Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

Received: 5 February 2021
Revised: 11 March 2021
Accepted: 11 March 2021

Detection of anti-SARS-CoV-2 antibodies in dried blood spots utilizing manual or automated spot extraction and electrochemiluminescence immunoassay (ECLIA)

Andre Knoop[1] | Hans Geyer[1] | Oliver Lerch[2] | Ana Rubio[1] | Yvonne Schrader[1] | Mario Thevis[1,3]

[1] Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne, Cologne, Germany

[2] Gerstel GmbH & Co. KG, Muelheim an der Ruhr, Germany

[3] European Monitoring Center for Emerging Doping Agents (EuMoCEDA), Cologne/Bonn, Germany

Correspondence
Andre Knoop, Institute of Biochemistry, Center for Preventive Doping Research, German Sport University Cologne, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
Email: a.knoop@biochem.dshs-koeln.de

Abstract

Serological test methods to detect anti-SARS-CoV-2 antibodies represent a major measure to manage the pandemic caused by the coronavirus disease 2019 (COVID-19). In this communication, test results obtained from minimal-invasively collected dried blood spot (DBS) specimens, which can be sampled 'at home' without the need of medically trained personnel, are compared to conventionally collected venous blood samples. DBS samples were prepared for analysis either manually or by a card extraction robot, and electrochemiluminescence assay (ECLIA) characteristics, assay readout values as well as stability data covering a period of more than 200 days are provided. Constant anti-SARS-CoV-2 antibody readouts of quality control DBS were obtained over the entire test period using DBS specimens stored under dry and dark conditions. In addition, test results obtained from individuals tested twice within 10 months post-infection indicated a consistent presence of antibodies.

KEYWORDS
antibody, automated DBS extraction, COVID-19, DBS, ECLIA, SARS-CoV-2

1 | INTRODUCTION

The unprecedented coronavirus disease 2019 (COVID-19) has necessitated versatile and highly adapted diagnostic and analytical tools to understand and manage the pandemic. After a series of unexplained cases of pneumonia occurred in Wuhan (China) in December 2019, the target pathogen severe respiratory syndrome corona virus 2 (SARS-CoV-2) was readily identified and genetically characterized,[1,2] and only approximately 12 months later the first[3] of several promising vaccine candidates passed approval procedures[4–7] in several countries. Nevertheless, the still continuously high/increasing numbers of infections and the appearance of new fast-spreading variants[8–11] have pushed health care systems to their limits, and the search for proportional but adequate measures to combat the pandemic and its global consequences has been of extraordinary complexity.

To date, various analytical testing procedures are approved and available, including molecular antigen detection (nucleic acid tests, NAT) as well as a versatile range of serological tests (antibody detection).[12] NAT approaches, especially the promptly developed real-time reverse-transcription-polymerase chain reaction (RT-PCR) assay[13,14] are the gold standard for confirming a SARS-CoV-2 infection. In addition, rapid test methods such as lateral flow immunoassays (LFIA) have been introduced for various purposes, *e.g.* for access control to sensitive areas.[15,16] Complementary, serological test methods can be applied to supplement standard RT-PCR assays to cover false negative results as a consequence of decreasing viral shed in the

Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

respiratory sampling area.[17] Moreover, these techniques are highly valuable to detect the humoral response in form of immunoglobulins (mostly IgG and/or IgM), which enable virus-tracking in case of epidemiologic studies and support follow-up analyses concerning the efficacy of initiated vaccination programs. The aforementioned immune response to SARS-CoV-2 exposure is described to be of successive antibody formation, presenting first IgM (after 3-7 days) followed by the long-term presence of IgG (after 7-25 days). The absolute intensity of antibody production is known to correlate with the clinical severity,[15,17] and whether or not IgGs and their concentration are indicative for potential immunity has been a topic of ongoing debates.

Herein, a routine procedure contributing to comprehensive anti-SARS-CoV-2 antibody tests from dried blood spots (DBS) is presented in continuation of previous studies.[18–22] The use of DBS as an alternative matrix offers clear benefits as no one-on-one contact for an invasive venipuncture is needed and sample transport and storage are tremendously facilitated by mail delivery and room temperature conditions. Due to their design and properties, enzyme-linked immunosorbent assays (ELISAs) and CLIAs are suitable screening methods to test larger populations within a short time.[16] Approximately 1000 samples have been analyzed in the context of (elite) sport test programs and research projects, employing manual sample preparation as well as automated DBS sample extraction. Feasibility and utility of automated DBS extraction[23–26] have been reported in the context of various applications, including SARS-CoV-2 antibody tests.[19]

2 | MATERIALS AND METHODS

2.1 | Chemicals and materials

Ethylenediaminetetraacetic acid dipotassium salt dehydrate (K2E) was purchased from Sigma Aldrich/Merck (Darmstadt, Germany). Hence, an aqueous 1.8 mg/mL solution for DBS extraction was prepared using deionized water obtained from a Barnsted GenPure device from Thermo Scientific (Bremen, Germany). For the sampling of capillary blood, 20 µL end-to-end capillaries coated with K2E and 1.5 mL PP micro tubes were acquired from Sarstedt (Nümbrecht, Germany). Additionally, 20 µL Mitra®-tips from Neoteryx (Torrance, CA, USA) were used. Moreover, DBS collection was conducted using Hemaxis DB 10 collection devices (DBS Systems SA, Gland, Switzerland) and Tasso-M20 devices (Tasso Inc., Seattle, WA, USA). For venous blood drawings, BD Vacutainer® Safety-Lok™ Blood Collection Sets and K2E (5.4 mg, 3 mL) and SST™ II Advance (5 mL) Plus Blood Collection Tubes from BD were used for plasma and serum collection, respectively. QIAcard FTA™ DMPK-C were purchased from VWR International GmbH (Bruchsal, Germany). MiniPax® absorbents packets were also obtained from Sigma Aldrich/Merck.

2.2 | Blood samples

All research samples (venous whole blood and DBS) were collected with approval of the local ethics committee (#054/2020, German Sport University Cologne, Germany) and written informed consent of the participants. A total of 434 contract DBS analyses were conducted in cooperation with Droplabs UG (Düsseldorf, Germany). Furthermore, 72 blood samples of PCR positive-confirmed specimens from hospital patients including EDTA whole blood, plasma, and serum were obtained from the *Plataforma Biobanco Pulmonar* of the *Institut d'Investigació Sanitària Illes Balears* (IdISBa, Spain).

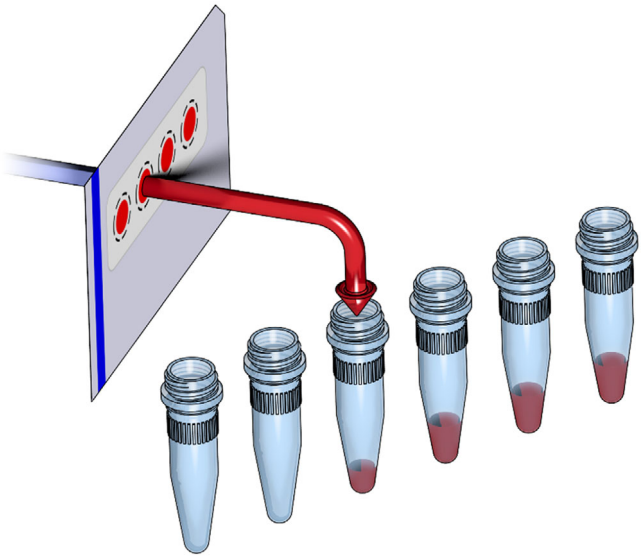

FIGURE 1 Schematic illustration of DBS extraction by the Flow Through Desorption™ (FTD) principle

2.3 | Sample preparation

Serum and plasma were prepared by centrifugation (1800 × *g*, 5 min) from venous whole blood collection tubes and were immediately applicable to ECLIA analysis (vide infra). For the analysis of DBS, up to 4 spots of approximately 20 µL of capillary blood were collected by a finger-prick onto the cellulose-based DBS cards. Directly after sampling, the DBS cards were stored together with a desiccant pack in suitable plastic bags at room temperature until analysis. For extraction purposes, the DBS was punched and quartered into 1.5 mL microcentrifuge tubes before addition of 100 µL of the EDTA solution and 10 min of ultra-sonication. The obtained blood extract was separated from the remaining cellulose material and transferred to fresh PP micro tubes for ECLIA measurement.

2.4 | Automated DBS extraction

Alternatively, the utility of a Dried Blood Spot Autosampler (DBS-A, GERSTEL, Muelheim, Germany) in support of an automatic sample extraction procedure based on the Flow Through Desorption™ (FTD) principle (Figure 1) was assessed. The operating instrumental setup consisted of two MultiPurpose Samplers (MPS) controlled via Maestro

Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

TABLE 1 DBS-A sample extraction protocol

#	Solvent	Volume [µL]	Aspiration [µL/min]	Dispensing [µL/min]
1	EDTA	85	10 000	2000
2	Air	250	10 000	250
3	Air	4000	10 000	10 000
4	EDTA	2000	10 000	4000
5	EDTA	2000	10 000	4000
6	Air	4000	10 000	10 000

software version 1.4.57.5 (both from GERSTEL, Muelheim, Germany). One (left) was customized and trained for DBS card sampling. It was equipped with autosampler card racks of 24 positions each and a sampling arm for DBS card handling. This unit was directly connected to serve the DBS unit where the extraction takes place. As pump control system, a High Pressure Dispenser (HPD) was promoting extraction and wash solutions. The second MPS (right) was equipped with an 80 µL sideport-syringe for transfer and filling of the sample extracts into ready-to-use vials for ECLIA.

The automated extraction workflow was programmed as follows: The left MPS inserts a card from a card rack into the DBS unit, where digital photography ensures the documentation of the spot quality, the spot localization, and (optionally) the barcode reading. The blood spot is sealed leak tight into the flow path at the determined position by a set of clamps of 8 mm diameter (alternatively 2, 4, or 6 mm) enabling full spot desorption of the 20 µL blood spots. For antibody extraction, an aqueous EDTA solution (1.8 g/L) is used. A volume of 85 µL is transported as a segmented volume through the capillaries (Table 1, step 1). To prevent dilution effects, the volume is pushed throughout the system and the card material by injection of air instead of other solvents or water. Immediately before passing the sample spot, the flow is reduced to 250 µL/min and the EDTA solution is heated to approximately 80°C by a built-in preheater before entering the clamp mechanism (step 2). The extract is transferred via the sideport-syringe of the right MPS to open vials (step 3). This procedure is followed by washing steps (step 4 and 5) with the EDTA solution and drying of the system by finally injecting air (step 6). The extraction protocol is summarized in Table 1. After desorption, washing and drying, another photo of the card is taken for quality control and documentation purposes, before the card is returned to the respective rack position. Due to the requirements of undiluted extraction the overall extraction time per spot is approximately 7 min. Assays accepting more dilute extracts will be compatible with simplified extraction protocols allowing for reduced runtimes of approximately 3 min per DBS.

2.5 | ECLIA detection of anti-SARS-CoV-2 antibodies

For the detection of anti-SARS-CoV-2 antibodies, a Cobas e411 analyzer for immunoassay testing (Roche Diagnostics GmbH, Mannheim, Germany) was applied, using an ECLIA Elecsys Anti-SARS-CoV-2 (REF 09203095190) Kit off-label. According to the manufacturer's product description[27,28] it is intended for qualitative detection of SARS-CoV-2 antibodies in human serum and plasma. The detection principle is based on an electrically induced chemiluminescent emission after formation of a sandwich complex where present anti-SARS-CoV-2 antibodies are targeted by two nucleocapsid antigens, one labeled with a tris(2,2′-bipyridyl)ruthenium(II)-complex. For all employed matrices, that is, serum and plasma as well as extracts obtained from DBS, the analysis is performed fully automated in batches of 30 samples with an overall run time of ca. 45 min.

2.6 | Assay characterization

In addition to the assay's performance characteristics documented in the manufacturer's factsheet, additional figures of merit concerning the test method's precision were determined for the analyses of DBS and Mitra®-tip extracts. For the assessment of the inter- and intra-day precision, batches of six QC sample replicates were prepared on three consecutive days ($n = 3 \times 6$). Moreover, the instrumental precision of the ECLIA was determined at three concentration levels. Therefore, samples were prepared as rDBS QCs and Mitra®-tip samples from different donors with high, medium and low serum concentrations, respectively. For each level, 3 spots were pooled and the yielded extracts were each analyzed sixfold ($n = 3 \times 6$). The precision values were calculated as the corresponding relative standard deviations.

3 | RESULTS AND DISCUSSION

3.1 | Study designs and test cohorts

3.1.1 | Characteristics of plasma and serum versus DBS

According to the manufacturer's instruction leaflet,[28] the test results should be assessed by means of a numerical cutoff index (COI). A COI of ≥1.0 should be interpreted as the threshold for a reactive test result and vice versa (< 1.0 non-reactive test result) in plasma or serum. As evaluated before,[22] DBS extracts generally allow for the measurement of anti-SARS-CoV-2 antibodies (using ELISA and LFIAs). With regards to lower absolute amounts of antibodies in 20 µL of (capillary) blood instead of serum or plasma, potential analyte loss and dilution during the extraction process, the cutoff-level for DBS samples was adapted to a conservative COI of ≥0.5 to account for a reduced overall assay sensitivity.

In the tested cohort, the observed "reactive" values ranged from the modified COI of ≥0.5 up to > 200 while values for non-reactive samples were found in a range of 0.04-0.07 for DBS and 0.06-0.1 for plasma/serum. Interestingly, extracted blank card spots (i.e. without blood applied and thus representing largely neat EDTA solution) resulted in elevated readout values of 0.1-0.2. ECLIA test results close

Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

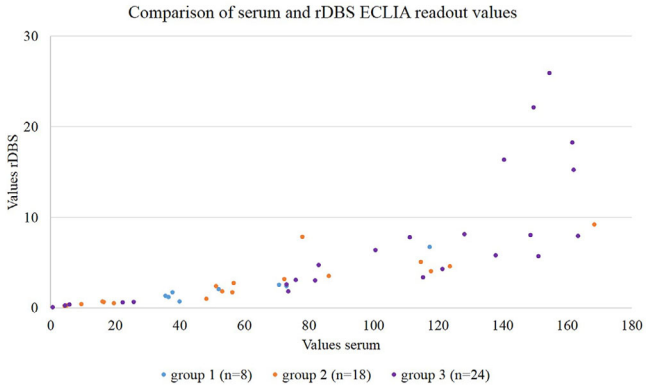

FIGURE 2 Highly significant ($P < .001$) linear correlation of serum and corresponding reconstituted DBS (rDBS) values

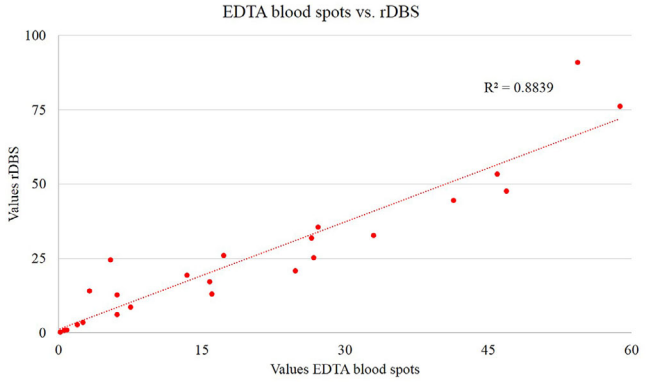

FIGURE 3 Linear/proportional correlation of EDTA whole blood spotted onto DBS cards and corresponding rDBS derived from serum of the same volunteers

to but still below the COI (0.2 to < 0.5) were considered as "inconclusive," which warranted follow-up tests such as a second analysis of another DBS spot or, if/where possible, a venous blood drawing for subsequent anti-SARS-CoV-2 antibody test(s).

Also, the correlation of values determined from serum and corresponding DBS was assessed. For that purpose, anti-SARS-CoV-2 antibody reactive serum samples were mixed with blood cells from anti-SARS-CoV-2 antibody-negative blood donors (set to a hematocrit of 45) and spotted at 20 µL / sample onto DBS cards. These DBS were subsequently regarded as reconstituted DBS (rDBS). In Figure 2, the correlation of serum and rDBS samples of 50 reportedly PCR-positive volunteers of three test groups is graphically presented. Data was tested by a generalized linear model and values were found to be highly significantly correlated ($P < .001$).

To assess the validity of rDBS results, EDTA whole blood samples were spotted onto DBS cards and directly compared to corresponding rDBS from the same subjects. By plotting both ECLIA values (Figure 3), a linear correlation was observed.

Data on the overall as well as the instrumental precision of the ECLIA for the analysis of DBS and Mitra®-tip extracts are summarized in Table 2. Both the overall method (rel. SD: DBS < 12.3% and Mitra® < 8.5%) and the ECLIA (rel. SD: DBS < 1.1% and Mitra®-tips < 2.2%) were found to be precise. The DBS and Mitra®-tip readouts obtained from identical blood samples prepared and analyzed in the context of the instrumental precision measurements at high, medium and low concentrations further confirmed comparable target analyte readouts from both sampling supports yielding Mitra®/DBS ratios of 0.91, 0.96 and 1.01, respectively (Table 2).

TABLE 2 Results of characterization experiments utilizing the Elecsys Anti-SAS-CoV-2 ECLIA for analysis of DBS and Mitra®-tips extracts

Instrument precision	DBS	Mitra®-tip	Readout value comparison (Mitra®/DBS)
High	0.6%	2.2%	0.91
Medium	0.6%	1.2%	0.96
Low	1.1%	0.7%	1.01
Overall precision			
Intraday	5.2-12.3%	4.6-8.5%	
Interday	8.7%	6.8%	

3.1.2 | Authentic DBS sample analyses

In the context of a pilot study, more than 500 DBS samples, collected from participants of a largely defined and limited environment (working colleagues and close acquaintances) were analyzed. With the exception of two study participants, antibodies were detected in all samples obtained from previously reported PCR-positive individuals (n = 16). Furthermore, 6 DBS specimens yielded anti-SARS-CoV-2 antibody reactive values ("unexpected findings"), and in two cases inconclusive results (ECLIA readouts of 0.3–0.49) were found but eventually confirmed as antibody-reactive through additional serum sample analyses.

In a second cohort, 434 anonymized contract analysis with 67 (EDTA-)plasma and serum samples and 367 DBS specimens on varying collection supports (Figure 4) were conducted, yielding 14 antibody-reactive (3.2%) and 3 (0.7%) inconclusive test results. While necessitating minor adaptations of the extraction protocol depending on the DBS sampling device, the ECLIA analysis remained unmodified for all specimens.

3.1.3 | Stability of anti-SARS-CoV-2 antibodies in DBS

For quality control purposes, a stock of various DBS cards was prepared by spotting 4 × 20 µL of EDTA blood from two different PCR- and antibody-tested volunteers to obtain negative and positive quality control samples, respectively. Those cards were packed in sealed plastic bags together with a desiccant to be stored in a refrigerator at 4°C

Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

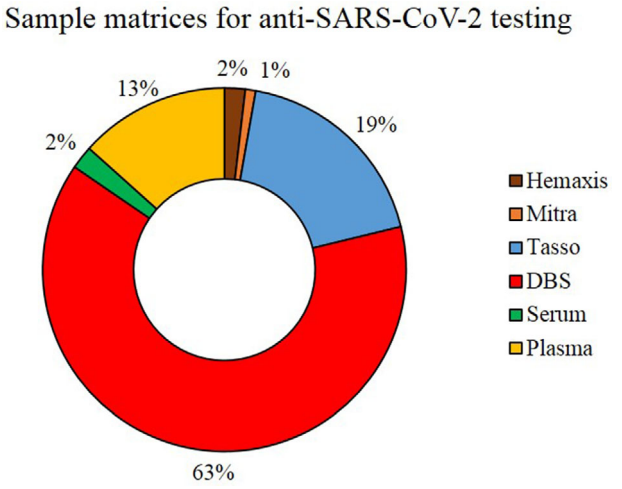

FIGURE 4 Distribution of different blood sample matrices tested for the presence of anti-SARS-CoV-2 antibodies. In total, 434 samples were analyzed: 275 card-based DBS, 80 Tasso-Kit-derived DBS, 58 plasma, 9 serum, 8 Hemaxis DBS, and 4 Mitra®-tip DBS

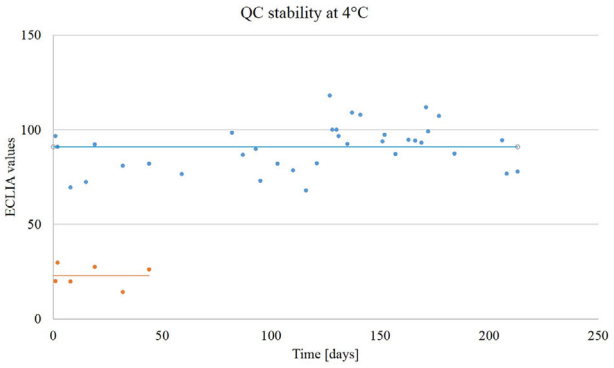

FIGURE 5 Time-ECLIA readout value plot of repeated analyses of anti-SARS-CoV-2 antibody-reactive positive QC samples using card-derived DBS and Mitra®-tips. Results suggest no degradation of the reactive species with median values of 90.9 (SD 12.4) and 22.9 (SD 5.3), respectively

until analysis. Over a period of more than six months, positive QC spots were freshly prepared on a daily basis with each batch of test samples. In addition, Mitra®-tips were loaded with another EDTA blood sample and repeatedly prepared and analyzed over a period of 44 days. The ECLIA results of both QC samples yielded reproducible data centering around the median of 90.9 for DBS and 22.9 for Mitra®-tips (Figure 5).

3.1.4 | Post-infection presence of antibodies

With the established approach, PCR-confirmed SARS-CoV-2-positive reported individuals were repeatedly tested for the presence of anti-SARS-CoV-2 antibodies over the course of up to 10 months as summarized in Table 3. Being considered as qualitative information only, the obtained data corroborate the long-term presence of anti-SARS-CoV-2 antibodies.

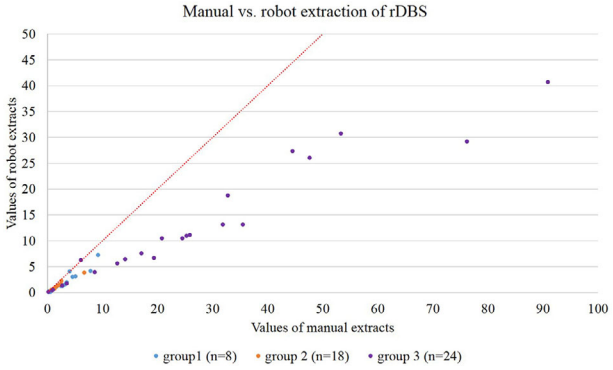

FIGURE 6 Linear correlation of rDBS QC samples prepared by automated spot extraction compared to manual sample preparation. The red dotted line is representing potential proportional 1:1 recovery

3.1.5 | Automated DBS extraction

In the course of establishing an automated DBS extraction method, the automated card handling and extraction process within a sequence of multiple samples was assessed for potential carry-over effects. Therefore, a sequence of samples consisting of negative QC, positive QC, blank (empty) spot, and negative QC was prepared and analyzed. All four samples yielded typically observed ECLIA readouts as reported above (data not shown), demonstrating the absence of analyte carry-over, and the employed cleaning steps within the extraction run were regarded as adequate. For reliable test results, repeatable and correct sample volumes (at least 60 µL of the extraction volume of 85 µL) were needed to be transferred into the sampling tubes for the ECLIA. All sample extracts were completely transferred without any losses nor further dilution effects.

Finally, the ELICA readouts of manually prepared rDBS QC samples were compared to extracts of the same sample handled by the DBS-A robot. As depicted in Figure 6, an average recovery of 57% was enabled by the DBS-A automated extraction when compared to the manual sample preparation protocol. Values were found to be well correlated despite the consistently lower recovery found for automated extraction.

Besides routine preventive maintenance (every 6-12 months) of the DBS-A robot concerning e.g. rotor seals, extraction clamps, collection syringe including needle and MPS bungee cords, no additional maintenance has been required to date due to the herein presented extraction protocol. Extraction clamps are visually inspected before starting a sequence and are cleaned with a wetted cotton swab, if indicated.

4 | CONCLUSION

Major benefits of minimally invasive blood collection especially in the context of serological diagnostics for infectious diseases are *inter alia* the quick and easy way of individual blood collection without the need of a venipuncture by healthcare professionals, the cheap and easy

Application Note
doi.org/10.1002/ansa.202100009
Chemistry Europe
European Chemical Societies Publishing

TABLE 3 Overview about test results for anti-SARS-CoV-2 antibodies (ABs) in reportedly PCR-positive individuals tested early 2020 and late 2020/early 20201. Where first anti-SARS-CoV-2 antibodies were determined with standard ELISA methods, no numerical comparator to the herein employed ECLIA test is available

	PCR test				
Sex	Yes/no	Pos/neg	date	First AB test value (date)	Latest AB test value (date)
♀	Yes	Pos	03/2020	ELISA-pos (04/2020)	4.91 (12/2020)
♂	Yes	Pos	03/2020	ELSIA-pos (04/2020)	5.89 (01/2021)
♀	No			ELISA-pos (04/2020)	3.05 (12/2020)
♂	Yes	Pos	04/2020	21.3 (06/2020)	5.6 (01/2021)
♀	Yes	Pos	04/2020	0.90 (08/2020)	0.482 (01/2021)
♂	Yes	Pos	11/2020	4.24 (12/2020)	27.41 (01/2021)
♂	No			ELISA-pos (04/2020)	2.06 (02/2021)
♂	Yes	Pos	03/2020	ELISA-pos (04/2020)	1.89 (02/2021)
♀	Yes	Pos	09/2020	0.945 (11/2020)	3.26 (02/2021)
♂	Yes	Pos	09/2020	0.226 (11/2020)	1.02 (02/2021)
♂	Yes	Pos	04/2020	ELISA-pos (04/2020)	1.18 (02/2021)
♂	Yes	Neg	04/2020	ELISA-pos (04/2020)	0.364 (02/2021)
♂	Yes	Pos	03/2020	ELISA-pos (04/2020)	0.730 (02/2021)
♀	Yes	Pos	03/2020	3.58 (07/2020)	1.09 (02/2021)
♂	Yes	Pos	03/2020	5.98 (07/2020)	2.37 (02/2021)
♀	Yes	Pos	03/2020	ELISA-pos (04/2020)	3.06 (02/2021)
♂	Yes	Pos	03/2020	ELISA-pos (04/2020)	2.12 (02/2021)
♀	Yes	Pos	03/2020	ELISA-pos (04/2020)	3.28 (02/2021)
♂	Yes	Pos	03/2020	ELISA-pos (04/2020)	2.44 (02/2021)
♀	Yes	Pos	03/2020	ELISA-pos (04/2020)	0.106 (02/2021)
♂	Yes	Pos	03/2020	ELISA-pos (04/2020)	0.183 (02/2021)
♀	Yes	Pos	03/2020	57.59 (07/2020)	5.41 (02/2021)

transportation, and storage stability as also supported by the herein presented data.

The need for extended anti-SARS-CoV-2 antibody test methods might further increase in the near future to address questions besides identifying past infections such as assessing the immune response to (different) vaccination strategies and therapeutics or the long-term monitoring of the immune and antibody status of formerly infected or vaccinated individuals. Here, the determination of the antibody titer will be a vital information that can potentially be produced with adequate immunological assays utilizing DBS, preferably with largely automatable extraction and analysis option, for which proof-of-principle data and strategies were presented.

A significant difference as observed between DBS and Mitra®-tip-based readout values of identical blood samples (ca. fourfold) necessitates further investigation and potentially adaptation of the herein employed sample preparation protocol, accounting for the substantially different physico-chemical properties of the DBS support materials. Nevertheless, the principle applicability of the discussed approach using microsampling devices featuring a hydrophilic porous DBS-sampling material is given, opening further opportunities in anti-SARS-CoV-2 antibody testing.

ACKNOWLEDGMENTS

The authors acknowledge support from the Federal Ministry of the Interior, Building and Community (Berlin, Germany), the Manfred-Donike-Institute for Doping Analysis (Cologne, Germany), and Dr. Thomas Piper.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DISCLOSURE

Oliver Lerch is an employee of Gerstel GmbH & Co. KG.

ORCID

Andre Knoop https://orcid.org/0000-0003-0040-8754

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.

2. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. 2020;579(7798):270-273.
3. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. *Nature*. 2020;586(7830):594-599.
4. European Medicines Agency. EMA recommends first COVID-19 vaccine for authorisation in the EU https://www.ema.europa.eu/en/news/ema-recommends-first-covid-19-vaccine-authorisation-eu. Accessed 29.12.2020.
5. US Food&Drug Administration. Pfizer-BioNTech COVID-19 Vaccine. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/pfizer-biontech-covid-19-vaccine. Accessed 29.12.2020.
6. European Medicines Agency. EMA recommends COVID-19 Vaccine Moderna for authorization in the EU. https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-moderna-authorisation-eu. Accessed 13.01.2021.
7. European Medicines Agency. EMA recommends COVID-19 Vaccine AstraZeneca for authorisation in the EU. https://www.ema.europa.eu/en/news/ema-recommends-covid-19-vaccine-astrazeneca-authorisation-eu. Accessed 01.02.2021.
8. Public Health England. PHE investigating a novel variant of COVID-19. https://www.gov.uk/government/news/phe-investigating-a-novel-variant-of-covid-19. Accessed 27.01.2021.
9. Tegally H, Wilkinson E, Giovanetti M, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv*. 2020.
10. Voloch CM, Silva FRD, de Almeida LGP, et al. Genomic characterization of a novel SARS-CoV-2 lineage from Rio de Janeiro, Brazil. *medRxiv*. 2020.
11. Volz E, Mishra S, Chand M, et al. Transmission of SARS-CoV-2 Lineage B.1.1.7 in England: insights from linking epidemiological and genetic data. *medRxiv*. 2021.
12. Theel ES, Slev P, Wheeler S, et al. The role of antibody testing for SARS-CoV-2: is there one? *J Clin Microbiol*. 2020;58(8):e00797-00720.
13. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill*. 2020;25(3):2000045.
14. Chan JF, Yip CC, To KK, et al. Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. *J Clin Microbiol*. 2020;58(5):e00310-00320.
15. D'Cruz RJ, Currier AW, Sampson VB. Laboratory testing methods for novel Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2). *Front Cell Dev Biol*. 2020;8:468.
16. Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): current status, challenges, and countermeasures. *Rev Med Virol*. 2020;30(3):e2106.
17. Özçürümez MK, Ambrosch A, Frey O, et al. SARS-CoV-2 antibody testing-questions to be asked. *J Allergy Clin Immunol*. 2020;146(1):35-43.
18. Moat SJ, Zelek WM, Carne E, et al. EXPRESS: development of a high throughput SARS-CoV-2 antibody testing pathway using dried blood spot specimens. *Ann Clin Biochem*. 2020:4563220981106.
19. Gaugler S, Sottas PE, Blum K, Luginbühl M. Fully automated dried blood spot sample handling and extraction for serological testing of SARS-CoV-2 antibodies. *Drug Test Anal*. 2020;13(1):223-226.
20. McDade TW, McNally EM, Zelikovich AS, et al. High seroprevalence for SARS-CoV-2 among household members of essential workers detected using a dried blood spot assay. *PLoS One*. 2020;15(8):e0237833.
21. Morley GL, Taylor S, Jossi S, et al. Sensitive detection of SARS-CoV-2-specific antibodies in dried blood spot samples. *Emerg Infect Dis*. 2020;26(12):2970-2973.
22. Thevis M, Knoop A, Schaefer MS, et al. Can dried blood spots (DBS) contribute to conducting comprehensive SARS-CoV-2 antibody tests?. *Drug Test Anal*. 2020;12(7):994-997.
23. McClendon-Weary B, Putnick DL, Robinson S, Yeung E. Little to give, much to gain-what can you do with a dried blood spot?. *Curr Environ Health Rep*. 2020;7(3):211-221.
24. Luginbühl M, Gaugler S. The application of fully automated dried blood spot analysis for liquid chromatography-tandem mass spectrometry using the CAMAG DBS-MS 500 autosampler. *Clin Biochem*. 2020;82:33-39.
25. Capiau S, Veenhof H, Koster RA, et al. Official International Association for Therapeutic Drug Monitoring and Clinical Toxicology Guideline: development and validation of dried blood spot-based methods for therapeutic drug monitoring. *Ther Drug Monit*. 2019;41(4):409-430.
26. Lange T, Thomas A, Walpurgis K, Thevis M. Fully automated dried blood spot sample preparation enables the detection of lower molecular mass peptide and non-peptide doping agents by means of LC-HRMS. *Anal Bioanal Chem*. 2020;412(15):3765-3777.
27. Roche Diagnostics GmbH. Elecsys Anti-SARS-CoV-2: Immunoassay for the qualitative detection of antibodies against SARS-CoV-2. https://diagnostics.roche.com/content/dam/diagnostics/Blueprint/en/pdf/cps/Elecsys-Anti-SARS-CoV-2-factsheet.pdf. Accessed 09.12.2020.
28. Roche Diagnostics GmbH. Elecsys Anti-SARS-CoV-2 Cobas. https://www.fda.gov/media/137605/download. Accessed 09.12.2020.

How to cite this article: Knoop A, Geyer H, Lerch O, Rubio A, Schrader Y, Thevis M. Detection of anti-SARS-CoV-2 antibodies in dried blood spots utilizing manual or automated spot extraction and electrochemiluminescence immunoassay (ECLIA). *Anal Sci Adv*. 2021;2:440–446. https://doi.org/10.1002/ansa.202100009