HIV-1/ HIV-2 Ab immunoassay

First immunoassay results

Positive

Negative for HIV-1 and HIV-2 Abs

Second immunoassay results
(different type from first)

Positive

Presumptive positive for HIV-1 or HIV -2 Ab, required additional testing

Negative

Inconclusive, additional testing needed
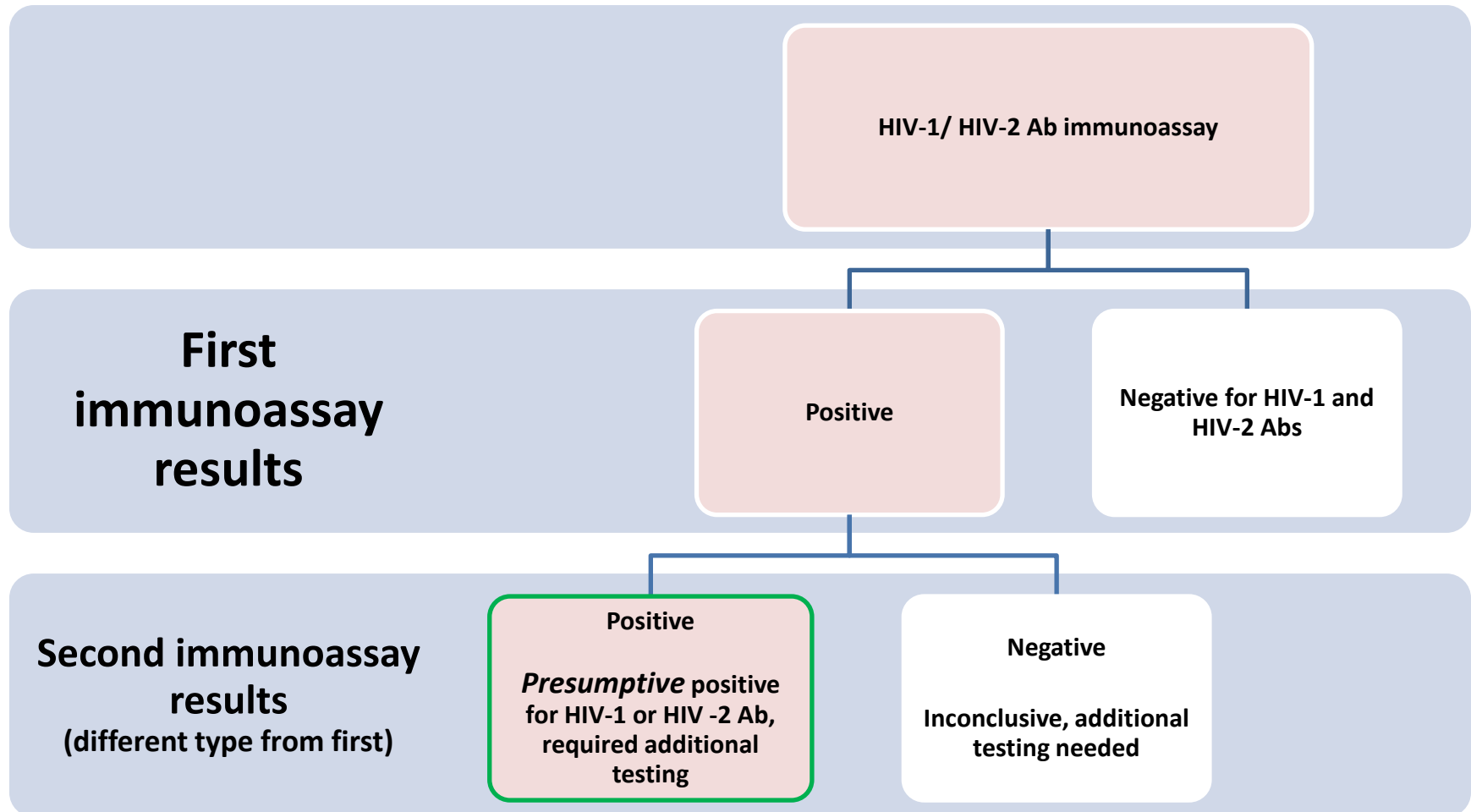
Algorithm 4: Antibody test with initial oral specimen

Oral HIV-1/HIV-2 Ab results

Different HIV-1/HIV-2 Ab immunoassay (blood)

HIV-1/HIV-2 Ab immunoassay (blood for 1st immunoassay)

HIV-1 or HIV-1/HIV-2 Ab immunoassay (oral fluid)

Positive

Negative for HIV-1 and HIV-2 Abs

Positive
Presumptive pos. for HIV-1 or HIV-2, additional testing needed

Negative

Positive
Inconclusive, additional testing needed

Negative
Negative for HIV-1 and HIV-2 Ab

Using Rapid HIV Testing Algorithms to Improve the Accuracy of HIV Testing, Receipt of Test Results, and Linkage to Care

- Delaney et al, CROI 2011

Intervention

- **Rapid test algorithm**
  - ☐ **Clients with a preliminary-positive test have blood drawn for standard (offsite) confirmatory testing**
  - ☐ **Up to 2 additional rapid blood tests**
  - ☐ **2 positive rapid tests = same day referral for HIV care**

- **Los Angeles: 4 sites   San Francisco: 5 sites**

Comparison

- **Rapid test with laboratory confirmation**
  - ☐ **Clients with a preliminary-positive test had blood drawn for standard offsite confirmatory testing**
  - ☐ **Appointment scheduled (usually for 7 days later) to receive confirmatory test results**
  - ☐ **Referral if confirmatory test positive**

- **Los Angeles: 12 sites; San Francisco: 11 sites**

Results

	Intervention Sites		Comparison Sites	
	N	%	N	%
False-positive rapid test	37	14.8%	124	13.6%
Confirmed positive	213	85.2%	791	86.4%
Positive on multiple rapid tests	213*	100.0%		
Received results	250	100.0%	430	47.0%

*Includes one client who tested (false) negative on the 2nd test before testing positive on a third rapid test

Conclusions

- **PPV:**
  - **rapid test algorithm 100%;**
  - **single rapid test 85%**
- **Engaged in care <90 days:**
  - **67% of clients who received referral**
  - **50% of clients who did not return for confirmatory results or receive referral**
- **Referral to care after reactive rapid test is essential**

CONTACT INFORMATION

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