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Short Communication: Dried Blood Spots Stored at Room Temperature Should Not Be Used for HIV Incidence Testing

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Abstract

The limiting antigen (LAg)-avidity assay is a serologic assay used for cross-sectional HIV incidence testing. We compared the results obtained with the LAg-avidity assay using dried blood spot (DBS) samples stored at room temperature (18°C–25°C) or stored frozen at −80°C with results obtained from matched plasma samples. Matched DBS and plasma samples (306 paired samples) were collected in the HIV Prevention Trials Network (HPTN) 068 trial in South Africa (2012–2014). The DBS were stored at room temperature before testing. Matched DBS and plasma samples (100 paired samples) from the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA) were collected in 2016 and were stored at −80°C. All DBS testing was performed in 2017. Differences in normalized optical density (ODn) were compared between matched DBS and plasma samples. For DBS samples stored at room temperature (HPTN 068), the average difference in ODn values for plasma versus DBS was 1.49 (95% confidence intervals [CI]: 1.36–1.62). In contrast, when DBS samples were stored at −80°C (CEPHIA), the average difference in ODn values for plasma versus DBS was −0.22 (95% CI: −0.32 to −0.13). DBS samples stored at room temperature should not be used for cross-sectional HIV incidence testing with the LAg-avidity assay.

Keywords: dried blood spot, incidence testing, sample storage

Estimating population-level HIV incidence is important for monitoring the HIV epidemic and understanding the reach and impact of community-level interventions for HIV prevention.[1,2] However, measuring HIV incidence is challenging. Currently, the gold standard for measuring HIV incidence is through longitudinal cohort studies, which are expensive and associated with selection bias. A more feasible approach for estimating HIV incidence is to use biomarkers to analyze samples from a cross-sectional survey.[3] The current algorithm used by the U.S. Centers for

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Disease Control and Prevention (CDC) includes the limiting antigen (LAg)-avidity assay (cutoff: normalized optical density [ODn] < 1.5) and HIV viral load (cutoff: >1,000 copies/mL).

Use of dried blood spots (DBS) may increase the feasibility of cross-sectional incidence testing, particularly for field research in resource-limited settings. Collection of DBS is less invasive than collecting plasma, less time intensive since limited processing is required, and can be easily performed in low-resource settings. However, information on the performance of the LAg-avidity assay using DBS is limited. A previous study showed that the LAg-avidity assay results obtained using plasma and DBS were highly correlated when DBS were stored at −80°C.[4] However, little has been documented on how alternate storage conditions affect DBS results using antibody-based assays for HIV incidence testing.[5,6] In this study, we compared the results obtained with the LAg-avidity assay, using DBS stored at room temperature and DBS stored frozen at −80°C. Samples were obtained from two sources: the HIV Prevention Trials Network (HPTN) 068 Study[7] and the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA).[4]

The first sample set included 306 matched plasma and DBS samples from 150 participants with known duration of infection enrolled in HPTN 068. These samples were prepared on Whatman® 903 Protein Saver Cards. Samples were collected per manufacturer's protocol, initially allowed to air dry overnight, and then moved to a sealed bag with desiccant. The samples were collected in South Africa from young women aged 13–20 years between 2012 and 2014. Plasma samples were stored at −80°C; DBS samples were stored at room temperature in closed plastic bags with desiccant.[8] The second sample set included 100 matched plasma and DBS samples from 100 participants who provided samples for CEPHIA. DBS samples from CEPHIA were prepared on

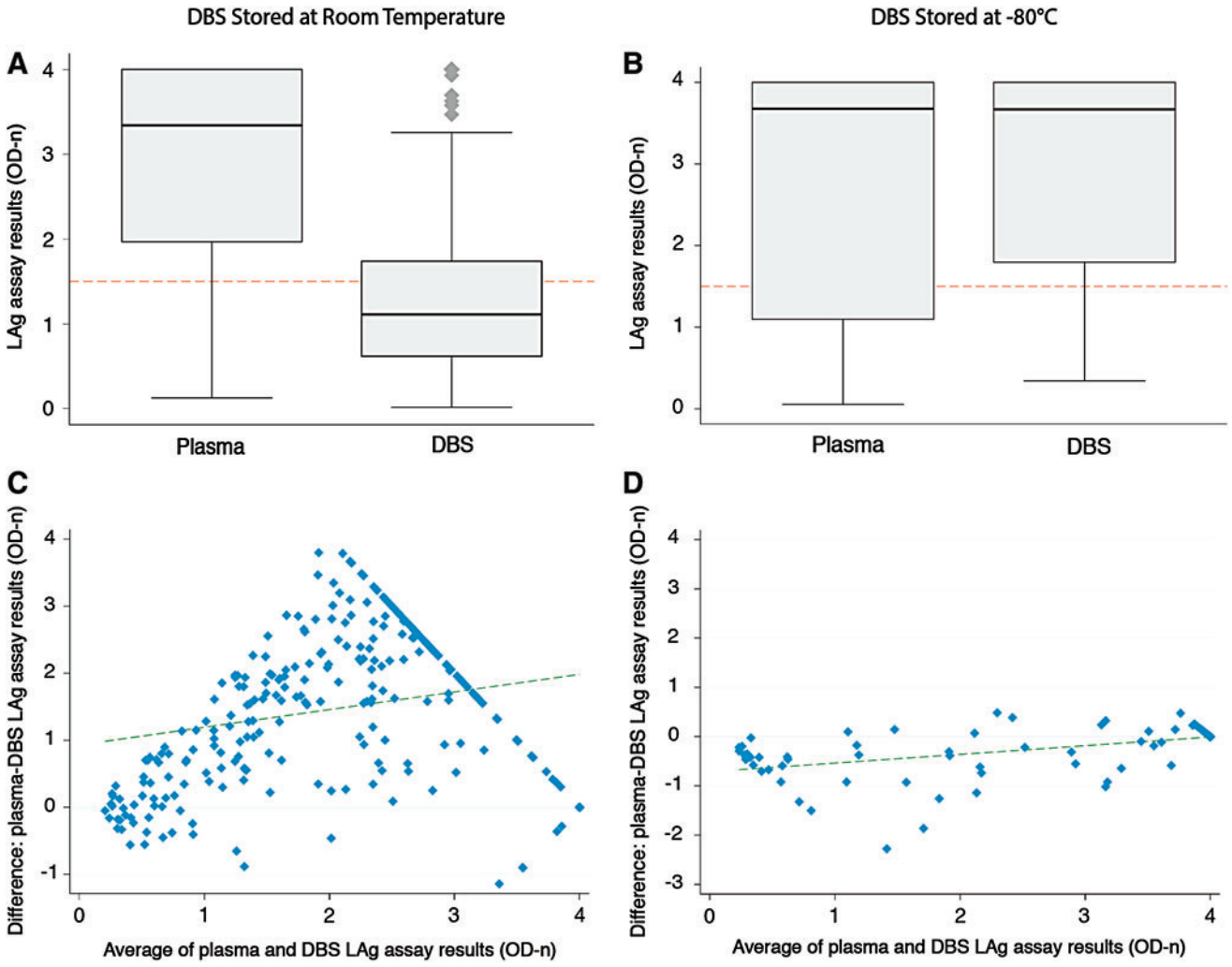

FIG. 1. Comparison of limiting antigen (LAg)-avidity results of paired plasma and dried blood spot samples stored in different conditions. Comparison of LAg-avidity assay results from paired plasma and dried blood spots samples. **(A)** The distribution of paired plasma and DBS LAg-avidity ODn values where the DBS were stored at room temperature. **(B)** The distribution of paired plasma and DBS LAg-avidity ODn values where the DBS were stored at −80°C. **(C)** Bland–Altman plot comparing the difference in plasma minus DBS LAg-avidity ODn results over the average of those values where the DBS samples were stored at room temperature. **(D)** Bland–Altman plot comparing the difference in plasma minus DBS LAg-avidity ODn results over the average of those values where the DBS samples were stored at −80°C. DBS, dried blood spots; LAg-avidity, limiting antigen avidity; ODn, normalized optical density. Color images available online at www.liebertpub.com/aid

Whatman 903 Protein Saver Cards at different testing sites; 75 samples were from a site in the United States and 25 samples were from two sites in Brazil. Plasma samples were stored at −80°C; DBS samples were initially stored at −20°C and later moved for long-term storage at −80°C in closed plastic bags with desiccant.

For testing, 6 mm punches were taken from each sample using the BSD600 PLUS sample puncher (Microelectronic Systems, Australia). The puncher dispenses the disk directly into the designated well; after each sample, two 6 mm blank punches were made to reduce the chance of contamination between sample punches. Punches were eluted in 500 μl of sample diluent provided with the Maxim HIV-1 LAg-Avidity Incidence DBS EIA (Rockville, MD), overnight at 4°C. All DBS samples were tested in 2017.

We compared the LAg-avidity assay ODn values for paired plasma and DBS specimens using descriptive statistics and a two-sample paired signed-rank test for continuous results. In addition, differences between continuous results were observed using Bland–Altman plots. Differences in binary outcomes were determined using Fisher's exact test. p-Values <.05 were considered statistically significant. All analyses were performed using STATA SE, version 14.2 (StataCorp, College Station, TX).

Among the matched plasma and DBS samples from HPTN 068, the ODn values were significantly lower for DBS stored at 25°C (median 1.11 [interquartile range (IQR): 0.62–1.74]) than for plasma stored at −80°C (median 3.34 [IQR: 1.97–4.00]; $p < .001$; Fig. 1A). Figure 1B shows the differences in ODn values for plasma versus DBS using a Bland–Altman plot. The average difference in ODn values for plasma versus DBS was 1.49 (95% confidence intervals [CI]: 1.36–1.62). Furthermore, as the average ODn for DBS and plasma increased, there was an increase in the difference between the ODn values for the two sample types.

In contrast, among the matched CEPHIA samples, the ODn values were not significantly different for DBS stored at −80°C (median 3.67 [IQR: 1.80–4.00]) compared to plasma stored at −80°C (median, 3.68 [IQR: 1.10–4.00]; $p < .09$; Fig. 1C). Figure 1D shows the difference between plasma and DBS results as the average plasma ODn increased, using Bland–Altman plots. The average difference in ODn values for plasma versus DBS was −0.22 (95% CI: −0.32 to −0.13). For these samples, minor differences in the range in ODn values were observed. These slight differences are consistent with the variance of the assay on replicate, and are unlikely to impact studies using DBS estimate incidence or compare interventions.

These findings suggest DBS used for testing with the LAg-avidity assay should not be stored at room temperature. DBS samples stored at room temperature had lower ODn values, indicating a decrease in antibody avidity. Samples with low antibody may more likely be misclassified as being from individuals with recent HIV infection, leading to overestimation of HIV incidence. For the HPTN 068 study samples, there were 31 samples from individuals known to be infected >1 year and a viral load >1,000 copies/mL. None of these individuals were misclassified when using plasma (0/31), whereas half the DBS samples were misclassified (17/31), $p < .01$. In contrast, for the CEPHIA samples, there were 34 such samples and the same unique patient time point misclassified for both plasma and DBS.

A key limitation of this study is the lack of repeat testing of DBS at various temperatures. Thus, additional studies are needed to determine how the duration of DBS storage at room temperature impacts LAg-avidity assay results. Future studies could include a time course experiment where the same sample is stored and evaluated at −80°C and then stored at room temperature. Systematic testing in time intervals after initiating the room temperature storage will determine the length of time that DBS samples could be stored at room temperature before LAg-avidity assay results start to decay. Additional studies are also needed to determine whether results observed in this study are also observed with other serologic incidence assays.[9]

Ultimately, we recommend storing DBS for HIV incidence testing at −80°C; this is consistent with guidelines from the Criteria and Laboratory Standards Institute (CLSI), which recommend storing DBS at −80°C for other types of HIV testing,[10] including viral loads, an assay needed for the interpretation of LAg and other testing algorithms.

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Author Disclosure Statement

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