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Short communication

Correlation of dried blood spots and plasma for quantification of Immunoglobulin (IgG) against Receptor binding domain and full length spike protein of SARS-CoV-2

Gaurav Kwatra^{a,b,c,*}, Marta Nunes^{a,b}, Nisha Dhar^{a,b}, Vicky Baillie^{a,b}, Natali Serafin^{a,b}, Stephanie Jones^{a,b}, Shabir A. Madhi^{a,b}

^a South African Medical Research Council: Vaccines and Infectious Diseases Analytics Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

^b Department of Science and Technology/ National Research Foundation: Vaccine Preventable Diseases, University of the Witwatersrand, Faculty of Health Science, Johannesburg, South Africa

^c Department of Clinical Microbiology, Christian Medical College, Vellore, India



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ABSTRACT

We compared plasma and dried blood spots for detection of SARS-CoV-2 IgG antibodies. There was a good correlation between IgG values measured by both sampling methods, $r = 0.935$ and 0.965 for Receptor Binding Domain and full-length spike protein of SARS-CoV-2. Bland-Altman assessment showed good agreement between two sampling methods. Dried blood spots is a more pragmatic method for collecting samples for sero-epidemiological surveys of SARS-CoV-2 infection.

Undertaking venesection to obtain blood samples to perform community based sero-surveys, including to quantify the force of infection by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) in different communities is resource intensive and requires skilled staff in phlebotomy. Furthermore, other logistical constraints to obtaining whole blood samples for sero-surveys include the need for transporting under controlled temperature from the time of venesection to the laboratory, processing of blood for serum/plasma separation and storage in the laboratory (Ostler et al., 2014). Dried blood spot (DBS) samples on filter cards has been used for sero-surveys of measles antibody, and also sero-surveys and diagnosis of HIV, hepatitis B and hepatitis C (Soulier et al., 2016; Tuailon et al., 2020; Uzicanin et al., 2011). In this study we evaluated the correlation and agreement of IgG mean fluorescent signal (MFI) against the Receptor Binding Domain (RBD) and full length spike protein of SARS-CoV-2 measured on paired DBS and plasma samples.

The study was conducted at Chris Hani Baragwanath Academic Hospital, Soweto, South Africa. The study participants included hospitalised adults between April 2020 and December 2020 with respiratory tract symptoms independent of duration, who tested positive for SARS-CoV-2 on nasal swab by PCR test, and for whom paired blood and DBS

specimen were collected. Sixteen participants were included in the study. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (reference number 200313).

Plasma was separated from whole blood by centrifugation at 2000 g for 10 min and stored at $-70\text{ }^{\circ}\text{C}$ until analysis. DBS's were collected using a single use lancet needle for pricking of the finger, with 2–4 DBS collected on filter cards (Munktel TFN, cat# 2.460.B00023) for each individual. The DBS were dried for 3 h at room temperature, then packed in plastic pouches with silica gel sachets and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

The elution of antibodies from DBS was performed as described (Mercader et al., 2006). Briefly, using a 6 mm hole punch, one spot was cut from the filter card and added to 600 μl of assay buffer, assuming 6 μl of plasma in each 6 mm blood spot. The spot was kept in a shaker at $2-8\text{ }^{\circ}\text{C}$ overnight for elution and the following day centrifuged at 2000 g for 10 min before analysis.

Paired plasma samples and DBS elutes were inactivated at $56\text{ }^{\circ}\text{C}$ for 1 h before analysis. The IgG concentrations against SARS-CoV-2 RBD and whole length spike proteins were measured on Luminex platform for

* Corresponding author at: Vaccines and Infectious Diseases Analytics Research Unit, Chris Hani Road, Chris Hani Baragwanath Academic Hospital, New Nurses Residence, 11th Floor West Wing, 2013, Bertsham, South Africa.

E-mail address: gaurav.kwatra@wits-vida.org (G. Kwatra).

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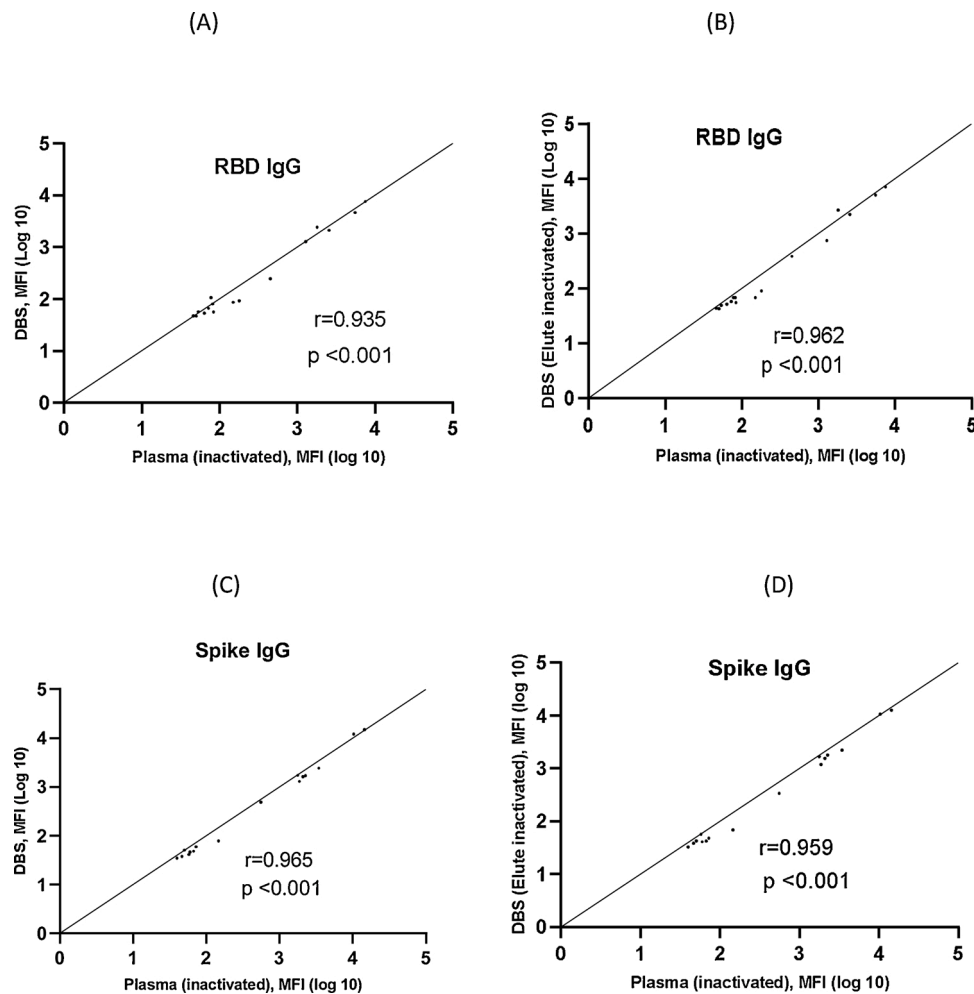


Fig. 1. Correlation between mean florescent intensity (MFI) between plasma and Dried blood spots (DBS) for SARS-Cov-2 RBD (A, B) and full length Spike protein (C, D) IgG antibody.

inactivated serum, inactivated DBS elute and normal DBS elute. The expression plasmid encoding for RBD and full length spike protein were obtained from the Krammer's laboratory (Mount Sinai, USA). The recombinant RBD protein was expressed as described previously (Stadlbauer et al., 2020) and was coupled to the magnetic microsphere beads (Bio-Rad, USA) using a two-step carbodiimide reaction as described (Simani et al., 2014). Assays were performed in duplicate, optimal plasma dilution for the assay was 1:100. The results for RBD and full length spike IgG were expressed in MFI. Bead fluorescence was read with the Bio-Plex 200 instrument using Bio-Plex manager 6.2 software (Bio-Rad).

Nasopharyngeal swabs were tested by PCR for the presence of SARS-CoV-2 which target two regions within the nucleocapsid gene and a third assay which detects the human RNase P gene 1, 2 as described previously (Lu et al., 2020). The correlation coefficient between MFI values obtained from plasma and DBS was determined by Spearman rank correlation. To assess agreement between two different methods Bland Altman analysis was performed. Data was analysed using GraphPad software Version 8.0.02.

The mean age (Standard Deviation) of the 16 participants at enrolment was 50.6 years (\pm 16.0 years). The plasma and DBS results for SARS-CoV-2 IgG are compared in Fig. 1. There was significant positive correlation between IgG MFI values measured on inactivated plasma and the normal DBS elute. The correlations for IgG measured by these two method were 0.935 (95 %CI: 0.814–0.978) for RBD and 0.965 (95 %CI: 0.895–0.988) for full length spike protein; $p < 0.001$ for both.

Comparing MFI measured on inactivated plasma and inactivated DBS elute yielded correlation of 0.962 (95 %CI: 0.889–0.986) for RBD and 0.959 (95 %CI: 0.879–0.986) whole length spike protein; $p < 0.001$ for both. Bland-Altman assessment also showed good agreement between IgG MFI values measured on inactivated plasma and the normal DBS elute with 6.25 % (1/16) of observations for both RBD IgG and spike IgG falling outside 95 % limits of agreement (LoA) (Fig. 2). The observed bias was $1.19 \pm$ Standard deviation (SD) 0.36 (95 % LoA; 0.48–1.90) and 1.25 ± 0.24 (95 % LoA; 0.77–1.72) for MFI measured on inactivated plasma and normal DBS elute for RBD and Spike IgG, respectively. Similarly, the observed bias was 1.29 ± 0.38 (95 % LoA; 0.54–2.05) and 1.37 ± 0.29 (95 % LoA; 0.78–1.95) for MFI measured on inactivated plasma and inactivated DBS elute for RBD and Spike IgG, respectively.

The heat inactivation of DBS elute did not affect IgG antibody determination for both RBD and full length spike. The correlations for IgG measured between normal and inactivated DBS elute were 0.987 (95 %CI: 0.962–0.995) for RBD and 0.967 (95 % CI: 0.904 – 0.989) for full length spike protein; $p < 0.001$ for both (Supplementary Fig. 1). Bland-Altman assessment also showed good agreement between IgG MFI values measured on normal DBS elute and inactivated DBS elute. The observed bias was 1.11 ± 0.24 (95 % LoA; 0.62–1.60) and 1.10 ± 0.15 (95 % LoA; 0.79–1.40) for RBD and Spike IgG, respectively (Supplementary Fig. 2).

Population-based sero-epidemiological surveys of SARS-CoV-2 infection could help inform the prevalence of past infection and assist in determining the ongoing susceptibility of communities for COVID-19.

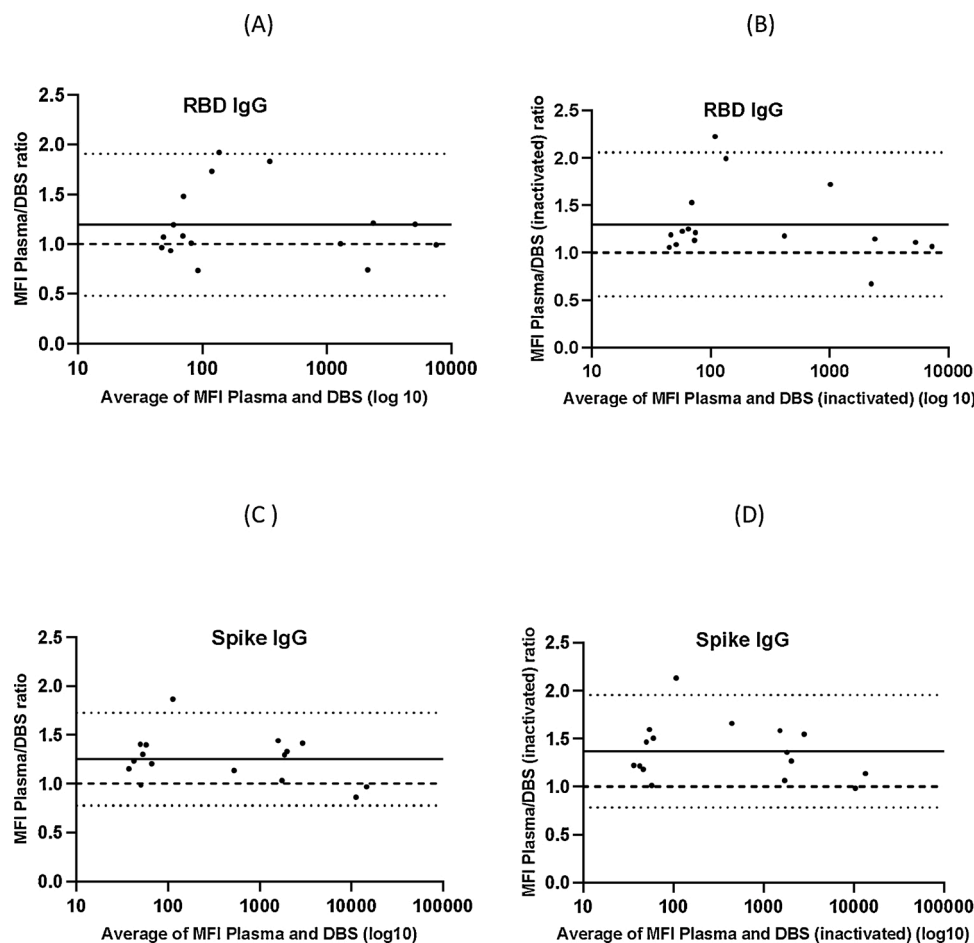


Fig. 2. Bland-Altman plot of plasma and dried blood spots (DBS) MFI ratios compared to average Plasma and DBS MFI for SARS-Cov-2 RBD (A, B) and full length Spike protein (C, D) IgG antibody. The line representing the bias is presented as a solid line, and the 95 % limits of agreement are presented as dashed lines.

Our results demonstrate the utility of DBS instead of whole blood obtained by venesection to quantify the presence of SARS-CoV-2 IgG against the RBD and full length spike proteins. Several other studies using ELISA-type immunoassays reported good correlation and agreement between Serum/Plasma and DBS sampling method to detect SARS-CoV-2 antibody detection (Amendola et al., 2021; Brinc et al., 2021; Weisser et al., 2021; Zava and Zava, 2021). A Study by Amendola et al., compared paired serum and DBS samples from health care workers and found high correlation and agreement between both sampling methods in detection of SARS-CoV-2 antibodies using ELISA (Amendola et al., 2021). In another study by Weisser et al., analysed paired serum and DBS samples, high correlation and agreement was observed for both Spike 1 and Nucleocapsid IgG (Weisser et al., 2021). A recent study by Brinc et al., reported high concordance in between Matched Plasma and DBS Fingerprick from PCR-confirmed COVID-19 patients using Roche Elecsys Anti-SARS-CoV-2 assay measuring antibodies to RBD (Brinc et al., 2021).

The DBS approach offers advantages over venesection, including being logistically easier to collect, transport, process and storage in the laboratory than is the case for whole blood samples obtained by venesection. Collection of DBS on filter paper is therefore a more pragmatic approach to collect samples for community-based sero-surveys and mitigates many of the challenges of whole blood collection (Ostler et al., 2014). Antibodies become stabilized once dried on filter cards, making it easier to ship DBS especially from remote sites to central facilities, and requires minimal processing at the laboratory upon receipt and subsequent testing (Su et al., 2018). Nevertheless, precautions must be taken with DBS collection, including that samples should be dried for at least

3 h and stored in Zip lock bags with desiccant to prevent atmosphere humidity damage (Smit et al., 2014). Also, the quality and integrity of the filter cards should be checked and maintained before using them for analysis, as they can vary because of incorrect or inadequate blood spots on the filter card (Su et al., 2018).

In conclusion, these data support the use of DBS's as a method to evaluate the prevalence of covid 19 sero-positivity in community-base sero-surveys as is currently being undertaken in South Africa.

Contributions

S A M, G K, M C N participated in conception of the study. M C N, N F, S J enrolled study participants and collected samples. G K, N D, V B performed the laboratory work. G K, N D analyzed the data. G K drafted the manuscript. All authors reviewed and edited the manuscript.

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Declaration of Competing Interest

SAM receive other grants related to Covid-19 research from Bill and Melinda Gates Foundation and South African Medical Research Council. All other authors report no potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jviromet.2021.114394>.

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