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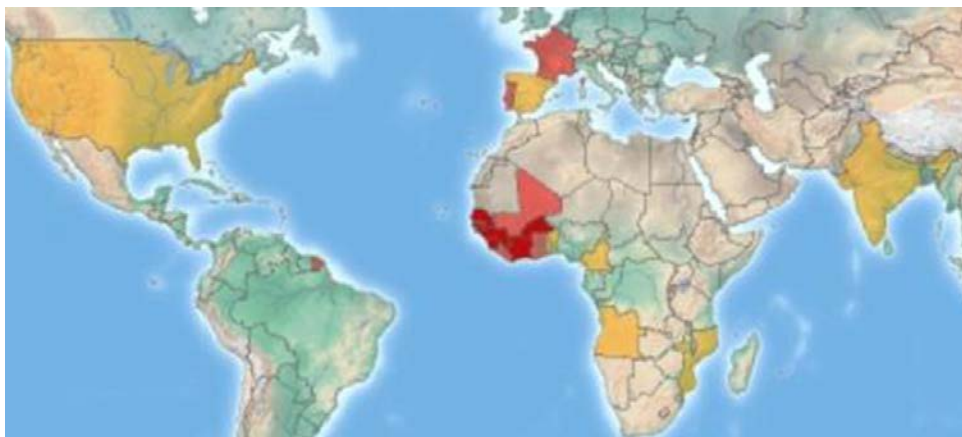
An Evaluation of Qualitative HIV-2 RNA Referral Testing

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**** The authors have no conflicts of interest to disclose ****

HIV-2 is a rare form of HIV with highest prevalence in West Africa



Visseaux et al 2016. Infection, Genetics and Evolution

U.S. Case Locations	#
New York City	77 (46%)
Northeast U.S.	33 (20%)
South	24 (15%)
Midwest	18 (11%)
West	14 (8%)
Total Cases 1987-2009	166

MMWR. July 29, 2011 / 60(29);985-988




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HIV-2 real time RT-PCR assay detects HIV-2 RNA and an internal control virus

1. LYSIS

0.1-0.9 mL serum/plasma



20ul internal control

2. EXTRACTION

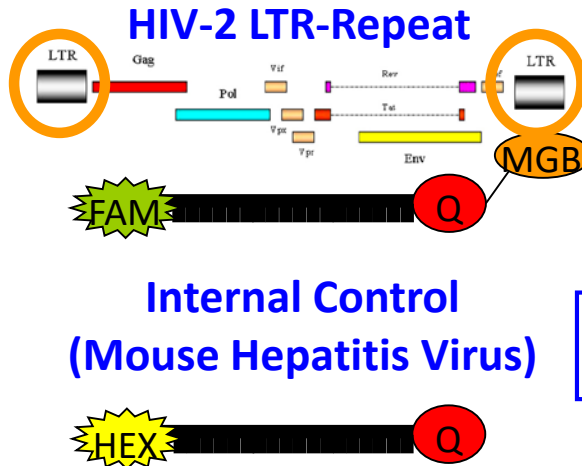


Total nucleic acid
Nuclisens
EasyMAG

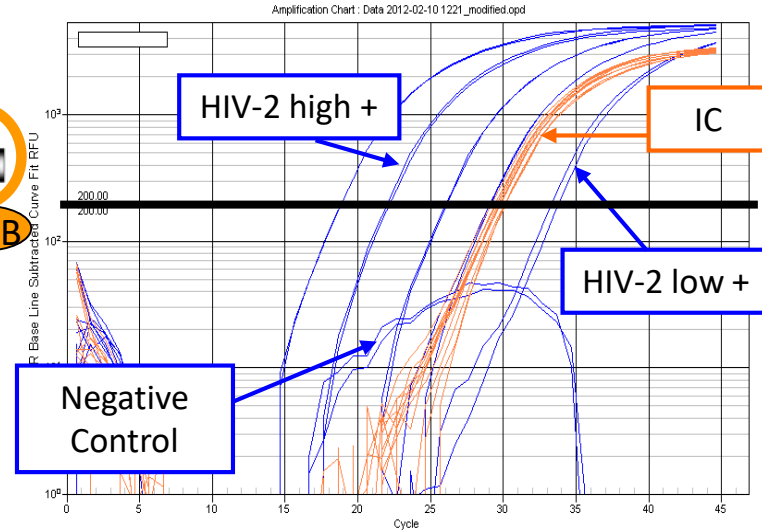
3. REVERSE TRANSCRIPTION

Random Hexamer

4. DUPLEX REAL TIME PCR



LOD: 7 IU/ml (0.9 mL), 64 IU/ml (0.1 mL)



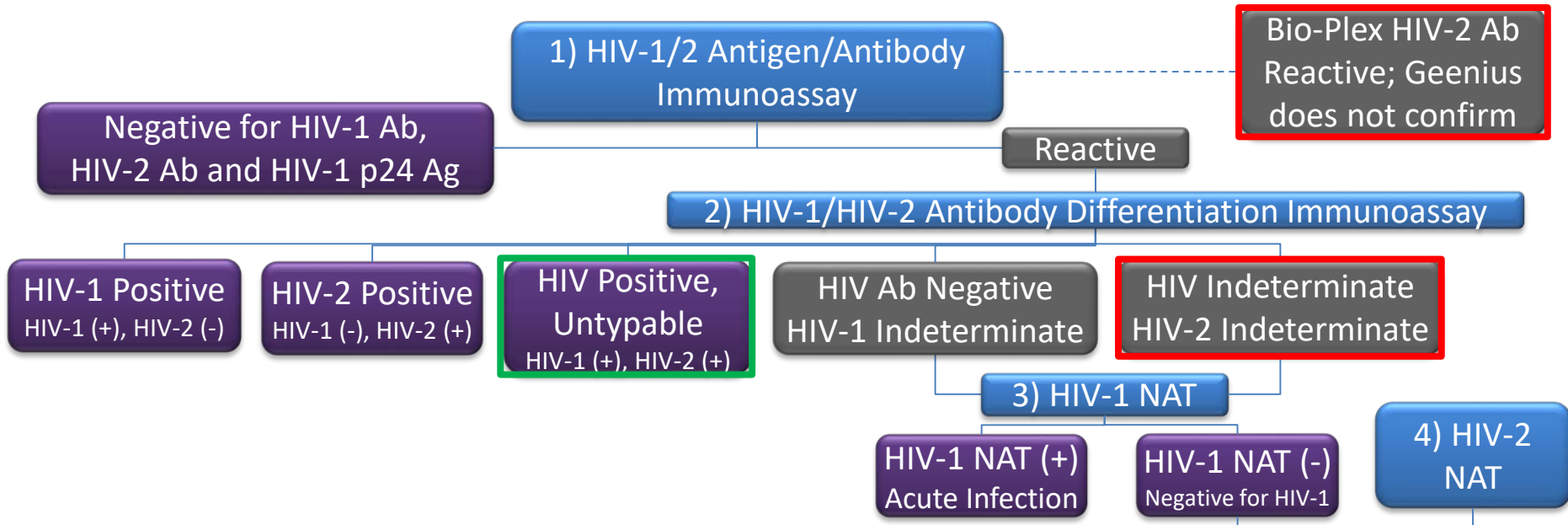
Styer 2013 JCV 58(s1): e127-e133



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HIV testing algorithm differentiates HIV-1 & HIV-2 but can give indeterminate HIV-2 Ab results



In June 2016, offered HIV-2 RNA testing to public health labs through APHL/CDC NAAT Demonstration Project



- APHL/CDC NAAT Demonstration Project
 - Specimens submitted from other U.S. public health labs
 - Results (1): HIV Ag/Ab reactive, Geenius HIV-2 or HIV Indeterminate OR (2) BioPlex HIV Ag/Ab R: HIV-2 Ab R, Geenius HIV-2 Ab NR or Indeterminate
 - NAAT referral labs: Wadsworth Center (HIV-1 & HIV-2) & Florida (HIV-1)
 - Test for HIV-1 RNA – if negative, reflex to HIV-2 RNA
- 90 specimens collected from June 2016 to Dec 2018
 - APHL/CDC NAAT Demonstration Project (48 specimens)
 - NYS clinicians, laboratories, contract facilities (42 specimens)



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Group 1: Unconfirmed HIV-2 Ab reactivity (n=56, 62%)

HIV Testing Algorithm Results		# Specimens	HIV-2 RNA
HIV Ag/Ab: Reactive Geenius: HIV-2 Indeterminate HIV-1 RNA: Not Detected		34 APHL/CDC 11 Other In 7 with known bands – all gp140	Not Detected
HIV Ag/Ab: Reactive Geenius: HIV Indeterminate HIV-1 RNA: Not Detected		6 APHL/CDC 2 Other In 2 with known bands – both gp36, gp41	Not Detected
HIV Ag/Ab (Bio-Plex): Undifferentiated Geenius: HIV-2 Ab not confirmed HIV-1 RNA: Not Detected		3 APHL/CDC	Not Detected



Group 2: Rule out Dual HIV-1/HIV-2 Infection (N=8, 9%)

HIV Testing Algorithm Results	# Specimens	HIV-2 RNA	
<p>HIV Ag/Ab: Reactive Geenius: HIV Positive Untypable HIV-1 RNA: Detected</p>	 <p>The image shows a Geenius HIV 1/2 test strip. It has two wells: '2' (Buffer) and '1' (Sample + Buffer). The result window shows six lines: line 1 (HIV-2) is red, line 2 (HIV-1) is red, and line 6 (IC) is red. Lines 3, 4, and 5 are blank. A barcode is visible below the result window.</p>	<p>3 APHL/CDC 2 Other</p>	<p>Not Detected</p>
<p>HIV Ag/Ab: Reactive Geenius: HIV Positive Untypable HIV-1 RNA: Unknown</p>	 <p>The image shows a Geenius HIV 1/2 test strip. It has two wells: '2' (Buffer) and '1' (Sample + Buffer). The result window shows six lines: line 1 (HIV-2) is red, line 2 (HIV-1) is red, and line 6 (IC) is red. Lines 3, 4, and 5 are blank. A barcode is visible below the result window.</p>	<p>3 Other</p>	<p>Not Detected</p>



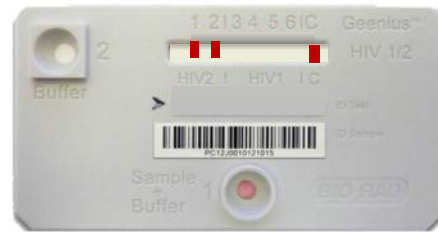
Group 3: Resolve HIV status (N=21, 23%)

HIV Testing Algorithm Results	# Specimens	HIV-2 RNA
<p>HIV Ag/Ab: Reactive Geenius: HIV-1 Positive HIV-1 RNA: Unknown</p>	<p>7 Other</p>	<p>Not Detected</p>
<p>HIV Ag/Ab: Reactive Geenius: HIV-1 Indeterminate Or HIV Ab Negative HIV-1 RNA: Not Detected</p>	<p>2 APHL/CDC 12 Other</p>	<p>Not Detected</p>



Group 4: Confirm HIV-2 Diagnosis (N=5, 6%)

HIV Testing Algorithm Results	# Specimens	HIV-2 RNA
<p>HIV Ag/Ab: Reactive</p> <p>Geenius: HIV-2 Positive</p> <p>HIV-1 RNA: Unknown</p>	<p>5 Other</p>	<p>3 Detected</p> <p>2 Not Detected</p>



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Conclusions

- Tested 90 specimens with qualitative HIV-2 RNA real time PCR, **detected HIV-2 RNA in 3 HIV-2 Ab positive samples**
- HIV-2 RNA testing helps to exclude infection in specimens with unresolved HIV-2 antibody reactivity
- Too many unnecessary tests?
 - Confusion about detection capabilities of Ag/Ab test
 - Misunderstanding of Geenius results vs. interpretation
 - False reactivity of Geenius gp140 band



Acknowledgements

- Bloodborne Viruses Lab – Wadsworth Center
 - Jean Stella, Robert Blum, Tom Miller, Tim Sullivan, Phil Rivenburg, William Carmichael, Patty Dingman, Renee Hallack, Lea Ryman
- Florida DOH – Bureau of Public Health Laboratories
 - Sally Fordan
 - Berry Bennett
- Funding
 - CDC Cooperative Agreement # 5NU600E000103
 - APHL HIV NAT Demonstration Project Contract
 - NYSDOH Wadsworth Center and AIDS Institute



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