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Evaluation of dried blood spot protocols with the Bio-Rad GS HIV Combo Ag/Ab EIA and Geenius™ HIV 1/2 Supplemental Assay

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Abstract

Objective: FDA-approved antigen/antibody combo and HIV-1/2 differentiation supplemental tests do not have claims for dried blood spot (DBS) use. We compared two DBS-modified protocols, the Bio-Rad GS HIV Combo Ag/Ab (BRC) EIA and Geenius™ HIV-1/2 (Geenius) Supplemental Assay, to plasma protocols and evaluated them in the CDC/APHL HIV diagnostic algorithm.

Methods: BRC-DBS p24 analytical sensitivity was calculated from serial dilutions of p24. DBS specimens included 11 HIV-1 seroconverters, 151 HIV-1-positive individuals, including 20 on antiretroviral therapy, 31 HIV-2-positive and one HIV-1/HIV-2-positive individuals. BRC-reactive specimens were tested with Geenius using the same DBS eluate. Matched plasma specimens were tested with BRC, an IgG/IgM immunoassay and Geenius. DBS and plasma results were compared using the McNemar's test. A DBS-algorithm applied to 348 DBS from high-risk individuals who participated in surveillance was compared to HIV status based on local testing algorithms.

Results: BRC-DBS detects p24 at a concentration 18 times higher than in plasma. In seroconverters, BRC-DBS detected more infections than the IgG/IgM immunoassay in plasma ($p = 0.0133$), but fewer infections than BRC-plasma ($p = 0.0133$). In addition, the BRC/Geenius-plasma algorithm identified more HIV-1 infections than the BRC/Geenius-DBS algorithm ($p = 0.0455$). The DBS protocols correctly identified HIV status for established HIV-1 infections, including those on therapy, HIV-2 infections, and surveillance specimens.

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Ethical approval

Specimens used in this study that were unlinked from personal identifiers or from commercial sources were determined by the CDC to be research not involving human subjects. Activities for NHBS were approved by local institutional review boards (IRB) for each participating city, and the protocol was approved by CDC. Samples sent to the CDC HIV Reference Lab for HIV Diagnostics were approved by IRB (protocol #1896).

Competing interests

M. Kathleen Shriver and Geoff Davis are employees of Bio-Rad Laboratories. The other authors have no conflicts of interest to declare.

Conclusions: The DBS protocols exhibited promising performance and allowed rapid supplemental testing. Although the DBS algorithm missed some early infections, it showed similar results when applied to specimens from a high-risk population. Implementation of a DBS algorithm would benefit testing programs without capacity for venipuncture.

Keywords

DBS testing; HIV diagnostics; Surveillance

1. Introduction

In 2014, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) released an updated HIV diagnostic algorithm for laboratory settings. The updated algorithm recommends screening with an HIV antigen/antibody (Ag/Ab) combo assay followed, when reactive, by an HIV-1/2 antibody differentiation supplemental assay. An HIV RNA test is used to resolve negative or indeterminate supplemental results [1–3]. The update considers testing efficiency, turnaround time, and cost with the aim of detecting more individuals in early stages of HIV infection. However, the Food and Drug Administration (FDA)-approved assays that can be used in the algorithm do not have claims for dried blood spot (DBS) testing. This limits the use of expanded HIV testing among people who refuse venipuncture and in settings where processing blood is difficult.

DBS have advantages over serum/plasma collection. They are minimally invasive, less expensive and easier to collect and ship to a central laboratory, they can be stored for a short period of time before shipping at ambient temperature with desiccant and humidity indicator cards [4], and they can be used for several applications. DBS have been successfully implemented for HIV viral load (VL), early infant diagnosis, drug resistance and recency testing [5–17]. However, there are limited studies showing the feasibility of using DBS for HIV diagnosis with current assays [18]. In the U.S., there are two assays approved for use with DBS: the AVIOQ HIV-1 Microelisa System (AVIOQ, Inc., NC) enzyme-linked immunoassay (EIA) for HIV-1 antibody (IgG/IgM/IgA) screening, and the GS HIV-1 Western blot (IgG) for supplemental testing (Bio-Rad Laboratories, CA).

Bio-Rad Laboratories (Redmond, WA) modified two FDA-approved protocols, the GS HIV Combo Ag/Ab EIA (BRC) and the Geenius™ HIV-1/2 supplemental assay (Geenius), for use with DBS [19,20]. A single eluate would facilitate screening and confirmation with less sample volume and reduced turnaround times; however, further evaluation is needed to demonstrate the utility of the proposed algorithm. We characterized the investigational screening assay, compared the performance of the two DBS-modified protocols to plasma protocols and evaluated the performance of DBS-modified protocols in the CDC/APHL diagnostic algorithm using different sample sets.

2. Materials and methods

DBS were made from EDTA-whole blood or simulated whole blood from early and established HIV-1 infections and known HIV-2 infections, or collected during surveillance.

Following the optimized protocols, a 6 mm punch (~11.5 μl whole blood [21]) of each DBS was eluted into an uncoated microwell or titer tube using 150 μl of GS HIV-1 Western Blot Working Strength Specimen Diluent/Wash (Bio-Rad catalog # 32574) overnight at 2–8 ∘C [19,20]. Seventy-five microliters of the DBS eluates were transferred to each well along with 25 μl of conjugate 1. For the DBS protocol, the first incubation was performed for 60 ± 5 min at room temperature on a shaking platform [625 rpm] instead of at 37 ∘C, and the cutoff was decreased from 0.200 to 0.150 plus the mean of the Cutoff Calibrators. Samples were tested in singlet unless indicated.

2.1. Bio-Rad Geenius™ HIV-1/2 Supplemental Assay

For BRC-positive specimens, 40 μl of DBS eluate along with one drop of the Geenius assay buffer (instead of 5 μl and two drops for plasma/serum) was added to the first well of the dual path platform. After 5 min at room temperature, the standard five drops of Geenius assay buffer were added to the second well. The results were read and interpreted after 20 min using the Geenius reader and software.

2.2. Abbott HIV-RNA VL for DBS

An Abbott Open Mode protocol for quantitative HIV-1 RNA VL from DBS was used in selected specimens that required nucleic acid testing for confirmation in the algorithm. The Abbott research use protocol was validated in the laboratory using four 6-mm punches with the Abbott RealTime HIV-1 VL kit (Abbott Molecular Inc., Des Plaines, IL).

3. Plasma testing

Matched plasma/serum were tested with the FDA-approved BRC and Geenius protocols. Selected plasma specimens were also tested with the GS HIV-1/2 Plus O EIA (BR + O; Bio-Rad Laboratories, Redmond, WA), and the COBAS® AmpliPrep/COBAS® TaqMan48 HIV-1 test, v2.0 VL assay (Roche Molecular Systems, Inc., NJ). All protocols were performed using the manufacturer’s instructions.

4. Specimens

4.1. Bio-Rad p24 serial dilutions

Three lyophilized p24 standards (Bio-Rad #72217), supplied by Bio-Rad Laboratories, were re-suspended following the manufacturer’s instructions. p24 was serially diluted in HIV-negative human plasma and each dilution was then mixed at a ratio of 1:1 with HIV-negative washed, group O red blood cells. Seventy-five microliters of the simulated whole blood were spotted onto protein saver Whatman 903 cards (GE Healthcare Life Sciences, Pittsburgh, PA), dried overnight, bagged with desiccants (MULTISORB Technologies, Buffalo, NY) and humidity indicator cards (Delta Adsorbents, Roselle, IL), and stored at −20 ∘C until testing or shipped frozen to the laboratories where testing was performed. Three laboratories tested the three standards at different times with different kit lots. Serial dilutions of the p24 standard were tested with BRC plasma protocol to verify the proper creation of the DBS p24 standards.

4.2. DBS from HIV-1 and HIV-2 infected individuals

HIV-1 DBS specimens included: (a) 105 prepared from EDTA-whole blood from a U.S. study that recruited HIV-positive adults who were not on antiretroviral treatment (ART) at enrollment and later confirmed as HIV-1 WB-positive [22]; (b) 26 de-identified HIV-1 WB-positive samples prepared from EDTA-whole blood sent to the CDC HIV Reference Lab for HIV diagnostics (IRB protocol #1896); and (c) 20 DBS prepared from EDTA-whole blood from HIV-1-infected individuals receiving ART purchased from Tennessee Blood Services (Memphis, TN).

HIV-2 DBS specimens included: (a) 25 from previously characterized HIV-2 plasma specimens from the Ivory Coast [23]. Plasma specimens were thawed and simulated whole blood was prepared as indicated above to prepare DBS; (b) seven prepared from EDTA-whole blood collected from six HIV-2-infected and one HIV-1/2 dually infected individual from the U.S. at The Wadsworth Center, New York State Department of Health.

4.3. Commercial HIV-1 seroconversion panels

Sixty plasma specimens from 11 commercial seroconversion panels (Zeptometrix, Inc., Buffalo, NY and BBI-SeraCare Diagnostics, Mildford, MA) were selected and DBS were prepared from HIV-infected whole blood as before.

4.4. DBS collected during surveillance

DBS were collected using fingerstick whole blood or EDTA-whole blood from 348 consenting persons who inject drugs (PWID) participating in National HIV Behavioral Surveillance (NHBS) in 19 U.S. cities [24]. Participants were screened with a HIV-1/2 rapid test using whole blood or oral fluid (OF). Preliminary positive results or self-reported HIV-positive individuals were confirmed for HIV status using DBS or OF HIV-1 Western blot or following the CDC/APHL HIV diagnostic algorithm using plasma. At each site, DBS were dried ≥4 h, bagged with desiccants and humidity indicators, stored, and shipped at ambient temperature to CDC within 10 days of collection. All DBS were stored frozen (−20 or −80 ○C) for different periods of time (up to 8 years in some cases) in the presence of desiccants until testing.

Specimens used in this study that were unlinked from personal identifiers or from commercial sources were determined by the CDC to be research not involving human subjects. Activities for NHBS were approved by local institutional review boards for each participating city, and the protocol was approved by CDC.

4.5. Analysis

Linear regression analysis was used to calculate the p24 analytical sensitivity in three laboratories using the same p24 DBS panels. DBS from known, antibody-positive specimens (including 20 ART-experienced) were used to calculate the HIV-1/HIV-2 sensitivity of the BRC assay and reactivity of Geenius. Characterization of the BRC-DBS protocol in early stages of seroconversion was evaluated using 60 matched DBS and plasma from commercial seroconversion panels. Matched plasma specimens were tested with the BRC, BR + O, and Geenius. Reactivity with DBS and plasma was analyzed with McNemar’s

test. The performance of the diagnostic algorithm was evaluated using DBS made from seroconversion specimens (early stages of HIV-1 infection) and DBS collected from HIV-positive and HIV-negative participants during surveillance. Results obtained with DBS collected during surveillance were compared to HIV status reached using different local diagnostic algorithms.

5. Results

5.1. p24 analytical sensitivity of BRC with DBS

Results from the testing of one of the p24 DBS panels are plotted in Fig. 1. This linear regression analysis shows that the p24 analytical sensitivity of the BRC-DBS protocol ranged from 207.8 to 293.3 pg/ml compared to 13.22 to 15.89 pg/ml reported with the plasma protocol [25] using different kit lots. Results obtained with other panels fell within the presented range (data not shown).

5.2. Characterization of the two investigational DBS protocols in established infections

The sensitivity of BRC-DBS in 151 HIV-1, 31 HIV-2 and one dual HIV-1/2-positive specimens was 100% (Table 1). Although 20 of 151 HIV-1 positives were exposed to ART for unknown time, ART did not suppress viremia in nine specimens (VL range: 48–73,244 copies/ml). Target was not detected in eight plasma specimens and three had detectable HIV-1 RNA below the measurable level of detection (<40 copies/ml). Geenius results, performed using the same eluate from the HIV-positive specimens, are also described in Table 1. All but one HIV-1-positive infections were correctly identified with Geenius regardless of the duration of storage under proper conditions. One DBS-eluate gave an invalid result, but specimen quantity was insufficient for retesting. HIV-2 infections from the Ivory Coast gave two HIV-untypable (undifferentiated) and one HIV-indeterminate result. The HIV-1/2 dual infection was correctly classified as HIV-untypable. Viral suppression (target not detected or <40 copies/ml) did not change antibody titers enough to affect reactivity in these DBS assays.

5.3. Characterization of the two investigational DBS protocols in early HIV-1 infections

In order to further validate the DBS protocols, performance of the BRC-DBS protocol in early stages of infection was evaluated by comparing results of plasma and simulated DBS from HIV-1 seroconverters. Of 60 DBS, BRC-DBS detected more infections than the IgG/IgM-only immunoassay (BR + O) in plasma (39 vs. 32, $p = 0.0133$), but fewer infections than the BRC-plasma assay (39 vs. 47, $p = 0.0133$). When comparing BRC with plasma and DBS, 13 were concordant negative (seven HIV-1 RNA-negative and six HIV-1 RNA positive VL ranging from 21 to 1.9×10^4 copies/ml); 39 were concordant positive (HIV-1 RNA VL ranged from target not detected to $>10^7$ copies/ml); and eight were positive with plasma but negative with DBS (HIV-1 RNA VL ranged from 3.3×10^3 to 1.8×10^5 copies/ml). The discrepant results between plasma and DBS were all Geenius HIV-negative with plasma. The BRC/Geenius-plasma algorithm identified more HIV-1 infections than BRC/Geenius-DBS algorithm during early HIV infection (22 vs. 15, $p = 0.0455$). When comparing Geenius reactivity with plasma and DBS, of 39 BRC-concordant positive results, 13 (33%) showed discrepant results between plasma and DBS with Geenius (Table 2).

5.4. Performance of the HIV diagnostic algorithm using DBS collected from high-risk individuals during HIV surveillance

Using locally approved diagnostic algorithms, 102 specimens were HIV-1-positive and 246 were HIV-negative. The BRC-DBS assay was reactive for all HIV-1 infections, including 35 specimens from individuals who were unaware of their HIV status but who tested preliminary positive with rapid tests, and later confirmed HIV-1 positive, and all 67 specimens from self-reported HIV-1-positive individuals (Table 3). Of these, Geenius confirmed 101 HIV-1 infections and one specimen was invalid. All 246 HIV-negative specimens were BRC-DBS non-reactive except one specimen preliminarily positive with a rapid test but HIV-1 Western blot-negative and three specimens rapid test negative (Table 3). Of these four BRC-reactive specimens, three were Geenius HIV-negative and one result was lost due to improper procedure. To address false reactivity, BRC-DBS was repeated in duplicate on the four discrepant results and VL was done on three of these. Three were BRC-DBS non-reactive, including the specimen that was preliminarily positive on the rapid test, and one was repeatedly reactive, but the VL showed target not detected (Table 3). Geenius was not repeated because of insufficient specimen quantity.

6. Discussion

Screening with an Ag/Ab combo assay followed by an HIV-1/2 differentiation supplemental assay is preferred to identify HIV infections as indicated in the CDC/APHL Diagnostic Algorithm [2]. However, these assays are not FDA-approved for use with DBS. This is the first study that describes the performance of the modified DBS screening protocol and evaluates a DBS diagnostic algorithm. The FDA-approved Ag/Ab and supplemental assays have good performance with plasma/serum [3,25–34], but few studies describe the performance of DBS with an Ag/Ab assay [35] and Geenius [18]. One study used an Ag/Ab assay not approved in the U.S. and another used one full 75 μl whole blood spot for confirmation. The protocols evaluated in our study used the same eluate from one 6 mm punch (~11.5 μl whole blood [36]) and all reagents are available from one vendor.

Although a single eluate would facilitate screening and confirmation with less sample volume, the initial volume is much lower than the plasma protocol thus decreased sensitivity would be expected. One punch at 40% hematocrit contains ~7 μl plasma and half of the eluate is used in the assay compared to 75 μl of plasma. Previous results showed that the use of more than one punch did not change the sensitivity of the assay [19], thus for sample conservation we used one punch. We show that BRC-DBS is less sensitive at detecting p24 than BRC with plasma/serum [25], but the results suggest that BRC-DBS can detect Ag and/or Ab early in the infection since it performed significantly better than an IgG/IgM only plasma assay in HIV-1 seroconverters. The use of BRC-DBS would be advantageous over Ab only-based assays approved for use with DBS. The HIV-1/HIV-2 sensitivity in known HIV-1 and HIV-2 antibody-positive specimens was comparable to plasma [25,26], even in virally suppressed specimens. Although when testing DBS from high-risk individuals the BRC-DBS did not identify any acute HIV-1 infections that could have been missed by Western blot, the evaluated and locally approved algorithms performed similarly. However, our results show that BRC-initially reactive specimens must be retested in duplicate as

indicated in the plasma protocol to improve specificity. Collection of good blood spots can be challenging, so conservation of sample is key.

Although the input volume for Geenius-DBS is also lower than the plasma protocol (40 μl of 150 μl eluate containing ~2 μl of plasma is instead of ~5 μl of plasma), Geenius identified all HIV-1 infections except one that was not repeated because of insufficient specimen quantity. Geenius has eight possible assay interpretations. The discrepancy observed using plasma and DBS among HIV-1 seroconverters may be from other factors, including specimen preparation, lower Ab titers during early stages of seroconversion challenging the sensitivity of the assay, and decreased sensitivity of the DBS assay from using less input volume. The observed discrepancies between plasma and DBS will increase the need for nucleic acid testing of DBS specimens since most of the changes were from HIV-1-positive in plasma to HIV-negative or HIV-1-indeterminate in DBS. Geenius correctly identified the majority of HIV-1 Ab-positive specimens with few specimens with HIV-untypable or HIV-indeterminate results which require further testing for confirmation as is indicated for plasma specimens.

Limitations of our study include the use of simulated whole blood. Additionally, some specimen sets were frozen under recommended conditions for long-term storage, but stability studies were not performed to evaluate impact of this storage on the testing.

The DBS diagnostic algorithm identified significantly fewer early HIV-1 seroconversion specimens than testing using plasma. BRC-DBS initially-reactive were not confirmed when repeat testing and/or nucleic acid testing were added to the algorithm. As with plasma, nucleic acid testing with DBS is needed to confirm discrepant results and identify infections in very early stages of seroconversion or to differentiate HIV-1 from HIV-2 infections. If the DBS screening test is non-reactive but a recent HIV exposure is suspected, then CDC guidelines for testing plasma specimens should be followed [2].

Our study highlights results from two promising DBS protocols for HIV testing. The implementation of a DBS HIV diagnostic algorithm would benefit testing programs with limited or no access to venipuncture or for testing of persons with trypanophobia.

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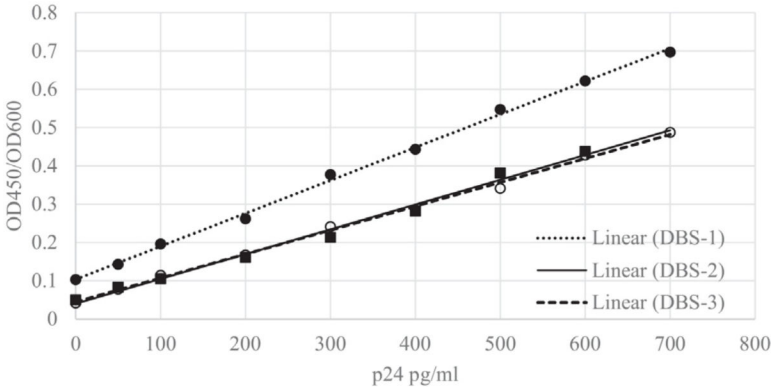

Fig. 1.

p24 analytical sensitivity of the Bio-Rad Combo investigational DBS protocol.

Run	Linear equation for each run	Endpoint p24 concentration (pg/ml)
DBS-1 (lot 1)	$0.291 = 0.0009x + 0.1039$	207.8
DBS-2 (lot 2)	$0.196 = 0.0006x + 0.0403$	259.5
DBS-3 (lot-2)	$0.221 = 0.0006x + 0.045$	293.3

Table 1

Reactivity with the two investigational DBS protocols for HIV-1 and HIV-2 specimens.

		GS HIV-1/2 Ag/Ab combo		Geenius HIV-1/2 supplemental					
HIV infections	**Total**	**Reactive**	**% [95% confidence interval]**	**HIV-1 positive**	**HIV-2 positive**	**HIV-2 positive w/XR-1**	**HIV untypable**	**HIV indeter-minate**	**Invalid**
HIV-1 established*	131	131	100 [97.15–100]	130					1**
HIV-2 established									
From USA	6	6	100 [88.97–100]		2	4			
From Ivory Coast	25	25			7	15	2	1	
HIV-1/2 dual	1	1	100 [20.65–100]				1		

HIV-2 pos w/XR-1: HIV-2 positive with HIV-1 cross-reactivity.

*Twenty specimens were from individuals who received antiretroviral therapy for unknown time where nine were not virally suppressed.

**Invalid result was not repeated because quantity was insufficient.

Table 2

Geenius reactivity with plasma and DBS specimens from HIV-1 seroconverters that were Bio-Rad Ag/Ab Combo-reactive in plasma and DBS.

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

Table 3

Performance of the HIV diagnostic algorithm using DBS collected during HIV surveillance.

		First DBS eluate							Second DBS eluate	
		BRC		Geenius				DBS VL	BRC-repeat	
Reported HIV status/rapid test result/HIV status	***n***	**NR**	**R**	**Negative**	**HIV-1 positive**	**Invalid**	**No result**		**NR**	**RR**
Unaware/negative/HIV-negative	245	242	3	2			1[b]	3 TND	2[a]	1[a]
Unaware/preliminary positive/HIV-negative	1[a]		1	1				QNS	1	
Unaware/preliminary positive/HIV-1-positive	35		35		35					
Self-reported positive/not done/HIV-1-positive	67		67		66	1[b]				
Total	348	242	106	3	101	1	1	3	3	1

Participants testing positive but who did not report a previous positive test were considered unaware of their status. HIV status was determined at each site using locally approved diagnostic algorithm (FDA-approved HIV tests). DBS, oral fluids (OF), or EDTA-whole blood were collected for HIV confirmation when preliminary positive results were obtained or when the participant self-reported HIV status and rapid tests was not conducted. HIV confirmation was performed using HIV-1 Western blot with DBS or OF (without nucleic acid testing) or following the CDC/APHL diagnostic algorithm using plasma (1 site). BRC, Bio-Rad GS HIV Ag/Ab combo EIA; DBS VL, Abbott m2000 HIV-1 viral load DBS assay; *n*, number of specimens; NR, non-reactive; R, reactive; TND, target not detected; QNS, quantity not sufficient.

[a]HIV-1 Western blot-negative.

[b]Insufficient quantity for repeat testing.