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

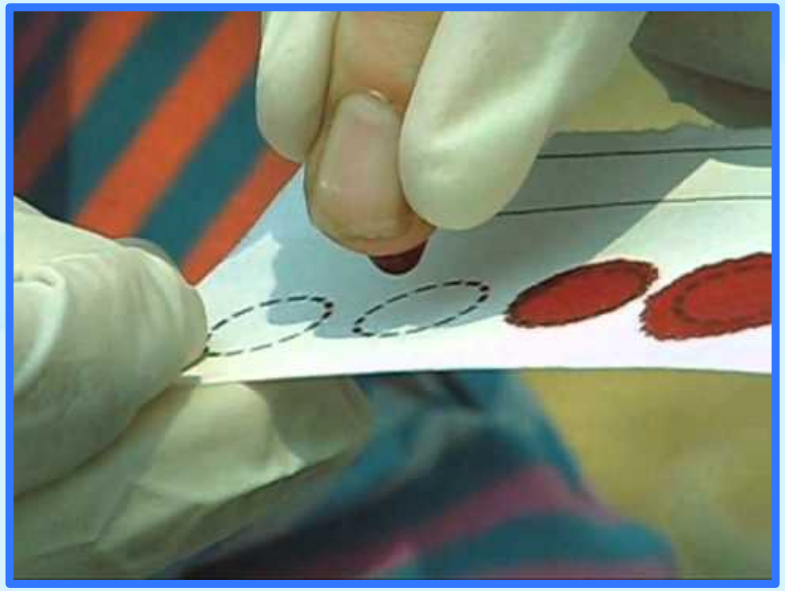
- **FDA-approved Ag/Ab Combo and HIV-1/2 differentiation assays used in the CDC/APHL diagnostic algorithm are not approved for use with dried blood spots (DBS)**
- **Bio-Rad developed two protocols for DBS with the GS HIV Combo Ag/Ab EIA (BRC) and the Geenius™ HIV-1/2 Supplemental assay (Geenius)**

	Bio-Rad Combo	Bio-Rad Geenius
Sensitivity HIV-1 n=65	65/65 (100%)	45/45 (100%)
Sensitivity HIV-2 n=6	6/6 (100%)	6/6 (100%)
Specificity negative n=95	95/95 (100%)	not tested
Analytical sensitivity	200-300 p24 pg/ml	not applied

- **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

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 GS HIV-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 GS HIV 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:

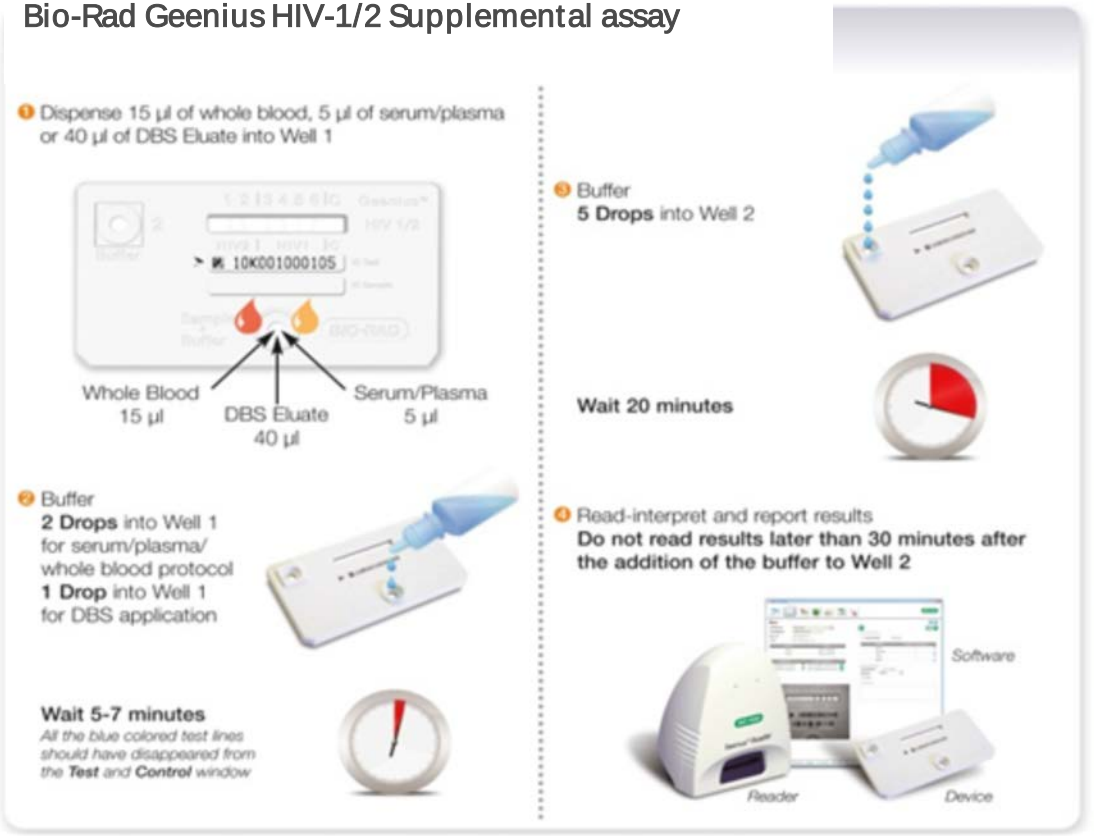
- Add 80 µl Working TMB to each well
- Cover and incubate 30 ± 5 min. at RT

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

Bio-Rad Geenius HIV-1/2 Supplemental assay



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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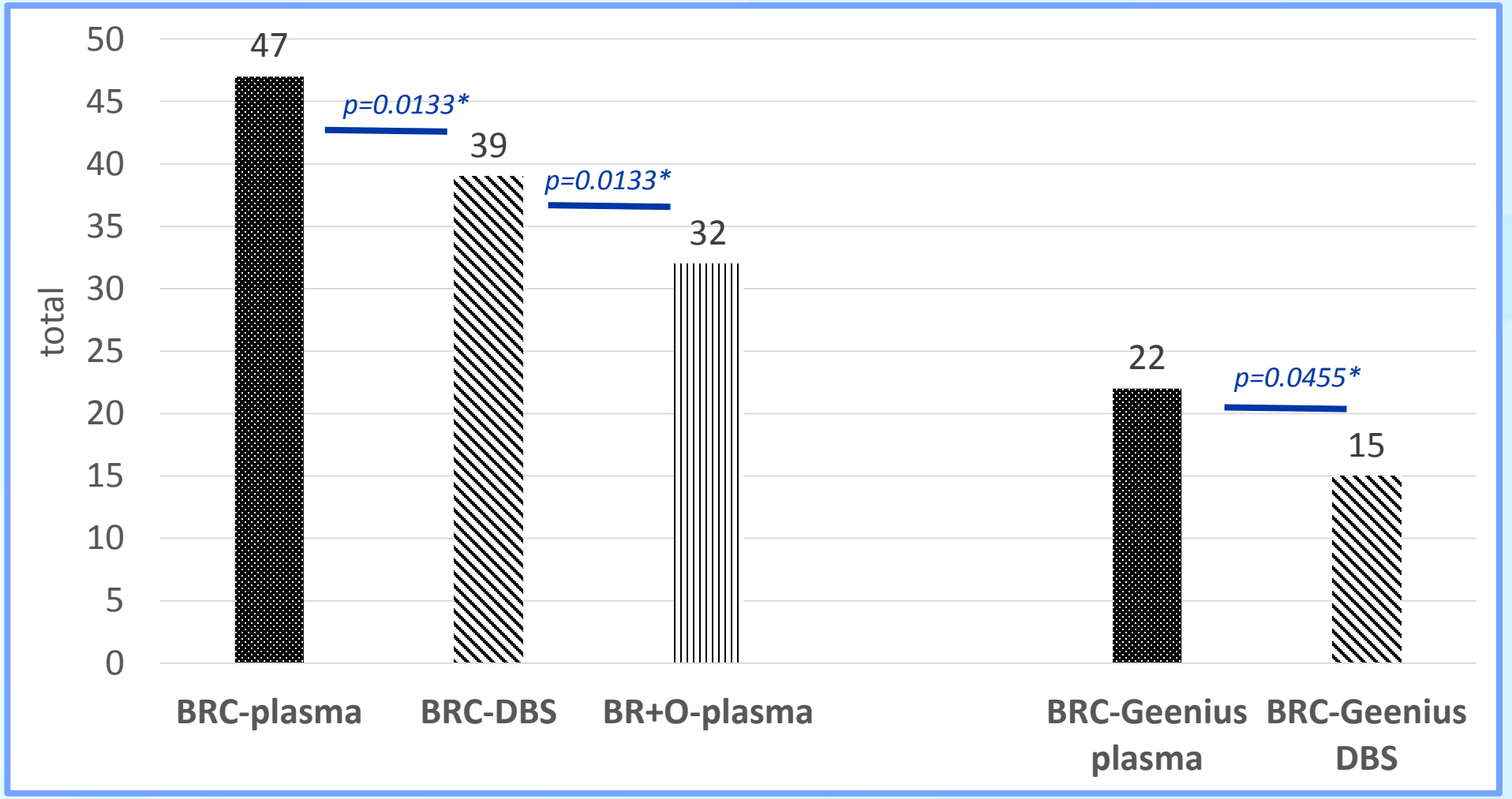
Diagnostic algorithm among HIV-1 seroconverters

- **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- $1.9X10^4$ copies/ml]
  - 8 BRC-plasma reactive/BRC-DBS non-reactive discordant
    - HIV-1 RNA positive VL= [$3.3x10^3$- $1.8x10^5$ copies/ml]
    - All Geenius-negative and Aptima-positive in plasma
  - 39 BRC-plasma and BRC-DBS concordant reactive
    - HIV-1 RNA positive VL=[TND- $>10^7$ copies/ml]

DBS protocols in 47 BRC-plasma reactive



Geenius reactivity in plasma and DBS

39 BRC-plasma R/BRC-DBS R		
Geenius-plasma	**Geenius-DBS**	**total**
negative	negative	12
negative	HIV-1 indeterminate	1
HIV-1 indeterminate	negative	3
HIV-1 indeterminate	HIV-1 positive	1
HIV-1 positive	negative	4
HIV-1 positive	HIV-1 indeterminate	4
HIV-1 positive	HIV-1 positive	14

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:**
  - Unware 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

		First DBS eluate						
		BRC		Geenius				Viral load
Reported HIV status/rapid test result/final result	n	NR	R	negative	HIV-1 positive	invalid	no result	m2000
unaware/negative/HIV-negative	245	242	3	2			1	3 TND
unaware/preliminary positive/HIV-negative	1[a]		1	1				qns
unaware/preliminary positive/HIV-1 positive	35		35		35			
self-reported positive/not done/HIV-1 positive	67		67		66	1		

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 $OD/CO_{initial}$=1.1, $OD/CO_{duplicate}$=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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