**Abstract**

Dried Blood Spots (DBS) are broadly used in SARS-CoV-2 surveillance studies, reporting either the presence or absence of SARS-CoV-2 antibodies. However, quantitative follow-up has become increasingly important to monitor humoral vaccine responses. Therefore, we aimed to evaluate the performance of DBS for the detection of anti-spike SARS-CoV-2 antibody concentrations using a commercially available assay, reporting in a standardized unitage (International Units/mL; IU/mL).

To assess the sensitivity and specificity of the ImmunoDiagnostics ELISA on serum and DBS for SARS-CoV-2 antibody detection, we analysed 72 paired DBS and serum samples. The SARS-CoV-2 S1 IgG ELISA kit (EUROIMMUN) on serum was used as the reference method. We performed a statistical assessment to optimize the cut-off value for DBS and serum and assessed the correlation between DBS and serum antibody concentrations.

We found that anti-spike SARS-CoV-2 antibody concentrations detected in DBS are highly correlated to those detected in paired serum (Pearson correlation 0.98; p-value<0.0001), allowing to assess serum antibody concentration using DBS. The optimal cut-off for antibody detection on DBS was found to be 26 IU/mL, with 98.1% sensitivity and 100% specificity. For serum, the optimal cut-off was 14 IU/mL, with 100% sensitivity and 100% specificity.

Therefore, we conclude that the ImmunoDiagnostics ELISA kit has optimal performance in the detection of SARS-CoV-2 antibodies on both DBS and serum. This makes DBS ideal for large-scale follow-up of humoral SARS-CoV-2 immune responses, as it is an easy but valuable sampling method for quantification of SARS-CoV-2 antibodies, compared to serum.

**Keywords**

SARS-CoV-2, antibodies, IgG, serology, international units/ml, dried blood spots (DBS), enzyme-linked immunosorbent assay (ELISA), serosurveillance, vaccination

**Background**

In the current severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic, large-scale serology studies are highly important to monitor vaccine- and infection-induced immune responses. We and others previously showed that dried blood spots (DBS) are a most valuable alternative to serum for the qualitative detection of SARS-CoV-2 antibodies [1-8]. However, quantitative assessment of SARS-CoV-2 antibody concentrations is needed for more profound insights into the dynamics of SARS-CoV-2 antibody responses. Moreover, reporting of results in a standardized World Health Organization recommended unitage (i.e., international units/ml (IU/ml)) is essential to evaluate and compare data [9]. To the best of our knowledge, commercial assays that quantify SARS-CoV-2 antibodies and report in IU/ml have not yet been validated using DBS. Therefore, we aimed to assess the diagnostic performance of DBS sampling for quantitative detection of SARS-CoV-2 antibodies using a commercial assay, i.e. the SARS-CoV-2 anti-spike S1 receptor-binding domain (S1RBD) IgG ELISA (ImmunoDiagnostics).

**Methods**

*Ethical considerations*

This study was approved by the Ethical Committee of the Ghent University Hospital (BC-07665). Each participant or legal representative signed an informed consent form after being informed about the study procedures.

*Study design and population*

In January and February 2021, we collected venous blood from nursing home residents and staff/caregivers from seven nursing homes in Flanders, Belgium. Blood was collected just before administration of the first vaccine dose (baseline, BL) and 14 days after the second dose (two-dose BNT162b2 vaccination regimen) (follow-up, FU), as part of a study on COVID-19 vaccination [10].

For the study presented here, we additionally collected paired DBS samples from residents from one nursing home at FU. Using an anticipated sensitivity and specificity of 85% and 95%, respectively, an α level of 0.05 and precision parameter ε of 0.10, we needed a minimum of 49 positive sera and 19 negative sera according to Buderer (1996) [11].

*Sample collection*

We obtained approximately 5 ml of venous blood from each participant by venepuncture in serum tubes. Tubes were transported to the Laboratory of Clinical Microbiology of the Ghent University Hospital (Ghent, Belgium) within six hours after collection, centrifuged at 3000 g for 8 minutes and stored at -20°C upon analysis. Capillary blood was collected onto DBS saver cards (EUROIMMUN, Lübeck, Germany) and stored the same day at -20°C upon analysis as previously described [1].

*SARS-CoV-2 antibody detection*

Both serum and DBS were analysed by the SARS-CoV-2 S1RBD IgG ELISA (ImmunoDiagnostics, Hong Kong) (ID ELISA). Serum was diluted according to the manual instructions (1:100) and a volume of 100 µl of the diluted serum was loaded on the coated ELISA well plate. DBS samples were processed as previously described (1) and placed in 250 µl 1x ID ELISA sample buffer. The further procedures for both DBS and serum were conducted manually as described in the instruction manual. Samples with an optical density (OD) that exceeded the OD of the highest standard were re-analysed using a 10- and 100-fold dilution. A set of SARS-CoV-2 antibody standards (included in the kit) was used to generate a 4PL logistic regression curve to calculate the antibody concentrations (IU/mL). According to the manual, 5 IU/mL is the recommended cut-off for positivity. ODs were measured on the Behring ELISA Processor III (Siemens AG, Munich, Germany) at 450 nm.

As the reference test, we detected SARS-CoV-2 antibodies in serum using the semi-quantitative SARS-CoV-2 S1 IgG ELISA kit (EUROIMMUN, Lübeck, Germany) (EI ELISA), which has been recommended by the Belgian Federal Agency for Medicines and Health Products, with a sensitivity of 90% (95% CI: 74.4%-96.5%) and specificity of 100% (95% CI: 95.4%-100%) [12, 13]. Serum (1:100 dilution) was analysed according to the manufacturer’s instructions. We considered samples with an OD ratio ≥ 0.48 as positive (Supplementary file 1).

*Statistical analysis*

The optimal cut-off value for ID ELISA seropositivity on DBS and serum was calculated by a Receiver Operating Characteristic (ROC) analysis as previously described [1]. The sensitivity and specificity of the ID ELISA on DBS and serum were calculated using the optimized cut-off and documented with the 95% confidence intervals (CI) (Wilson-Brown method) [14]. To assess the correlation between the antibody concentrations in DBS vs. serum (ID ELISA), we calculated the mean concentrations and the Pearson correlation. The correlation between antibody concentrations in DBS and serum was assessed by a regression analysis and used to calculate DBS-converted serum (DCS) concentrations (IU/ml). Mean antibody concentrations between the different sample types were compared using a Wilcoxon test. Statistical analyses were performed using GraphPad Prism 9 (GraphPad Software Inc., San Diego, U.S.).

**Results**

A total of 72 paired serum and DBS samples were analysed, of which 62 paired samples were collected from nursing home residents. Since 54 of these were found positive by the reference test (Table 1), we additionally spiked 10 DBS cards with sera from unvaccinated caregivers (staff) previously found negative for SARS-CoV-2 antibodies, to have sufficient negative samples (10). The mean age of residents was 89 (standard deviation (SD): ± 6) and 79% were female. The mean age of caregivers was 64 (SD: ± 15) and 70% were female.

*Cut-off optimization*

A ROC analysis was done for the ID ELISA on DBS and serum compared to the reference test. The area under the curve was 0.999 (95% CI; 0.996-1.000; p-value<0.0001) and 1.000 (95% CI, 1.000-1.000; p-value<0.0001) for DBS and serum, respectively. The optimal Youden’s cut-off point for seropositivity on DBS was ≥ 26 IU/mL and ≥ 14 IU/mL for serum.

*Sensitivity and specificity*

Table 1 shows the number of true/false-positive and -negative ID ELISA results for DBS and serum, in comparison to the reference test. The ID ELISA was found to have a sensitivity of 98.1% (95% CI, 90.2% - 99.7%) and specificity of 100.0% (95% CI, 82.4%-100.0%) for antibody detection on DBS using the optimized cut-off. For serum, the ID ELISA had a sensitivity of 100.0% (93.4% - 100.0%) and a specificity of 100.0% (82.4%-100.0%) using the optimized cut-off.

**Table 1**. **Number of true/false-positive and -negative SARS-CoV-2 IgG results on DBS and serum using the ID ELISA compared to the reference test (EI ELISA on serum).** DBS: Dried Blood Spots; ID ELISA: ImmunoDiagnostics ELISA; EI ELISA: EUROIMMUN ELISA.

|  |  |
| --- | --- |
|  | **Reference test (EUROIMMUN SARS-CoV-2 S1 IgG serum)** |
| **ID ELISA on DBS**  | Positive | Negative |
| Positive | 53 | 0 |
| Negative | 1 | 18 |
| **ID ELISA on serum**  |  |  |
| Positive | 54 | 0 |
| Negative | 0 | 18 |

*Correlation between SARS-CoV-2 antibody concentration in DBS and serum (ID ELISA)*

We detected mean antibody concentrations of 3781 IU/ml (95% CI, 1791-5771) and 2905 IU/ml (95% CI, 1293-4516) for DBS and serum, respectively The Pearson correlation between antibody concentrations (IU/mL) detected in DBS and serum was 0.98 (95% CI, 0.96-0.99; p-value<0.0001). The regression curve (fitted through the origin) was described by the equation *y = 0.788x.* The slope of this equation was used to calculate DBS-converted serum concentrations (DCS). The mean DCS concentration was 2987 IU/mL (95% CI, 1411-4545) (Figure 1). The Bland-Altman plot of serum vs. DCS did not show an over- or underestimation of antibody concentrations of one method vs. the other (Figure 2).

<<< Insert Figure 1 here >>>

**Figure 1. SARS-CoV-2 antibody concentrations in DBS, serum and DCS (left) and the correlation of antibody concentrations in DBS vs. serum measured using the ID ELISA (right). Left**. Spaghetti plot of antibody concentrations in DBS and serum and DBS-converted serum concentrations (DCS). The two broad horizontal lines show the positivity cut-off for DBS (top line) and serum (bottom line). Mean antibody concentrations per sample type are depicted in the graph. **Right.** Correlation plot of concentration in DBS and serum. The horizontal and vertical line show the positivity cut-off in serum and DBS, respectively. The diagonal line represents the regression line, fitted through the origin. The equation of the line is given in the graph. DBS: dried blood spots; DCS: DBS-converted serum concentrations; IU/ml: international units/ml; S1RBD IgG: anti-SARS-CoV-2 subunit 1 spike protein receptor-binding domain immunoglobulin G; all concentrations are shown on a logarithmic scale.

<<< Insert Figure 2 here >>>

**Figure 2**. **Bland-Altman plot of antibody concentrations in serum (EUROIMMUN ELISA) vs. DCS (ImmunoDiagnostics ELISA) (IU/ml).** The grey dotted lines repredent the 95% limits of agreement. DCS; DBS-converted serum concentrations.

**Discussion**

To monitor antibody responses upon COVID-19 vaccination and infections, large-scale studies reporting quantitative antibody levels in a standardized unitage are needed. In this context, DBS are an ideal sample collection method, as they are minimally invasive, low-cost and have minimal logistic constraints. However, DBS have not been validated for quantitative SARS-CoV-2 assays. Here, we demonstrated that DBS can be used to do so, and found that the ID ELISA had 98.1% sensitivity and 100% specificity in the detection of antibodies on DBS, after cut-off optimization. The sensitivity and specificity in serum were 100%. Moreover, we found that S1RBD IgG antibody concentrations in DBS and serum were highly correlated, allowing to assess serum antibody concentration using DBS by correction through a conversion factor. The antibody concentrations detected in DBS were slightly higher than in paired serum, which could be explained by the different sample preparation steps.

Given that DBS sampling followed by ID ELISA allows antibody quantification in a WHO recommended unitage, we conclude this is an optimal strategy for large-scale SARS-CoV-2 serology studies. Similar to our results, two other studies similarly showed that SARS-CoV-2 antibody concentrations in DBS were highly correlated to this of serum, however, using a different commercial assay [15, 16].

**Declarations**

**Conflict of interest**

The authors have no conflict of interest to declare**.**

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**Contributions**

Conceptualization: P.C., E.M.; data analysis: E.M., P.C.; data interpretation: E.M., P.C., A.C.; funding acquisition: P.C., S.C.; laboratory analysis: E.M.; A.C.; project administration: W.W., P.C.; sample collection: S.H.; draft: E.M.; review and editing: P.C., E.P., S.H., W.W., L.V, S.C., A.D.S, A.C. All authors have read and agreed to the published version of the manuscript.

**Ethics**

The current study was approved by the Ethical Committee of the Ghent University Hospital (BC-07665) and conducted in accordance to the Declaration of Helsinki. Informed consent was obtained from every participant.

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