Evaluation of the Performance of the Bio-Rad GS HIV Combo Ag/Ab EIA and Bio-Rad Geenius™ HIV-1/2 Supplemental Assay Using Dried Blood Spots as an Alternative Specimen Type

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Background

- FDA-approved Ag/Ab Combo and HIV-1/2 differentiation assays used in the CDC/APHL diagnostic algorithm are not approved for use with dried blood spots (DBS)
- Bio-Rad developed two protocols for DBS with the GS HIV Combo Ag/Ab EIA (BRC) and the Geenius™ HIV-1/2 Supplemental assay (Geenius)

In HIV-1 seroconverters
- BRC DBS assay detected 62.5% compared to 70.8% using the plasma assay and earlier than when using a IgG/IgM EIA
- Geenius DBS assay gave similar results to plasma assay and detected more infections than Bio-Rad Multispot and Western blot

<table>
<thead>
<tr>
<th></th>
<th>Bio-Rad Combo</th>
<th>Bio-Rad Geenius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity HIV-1 n=65</td>
<td>65/65 (100%)</td>
<td>45/45 (100%)</td>
</tr>
<tr>
<td>Sensitivity HIV-2 n=6</td>
<td>6/6 (100%)</td>
<td>6/6 (100%)</td>
</tr>
<tr>
<td>Specificity negative n=95</td>
<td>95/95 (100%)</td>
<td>not tested</td>
</tr>
<tr>
<td>Analytical sensitivity</td>
<td>200-300 p24 pg/ml</td>
<td>not applied</td>
</tr>
</tbody>
</table>
OBJECTIVE

- To further evaluate DBS specimen suitability for use with the developed assays
DBS protocols

A- One 6 mm punch was eluted using 150 µl of additional Working Strength GSHIV-1 Western blot Specimen diluent/Wash buffer (Bio-Rad)

B- O/N incubation at 2-8º C, brought to RT, mixed and used

Bio-Rad GSHIV Combo Ag/Ab EIA with DBS

Step 1:
- Add 25µl Conjugate 1 + 75µl control or eluate to each well
- Cover and incubate 60 ± 5 min. at RT on a shaking platform (625 rpm)

Wash a minimum of 5 times with 30-60 second soaks

Step 2:
- Add 100 µl Working Conjugate 2 to each well
- Cover and incubate 30 ± 5 min. at RT

Wash a minimum of 5 times with 30-60 second soaks

Step 3:
- Add 80 µl Working TMB to each well
- Cover and incubate 30 ± 5 min. at RT
- Add 100 µl Stopping Solution to each well

Read within 30 min. at 450 nm, with the 615-630 nm filter as a reference

Cutoff = Mean of Cutoff Calibrators + 0.150
Sample sets and analysis

- 60 DBS prepared from simulated whole blood from 11 commercial HIV-1 seroconversion panels
  - DBS results were compared to results from matched plasma tested with Bio-Rad GS HIV-1/HIV-2 PLUS O EIA (BR+O) and Geenius

- 105 DBS from persons with established HIV-1 infections stored for 7-8 years at -20°C
  - Reactivity after long-term storage was analyzed

- 348 DBS from persons who inject drugs who were screened with rapid test during HIV surveillance in the US (20 cities)
  - DBS were made from whole blood from an EDTA tube or fingerstick
  - DBS results were compared to HIV diagnosis reported at each site during surveillance
RESULTS
Diagnostic algorithm among HIV-1 seroconverters

- 60 plasma and DBS from commercial seroconversion panels
  - 13 BRC-plasma and BRC-DBS concordant non-reactive
    - 7 HIV-1 RNA negative
    - 6 HIV-1 RNA positive VL = [21 - 1.9 \times 10^4 \text{ copies/ml}]
  - 8 BRC-plasma reactive/BRC-DBS non-reactive discordant
    - HIV-1 RNA positive VL = [3.3 \times 10^3 - 1.8 \times 10^5 \text{ copies/ml}]
    - All Geenius-negative and Aptima-positive in plasma
  - 39 BRC-plasma and BRC-DBS concordant reactive
    - HIV-1 RNA positive VL = [\text{TND-} > 10^7 \text{ copies/ml}]
DBS protocols in 47 BRC-plasma reactive

- BRC-plasma: 47
- BRC-DBS: 39
- BR+O-plasma: 32
- BRC-Geenius plasma: 22
- BRC-Geenius DBS: 15

Significance levels:
- BRC-plasma vs. BRC-DBS: p=0.0133*
- BRC-DBS vs. BR+O-plasma: p=0.0133*
- BRC-plasma vs. BRC-Geenius plasma: p=0.0455*
# Geenius reactivity in plasma and DBS

<table>
<thead>
<tr>
<th>Geenius-plasma</th>
<th>Geenius-DBS</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>negative</td>
<td>12</td>
</tr>
<tr>
<td>negative</td>
<td>HIV-1 indeterminate</td>
<td>1</td>
</tr>
<tr>
<td>HIV-1 indeterminate</td>
<td>negative</td>
<td>3</td>
</tr>
<tr>
<td>HIV-1 indeterminate</td>
<td>HIV-1 positive</td>
<td>1</td>
</tr>
<tr>
<td>HIV-1 positive</td>
<td>negative</td>
<td>4</td>
</tr>
<tr>
<td>HIV-1 positive</td>
<td>HIV-1 indeterminate</td>
<td>4</td>
</tr>
<tr>
<td>HIV-1 positive</td>
<td>HIV-1 positive</td>
<td>14</td>
</tr>
</tbody>
</table>
Reactivity in DBS stored frozen for 7-8 years

- Individuals were OQ-FS whole blood preliminary positive

- Plasma tested with IgG/IgM EIAs, Ag/Ab Combo IA, Supplemental test, NAT and HIV-1 Western blot
  - 105 established HIV-1 infections

- DBS collected 2007-2008 and stored at -20° C until testing
  - All BRC-Reactive
  - All Geenius HIV-1 positive:
    - 41 gp160 gp41
    - 19 gp160 p24 gp41
    - 17 p31 gp160 gp41
    - 27 p31 gp160 p24 gp41
    - 1 gp36 gp160 p24 gp41
DBS and surveillance in 20 cities in the USA

- **Individuals:**
  - Unaware of HIV status get tested with rapid test (FS-whole blood or OF) or IA (EDTA whole blood), confirmation is performed when preliminary positive
  - Self-reported HIV positive may or may not get tested, but confirmation is performed in plasma, DBS or OF (3rd or 4th gen IA, WB, NAT)
  - If consent is given, DBS are collected, dried, stored in bags with desiccants and humidity indicator card, and shipped to CDC at ambient temperature within 10 days of collection

- **HIV diagnosis is performed at each site**
  - Different tests and specimen types are used

- **DBS are stored at -20° C at CDC until testing**
Performance of the HIV diagnostic algorithm with DBS collected during HIV surveillance

<table>
<thead>
<tr>
<th>Reported HIV status/rapid test result/final result</th>
<th>n</th>
<th>BRC</th>
<th>Geenius</th>
<th>Viral load m2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>unaware/negative/HIV-negative</td>
<td>245</td>
<td>242</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>unaware/preliminary positive/HIV-negative</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>unaware/preliminary positive/HIV-1 positive</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>self-reported positive/not done/HIV-1 positive</td>
<td>67</td>
<td>67</td>
<td>66</td>
<td>1</td>
</tr>
</tbody>
</table>

n: number of specimens; NR: non-reactive; R: reactive; TND= Target not detect

a: Western blot-DBS negative, Bio-Rad avidity-DBS 'invalid'

- Initial testing with BRC and Geenius using one 6 mm punch identified:
  - 99.0% of the HIV-1 infections diagnosed at each site (different algorithms)
  - Four BRC-reactive samples (OC/CO:=[1.1-3.8]) were Geenius HIV-negative or had no result among HIV-negative samples, thus NAT will be needed

- Repeat of BRC using a second eluate in duplicate:
  - Three samples were non-reactive
  - One remained reactive OD/CO\(_{\text{initial}}\) =1.1, OD/CO\(_{\text{duplicate}}\) =1.2 (VL TND)
    - Geenius was not repeated
Summary BRC-DBS

- 100% HIV-1 sensitivity (n=127)
- 100% HIV-2 sensitivity (n=6)
- 98.4% specificity (n=245)
  - Initially reactive repeated in duplicate improved specificity to 99.6%
- Analytical sensitivity = 200-300 p24pg/ml
- Detection was not affected by antiretroviral therapy
- BRC detected few more early HIV-1 infections than an IgG/IgM IA with plasma
- BRC worked with specimens stored for years at -20°C
Summary Geenius-DBS

- 95.3% HIV-1 sensitivity (n=106)
- 100% HIV-2 sensitivity (n=6)
- Detection was not affected by antiretroviral therapy
- Geenius worked with specimens stored for years at -20° C
- DBS algorithm detected fewer Geenius HIV-1 positive than plasma algorithm in early HIV-1 infections
- Among BRC-reactive samples, 67% showed concordant results between plasma and DBS
Conclusions

- The DBS algorithm was less sensitive than plasma in early HIV-1 infections, but the BRC-DBS was more sensitive than an IgG/IgM IA with plasma.

- An eluate from one 6 mm punch can be used for both assays.
  - Repeat testing may be needed to increase BRC-DBS specificity.
  - NAT with DBS will be needed to confirm infection.

- The results are promising when applied in a high-risk population.

- Implementation of a DBS diagnostic algorithm would benefit HIV surveillance and individuals reluctant to have blood draws.
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