Could HIV-1 RNA be an option as the second step in the HIV diagnostic algorithm?

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Disclaimer: This evaluation was conducted using a non-FDA approved indication for use.
Tradenames are used for informational purposes and do not constitute an endorsement by CDC.
Disclosure: No relevant financial relationships
- Implementation of the HIV diagnostic algorithm in 2014
- Use of an HIV-1/2 antibody differentiation assay
  - HIV-2 infections are rare in the US
- Use of nucleic acid testing to increase detection of acute infections
  - Only one FDA-approved assay for diagnosis
  - Frequently, VL assays are used as third test
BioPlex® 2200 HIV Ag-Ab Assay (BPC)

- Bio-Rad Laboratories (2015)
- Multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation
  - HIV-1 p24 antigen
  - HIV-1 (group M and O) antibodies
  - HIV-2 antibodies
- An aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute HIV-1 infection in human serum or plasma
- Pediatric subjects ≥ 2 yo and pregnant women
### BPC results

<table>
<thead>
<tr>
<th>Index (IDX)</th>
<th>Retest</th>
<th>Retest Result</th>
<th>Final Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.00 for all analytes</td>
<td>No</td>
<td>Not Applicable</td>
<td>Non- Reactive</td>
</tr>
<tr>
<td>&gt; 1.00 for at least one analyte</td>
<td>Yes</td>
<td>Both retest results have an Index (IDX) &lt; 1.00 for all analytes</td>
<td>Non- Reactive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Index (IDX) of at least one retest result is &gt; 1.00 for the analyte(s) that was initially reactive</td>
<td>Reactive for HIV Ag-Ab with Reactive for HIV-1 Ag and/or Reactive for HIV-1 Ab and/or Reactive for HIV-2 Ab or Reactive, Undifferentiated</td>
</tr>
</tbody>
</table>

- CDC lab evaluation showed BPC performance similar to other FDA-approved lab-based Ag/Ab immunoassays in early HIV-1 infections
Aptima HIV-1 Quant Assay on the Panther system (APT-Quant)

- Hologic (2016)
- CE-IVD marked for HIV diagnosis and monitoring (dual claim)
- High-throughput fully automated testing platform with random access
- Transcription mediated amplification (TMA) and dual target approach (LTR and integrase)
- Reported limit of detection: ~ 13 copies/ml
- Linear range of quantification: 30- $10^7$ copies/ml
In 417 samples from HIV-1 U.S. seroconverters, APT-Quant detected virus in more samples including seronegative phase than Hologic HIV-1 RNA Qualitative (APT-Qual).

- In HIV-1 established infections, both tests performed similarly.
- APT-Quant non-inferior to the FDA-approved diagnostic test.
Objective

To compare the performance of a two-test diagnostic algorithm consisting of screening with a Ag/Ab HIV-1/2 differentiation immunoassay, followed by HIV-1 NAT to the currently recommended three-test algorithm.
HIV samples and analysis

- **Specificity**
  - BPC: 596 HIV-negative samples
  - APT-Quant: 478 Hologic Aptima HIV-1 RNA Qualitative (APT-Qual) nonreactive and HIV-1 antibody negative samples
  - APT-Quant carry over contamination experiment in open platform

- **Comparison of HIV diagnostic algorithms**
  - 46 U.S. seroconverters (subtype B) with 255 longitudinal samples before and 73 after initiation of antiretroviral therapy (ART) and after BPC-seroreactivity
  - 105 Cameroonian ART-naïve established infections
    - 3 HIV-1 Group O and 102 HIV-1 Group M non-B subtypes

- **HIV testing was performed as part of studies with Bio-Rad and Hologic that provided kits**
Specificity

- **Bio-Rad BioPlex 2200 Ag/Ab Combo:** 99.7% [95% CI 98.8-99.9%]

- **Hologic Aptima HIV-1 Quant:** 99.8% [95% CI 98.8- 99.9]
  - Four sequences of nine HIV-negative plasma followed by plasma with $10^7$ HIV-1 RNA cop/ml tested in the Panther system
  - No carry over contamination was observed on the open system
Three-test algorithm results with early stages of HIV-1 infection before ART initiation
Three-test algorithm results with early stages of HIV-1 infection before ART initiation

- BPC detected p24 Ag reactive samples after HIV-1 RNA positivity
### Three-test algorithm results with early stages of HIV-1 infection before ART initiation

<table>
<thead>
<tr>
<th>BioPlex 2200 Ag/Ab assay</th>
<th>Geenius HIV-1/2 assay</th>
<th>APTIMA-Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 Ag</td>
<td>HIV-1 Ab</td>
<td>HIV-2 Ab</td>
</tr>
<tr>
<td>R</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>R</td>
<td>NR</td>
<td>NR</td>
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<td>R</td>
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<td>R</td>
<td>NR</td>
</tr>
<tr>
<td>NR</td>
<td>R</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR: non reactive; R: reactive

- BPC and Geenius agreed on HIV-1 antibody reactivity
- HIV-2 antibody reactivity was not observed with either test
Three-test algorithm results with early stages of HIV-1 infection before ART initiation

- 79 samples from seroconverters were from early stages of HIV-1 infection
- The three-test algorithm detected 76/79 (96.2%)

<table>
<thead>
<tr>
<th>BioPlex 2200 Ag/Ab assay</th>
<th>Geenius HIV-1/2 assay</th>
<th>Final intreparation v1.1</th>
<th>APTIMA-Qualitative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>Ab</td>
<td>Ab</td>
<td>HIV-1 Ag</td>
</tr>
<tr>
<td>R</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>R</td>
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<tr>
<td>NR</td>
<td>R</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR: non reactive; R: reactive
Comparison of the algorithms on early stages of HIV-1 infection before ART initiation

The two-test algorithm detected 78/79 (98.7%)

- VL range: <1.47 to >7 log(cop/ml)
- *2 samples APT-Qual NR were APT-Quant R with VL < 1.47 and 4.89 log (cop/ml)

Similar performance of both algorithms (McNemar’s p=0.4795)
Three-test algorithm results with late stages of HIV-1 infection before ART initiation

<table>
<thead>
<tr>
<th>BioPlex 2200 Ag/Ab assay</th>
<th>Geenius HIV-1/2 assay</th>
<th>Final interpretation v1.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1</td>
<td>HIV-1</td>
<td>HIV-1 Positive</td>
</tr>
<tr>
<td>HIV-2</td>
<td>HIV-2</td>
<td></td>
</tr>
<tr>
<td>Ag Ab Ab</td>
<td>R R NR</td>
<td>20</td>
</tr>
<tr>
<td>NRDHAL R R</td>
<td>HIV-1 Positive</td>
<td>19</td>
</tr>
<tr>
<td>NR R NR</td>
<td>HIV-1 Positive</td>
<td>241</td>
</tr>
<tr>
<td>NR R NR</td>
<td>HIV untypable</td>
<td>1</td>
</tr>
</tbody>
</table>

NR: non reactive; R: reactive; NRDHA: not reportable due to high antibody titer

- 176 samples from seroconverters were Geenius HIV-1 positive
- All Cameroonian established HIV-1 infections were Geenius HIV-1 positive
  - One sample was also Geenius HIV-2 reactive (untypable), but further testing showed no evidence of HIV-2 infection
The two-test algorithm detected 279/281 (99.3%)
- VL range: <1.47 to >7 log(cop/ml)
- 7 samples were APT-Quant R with VL < 1.47 log (cop/ml)
- *2 samples were APT-Quant and APT-Qual NR

Similar performance of both algorithms (McNemar’s p=0.4795)
Three-test algorithm results with HIV-1 infections after ART initiation

- 9 samples from seroconverters showed seroreversion
- Three-test algorithm detected 73/73 (100%)
The two-test algorithm detected 64/73 (87.7%)
- VL range: Target not detected (TND)- 6.9 log (cop/ml)
- 3/9 that seroreverted and 6/64 Geenius HIV-1 positive samples were APT-Quant TND

Three-test algorithm performed better after ART initiation
- McNemar’s $p=0.0077$
Limitations of the study

- NAT was done in singlet
- APT-Qual and APT-Quant were not performed in parallel for a set of ART-naïve seroconversion panels
- Geenius HIV-1/2 differentiation assay was performed using software v1.1 prior the update to address HIV-2 indeterminate results
- Small number of samples from ART-treated persons
Summary results

- The BPC/APT-Quant algorithm performed similar to the BPC/Geenius/APT-Qual in ART-naïve samples at different stages of HIV-1 infection.
- The three-test algorithm performed better than the two-test algorithm in samples with lower viremia due to ART.
- BPC accurately identified early and established HIV-1 infections.
- Despite the limitations, BPC and Geenius v1.1 showed great concordance for HIV-1 antibody differentiation.
Conclusions

- APT-Quant, an automated HIV-1 RNA assay, works well for diagnosis and quantification as a second step in the proposed algorithm in different stages of HIV-1 infection
  - No FDA-approved dual claim assay
- APT-Quant performance decreases after the IgG response is elicited and with suppressed viremia due to ART
  - Use of HIV antibody test after undetectable viral load results
- Confirmation with a dual claim RNA assay is advantageous for patient care
- However, additional factors such as the implications of off-label use and cost associated with the implementation of a second-step quantitative NAT algorithm need to be explored
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Disclaimer

The findings and conclusions in this presentation are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.