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

HIV-1/2 antigen/antibody immunoassay

(+)

HIV-1/2 antibody differentiation immunoassay

(-)

HIV-1 Positive
HIV-1 (+)
HIV-2 (-)
HIV-1 Ab detected

HIV-2 Positive
HIV-1 (-)
HIV-2 (+)
HIV-2 Ab detected

HIV Positive
HIV-1 (+)
HIV-2 (+)
HIV Ab detected

HIV Negative or HIV, HIV-1, HIV-2 Indeterminate
HIV-1 (-) or Indeterminate & HIV-2 (-) Indeterminate

HIV-1 NAT

NAT (+)
Acute HIV-1 Infection

NAT (-)
Negative for HIV-1

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

Updated January 2018
HIV Infection and Laboratory Markers

Days Since Infection

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

- HIV-1/2 antigen/antibody immunoassay
  - Reactive
    - HIV-1/HIV-2 antibody differentiation immunoassay
      - HIV Positive Untypable
        - HIV-1 (+)
        - HIV-2 (+)
  - Positive for HIV-1 and HIV-2 Antibodies

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
Testing at Wadsworth

HIV-1/2 antigen/antibody immunoassay

Reactive

HIV-1/HIV-2 antibody differentiation immunoassay

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

HIV-2 NAT

Undetected

Any explanations?
Any additional testing?
Case 1: Misconceptions, Discordance and Reality

Summary of Testing

<table>
<thead>
<tr>
<th>Testing Performed at</th>
<th>HIV Ag/Ab (s/co)</th>
<th>HIV-1/2 Ab Diff</th>
<th>HIV-1 RNA Qual</th>
<th>HIV-1 RNA Quant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital Laboratory</td>
<td>Reactive</td>
<td>HIV Positive, Untypeable</td>
<td>N/A</td>
<td>Positive</td>
</tr>
<tr>
<td>Wadsworth</td>
<td>Reactive</td>
<td>HIV-1 Positive</td>
<td>N/A</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

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/mL
- Validated and approved by NYS Clinical Lab Evaluation Program (CLEP)
Case 2: But is this one HIV-2?

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

- 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

HIV-1/2 antigen/antibody immunoassay

(+)

HIV-1/2 antibody differentiation immunoassay

HIV-1 Positive
HIV-1 (+)
HIV-2 (-)
HIV-1 Ab detected

HIV-2 Positive
HIV-1 (-)
HIV-2 (+)
HIV-2 Ab detected

HIV Positive
HIV-1 (+)
HIV-2 (+)
HIV Ab detected

HIV Negative or HIV, HIV-1, HIV-2 Indeterminate
HIV-1 (-) or Indeterminate & HIV-2 (-) Indeterminate

HIV-1 NAT

NAT (+)
Acute HIV-1 Infection

NAT (-)
Negative for HIV-1

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

Updated January 2018
Case 2: But is this one HIV-2?

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

**Conclusion:** HIV-2 Positive; Strong Ab cross-reactivity to HIV-1 Ags
Case 3: What at first appears simple…

Detection of Acute HIV-1 Infection

But...2 weeks later a 2\textsuperscript{nd} sample from the same patient is submitted for testing
Case 3: What at first appears simple...has gotten more complicated

<table>
<thead>
<tr>
<th>Sample</th>
<th>HIV Ag/Ab (s/co)</th>
<th>HIV-1/2 Ab Diff</th>
<th>HIV-1 RNA Qual</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Sample</td>
<td>Reactive (1.34)</td>
<td>HIV-1 Indeterminate</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

$s/co < 1$: nonreactive, $s/co > 1$: reactive

What is a potential explanation?
What would you do next?
### Case 3: What at first appears simple...has gotten more complicated

<table>
<thead>
<tr>
<th>Sample</th>
<th>HIV Ag/Ab (s/co)</th>
<th>HIV-1/2 Ab Diff</th>
<th>HIV-1 RNA Qual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Reactive (1.04)</td>
<td>Nonreactive</td>
<td>Detected</td>
</tr>
<tr>
<td>Sample 2 (not tested at Wadsworth)</td>
<td>Nonreactive (0.93)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Reactive (1.34)</td>
<td>HIV-1 Indeterminate</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

`s/co <1: nonreactive, s/co > 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