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HIV Testing Overview

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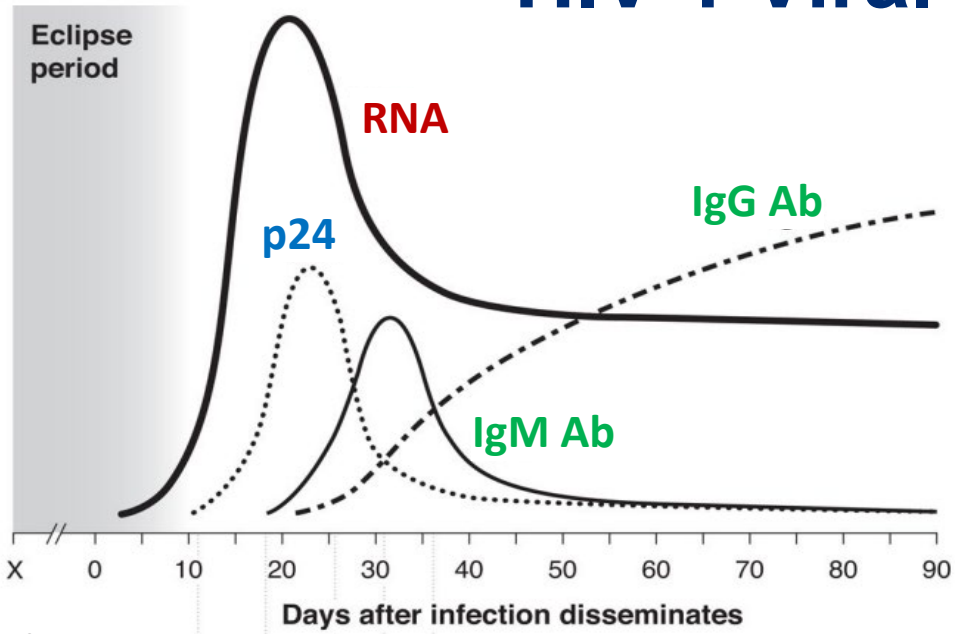
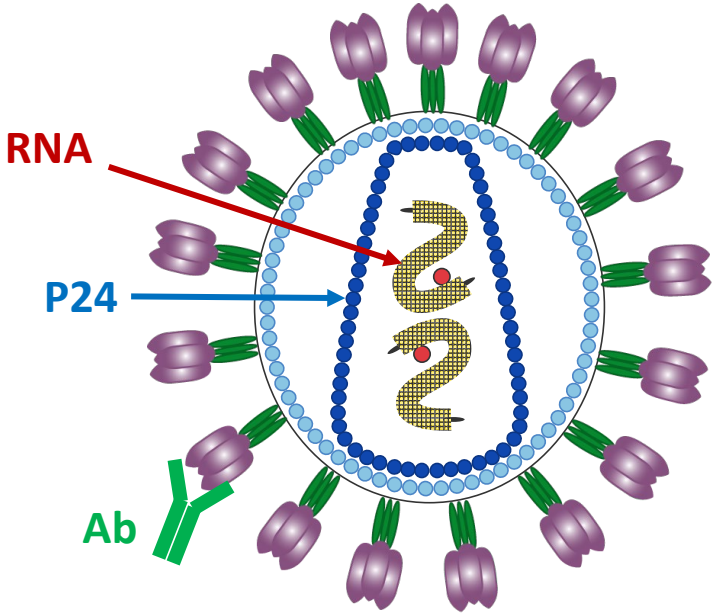
Learning objectives

- Become familiar with the window periods of HIV diagnostic test methods
- Understand test results, interpretations and next steps of the HIV Diagnostic Testing Algorithm
- Learn about testing challenges, including additional algorithms, testing in the context of antiretrovirals, and self-testing

HIV-1 Viral Markers





By Original: US National Institute of Health Vector: Blleininger: - Niaid-hiv-virion-mod.jpg, Public Domain, https://commons.wikimedia.org/w/index.php?curid=12073375

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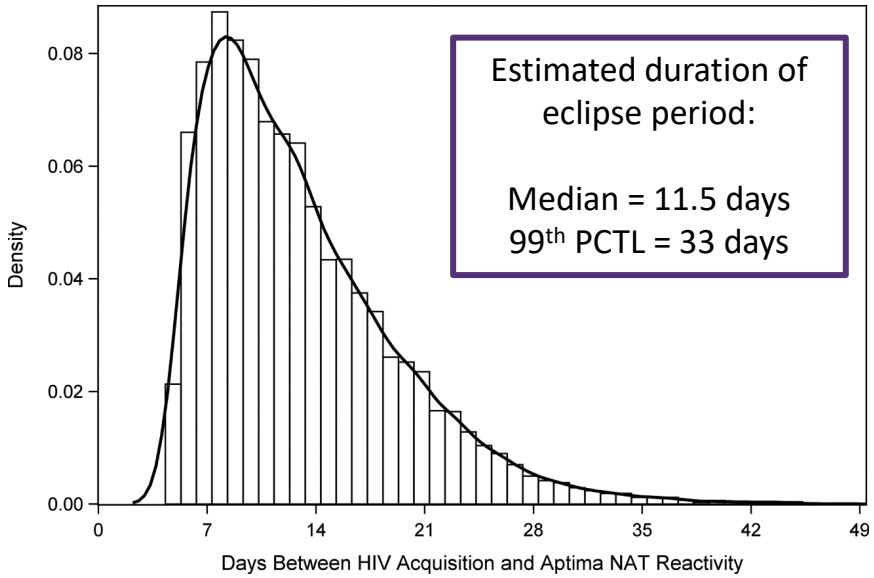
Modified from Sexually Transmitted Diseases44(12):739-746, December 2017. doi: 10.1097/OLQ.0000000000000719

Window Period: HIV-1 Exposure to Test Reactivity

Eclipse = HIV-1 exposure to RNA detection



Category	Median (IQR; Days)	99th PCTL (Days)
Ab/Ag laboratory	17.8 (13.0, 23.6)	44.3
IgG/IgM-sensitive laboratory	23.1 (18.4, 28.8)	49.5
IgG-sensitive rapid screening	31.1 (26.2, 37.0)	56.7
IgG-sensitive supplemental	33.4 (28.5, 39.2)	58.2
Western blot (viral lysate)	36.5 (31.0, 43.2)	64.8

Ab = antibody
Ag = antigen

From: Delaney et al. Clin Infect Dis. 2017;64(1):53-59. doi:10.1093/cid/ciw666

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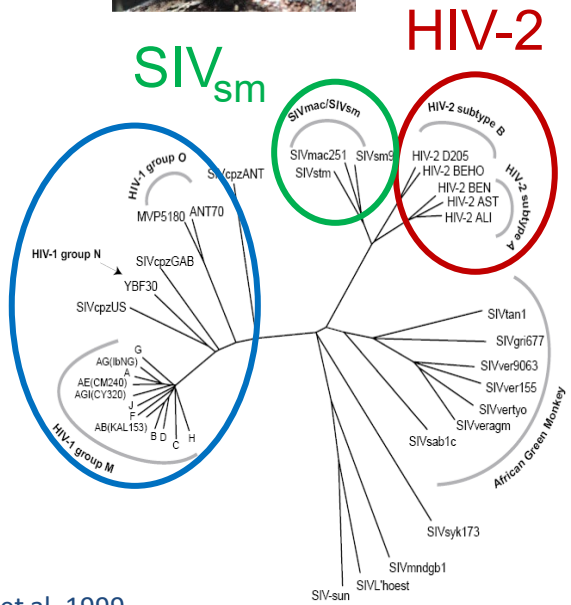
HIV-2: rare in U.S. but important to detect



Adapted from Kuiken,et al. 1999
Los Alamos National Laboratory

- U.S. HIV infections 2010-2017
  - HIV-1: 327,502 (99.94%)
  - HIV-2: 198 (0.06%)
- **HIV-2 resistant to certain antiretrovirals**

- HIV-1 & HIV-2
  - Genomes <50% homologous
  - Antibodies (Abs) cross react
- Current testing algorithm detects and differentiates HIV-1 & HIV-2 Abs

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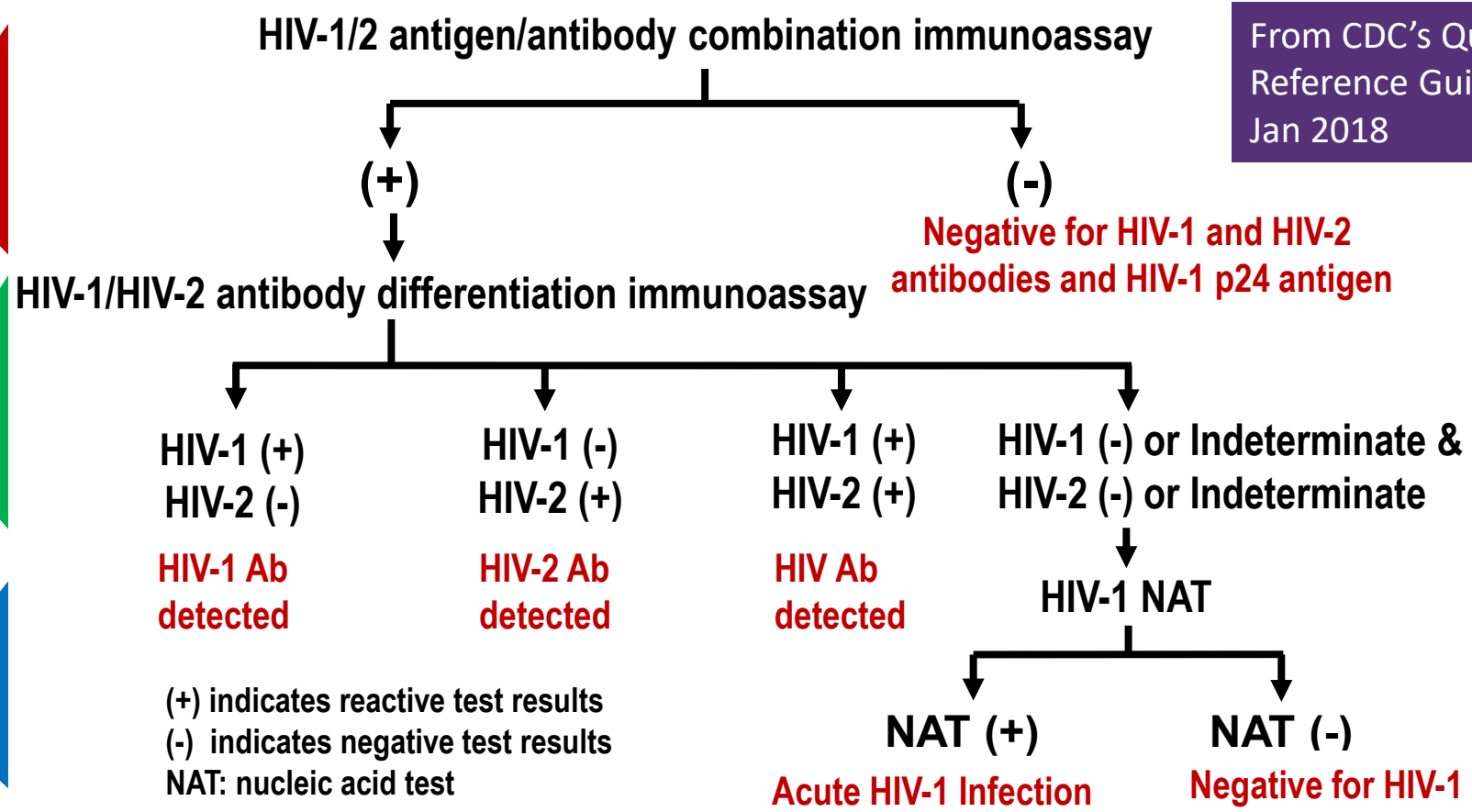
From CDC's Quick Reference Guide, Jan 2018

Step 1

Step 2

Step 3



(+) indicates reactive test results
(-) indicates negative test results
NAT: nucleic acid test

https://www.cdc.gov/hiv/testing/laboratorytests.html

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Step 1

HIV Ag/Ab Combo IAs

Test (Manufacturer)	Yr FDA approved	Method
Architect HIV Ag/Ab Combo (Abbott)	2010	CMIA
GS HIV Ag/Ab Combo EIA (Bio-Rad)	2011	EIA
ADVIA Centaur HIV Ag/Ab Combo (Siemens) *Centaur and Atellica instrument platforms*	2015	CMIA
Elecsys HIV combi PT (Roche Diagnostics)	2017	ECLIA
VITROS HIV Combo (Ortho Clinical Diagnostics)	2017	Immunometric
Alinity i HIV Ag/Ab Combo (Abbott)	2019	CMIA
LIAISON XL MUREX HIV Ab/Ag HT (Diasorin)	2020	CMIA
Determine HIV-1/2 Ag/Ab Combo (Abbott)	2013	LF rapid test
BioPlex 2200 HIV Ag-Ab (Bio-Rad Laboratories)	2015	Multiplex flow
Elecsys HIV Duo (Roche Diagnostics)	2020	ECLIA

All are designed to detect HIV-1 p24 antigen and IgM/IgG antibodies to HIV-1 and HIV-2

No analyte differentiation

None are designed to detect HIV-2 antigen

Analyte differentiation

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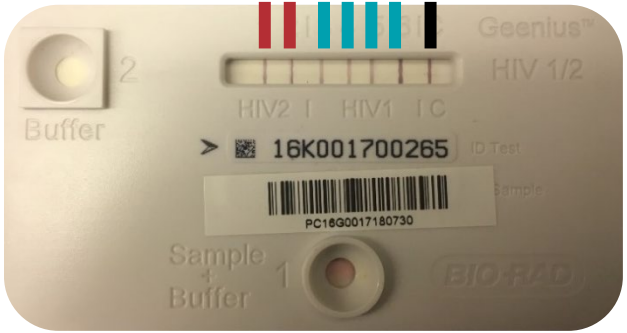
Step 2

Geenius™ HIV1/2 Supplemental Assay

- Intended for supplemental use
- Sample: 5ul of serum/plasma
- Detects IgG antibodies
  - Two HIV-2 bands (gp36, gp140)
  - Four HIV-1 bands (p31, gp160, p24, gp41)
  - Internal control band
- Geenius™ Software and Reader



https://www.bio-rad.com/en-us/product/geenius-hiv-1-2-supplemental-assay?ID=NGUT8KE8Z

30 minutes to result

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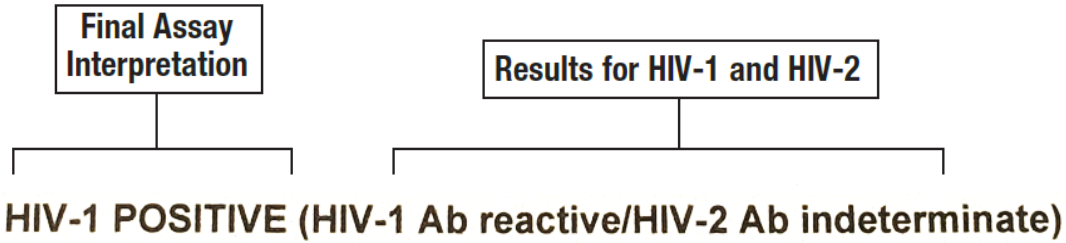
Step 2

Geenius™ Results and Interpretations



APHL recommends that all labs
1. include the Geenius Final Assay Interpretation, AND
2. exclude Geenius individual HIV-1 and HIV-2 results from the lab report

- Geenius software detects reactivity to individual bands and analyzes the relative strength to produce the **Final Assay Interpretation**
- Package insert: **"The Final Assay Interpretation should always be reported to the ordering provider"**

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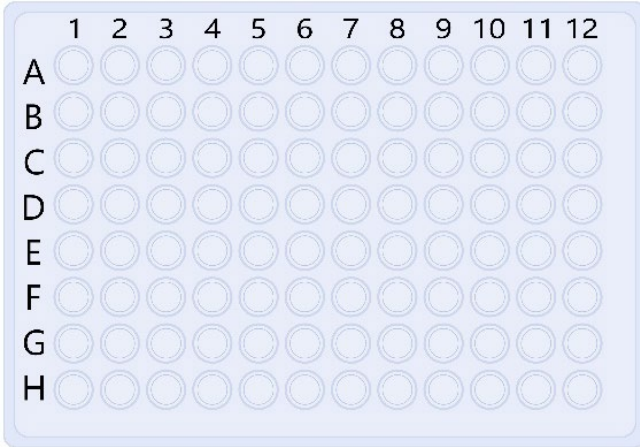
Step 2 VioOne™ HIV Profile™ Supplemental Assay

- Intended for supplemental use
- Sample: 160ul of serum/plasma
- ELISA format:
  - Four HIV-1 antigens
  - One HIV-2 antigen
- Microplate washer & reader required

FDA-Approved Oct 2020



No viral Ag
HIV-1 p65 (*pol*)
HIV-1 gp160 (*env*, low)
HIV-1 gp160 (*env*)
HIV-1 gp41 (*env*, M,O)
HIV-1 p24 (*gag*)
No viral Ag
HIV-2 gp36 (peptide)

Manufactured by Avioq, Inc.

2-3 hrs to result

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Step 2

Geenius Final Assay Interpretation	VioOne HIV Profile Result Interpretation
HIV (Antibody) Negative	HIV Negative
HIV-1 Indeterminate	HIV-1 Indeterminate
HIV-2 Indeterminate	No Equivalent
HIV Indeterminate	No Equivalent
HIV-1 Positive	HIV-1 Positive
HIV-2 Positive	HIV-2 Positive
HIV-2 Positive w/ HIV-1 Cross Reactivity	HIV-2 Positive with Reactivity to HIV-1 Antigens HIV-1 gp41 S/CO ≤ HIV-2 gp36 S/CO
No Equivalent	HIV-1 Positive with Reactivity to HIV-2 Antigen HIV-1 gp41 S/CO > HIV-2 gp36 S/CO
HIV Positive Untypable	No Equivalent

Sources: https://www.fda.gov/media/130312/download, https://www.fda.gov/media/143115/download

Step 3

HIV-1 RNA diagnostic testing

- Before 2020, only one RNA assay FDA approved for aiding in diagnosis
  - Aptima HIV-1 RNA Qualitative assay (Gen-Probe/Hologic)
- Since 2020
  - Aptima HIV-1 RNA Quant Dx (Hologic): Qual/Quant
  - Alinity m HIV-1 AMP Kit (Abbott): Qual/Quant
  - Cobas HIV-1/HIV-2 Qualitative (Roche): Qual only
    - Differentiates HIV-1 and HIV-2
- New assays: Automated, faster, less prone to error

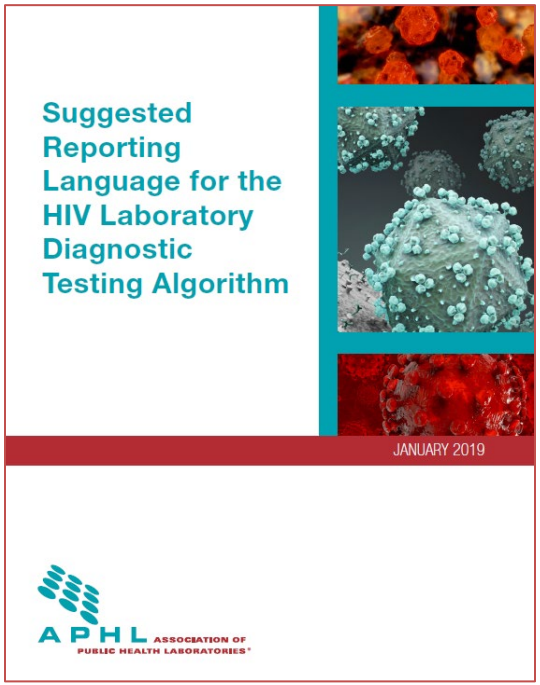
When do you need additional HIV-2 testing?

- If specimen is repeatedly HIV-2 or HIV indeterminate and HIV-1 NAT negative
  - Refer sample for testing with Cobas HIV-1/HIV-2 Qualitative assay or a validated HIV-2 supplemental test (Ab or NAT) **or**
  - Recommend repeating algorithm with a new specimen in 2 to 4 weeks
- HIV-2 qualitative NAT is available to public health labs enrolled in APHL HIV NAT Reference Centers Project

https://www.aphl.org/aboutAPHL/publications/Documents/ID-2019Jan-HIV-Lab-Test-Suggested-Reporting-Language.pdf

HIV Reporting Language

- Updated January 2019
- Addresses:
  - CDC Technical Update on the use of the Determine™ HIV 1/2 Ag/Ab Combo assay
  - CDC's January 2018 update to their Quick Reference Guide
  - Clarification and updated recommendations for reporting Geenius™ test results



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Guidance for Reporting Results from the HIV Laboratory Diagnostic Testing Algorithm for Serum and Plasma Specimens[a]

Test Outcomes	Test Sequence			Laboratory Algorithm Interpretation[d]	Interpretation for Provider Use[e]	Further Actions[f]
	Step 1	Step 2	Step 3			
	HIV-1/HIV-2 Ag/Ab IA[b]	HIV-1/HIV-2 Antibody Differentiation IA[c]	HIV-1 NAT			
	Nonreactive	n/a	n/a	HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection.	HIV negative	If recent HIV exposure is suspected or reported, conduct HIV-1 NAT or request a new specimen and repeat the algorithm according to CDC Guidelines.[g]
	Reactive	HIV-1 Positive	n/a	Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present.	HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
	Reactive	HIV-2 Positive	n/a	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	HIV-2 Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
	Reactive	HIV-2 Positive with HIV-1 Cross reactivity	n/a	Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.	HIV-2 Positive. This result is distinct from HIV positive untypable (undifferentiated).	Link patient to HIV medical care and provide appropriate prevention counseling.[h]
	Reactive	HIV Positive untypable (undifferentiated)	n/a	Positive for HIV-1 and HIV-2 antibodies. Laboratory evidence of HIV-1 and/or HIV-2 infection is present.	HIV Positive	Link patient to HIV medical care and provide appropriate prevention counseling.[h] Provider may consider additional testing for HIV-1 RNA or DNA and HIV-2 RNA or DNA to verify or rule out HIV-1/HIV-2 dual infection. Request additional specimen if original specimen volume is insufficient.
	Reactive	HIV-1 indeterminate or, HIV-2 indeterminate[i] or, HIV indeterminate	Detected	Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an acute HIV-1 infection.	Acute HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling immediately[h] to expedite prevention practices.
	Reactive	HIV-1 indeterminate	Not detected	HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected.	HIV Negative	If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance.[g]
	Reactive	HIV-2 indeterminate[i]	Not detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.	HIV-1 Negative, HIV-2 inconclusive	Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.
	Reactive	HIV Indeterminate	Not detected	HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.	HIV-1 Negative, HIV-2 inconclusive	Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.
	Reactive	HIV Antibody Negative	Detected	Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an acute HIV-1 infection.	Acute HIV-1 Positive	Link patient to HIV medical care and provide appropriate prevention counseling immediatelyh to expedite prevention practices.
	Reactive	HIV Antibody Negative	Not detected	HIV antibodies were not confirmed and HIV-1 RNA was not detected.	HIV Negative	If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance.[g]
	Reactive	HIV Antibody Negative or Indeterminate	Invalid or not performed	Inconclusive	Inconclusive	Request an additional specimen and repeat the algorithm. Ensure HIV-1 NAT is performed, if indicated by results of HIV-1/HIV-2 Ag/Ab IA and HIV-1/HIV-2 Ab differentiation IA.

https://www.aphl.org/aboutAPHL/publications/Documents/ID-2019Jan-HIV-Lab-Test-Suggested-Reporting-Language.pdf

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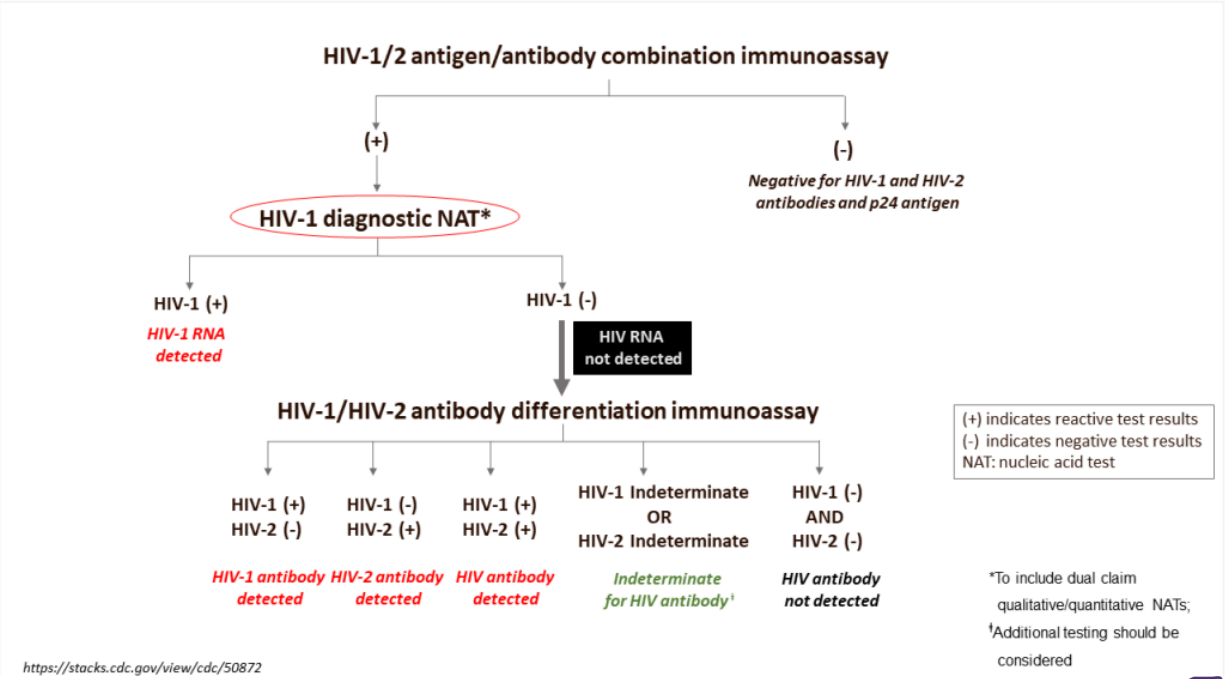
Challenge #1: Use of NAT as Step 2 of an HIV Testing Algorithm

- Three tests currently FDA approved for 'aiding in diagnosis'
  - Aptima HIV-1 RNA Quant Dx (Hologic): Qual/Quant
  - Alinity m HIV-1 AMP Kit (Abbott): Qual/Quant
  - Cobas HIV-1/HIV-2 Qualitative (Roche): Qual only HIV-1/HIV-2

- Would it be more efficient to use RNA test as Step 2 instead of HIV-1/HIV-2 differentiation immunoassay?

Alternative use of an HIV-1 NAT at the Second Step of an HIV Diagnostic Algorithm

- Includes dual claim Qual/Quant NATs



Presented at: 2022 Advancing HIV, STI and Viral Hepatitis Testing Conference (Mar 29 –Apr 1, 2022) by Dr. Jeff Johnson (CDC)

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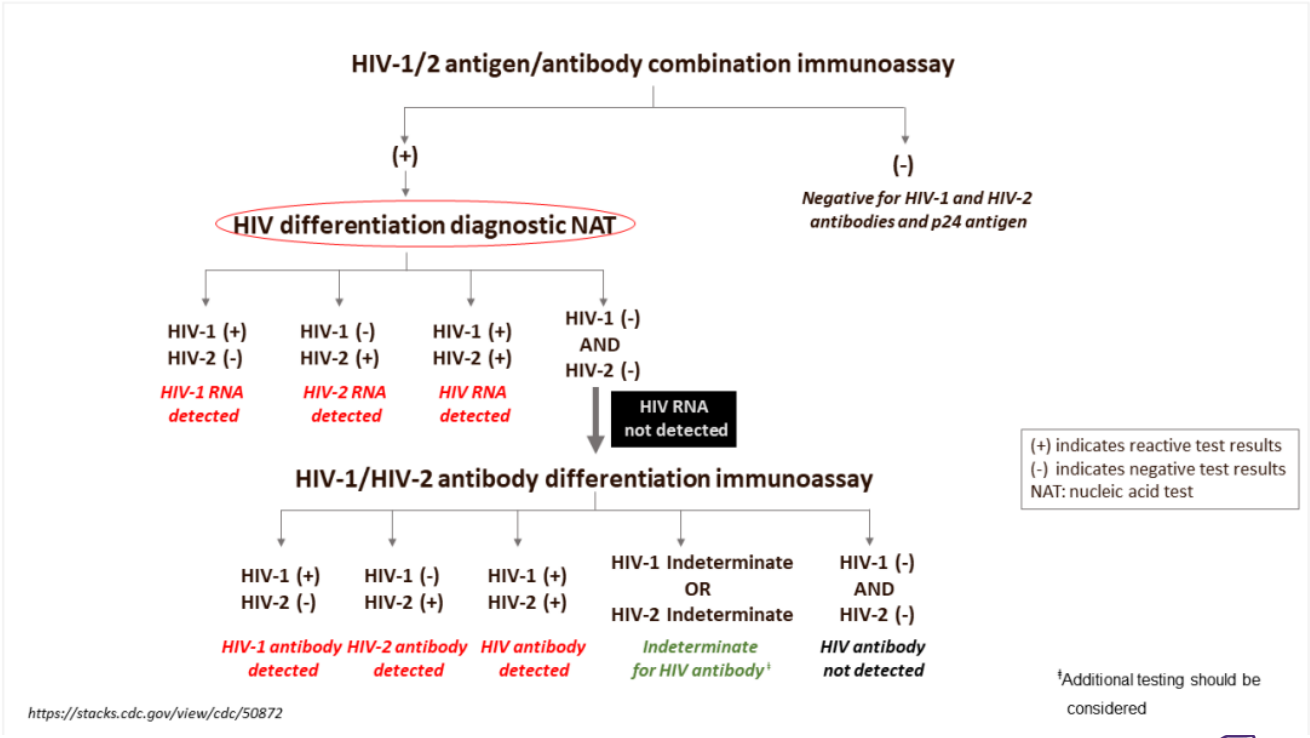
Alternative use of an HIV Differentiation NAT at the Second Step of an HIV Diagnostic Algorithm



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Benefits & Limitations of NAT as Step 2

Potential Benefits:

- Could reduce TAT
- Could allow viral load at diagnosis

Potential Limitations:

- Will not distinguish acute infection
- If NAT is negative, need guidance on interpretation and next steps
- More stringent specimen handling requirements

Sources: https://hivtestingconference.org/wp-content/uploads/2022/04/BernardBranson_DiscussionPanel1.pdf
Pitasi et al 2020 https://pubmed.ncbi.nlm.nih.gov/31895304/

Challenge #2: Diagnostic testing in the context of antiretrovirals (ARV)

Early Antiretroviral Treatment

- Start treatment as early as possible after diagnosis
- Reduces viral reservoir, reduces transmission and improves disease prognosis

Pre-exposure Prophylaxis (PrEP)

- Daily/bi-monthly treatment to reduce risk of acquiring HIV
- Recommended for HIV-negative people at high risk
- Oral and injectable forms

Sources: Manak et al 2019 https://pubmed.ncbi.nlm.nih.gov/31217270/
https://www.cdc.gov/hiv/clinicians/prevention/prescribe-prep.html

Diagnostic testing with antiretrovirals (ARV)

- If someone becomes infected and continues on PrEP or has early antiretroviral treatment…
  - Emergence of HIV markers (RNA, Ag, Ab) may be delayed
  - Virus levels may be near limit of detection
  - Waffling between pos and neg results over successive samples
- Performance data for FDA-approved tests did not include people on PrEP

Source: Branson 2019 https://pubmed.ncbi.nlm.nih.gov/31239094/

Challenge #3: Self-Testing for HIV

	Category 1	Category 2	Category 3
Specimen Collection Setting	Non-Clinical/Home	Non-Clinical/Home	At a Collection Facility
Specimen Testing Setting	Non-Clinical/Home	Consumer Ships to a Clinical Laboratory	Clinical Laboratory
Examples	OraQuick In-Home HIV Test (FDA-Approved)	Any box/kit sent to a consumer for collection (e.g. Let's Get Checked, My Lab Box)	Some commercial labs offer direct to consumer testing (e.g. Quest)

Considerations for Self-Testing

- May help people get tested and learn their status
- Oral fluid and DBS testing less sensitive than plasma/serum
  - May be less appropriate for people on PrEP
- One FDA-approved test (OraQuick), rest are ‘lab-developed’
- Consumers may not understand results or test limitations
- Verifying specimen suitability and identity of source patient may be difficult

Final Points

- More test options are available for each step of the recommended algorithm
- An alternative diagnostic algorithm with a NAT as Step 2 has been proposed
- Early ART and PrEP may delay HIV markers and cause false negative or fluctuating results
- Self-testing for HIV will allow more people to access testing but may be less sensitive and difficult for consumers to interpret

Questions?