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Dried Blood Spots to Monitor the Anti-SARS-CoV-2 Antibody Response after Infection or Vaccination

Getrocknete Blutstropfen zur Überwachung der Anti-SARS-CoV-2-Antikörperantwort nach Infektion oder Impfung

ORIGINALIA

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Summary

› **Rationale:** Anti-SARS-CoV-2 antibody responses elicited by infection or vaccination vary among individuals and over time. Their knowledge is of particular importance in, amongst others, elite sport. Can dried blood spots serve as a minimally-invasive, low-cost, decentralized tool to monitor the quantitative antibody response and thus represent an alternative to full blood tests?

› **Methods:** Cross-validation of dried blood and venous blood samples of 27 individuals post-infection and 96 individuals post-vaccination, longitudinal antibody monitoring of 27 individuals after vaccination using different vaccines and vaccine schedules and detection of seropositive individuals in a cohort of 557 people using self-collected DBS (dried blood spots) and two commercial immunoassays.

› **Findings:** Plasma and DBS values were highly correlated allowing for extrapolation of plasma values from DBS using a factor of a least 10 following the presented procedure. Capillary volumetric sampling and self-sampling produced reliable results. After vaccination, participants showed heterogenous antibody responses but a consistent increase after the second dose. DBS allowed for the analysis of a huge sample volume in a timely manner by limited laboratory personnel.

› **Discussion:** DBS offer the possibility of infection and vaccination traceability of individuals and cohorts via minimally-invasive self-sampling. This way, they allow to screen and monitor the presence and evolution of anti-SARS-CoV-2 antibodies in a qualitative and quantitative manner. Using two commercial, automated assays enables large-scale and frequent testing, global implementation and comparability of results.

KEY WORDS:
Coronavirus, Pandemic, Diagnostics, Immunity, DBS

Zusammenfassung

› **Problemstellung:** Durch Infektion oder Impfung hervorgerufene Anti-SARS-CoV-2-Antikörper zeigen interindividuell variable Niveaus sowie Verläufe, deren Kenntnis auch im Leistungs- und Spitzensport von Bedeutung ist. Können getrocknete Blutstropfen (dried blood spots - DBS) als minimal-invasives, kostengünstiges und dezentral einsetzbares Instrument zur quantitativen Überwachung der Antikörperantwort dienen und so eine Alternative zur Vollblutentnahme darstellen?

› **Methoden:** Kreuzvalidierung getrockneter Blutstropfen und venöser Vollblutproben von 27 Probanden nach Infektion und 96 Probanden nach Impfung, longitudinale Überwachung der Antikörperantwort von 27 Probanden nach Impfung mit verschiedenen Impfstoffen, Detektion seropositiver Personen in einer Kohorte mit 557 Teilnehmern mittels selbstentnommener Blutstropfen (dried blood spots-DBS) und zweier kommerzieller Immunoassays.

› **Ergebnisse:** Zwischen DBS- und Plasmaproben wurde eine lineare Korrelation beobachtet. Unter Anwendung des vorgestellten Verfahrens konnten Plasmawerte auf Basis von DBS-Werten mittels eines Umrechnungsfaktors von mindestens zehn extrapoliert werden. Volumetrische Probenahme und Selbstentnahme lieferten zuverlässige Ergebnisse. Nach Impfung zeigten die Teilnehmer heterogene Antikörperreaktionen, jedoch stets einen Anstieg nach der zweiten Impfdosis. Die Verwendung von DBS ermöglichte die Analyse eines großen Probenvolumens in kurzer Zeit mit begrenzten personellen Ressourcen.

› **Diskussion:** DBS bieten die Möglichkeit der Infektions- und Impferfolgsverfolgbarkeit von Einzelpersonen und Kohorten durch minimalinvasive Selbsterprobung. Auf diese Weise ermöglichen sie die qualitative und quantitative Überwachung des Vorliegens und der Entwicklung von Anti-SARS-CoV-2-Antikörpern. Die Verwendung zweier kommerzieller, automatisierter Assays ermöglicht groß angelegte und häufige Tests, eine weltweite Implementierung und Vergleichbarkeit der Ergebnisse.

SCHLÜSSELWÖRTER:
Coronavirus, Pandemie, Diagnostik, Immunität, DBS

Introdution, Problem, Goal of Study

Despite the unprecedented speed and scientific collaboration in investigating the highly infectious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for coronavirus disease 2019 (COVID-19) and the massive restrictions on public and private life, containing the spread of the virus has not yet been successful. Since its outbreak in December 2019, more than 260 million cases and 5 million deaths were registered worldwide – with actual numbers exceeding documented ones by far (4, 40).

As a consequence of non-pharmaceutical interventions to curb the spread of SARS-CoV-2, athletes were faced with closed training facilities, reduced contact with training groups or coaches, cancelled competitions or postponed 2020 Olympic ›

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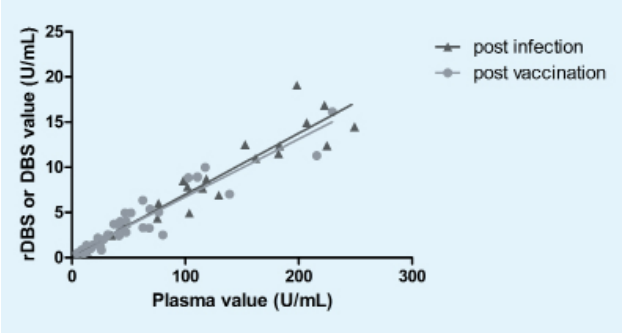

Figure 1

Cross-validation of plasma (rDBS) and dried blood samples. Linear correlation of anti-S antibodies in plasma and DBS ($p < 0.0001$) after infection ($n = 24$, $R^2 = 0.8970$) and vaccination ($n = 44$, $R^2 = 0.8938$). Samples were collected between 19 and 99 days after infection or vaccination. All samples were analyzed with the Elecsys Anti-SARS-CoV-2 S assay targeting the S antigen.

Games. These measures caused uncertainty about the athlete's career progress, frustration, a decrease in performance capacity and existential as well as financial anxiety (7). In light of rising case numbers in late 2021, a déjà-vu of the aforementioned scenario is imminent underlining the necessity of versatile diagnostic tools to detect and contain the virus.

Antibodies, notably neutralizing antibodies, are considered a promising biomarker which could serve as a quantifiable correlate of protection (COP) against an infection or re-infection with SARS-CoV-2 (10,14). However, no protective threshold could be established so far. Uncertainty prevails about the duration of post-infection or post-vaccination immunity, as a decrease of antibody concentration and immunity was observed over time (5, 22, 31, 35, 37).

After infection, antibody responses are heterogenous but correlate with disease severity and viral load (17, 21). Seroconversion in serum and saliva usually occurs within one to three weeks after infection (17) even though several studies report no seroconversion at all (17, 18, 21). The antibodies' main target structures are the spike (S) and nucleocapsid (N) antigen. Neutralizing antibodies usually target the spike protein (36) which is also the antigenic structure all vaccines licensed in Germany code for. So far, two mRNA-based (BNT162b2 from BioNTech/Pfizer and mRNA-1273 from Moderna) and two recombinant vector vaccines (ChAdOx1 nCoV-19 from AstraZeneca and Ad26.COV2.S from Johnson & Johnson/Janssen) are in use.

Serological tests can assess the antibody response after infection or vaccination, serve as a complement to false-negative PCR samples, detect unknown infections, identify potential plasma donors and individuals who are immunologically naïve and thus susceptible to SARS-CoV-2 (24). Over the course of the pandemic, numerous immunoassays have been developed, ranging from ELISAs to lateral flow and automated chemiluminescence immunoassays, with the latter allowing for the analysis of high sample sizes in a short amount of time (41).

However, phlebotomy necessary for serological tests requires medically trained personnel and is associated with a risk of infection for the person testing as well as the person being tested limiting its applicability for large cohorts or long-time monitoring due to economic and time-wise restrictions.

Dried blood spots (DBS) could bypass the problems described above while contributing further advantages as they can be obtained contactlessly, autonomously and in remote locations, are minimally invasive and require the collection of only a small volume of blood. DBS can be shipped using regular mail and be stored at room temperature. They have already been used to detect antibodies against several viral diseases (2). For athletes, the use of dried blood spots has proven successful in the context of doping controls (34). For SARS-CoV-2, antibody stability has been evident for several months (15). Its qualitative use has been demonstrated (15, 33) and has been applied in seroprevalence studies (6, 8, 11, 13, 26, 29, 42). First studies could also show a quantitative detection of anti-SARS-CoV-2 antibodies in DBS and its suitability for post-vaccination analysis (3, 12, 16, 19, 20).

Since the ratio between venous and capillary samples always has to be determined anew (39) this study aimed to conduct a cross-validation of DBS and whole blood using a commercial serum assay analyzing samples of previously infected or vaccinated subjects. Afterwards, two proof-of-principle studies using self-collected DBS were carried out: First, the antibody response of vaccinated people was monitored over several weeks. Second, a defined cohort was scanned for seropositive people. As a secondary question, the feasibility and reliability of self-collection was investigated.

Materials and Methods

Chemicals and Materials

A 1.8 mg/mL EDTA solution was prepared from ethylenediaminetetraacetic acid dipotassium salt dihydrate (K2E) from Sigma-Aldrich/ Merck (Darmstadt, Germany) and deionised ultrapure water from a Thermo Scientific™ Barnstead™ GenPure™ device (Bremen, Germany). Solofix® Safety Neonat lancets were purchased from B. Braun Petzold (Melsungen, Germany). For volumetric collection, 20 µL volume-absorbing end-to-end capillaries coated with K2E were used from Sarstedt (Nümbrecht, Germany). Cellulose-based DBS cards (QIAcard® FTA® DMPK-C) were purchased from VWR International (Bruchsal, Germany). MiniPax® absorbent packs were purchased from Sigma-Aldrich/ Merck. BD Vacutainer® Safety-Lok™ blood collection sets and K2E (5.4 mg, 3 mL) tubes were used for venous blood collections.

Blood Samples

All research samples were collected with approval of the local ethics committee (No. 54/2020, DSHS Cologne) and participants' written informed consent. A total of 27 plasma samples were collected from subjects post-infection (six women, 20-50 years). A total of 96 dried blood and whole blood samples were collected after vaccination with BNT162b2 or ChAdOx1 nCoV-19 (68 women, 19-69 years). A total of 27 subjects (23 women, 27-69 years) received DBS packages and instruction leaflets and collected DBS samples in parallel to their vaccination series. The DBS packages were sent back to the laboratory by post. Six subjects respectively were vaccinated twice with BNT162b2, mRNA-1273 or ChAdOx1 nCoV-19. Nine subjects received ChAdOx1 nCoV-19 followed by BNT162b2 (n=6) or mRNA-1273 (n=3). Surveillance began before the first vaccination and included both vaccination dates. During the first six weeks, DBS samples were taken on a weekly basis. Afterwards, surveillance continued at reduced frequency according to the vaccine and vaccine schedule. After the second dose, the monitoring was conducted weekly for at least three weeks. A total of 557 DBS samples were collected from employees of the German Sport University. DBS kits were handed out to volunteers who returned their self-collected samples by post.

Recreating rDBS from Plasma

As only plasma samples were obtained from formerly infected individuals, antibody-positive whole blood was reconstituted. By adding 65.5 µL of blood cells from an anti-SARS-CoV-2 antibody-negative blood donor to 80 µL of plasma of formerly infected volunteers, a hematocrit of 45 percent was established. Four spots of reconstituted blood of 20 µL each were applied to DBS cards and designated as reconstituted DBS (rDBS).

Sample Collection and Preparation

DBS were collected by a finger prick applying up to four spots of 20 µL each onto a DBS card using a volumetric capillary. The first drop of blood was removed to avoid high amounts of tissue fluid. Until analysis, DBS cards were stored at room temperature in plastic bags together with a desiccant. For analysis, the DBS spots were quartered and placed into microcentrifuge tubes. After addition of 100 µL EDTA, the samples were extracted in an ultrasonic bath for ten minutes following an in-house protocol (15, 33). Subsequently, the obtained blood extract was separated from the cellulose material. Plasma samples were prepared by centrifugation (1800×g, five minutes) from venous blood collection tubes and subsequently applicable for analysis.

ECLIA Detection

A Cobas e411 analyzer for immunoassay tests (Roche Diagnostics GmbH, Mannheim, Germany) and two Elecsys Anti-SARS-CoV-2 tests from Roche were used for the detection of anti-SARS-CoV-2 antibodies targeting the N (REF 09203095190) or the receptor-binding domain (RBD) of the S antigen (REF 09289267190). The detection principle of the electrochemiluminescence immunoassay (ECLIA) is based on an electrically induced, concentration-dependent chemiluminescence emission using a double antigen sandwich format. Analysis is performed automatically for all samples in batches of 30 samples with a total run time of approximately 45 minutes. Using the N assay, samples above a numerical cut-off index (COI) of 1.0 are considered reactive. Using the S assay, samples above 0.8 U/mL are considered reactive with a measuring range between 0.4 and 250 U/mL. Roche describes a correlation with surrogate neutralization tests and no cross-reactivity with endemic human coronavirus convalescent sera for both assays (27, 28). As the assays are intended for the detection of anti-SARS-CoV-2 antibodies in human serum and plasma, the analysis of DBS eluate was off-label.

Statistical Analysis

Data was analyzed using Microsoft Excel 2007, R 3.4.3 and GraphPad software version 5.01.

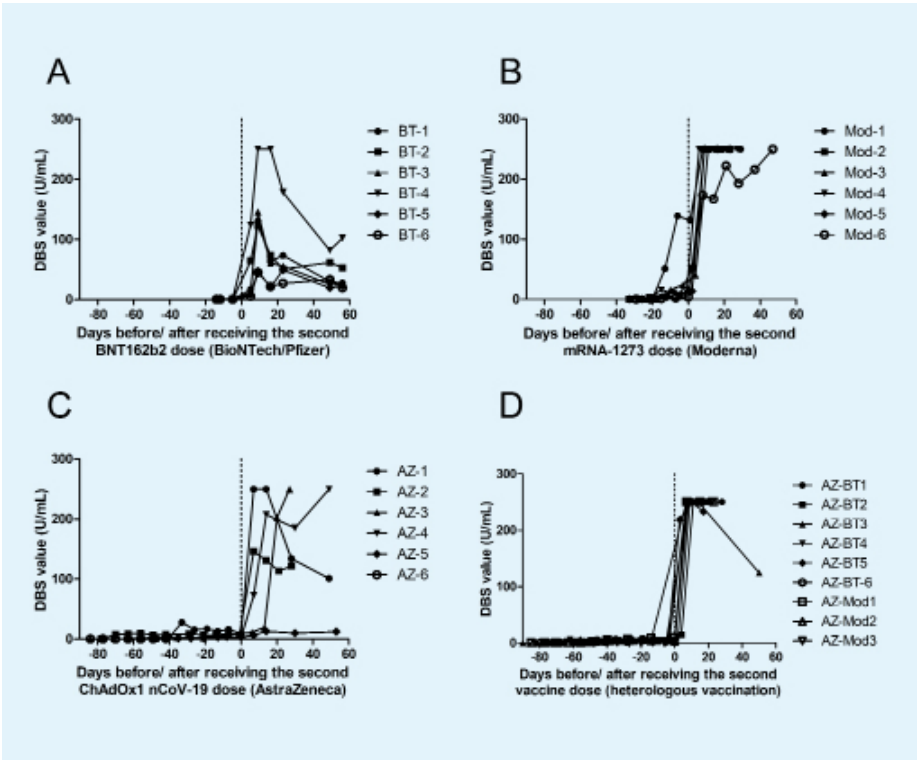

Figure 2

Change of anti-S antibodies measured in DBS before and after vaccination with BNT162b2 (A), mRNA-1273 (B), ChAdOx1 nCoV-19 (C) or ChAdOx1 nCoV-19 in combination with an mRNA vaccine (D). As intervals between first and second vaccine dose varied, the date of the second vaccine dose was set as zero. All samples were analyzed with the Elecsys Anti-SARS-CoV-2 S assay targeting the S antigen. Results point out inter-individual differences in antibody response and a significant increase after the second dose.

Results

Cross-Validation of Anti-SARS-CoV-2 Antibodies in DBS and Plasma after Infection or Vaccination using the Commercial Elecsys Anti-SARS-CoV-2 S Assay

Due to the limited measurement range of the S assay (0.4-250 U/mL) and lower antibody concentrations in DBS compared to plasma, several DBS samples showed values below the lower, several plasma samples values above the upper range limit. A total of 24 rDBS/ plasma samples post infection and 44 paired DBS/ plasma samples post vaccination showed paired values within the measurement range. After eliminating one outlier by applying the Grubb's test, a significant linear correlation ($p < 0.0001$) between venous blood samples and DBS was observed ($R^2 = 0.8970$ post infection, $R^2 = 0.8938$ post vaccination) (Figure 1).

To infer the antibody concentration in a venous blood sample based on the concentration detected in DBS, we observed a conversion factor of at least ten, fluctuating predominantly between 10 and 20. Testing the data with a generalized linear model, this factor turned out to be independent of the underlying antibody concentration.

To test capillary DBS for precision, venous DBS were prepared by applying 20 µL venous blood from 44 different samples onto DBS cards. By comparing these values with the corresponding capillary DBS, a linear correlation ($R^2 = 0.9523$) was observed.

To test the quality of self-sampled DBS, 10 DBS samples collected under supervision were compared with 10 samples collected without supervision. Testing the data with an unpaired t-test, no significant difference was observed. ›

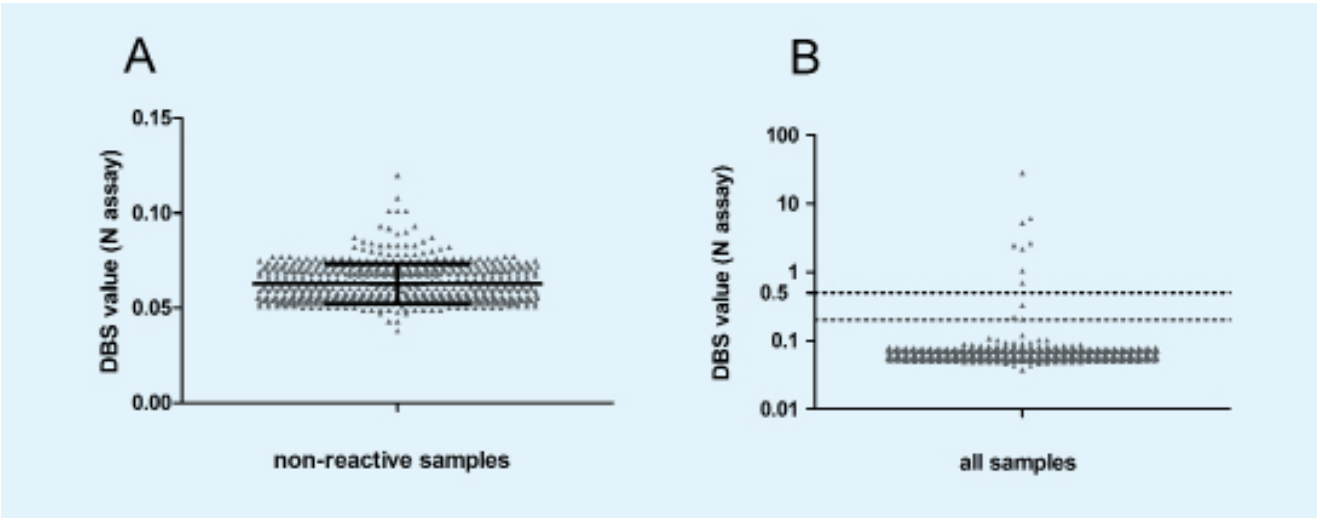

Figure 3

Antibody response of non-reactive samples (A) and all samples (B, logarithmic scale). Reactive and inconclusive samples can be clearly discriminated from non-reactive samples. All samples were analyzed with the Elecsys Anti-SARS-CoV-2 assay targeting the N antigen.

Proof-of-Principle: Using DBS to Monitor the Antibody Response after Vaccination

Self-collected DBS from 27 individuals were obtained on a regular basis from prior to vaccination up to at least three weeks following the second dose. This way, the antibody response after different vaccines and vaccine regimens was examined over time. The samples were handed in reliably and in good quality. Before the monitoring began, all participants were tested for the presence of antibodies using N and S assays. They showed no reactivity.

Whereas the individual responses varied, all participants showed a major increase of antibody concentration after receiving the second vaccine dose. The highest antibody values – exceeding the upper range limit of the assay – were observed in participants who received two doses of mRNA-1273 or one dose of ChAdOx1 nCoV-19 in combination with an mRNA-based vaccine (Figure 3). Due to the small sample size the values are only depicted descriptively.

Proof-of-Principle: Using DBS to detect Anti-SARS-CoV-2-Antibody-Positive Individuals

In order to investigate whether the analysis of anti-SARS-CoV-2 antibodies in self-sampled DBS reliably identifies antibodies after infection or vaccination and allows a timely analysis of a high sample volume, a study with 557 employees of the German Sport University was conducted.

All samples were analyzed with the N assay using pseudonyms. A conservative adjustment of the manufacturer's COI to 0.5 was made due to the use of whole blood instead of serum or plasma, a lower volume on DBS cards and a potential loss or dilution during extraction. Samples from vaccinated participants were also analyzed using the S assay. With a few exceptions, the samples were analyzed on the day of receipt.

The observed values of reactive DBS samples ranged from 0.698 to 28.2, while the values for non-reactive samples ranged from 0.04 to 0.12. Test results close to but below the COI (0.2 to 0.5) were considered inconclusive and were re-analyzed by testing another dried blood spot or venous blood sample (Figure 3).

Eight participants, four of whom had no positive test beforehand, showed reactive samples which could be confirmed by analyzing a second DBS. Three participants, two of whom had no positive test beforehand, showed inconclusive samples which could be identified as reactive by analyzing a second DBS and plasma sample. Four participants showed no reactivity repeatedly despite having had a positive PCR result in the past.

After vaccination, antibodies could be detected from three weeks onwards.

Discussion

So far, the need for venous blood drawings represents a serious limitation to serological tests. In this study, we examined the alternate use of DBS as a self-applicable and minimal-invasive tool to screen and monitor the qualitative and quantitative antibody response after infection or vaccination.

The linear correlation between quantitative DBS and plasma values allowed for the extrapolation of a minimum plasma value on the basis of DBS. As the conversion factor showed some variability, e.g. due to expected inaccuracies in volumetric blood sampling or incomplete desorption from the cellulose material, the exact calculation of plasma values was not possible.

Unsurprisingly, detected antibody levels in DBS were lower than in plasma due to the use of a smaller volume, full blood instead of plasma and potential losses during desorption resulting in reduced sensitivity of the method. However, as most DBS samples after infection and all DBS samples after second vaccination showed values within or above the assay's measurement range and no immunity threshold for SARS-CoV-2 has been defined so far, this might be of limited practical concern. Extraction gain could be enhanced by using higher volumes of capillary blood and less volume of extraction medium (34). In order to increase the sample throughput, simplify routine analysis and reduce costs, automatization of DBS analysis could be useful (15).

Most participants were able to collect and return DBS independently and in a timely manner. Self-sampled volumetric DBS showed high precision when compared with DBS prepared using a pipette. As some participants documented difficulties in capillary sampling, e.g. due to insufficient blood flow, blood clotting or tedious handling, the implementation of quantitative sampling tools could be beneficial (20). These tools have the additional advantage of being hematocrit-independent, albeit counteracting the financial advantage of

capillary DBS. For non-volumetric sampling, the collection of several spots should be maintained as occasional spots showed insufficient spot size and had to be pooled with additional spots in order to allow for analysis.

Whereas anti-S antibodies can be elicited via infection or vaccination, anti-N antibodies are only present after infection. Targeting these antigens using two different assays enabled the differentiation between infection and vaccination. This way, it was possible to exclude any previous infection before including participants in the vaccination monitoring and to attribute reactive samples in the screening study to previous infection or vaccination. The use of two automated assays allowed for a high throughput of samples in a timely manner making its expansion for even larger cohorts a realistic scenario. The use of two commercial ECLIAs would furthermore allow to implement the technique in many laboratories around the world thereby facilitating the comparability of results (3).

It was feasible to monitor the development and persistence of antibodies after vaccination even though any further interpretation of results was not possible due to small sample sizes and the lack of age or gender stratification. Although individual antibody dynamics differed, similar trends were observed over time, most particularly a significant increase after the second vaccine dose. It was remarkable, that the combination of ChAdOx1 nCoV-19 with an mRNA vaccine resulted in faster antibody increases and higher antibody concentrations exceeding the upper range limit than two doses of ChAdOx1 nCoV-19 or BNT162b2. As the antibody concentration after two doses of mRNA-1273 exceeded the upper range limit as well, a comparison is not possible in this case.

The observation is in line with current knowledge underlining a similar or even higher effectiveness of heterologous prime-boost vaccinations in preventing infection and stronger neutralizing activity compared to two doses of one vaccine alone (1, 23, 25) As a decline of antibody concentration could be observed several weeks after the second BNT162b2 dose, a longer follow-up and inclusion of booster doses could be investigated in follow-up studies (20).

When screening a cohort of more than 500 people, it was possible to detect anti-SARS-CoV-2 antibody-positive individuals in a timely manner. However, the study design did not allow to account for false negative samples. Formerly PCR-positive participants could be detected reliably. In the face of missing data, the presented method could be used to screen large cohorts of athletes within a short amount of time for past infection, e.g. to assess the impact of training or sport events on the occurrence of infection. Antibody screening could easily be combined with routine drug testing via DBS. It was interesting to note that a former positive PCR did not result in antibody persistence, a fact that is nowadays an established phenomenon for so-called non-responders and which is associated with age and low viral burden during infection (38).

Once a protective antibody threshold may be established, it could be used to detect particularly vulnerable athletes in advance of group events. Until today, it is still an open question, in which way antibody titers can serve as a COP, especially as cellular immunity plays an essential role in fighting off SARS-CoV-2, too (30). Anti-S antibodies have shown to be a good correlate of neutralization (9). Roche describes a high correlation of its assays with neutralization assays circumventing the need for biosafety level 3 laboratories (27, 28). Due to the emergence of virus variants, the presence of antibodies is not necessarily predictive for immune protection making adaptations of the assays potentially necessary. Another question is, whether re-infections occur due to lower levels of antibodies or reduced breadth in neutralizing emerging viral variants (32).

Using DBS eluate instead of serum or plasma can simplify serological studies by reducing the need of phlebotomy and thereby expanding its applicability beyond personnel and geographical restrictions. This holds true even more, when medically trained personnel is scarce due to the burden of a global pandemic. A lowered threshold for testing and surveillance in combination with high-throughput assays are crucial to examine the true dimensions of the pandemic, e.g. the estimated number of unknown cases or true case fatality rate, and identify particularly affected groups of people.

Conclusion

In this study, the utility of DBS in contributing to managing the SARS-CoV-2 pandemic was assessed and further expanded (33). Knowing how to use DBS in a large-scale and timely manner will also be of future use, both for characterizing evolving features and corresponding immune responses of SARS-CoV-2 as well as potential new future situations necessitating fast and comprehensive analytical approaches to contain quickly spreading diseases.

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Conflict of Interest

The authors have no conflict of interest.

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