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Technical Note

Validation of the use of dried blood spots in a chikungunya virus IgG serological assay

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ABSTRACT

Dried blood spot (DBS) sampling is a simple, fast, and minimally invasive blood collection method that is particularly useful for diagnostic or epidemiological studies in hard-to-reach populations. Nevertheless, the use of DBS in assays that have been optimized with gold-standard samples (serum or plasma) must be optimized to yield reliable results. Here, we describe the validation of DBS in a commercial assay to measure IgG against chikungunya virus (CHIKV IgG ELISA; Euroimmun, Lübeck, Germany). During a health survey of people experiencing homelessness in Salvador, Brazil, between September 2021 and February 2022, a subset (75/523; 14.3%) of the study participants had paired capillary (for DBS preparation) and venous (for serum separation) blood samples collected. A pilot optimization test was initially performed with 17 paired samples to compare the CHIKV IgG ELISA absorbance values between serum and three different dilutions of DBS. Based on the preliminary results, the best DBS dilution was selected for a final evaluation comparing paired serum and DBS samples from 58 participants. The sensitivity and specificity of the CHIKV ELISA of DBS compared to sera were 100% (95% C.I.: 85.8–100%) and 100% (95% C.I.: 93–100%), respectively. In the linear regression analysis, a coefficient of determination (R^2) value of 0.98 indicated the excellent performance of DBS in predicting the serum levels of IgG CHIKV antibodies. Our findings suggest that DBS at an optimized dilution is reliable for investigating the prevalence of CHIKV IgG antibodies during population surveys in the commercial assay tested here.

1. Introduction

Dried blood spot (DBS) sampling is a simple, fast, and minimally invasive sample collection method that does not require trained phlebotomists or a cold chain for sample transportation (Malsagova et al., 2020; Tuaillon et al., 2020). These features make DBS sampling particularly useful for assaying newborns and hard-to-reach, large, or vulnerable populations for diagnostic or epidemiological (serosurveys) purposes (Malsagova et al., 2020; Tuaillon et al., 2020). The method involves collecting capillary blood through a fingerprick (or a heel-prick in newborns), making a small puncture using a lancet, and applying the blood drops to appropriate blotting/filter papers (Tuaillon et al., 2020). The filter paper cards with blood drops are then allowed to dry and can be stored at the desired temperature until further use.

Commercial serology assays are typically validated using gold-standard samples such as serum or plasma. Therefore, the use of DBS samples in these assays requires optimization. Several factors can influence the outcome of an assay using DBS, including the specific type of filter paper used for sample collection (Malsagova et al., 2020), and the volume and type of buffer used to elute samples from DBS (Tuaillon

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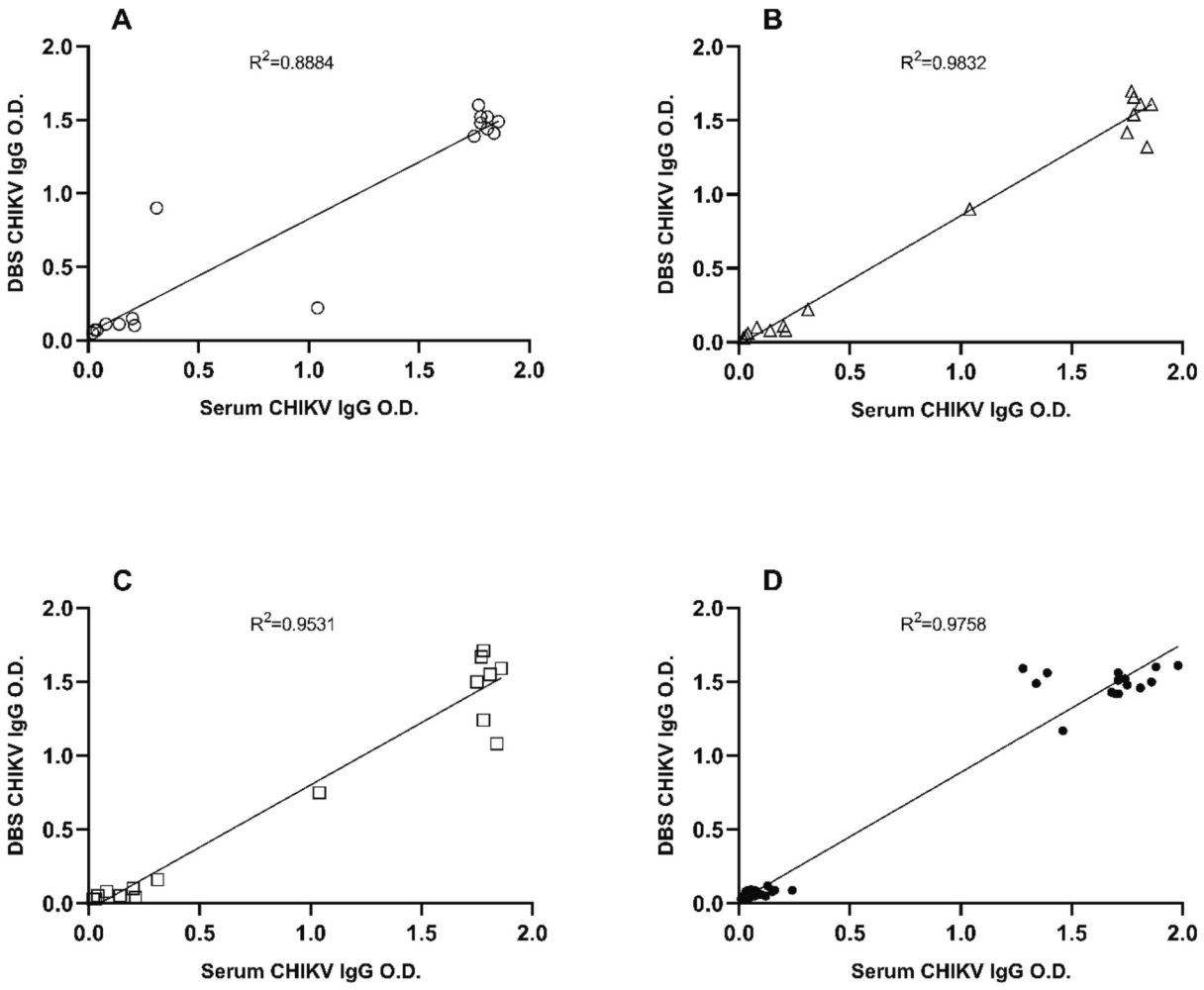

Fig. 1. Linear regression analysis with absorbance values of CHIKV IgG ELISA paired serum and dried blood spot (DBS) samples. In A-C, three different dilutions of DBS were tested using 17 paired samples, where one 3-mm disc of DBS was eluted in 100 (A), 150 (B) or 200 (C) uL of dilution buffer. In D, a final test was performed with 58 paired samples to compare the O.D. of the best DBS dilution from the pilot test (one 3 mm-disc in 150 uL of dilution buffer) with the O.D. of serum. O.D.: Optical density.

et al., 2020).

Here, we describe the optimization and validation of DBS in a commercial chikungunya virus (CHIKV) IgG ELISA, using samples collected from a vulnerable and hard-to-reach population. Although DBS has been employed for the assessment of previous exposure to CHIKV through serology (Moss et al., 2018), a standardized protocol for detecting CHIKV antibodies in population-based serological surveys is not available.

2. Methods

2.1. Study population and sample collection

The participants were people experiencing homelessness (PEH) in Salvador, Brazil, enrolled between September 2021 and February 2022 as part of a collaborative survey conducted by the Federal University of Bahia, the Oswaldo Cruz Foundation, and the Department of Health of Salvador. The study aimed to characterize sociodemographic features (such as access to social assistance and healthcare) and various health conditions (including mental, sexual, reproductive, and work-related health aspects, as well as chronic and infectious illnesses) of this vulnerable population. The study protocol was approved by the Institutional Review Board (IRB) under protocol No. (CAAE) 42517021.0.0000.0040 and all participants signed an Informed Consent Form before data and sample collection.

For the present study, blood samples collected from a subset (75/523; 14.3%) of the surveyed population were used in the DBS validation experiments. From each of the 75 participants, 10 mL of blood sample was collected by venipuncture into tubes with no anticoagulant. After being transported to the laboratory of Pathology and Molecular Biology at Gonçalo Moniz Institute, Oswaldo Cruz Foundation, tubes were centrifuged at 3000 x*g*. for 15 min for serum separation. Regarding the DBS samples, capillary blood drops were obtained from the same 75 participants by fingerprick, using a sterile lancet. Eight drops of blood per participant were applied to a Whatman® 903 Proteinsaver Card (Sigma-Aldrich, St. Louis, MO, USA). Each blood drop, representing nearly 75 µL, formed a circle of approximately 12 mm in diameter in the card, which was labeled with the participant's unique code. Cards were taken to the laboratory and allowed to dry for at least 4 h at room temperature before being put into individual plastic bags containing desiccant sachets. Serum samples and DBS cards were stored at -20 °C, and optimization assays were performed within <6 months of sample collection.

2.2. Comparison of CHIKV IgG ELISA results with paired serum and DBS samples

The use of DBS to reliably quantify CHIKV IgG in a commercial

Table 1
Ratio of paired chikungunya virus (CHIKV) IgG ELISA absorbance values of serum versus dried blood spot (DBS) samples among participants who tested positive.

	O.D. Serum	O.D. DBS Dilution 1	O.D. DBS Dilution 2	O.D. DBS Dilution 3		O.D. Ratio of Serum/DBS Dilution 1	O.D. Ratio of Serum/DBS Dilution 2	O.D. Ratio of Serum/DBS Dilution 3
Pilot test	1.81	1.52	1.61	1.55		1.19	1.12	1.17
	1.81	1.44	1.61	1.55		1.26	1.12	1.17
	1.78	1.48	1.66	1.71		1.20	1.07	1.04
	1.77	1.6	1.70	1.67		1.11	1.04	1.06
	1.75	1.39	1.42	1.5		1.26	1.23	1.17
	1.04	0.22	0.9	0.75		4.73	1.16	1.39
	1.78	1.52	1.54	1.24		1.17	1.16	1.44
	1.84	1.41	1.32	1.08		1.30	1.39	1.70
	1.86	1.49	1.61	1.59		1.25	1.16	1.17
					Mean/S.D.	**1.41/1.17**	**1.16/0.10**	**1.24/0.21**
Final test	1.71	–	1.51	–		–	1.13	–
	1.88	–	1.60	–		–	1.18	–
	1.34	–	1.49	–		–	0.90	–
	1.39	–	1.56	–		–	0.89	–
	1.28	–	1.59	–		–	0.81	–
	1.71	–	1.56	–		–	1.10	–
	1.86	–	1.50	–		–	1.24	–
	1.75	–	1.48	–		–	1.18	–
	1.70	–	1.42	–		–	1.20	–
	1.74	–	1.52	–		–	1.14	–
	1.71	–	1.42	–		–	1.20	–
	1.81	–	1.46	–		–	1.24	–
	1.68	–	1.43	–		–	1.17	–
	1.46	–	1.17	–		–	1.25	–
	1.98	–	1.61	–		–	1.23	–
					Mean/S.D.		**1.18/0.14**	

O.D.: optical density. DBS: dried blood spots. S.D.: standard deviation. Dilution 1: one 3-mm disc eluted in 100 ul of dilution buffer; dilution 2: one 3-mm disc eluted in 150 ul of dilution buffer; Dilution 3: one 3-mm disc eluted in 200 ul of dilution buffer.

CHIKV IgG ELISA (Cat. # EI 293a-9601 G, Euroimmun, Lübeck, Germany) was assessed by comparing DBS with the gold-standard serum samples. First, an ELISA was performed with serum samples from 30 participants to determine their CHIKV serostatus (positive or negative). Then, a pilot test was run with paired serum and DBS samples of 17 (8 negative and 9 positive for CHIKV IgG) of the 30 pre-tested participants to compare the results from serum and three different DBS dilutions and determine the best DBS dilution. One of the tested dilutions (dilution 1, described below) was selected based on the Euroimmun protocol optimized with PerkinElmer-226 filter paper DBS (Euroimmun, n.d.).

Dilutions of DBS were prepared using 3-mm discs (cut with a 3-mm puncher) and different volumes of the sample dilution buffer included in the Euroimmun kit, as following: two 3 mm-discs in 200 uL (dilution 1; equivalent to one 3 mm-disc in 100 uL); one 3 mm-disc in 150 uL (dilution 2); and one 3 mm-disc in 200 uL (dilution 3). The first dilution used two 3 mm-discs in 200 uL instead of one disc in 100 uL to yield sufficient sample volume for the ELISA test. After adding the elution buffer, DBS were allowed to elute for 1 h on a shaker (approximately 400 RPM/min) at room temperature. The elution process was carried out in uncoated 96-well plates.

CHIKV IgG ELISAs were performed immediately after DBS elution (i.e., the eluted samples were not stored before usage), using 100 uL/well of the eluted DBS from the 17 participants in parallel with their paired serum samples (100 uL/well of 1:101 diluted serum), following the manufacturer's instructions. The assays' reference values were determined by comparing the extinction coefficient of the sample to that of the calibrator. According to the manufacturer's instructions, samples with a ratio value <0.8 were defined as negative, those with a ratio between 0.8 and < 1.1 as indeterminate, and those with a ratio ≥ 1.1 as positive. Samples with indeterminate results were retested following our standard laboratory procedures, and the second result was considered definitive.

Linear regression analysis was performed with paired raw absorbance (optical density-O.D.) values obtained from serum and each dilution of DBS samples. For positive samples, the O.D. ratio of serum versus each dilution of DBS was also calculated to assess which DBS dilution resulted in values more similar to those obtained with serum samples; in this case a serum/DBS O.D. ratio closest to 1 was considered the best.

Based on the pilot test results, the optimal DBS dilution was chosen for a final evaluation with samples from the remaining 58 participants. For this, paired serum and DBS samples were utilized in the CHIKV IgG ELISA, using only the optimal dilution for the DBS samples. Like in the pilot test, a linear regression analysis was conducted using the O.D. values obtained from both serum and DBS samples, and the serum/DBS O.D. ratio was calculated for positive samples.

To determine the sensitivity and specificity of the CHIKV IgG ELISA using DBS in comparison to sera, the results from the pilot test (17 samples) and the final test (58 samples) with dilution 2 were combined.

GraphPad Prism was used for the linear regression analysis and Kappa coefficient calculation, whereas STATA was used to calculate the sensitivity and specificity, along with their respective confidence intervals.

3. Results

The pilot test regression analysis indicated that the best results were obtained with dilution 2 ($R^2 = 0.9832$), whereas the least optimal results were obtained with dilution 1 ($R^2 = 0.8884$) (Fig. 1A-C). Consistent with this finding, dilution 2 was the one with the mean ratio of serum/DBS O.D. values closest to 1 and with the lower standard deviation (SD) for the mean (Table 1).

The final evaluation was performed with dilution 2 only (one 3-mm disc in 150 uL of dilution buffer). The regression analysis with 58 paired serum and DBS samples resulted in an $R^2 = 0.9789$ (Fig. 1D), indicating an excellent performance of the DBS samples in dilution 2 in predicting

Table 2
Number and percentage of samples with negative and positive results in the chikungunya virus (CHIKV) IgG ELISA using paired serum and dried blood spot (DBS).

		Serum result		
		Positive N (%)	Negative N (%)	Total
DBS result	Positive	24 (32)	0 (0)	24
	Negative	0 (0)	51 (68)	51
	Total	24	51	75

the O.D. values from the paired serum samples. The absorbance ratios of serum/DBS are shown in Table 1. The mean ratio of serum/DBS O.D. was 1.18.

The number of samples testing negative and positive in the CHIKV IgG ELISA when using serum and DBS in dilution 2 is shown in Table 2. Of note, one serum sample that tested indeterminate at first, tested negative in the repetition; thus, no samples were considered indeterminate in the final results. The sensitivity and specificity of the ELISA using DBS compared to sera were 100% (95% C.I: 85.8–100%) and 100% (95% C.I.: 93–100%), respectively. The agreement of the CHIKV IgG ELISA performed on the serum and the DBS was 100% (Kappa = 1.0; 95% C.I.: 1.0–1.0).

4. Discussion

While information for detecting antibodies against non-arbovirus pathogens in DBS is more abundant, details on the methodology of the use of DBS to detect arbovirus IgG are very scarce in the literature. Moreover, the available methodologies for eluting antibodies from DBS vary in terms of the relative volume (in regard to the disc size) and type of elution solution (Moss et al., 2018; Anders et al., 2012; Balmaseda et al., 2008; Daag et al., 2021; Maldonado-Rodriguez et al., 2017; Matheus et al., 2008; Tran et al., 2006), and, in several cases, at least one of these key pieces of information is missing. Although the number of samples tested here was relatively small, our findings were robust, ensuring that the DBS elution strategy led to reliable results when compared with the gold-standard serum sample. It is important to note that the dilution leading to the best results (dilution 2) differed from the one (dilution 1) recommended by Euroimmun. The available Euroimmun's protocol was based on optimizations using DBS in PerkinElmer-226 filter paper (Euroimmun, n.d.), which is different than the paper used in the present study. The validation of DBS sampling to measure CHIKV IgG through the Euroimmun ELISA will allow us to reliably assess the prevalence of CHIKV IgG in DBS samples from the entire PEH population. Furthermore, it will provide support for the utilization of DBS in other seroprevalence studies aimed at investigating previous exposure to CHIKV.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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