



**Department
of Health**

**Wadsworth
Center**

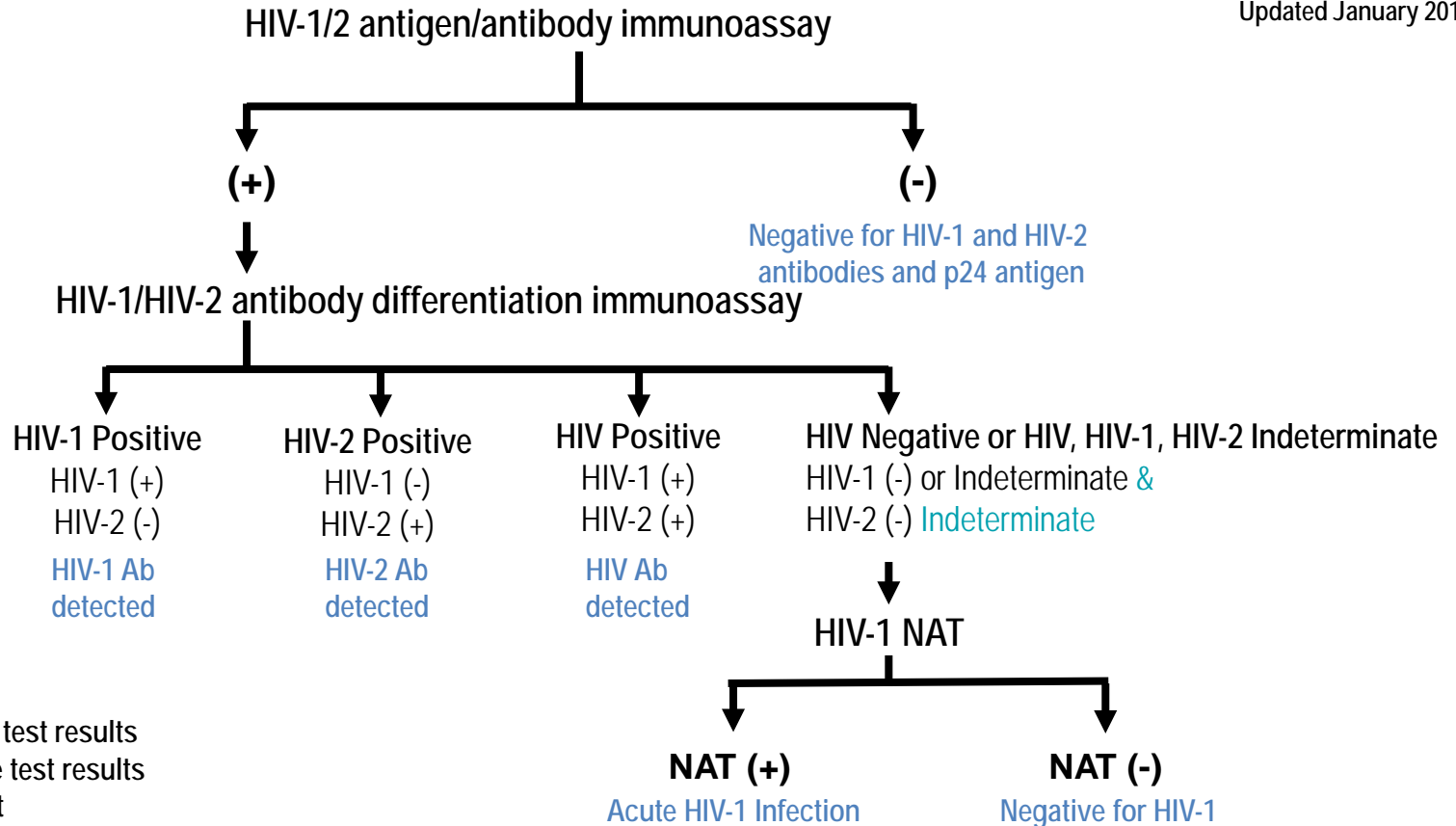
Adventures in Discordance- HIV Testing

Anne Gaynor, PhD

On behalf of Monica M. Parker, PhD
Director, Bloodborne Viruses Laboratory
Wadsworth Center, NYSDOH

HIV Laboratory Testing Algorithm

Updated January 2018



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

HIV Infection and Laboratory Markers

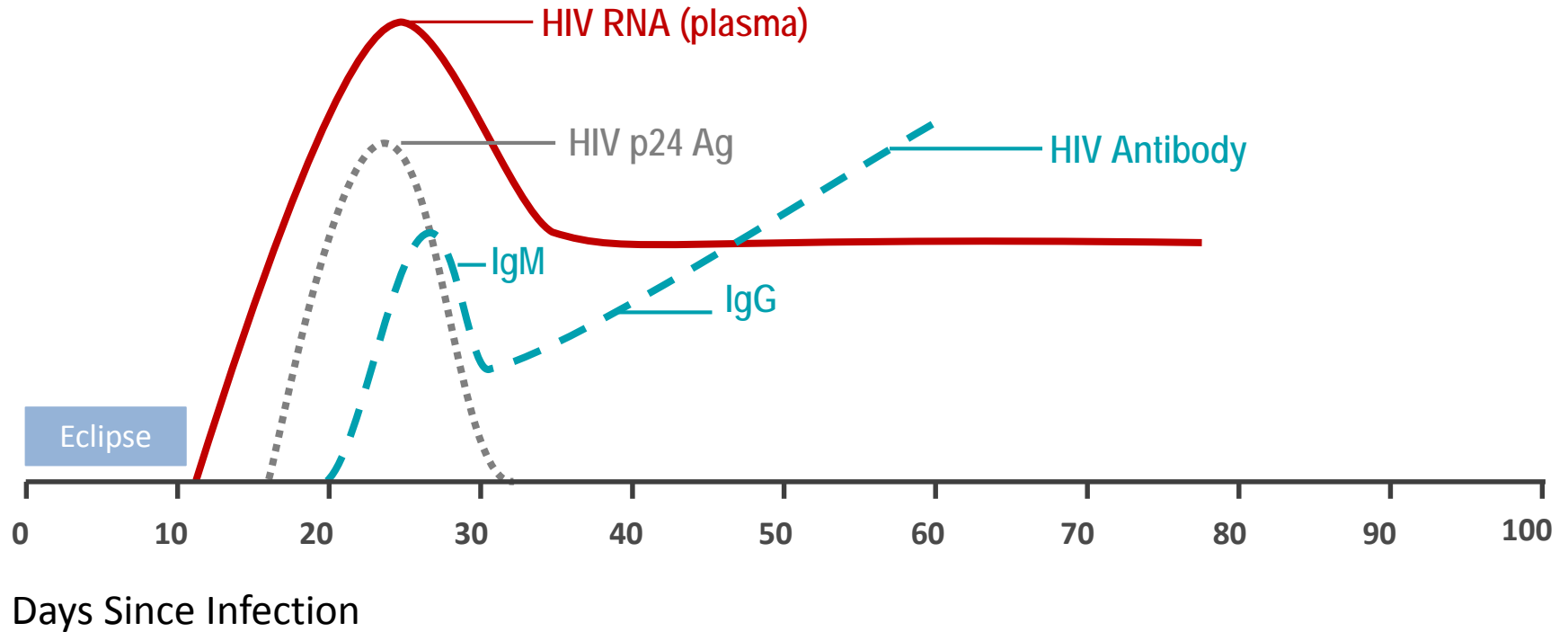


Figure adapted from Delaney et al., CID 2017:64 and provided by M. Owen, NCHHSTP, CDC

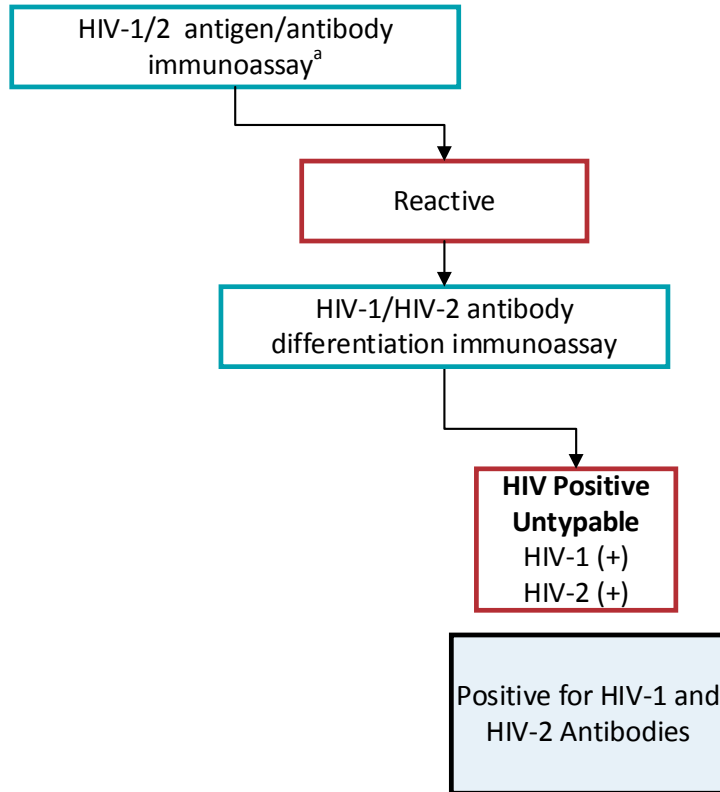
NY State HIV Testing

- Provides HIV-1 NAT Testing for any clinical hospital within NYS
- Provides HIV-2 NAT and Viral Load for persons with HIV-2 in NYS
- Will also repeat algorithm
 - upon request
 - for a new case of HIV-2
 - as needed to ensure quality test results



Case 1: Misconceptions, Discordance and Reality

Testing at Hospital Laboratory



Patient Details:

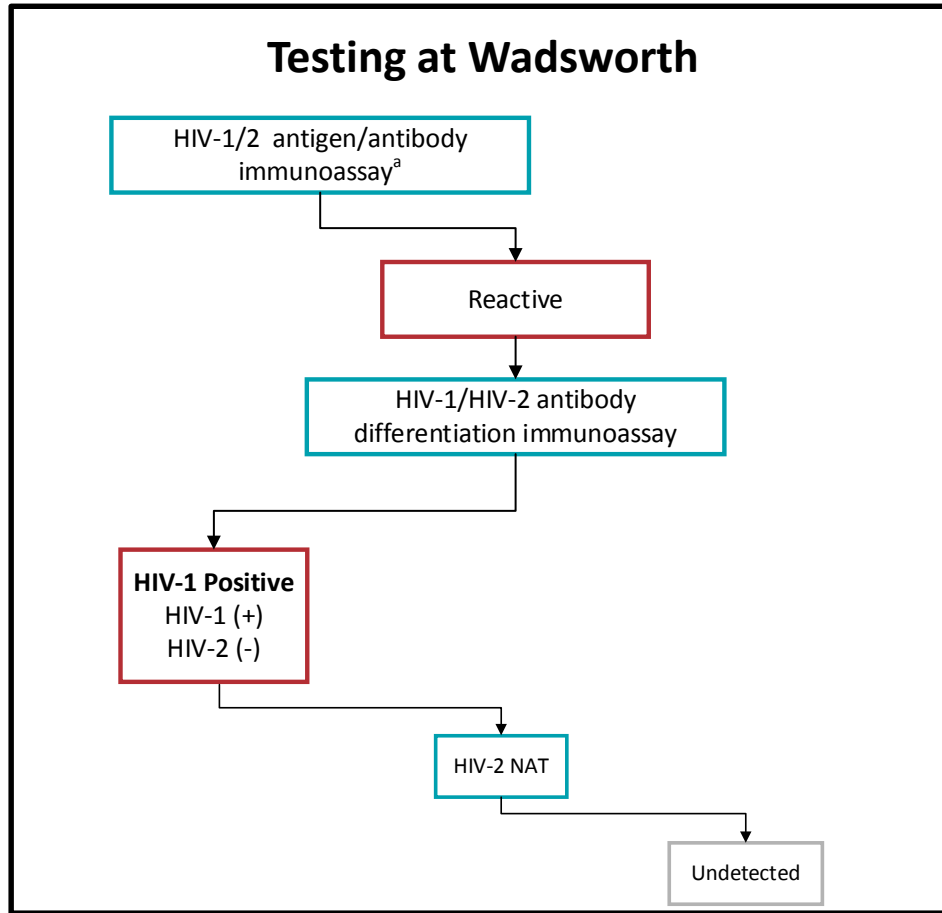
- From Ghana
- Previous Diagnosis of HIV-2

What additional testing/workup would you do?

What was ordered:

- Hospital requested HIV-1 Viral Load in-house.
- Hospital sends sample to Wadsworth for HIV-2 Viral Load due to suspect case

Case 1: Misconceptions, Discordance and Reality



Any explanations?
Any additional testing?



Case 1: Misconceptions, Discordance and Reality

Summary of Testing

Testing Performed at	HIV Ag/Ab (s/co)	HIV-1/2 Ab Diff	HIV-1 RNA Qual	HIV-1 RNA Quant
Hospital Laboratory	Reactive	HIV Positive, Untypeable	N/A	Positive
Wadsworth	Reactive	HIV-1 Positive	N/A	Not Detected

Conclusion: HIV-1 Positive; Ab cross-reactivity

- HIV-1 positive bands:[HIV-1 gp41+, gp160+; HIV-2 gp36+]
- gp41 and gp36 are homologs with known cross-reactivity
- HIV-1 antibodies confirmed, HIV-2 antibodies never confirmed
- HIV-1 RNA detected
- HIV-2 RNA not detected



HIV-2: Information and Detection

- Endemic in West Africa; Very low prevalence in U.S.
 - Majority of U.S. cases in Northeast, especially NYC which is home to many West African immigrants
- HIV-2 Abs may cross-react with HIV-1 Ags, but genomic sequence is <50% homologous to HIV-1
- In the past, HIV-2 often misdiagnosed as HIV-1 by Western blot
- HIV-1/2 Ab differentiation test (step 2) accurately confirms HIV-2 antibodies
 - But, can also produce HIV-2 indeterminate and HIV indeterminate results



HIV-2 NAT

- No HIV-2 NAT test kits available (qual or quant)
- HIV-1 NATs will not detect HIV-2
- Wadsworth has HIV-2 RNA real-time PCR LDTs
 - Qualitative LOD = 7 IU/mL
 - Quantitative LLOQ = 41 IU/MI
- Validated and approved by NYS Clinical Lab Evaluation Program (CLEP)



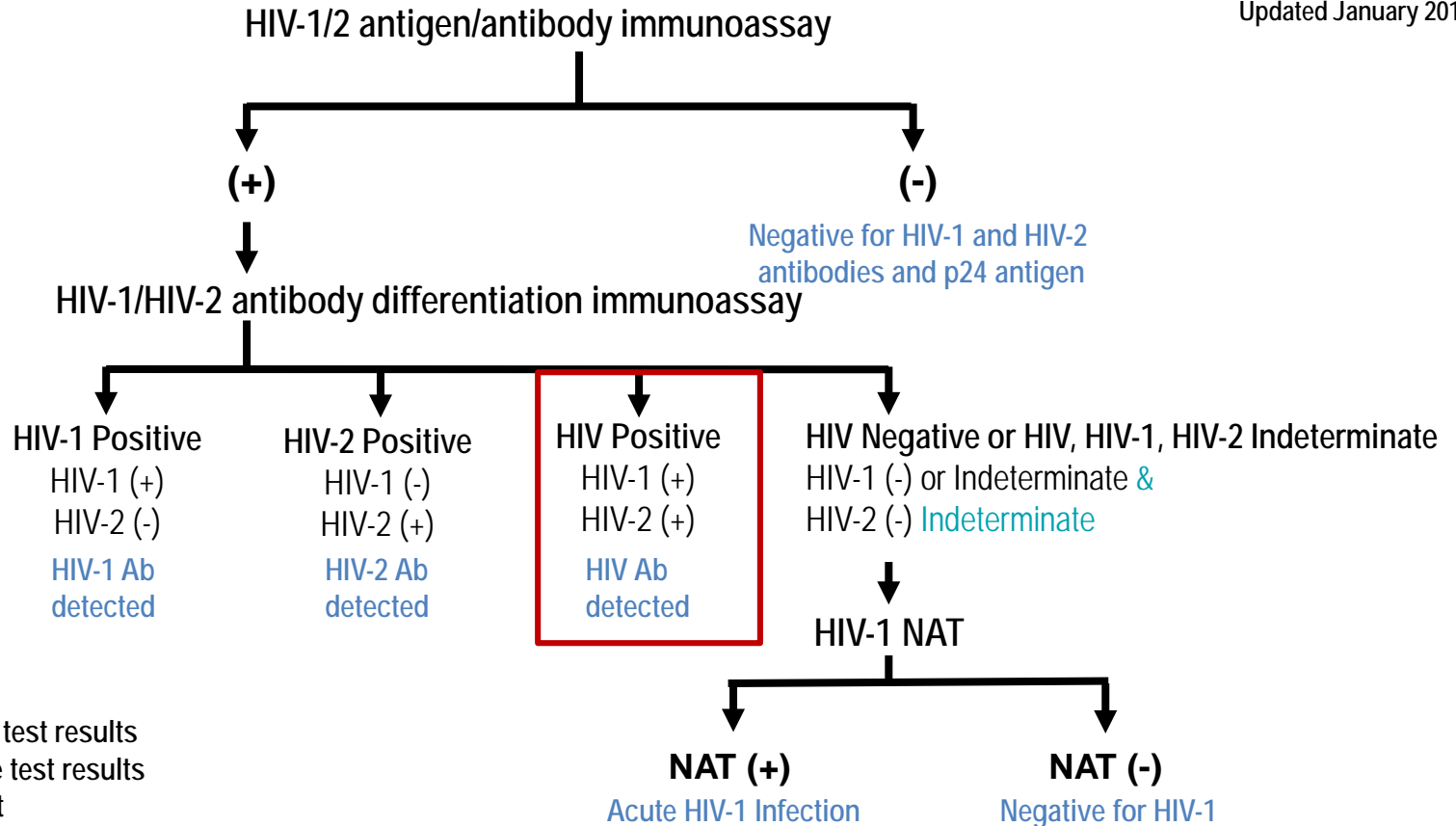
Case 2: But is this one HIV-2?

Test – Wadsworth BVL	Result
HIV Ag/Ab IA	Reactive (s/co 467)
HIV-1/2 Ab Diff	HIV Positive, Untypable [HIV-1 31+, gp41+, gp160+; HIV-2 gp36+, gp140+]

- New patient from Burkina Faso
- Requested HIV-2 Viral Load (noted HIV-2 pos WB in 2011)
- NY called the hospital doctor: also reported a positive HIV-1 WB ~10 years ago.

HIV Laboratory Testing Algorithm

Updated January 2018



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

Case 2: But is this one HIV-2?

Test – Wadsworth BVL	Result
HIV Ag/Ab IA	Reactive (s/co 467)
HIV-1/2 Ab Diff	HIV Positive, Untypable [HIV-1 31+, gp41+, gp160+; HIV-2 gp36+, gp140+]
HIV-1 RNA Qual	Not detected
HIV-2 RNA Qual	Detected
HIV-2 RNA Quant	3361 IU/mL

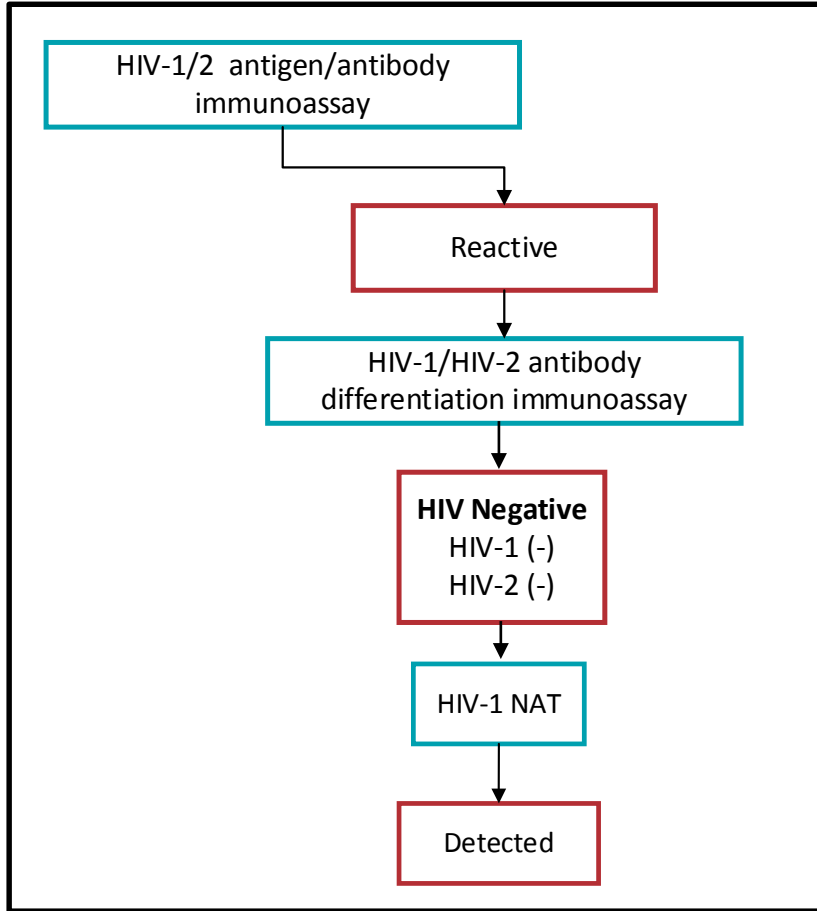
Conclusion: HIV-2 Positive; Strong Ab cross-reactivity to HIV-1 Ags



Department
of Health

Wadsworth
Center

Case 3: What at first appears simple...



Detection of Acute HIV-1 Infection

But...2 weeks later a 2nd sample from the same patient is submitted for testing



Case 3: What at first appears simple...has gotten more complicated

Sample	HIV Ag/Ab (s/co)	HIV-1/2 Ab Diff	HIV-1 RNA Qual
New Sample	Reactive (1.34)	HIV-1 Indeterminate	Not Detected

s/co < 1: nonreactive, s/co \geq 1: reactive

What is a potential explanation?
What would you do next?



Department
of Health

Wadsworth
Center

Case 3: What at first appears simple...has gotten more complicated

Sample	HIV Ag/Ab (s/co)	HIV-1/2 Ab Diff	HIV-1 RNA Qual
Sample 1	Reactive (1.04)	Nonreactive	Detected
Sample 2 (not tested at Wadsworth)	Nonreactive (0.93)	N/A	N/A
Sample 3	Reactive (1.34)	HIV-1 Indeterminate	Not Detected

s/co < 1: nonreactive, s/co \geq 1: reactive

Any additional guesses?



Case 3: What at first appears simple...has gotten more complicated

- High-risk individual on PreP (pre-exposure prophylaxis)
- 3 specimens tested over 3-week period
 - When first specimen came back positive, suspected the result was false-positive
 - Second sample was negative but provider was still uncertain
 - Worried about potential breakthrough infection
- Breakthrough infection is suspected, but person is still on PreP
- Serconversion may be delayed and virus detection impeded by ARVs



An Increasing Diagnostic Challenge

- As PreP use increases, this situation may arise more frequently
- Also ARV treatment of early/acute infection may delay Ab response
 - Difficult to verify infection, if needed
- HIV-1 DNA PCR test may be needed in these cases – no FDA-approved kits
 - This sample sent to Quest for HIV-1 DNA PCR

More diagnostic tools needed

- PreP and ARV prophylaxis in HIV-exposed infants pose diagnostic challenges
 - Viral suppression may impede RNA detection
- Naturally low RNA levels for HIV-2 can also leave doubt regarding status when RNA test is negative



Acknowledgements

**NYSDOH-Wadsworth Center
Bloodborne Viruses Laboratory**

Monica Parker
Tim Sullivan
Linda Styer