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Use of dried blood spot samples for SARS-CoV-2 antibody detection using the Roche Elecsys® high throughput immunoassay

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ABSTRACT

Dried blood spot samples (DBS) provide an alternative sample type to venous blood samples for antibody testing. DBS are used by NHS for diagnosing Hepatitis C and by Public Health England for large scale HIV and Hepatitis C serosurveillance; the applicability of DBS based approaches for SARS-CoV-2 antibody detection is uncertain. The study aimed to compare antibody detection in DBS eluates using the Roche Elecsys® immunoassay with antibody detection in paired plasma samples, using the same assay. The study was in one Police and one Fire & Rescue facility in England; it comprised of 195 participants within a larger sample COVID-19 serodiagnostics study of keyworkers, EDSAB-HOME. Outcome measures were sensitivity and specificity of DBS (the index test) relative to plasma (the reference test), at an experimental cut-off; quality of DBS sample collected; estimates of relative sensitivity of DBS vs. plasma immunoassay in a larger population. 18/195 (9.2%) participants tested positive using plasma samples. DBS sample quality varied markedly by phlebotomist, and low sample volume significantly reduced immunoassay signals. Using an experimental cut-off, sensitivity and specificity of DBS were 89.0% (95% CI 67.2, 96.9%) and 100.0% (95% CI 97.9, 100%) respectively compared with using plasma. The limit of detection for DBS is about 30 times higher than for plasma. DBS use for SARS-CoV-2 serology, though feasible, is insensitive relative to immunoassays on plasma. Sample quality impacts on assay performance. Alternatives, including the collection of capillary blood samples, should be considered for screening programs.

1. Introduction

Testing for SARS-CoV-2 antibodies is important to understand how the infection has spread in the population. However, extensive population testing is not feasible using the currently available, highly sensitive immunoassays, due to the need to take venous blood samples. As such, serosurveillance using home sampling is currently limited to the use of lateral flow immunoassays (LFIA), which often have limited sensitivity [1–5].

Dried blood spot (DBS) samples provide an alternative sample type to venous blood samples for antibody testing, and have been used extensively in screening for other viruses including Hepatitis B, Hepatitis C and HIV [6]. Recent studies have demonstrated the feasibility of using DBS for home blood collection for SARS-CoV-2 antibody screening, with or without virtual supervision [7] as well as in infants [8]. Small scale feasibility studies have evaluated DBS samples for SARS-CoV-2 antibody detection in high risk populations, using plate based enzyme immunoassays, with promising results [9–12]. Field studies indicate the DBS approach is likely to be acceptable [13].

Recently, laboratories, including those in the United Kingdom (UK)’s National Health Service (NHS), have put in place high-throughput immunoassays for SARS-CoV-2 antibodies, which have to date used venous blood samples [14]. However, when considering population screening or individual risk assessment using antibody tests, an approach offering the potential advantages of easy and safe collection [15] without the need for phlebotomy training and with the possibility for self-collection

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of samples [16], stability of the sample at ambient temperature once dried [17] and simple transport to the testing laboratory exempt from UN 3373 transport regulations [18] would be attractive. The taking and transporting of DBS samples has proved successful in screening for other viruses [19], and combined with testing on high throughput automated instrumentation could allow wider access to SARS-CoV-2 serology.

Here we describe a pilot study evaluating the potential for using DBS for SARS-CoV-2 antibody testing using the Roche Elecsys ® Anti-SARS-CoV-2 immunoassay. We chose this platform because it is widely available across the UK’s NHS, and has one of the best performance characteristics of existing assays, as evidenced by a large-scale study [20]. The protocol used is an adaptation of that currently used by the Public Health England (PHE) Manchester virology laboratory for high-throughput screening of Hepatitis C.

2. Methods

2.1. Participants studied

Samples were collected from volunteers attending two study locations over 2 days (3rd and 4th June 2020): Site 1 - Lancashire Police Headquarters and Site 2 - Lancashire Fire and Rescue Service. This recruitment was part of a larger ethically-approved study of UK key workers, Evaluating Detection of SARS-CoV-2 Anti Bodies at HOME (EDSAB-HOME); full recruitment methods have been described [1,21]. In brief, volunteers were key workers who had been working on site during the pandemic, and who were neither experiencing any COVID-19 compatible symptoms, currently or within the former seven days. Eligibility was unaffected by any prior COVID-19 compatible symptoms or SARS-CoV-2 nasal and throat swabs, or antibody tests. The overall approach, details of ethical approvals, and analysis are pre-specified in the study protocol available at http://www.isrctn.com/ISRCTN56609224.

All EDSAB-HOME participants consented to provide a venous blood sample. All of those who attended a study clinic at Site 1 or 2 on the 3rd or 4th June were asked whether they would like to provide an additional DBS sample (this pilot study was ethically approved within the larger EDSAB-HOME protocol); as such, they form a convenience sample. Sample collection was performed by 7 different phlebotomists, 5 at Site 1 and 2 at Site 2. The phlebotomists were experienced in the collection of venous blood samples but had no prior training in the collection of DBS samples, and a short on-site training session was provided.

2.2. Ethical statement

The EDSAB-HOME study was approved by NHS Research Ethics Committee (Health Research Authority, IRAS 284980) on 02/06/2020 and PHE Research Ethics and Governance Group (REGG, NR0198) on 21/05/2020.

2.3. DBS collection system

High flow BD Microtainer® lancets were used for puncturing fingertip skin. We aimed to collect 4 full size spots onto a custom designed collection card. The card comprised PerkinElmer 226 grade collection paper and circle outlines for the collection of 5 separate 25 μL blood spots, 4 of which are on a strip of paper attached with a perforated edge (Fig. 2) to enable removal using a disposable set of tweezers. Samples were stored at room temperature in ziplock bags containing a dessicant pack, and transported to PHE Manchester virology laboratory overnight. Samples were processed for elution on the 11th and 12th June, and stored at −80 °C until tested on 18th June when reagents and machine time were available.

This process is currently in use for analysis of Hepatitis C and HIV antibodies. Validation has been performed for antisera against both these agents showing that elution from dried blood and subsequent storage at −80 °C for up to 2 weeks has minimal impact on immunoassay titres relative to immediate analysis, however we did not perform this validation for SARS-CoV-2 antibodies prior to this pilot study.

2.4. Venous blood analysis (reference test)

Venous blood samples were collected in 6 ml volumes in EDTAVacutainer® tubes (Beckton Dickinson) at the same time as collection of DBS samples and were sent to the PHE Serology Unit (SEU) Manchester at the end of each day (3rd and 4th June) at room temperature. Plasma samples were separated the following morning from the EDTA blood after centrifugation at 1200 g for 15 minutes, and stored at −80 °C until sending to the PHE Rare and Imported Pathogens Laboratory (RIPL) Porton Down for testing (in ambient conditions overnight). All plasma samples were tested with the Roche Elecsys ® Anti-SARS-CoV-2 immunoassay run on the Roche cobas e 801 analytical unit, following the manufacturer’s instructions (COI > 1.0 was considered positive). The Roche Elecsys ® Anti-SARS-CoV-2 immunoassay uses a recombinant protein representing the nucleocapsid (N) antigen and provides detection of antibodies (including IgG) and is validated for use on human serum and plasma. Results from the venous blood analysis were treated as the reference standard in this study and was chosen as this assay was the most sensitive and specific approach available for SARS-CoV-2 serological testing at PHE [20]. Samples were sent from the PHE SEU in Manchester on 17th June 2020; due to limited availability of the Roche machines, plasma samples were analysed at PHE, RIPL, Porton Down between 29th June and 3rd July (as part of a large batch of EDSAB-HOME study samples). Between receipt and analysis, all plasma samples were stored at 4°C.

2.5. Dried blood spot analysis (index test)

The size of the 4 spots collected on the card were assessed and recorded. A full-size spot filling the entire collection circle represents a DBS spot of 25 μL in size. Samples were rated as “good” if 4 full size spots were collected on the card, see Fig. 2a. Spots were considered “small” if on visual inspection one or more appeared to fill only 50% or more of the collection area, and “very small” if one or more appeared to fill less than 50%, see Fig. 2b. A paper strip containing the 4 blood spots, the edges of which are pre-perforated, was removed using single use sterile forceps and placed in a 50 ml skirted centrifuge tube (Eppendorff) containing 1 mL of PBS/Tween 0.05% (Sigma) elution buffer. Tubes were placed flat on an orbital shaker for overnight elution at room temperature. Eluates were aspirated and transferred to false bottom tubes (Roche Diagnostics) and 12 μL tested with the Roche Elecsys ® Anti-SARS-CoV-2 immunoassay run on the Roche e801 analyzer. Apart from using eluate instead of plasma, the protocol followed was that recommended by the manufacturer. Removal of the strip is a rapid step (~30 seconds) and the whole process takes about 1 minute hands-on per sample.

Index tests were performed before the reference tests; those performing the index tests were blind to the reference test results, and vice versa. No clinical information on the samples was available to those performing either the index or reference test.

2.6. Statistical Analysis

We described the quality of DBS samples overall, and by each individual phlebotomist. Quantitative results from index and reference assays were depicted graphically. Among cases positive on the reference test, linear models were fitted using the R glm function modelling \( \log_2(y) = \log_2(x) + c + \text{error} \), where \( y \) is the Roche Elecsys ® immunoassay signal in the paired plasma sample, \( x \) is the Roche Elecsys ® immunoassay signal in the DBS eluate, and \( c \) is a categorical variable representing spot size, which is either 0 (if the spot size is ‘good’) vs. 1 if it is small or very small.

For the index test, we derived an experimental cut-off, which is ten
median absolute deviations about the median in samples negative by the reference test. An estimated limit of detection was computed from the regression model above as the most likely reference test value at which the index test values were at the experimental cut-off.

Sensitivity and specificity were computed by comparing the results of the dichotomised index test, using the experimental cut-off, relative to the dichotomised reference test result, using the manufacturer’s cut-off. All computations used R version 4.02. Receiver operating characteristic (ROC) analysis was performed using the pROC package, linear modelling used the glm function, and confidence intervals around proportions were computed using Wilson’s method with the Hmisc package binconf function.

In a separate analysis, we examined the distribution of the reference test (Roche Elecsys immunoassay on plasma) in 2,652 samples from the EDSAB-HOME study, computing the immunoassay cumulative frequency distribution, and comparing it with the predicted limit of detection of DBS samples.

3. Results

3.1. Participants

We planned to recruit 200 individuals with paired venous and DBS samples from participants on 3rd and 4th June 2020. Of the 475 individuals attending EDSAB-HOME study on those dates, 195 samples were collected, of which 18 were positive using the reference assay (Fig. 1); 4 of the individuals reported having previously had a positive PCR test, while the remaining had not. Reasons for not collecting samples included subject or phlebotomist electing not to do so (which was permitted under the protocol). The demographics of those providing DBS samples were similar to those who did not from the same sample days (Table 1) and the larger EDSAB-HOME study cohort in Police & Fire Stream (Supplementary Table 1).

3.2. Quality of DBS samples

Overall, around two thirds of the DBS samples were rated as “good” (n = 121, 62.1%); 42 (21.6%) were “small” and 32 (16.3%) were “very small” (Fig. 2). The quality of sample collected varied by phlebotomist collecting the samples (Fig. 3) ranging from 26/27 samples rates as “good” size (Phlebotomist 7) to 0/21 rated as a “good” size (Phlebotomist 4).

3.3. Accuracy of the test applied to DBS samples relative to plasma samples

When we applied the manufacturer’s cutoff value, optimised for plasma, to the DBS eluates, the sensitivity relative to plasma was poor (44%, 95% CI 24.5, 66.2) (Table 2). We therefore considered whether the cutoff used for plasma was appropriate for our DBS eluates.

The relationship between the immunoassay ratio from venous plasma vs. DBS is shown for the 195 samples in Fig. 4A. Although samples with negative reference tests gave very similar immunoassay ratios in both index and reference tests (black dots), positive samples generated lower results (median 0.7 vs. 18 units). However, for every unit increase in immunoassay signal in the Roche immunoassay performed on plasma (the reference test), the immunoassay signal increased linearly provided the results were above the limit of detection (Fig. 4A). Linear modelling quantified both the relationship between the immunoassays signals in the two tests (the slope of the line in Fig. 4A) and the limit of detection (Table 4). The limit of detection of the immunoassay performed on DBS test was estimated as corresponding to 5.9 antibody index units in tests on plasma (Fig. 4A). If we compute a limit of detection for the immunoassay on plasma in the same way we computed it for the immunoassay on DBS eluates, the cutoff is about 0.17, or about 33 fold lower. This limit of detection, derived from a regression model is based on “good” quality spots. Since index test ratios were 45% (95% CI 23, 82%) smaller in those with smaller spots vs. the 62% of “good” spots (Table 4), the test will be even less sensitive if smaller spots are obtained.

Nevertheless, in this data set, the high correlation between tests results (Fig. 4A), the small number of test results with low reference test results, and a predominance of “good” size spots, generates a high area-under-curve in ROC analysis (c = 0.985, Fig. 4B). Using the experimental cut-off derived above, of 18 positive samples using the reference test, 16 were positive in the index test giving a sensitivity estimate of

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### EDSAB-HOME KEYWORKER COHORT

**Recruitment in June 2020**

- Completed an online questionnaire: n = 3,087
- Did not attend study clinic: n = 220
- Requested removal from study prior to analysis: n = 5
- Attended study clinic: n = 2,867

**Attended Police or Fire & Rescue study clinics on 4 or 5 June 2020:**
- 475
- Sample missing/unlabelled: n = 4
- Insufficient sample: n = 1

**DBS samples taken:**
- n = 186
  - Reference test positive: n = 18
  - Reference test negative: n = 177

**Health care worker and Fire & Police general study population:**
- n = 2,652
  - Reference test positive: n = 565
  - Reference test negative: n = 2,087

**Attended Health care worker previously positive PCR study clinic:**
- n = 156
  - Never PCR positive: n = 1

**Stream C Health care worker previously positive PCR study clinic:**
- n = 154
  - Not analysed here.

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Fig. 1. Flow diagram.

Figure 1 Flow diagram illustrating where DBS samples were taken within the EDSAB-HOME study.
89.0% (95% CI 67.2, 96.9%), while maintaining a specificity of 100% (95% CI 97.9, 100%) (Table 2). Demographics and clinical symptoms of those testing positive on both the index test and reference test can be found in Table 3.

To understand whether this sensitivity is likely to be applicable to other samples, we examined the reference test (Roche Elecsys® immunoassay on plasma) in 2,652 samples from the EDSAB-HOME study (Fig. 1); there is no overlap between these samples and those on whom DBS samples were taken. Of these, 595 (22%) were positive on the reference assay, of which 62/595 (10.4%) were below the predicted limit of detection of DBS assays (Fig. 4C).

4. Discussion

In this study, we showed that the DBS based approach used is an insensitive approach relative to assays on venous plasma, with the limit of detection of the assay estimated to be about 30 times lower with DBS samples. This is not unexpected, because the DBS eluate is at best 10% plasma. In reality, elution will be incomplete, and (as we showed) smaller spots result in lower concentrations of antibody. Nevertheless, in the small sample tested, the sensitivity of DBS at our experimental cut-off relative to the reference test was 89%, with wide confidence intervals due to the small study size. This is compatible with an observation, in a much larger independent cohort of 595 seropositive individuals, that about 10% of the population studied have antibody concentrations which we estimate would not be detectable by DBS.

This study has several important limitations. Firstly, DBS has only been tested on a relatively small sample set, only 18 of which were positive on the reference standard, and only four of whom had reported a previous PCR test and the majority of those testing positive reported having tested positive by other methods. Nevertheless, the results are promising and suggest that DBS is a useful approach for screening large numbers of individuals for SARS-CoV-2 antibodies.
having previously had COVID-19 compatible symptoms. Secondly, sensitivity and specificity estimates of the index test were based on cut-offs derived from the data, and so should be considered exploratory. Thirdly, we assessed samples taken by trained phlebotomist. Variation in performance was observed between phlebotomists (and presumably would also occur between self-taken samples); as such, usability studies to quantify the extent of this variation would be essential to understand how the tests perform when self-administered by the target population.

In considering the generalisability of our study, the implications of the relative insensitivity of DBS approaches, and comparing our observations with those of others assessing DBS accuracy, there are some parallels with observations we made with another antibody detection technology (lateral flow testing) which is also insensitive relative to laboratory immunoassays [1]. In our recent work, we showed that the sensitivity of the lateral flow test appears higher if individuals with previous PCR positivity are examined (particularly those who had been eligible for testing at the start of the pandemic), because these people have more severe disease and higher antibody levels which are not representative of antibody levels in the general population, leading to a so called spectrum bias [23]. Comparisons of DBS accuracy using approaches using pre-pandemic samples vs. samples from individuals who are PCR positive may also overinflated estimates of DBS accuracy. Put alternatively, the analytical sensitivity of the test depends on the distribution of antibody concentrations in the target population; here, we used sequential sampling in a key worker population to try to obtain samples with an antibody distribution representative of that population, but we note that if other cohorts are studied in which antibody concentrations have declined compared with the one we studied, DBS will perform worse than we observe here.

Our sampling strategy, used in an attempt to minimise spectrum bias [1], may explain the lower sensitivity we observed relative to (for example) the study by Karp et al, in which they observed a 100% sensitivity of DBS compared to plasma, however all their positive samples were taken in the spring and confirmed with PCR; as such, these individuals were more likely to have experienced more severe COVID-19 (as testing was limited to these individuals at the time) and had limited time for antibody levels to decline [24]. Similar considerations apply when comparing our results with those of Morley et al, who report DBS analytical performance to be comparable to matched serum samples, with a sensitivity of 98.1% and specificity of 100%, when compared to

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**Fig. 4.** Immunoassay signals from plasma and DBS samples.

Figure 4 (A) The relationship between antibody ratios obtained using Roche Elecsys immunoassays for plasma (reference text, x-axis) vs. paired dried blood spots (index test, y-axis). Samples positive in the plasma immunoassay are in red. Solid blue line: manufacturer’s cutoff for plasma samples (1). Red dotted line: experimental cutoff ten median absolute deviations above the reference test negative median for DBS (index test) samples (n = 177). Blue dotted line: experimental cutoff ten median absolute deviations above the reference test negative median for plasma (reference test) samples. Solid black line: regression of index test immunoassay ratios on reference test immunoassay ratios. Arrow: estimate of limit of detection. (B) Receiver operator curve, showing sensitivity/specificity of DBS usage, relative to an immunoassay on plasma reference standard. (C) cumulative proportion of 595 seropositive cases from Police, Fire and Healthcare workers collected by EDSAB-HOME. There is no overlap with cases included in panel A. The estimated limit of sensitivity of the index test is shown as a dotted line.
and monitored and provided the population antibody distribution is periodically monitored using plasma immunoassays to allow correction for DBS insensitivity. When considering large-scale DBS testing programmes, additional factors which might impact on programme success include the more complex processing of DBS samples relative to plasma samples upon arrival in the testing laboratory. However, this may not be a substantial barrier as options for automation have been presented by Gaugler et al [26].

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CRediT authorship contribution statement

Ranya Mulchandani: Conceptualization, Formal analysis, Investigation, Validation, Writing - original draft, Writing - review & editing.
Ben Brown: Formal analysis, Validation, Writing - original draft, Writing - review & editing. Tim Brooks: Writing - review & editing.
Amanda Semper: Writing - review & editing. Nicholas Machin: Writing - review & editing. Ezra Linley: Writing - review & editing. Ray Borrow: Writing - review & editing. David Wylie: Formal analysis, Investigation, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest
The authors report no declarations 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.jcv.2021.104739.

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