Analysis of SARS-CoV-2 antibodies from dried blood spot samples with the Roche Elecsys Immunochemistry method

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ABSTRACT

Objectives: The aim of this study was to evaluate the application of the Roche Elecsys anti-SARS-CoV-2 assay to capillary dried blood samples for high throughput analyses on Roche COBAS 6000 systems.

Design and methods: The performance of the Elecsys anti-SARS-CoV-2 assay was assessed using three sets of dried blood spot samples. Method correlation was performed using spiked blood samples. Sensitivity and specificity were calculated using paired donor samples. An additional cohort of 50 individuals, including COVID-19 convalescent cases, was used for the evaluation of at-home collection for mail transport, and stability studies.

Results: The Elecsys anti-SARS-CoV-2 assay using dried blood spot samples showed an excellent agreement of 98.9% with results obtained using their paired serum samples, and 86.7% accuracy with dried blood spots collected after 9 days from diagnostic (PCR) tests.

Conclusions: Capillary dried blood spot samples can be confidently used on Roche COBAS automated analyzers to monitor the epidemiology of COVID-19, and are suitable for use in large-scale screening programs.

1. Introduction

The Elecsys Anti-SARS-CoV-2 is a commercial immunoassay intended for identifying individuals exposed to the COVID-19 virus by detecting total antibodies in venous serum or plasma [1,2]. However, the use of conventional venous samples represents a logistic barrier, and an obvious limitation for screening large populations. It requires people to travel to a patient service center for phlebotomy, or a healthcare worker must travel to multiple locations to collect blood to transport back to a central laboratory. The use of dried blood spots has shown to be a successful tool for COVID-19 epidemiology research using ELISA-based assays [3–5]. Recently, Vibrant Laboratories (San Carlos, CA) has developed the first commercial dried blood spot system for COVID-19 serology; nonetheless, samples must be collected by a healthcare provider. An alternative approach is to use high throughput analyzers already available in most central laboratories, and extend their validation from conventional venous samples to dried blood spots [6]. Dried blood spot sampling requires no professional training, can be performed by individuals in their own home, and the samples can be shipped to the laboratory by regular mail. Furthermore, dried blood samples are typically stable for a longer time at ambient temperature, and the biohazard risk is reduced since many viruses lose infectivity upon sample drying [7]. Our laboratory initially performed COVID-19 serology screening using dried blood spots with available commercial ELISA methods. However, the drawbacks of an ELISA include the long turnaround
time for results, and the labor involved. In order to reduce turnaround, reduce labor and increase screening capacity, our laboratory has validated the Roche Elecsys Anti-SARS-CoV-2 assay for use with dried blood spots on Roche COBAS 6000 systems.

2. Materials and methods

2.1. Analyzer system

The Elecsys Anti-SARS-CoV-2 is a double-antigen electrochemiluminescent immunoassay using a recombinant nucleocapsid protein of the virus, geared towards the detection of late, mature, high affinity antibodies independent of the subclass. It is a total SARS-CoV-2 antibody assay (IgA, IgM, and IgG) detecting predominantly, but not exclusively, IgG. We installed this assay on the Roche COBAS e601 module in a COBAS 6000 automated system. Results are reported based on numeric values in form of a cutoff index (COI) as well as in form of qualitative results non-reactive (COI < 1.0; negative) and reactive (COI ≥ 1.0; positive). All assays were performed using multiple reagent lots for verification of consistency at lower COI readings when using dried blood samples. Reagents used in this study include lot # 00496298, 00499300, and 00507260. Control materials were prepared in accordance with Roche SARS-CoV-2 specifications and run daily.

2.2. Clinical specimens

All studies were conducted using anonymized residual samples from routine health awareness testing, and approved by the ethics committee at CoreMedica Laboratories in accordance with the Declaration of Helsinki. Dried blood spot samples were either prepared from whole blood samples, or obtained from former test requests. Capillary blood was collected by fingerstick using a disposable contact-activated lancet (BD Medical Systems # 366594), and then captured on a Whatman 903 card (Cytiva # 10534612). Whatman 903 cards are in compliance with the requirements of the CLSI LA4-A5 consensus standard, and listed as an FDA Class II Medical Device. In Europe, they are sold as a “CE” marked “in vitro diagnostic” in compliance with 98/79/EC IVD Directive. One drop of blood (ca. 0.04 mL) was applied to each circle on the Whatman 903 cards, and dried for 30 min prior to transport or storage. For each card, four paper disks (3 mm punches) were removed using a Harris Uni-Core punch (Ted Pella # 15078), and placed into an 8.5 mL polystyrene tube (Sarstedt # 55.495). Before analysis, each sample was first prepared for hydration and elution of antibodies from the solid matrix by adding 0.25 mL of phosphate buffered saline (Sigma #P4417) using an Eppendorf repeater pipette equipped with a 25 mL Combitip (Eppendorf # 0030089472). The tubes were incubated at room temperature without agitation for 4 h for proper sample elution. Each primary elution tube had a Hitachi sample cup (Roche # 10394246001) sitting on top to avoid concentration of the sample. After elution, each specimen was gently shaken, poured into the Hitachi sample cup, and returned to the same position on top of the primary tube. This procedure fully reconstitutes each dried blood specimen into solution so it becomes ready for analysis using the standard Roche COBAS standard serum procedure.

2.3. Method comparison

An antibody-positive pool, approximately 20 mL, was prepared by combining leftover positive-antibody plasma samples with a COI value of 96. A set of 10 antibody-negative whole blood samples collected in 5 mL standard purple tops (BD # 367861) were partially depleted of their plasma and spiked with a similar volume of the positive pool to yield samples with a COI value in the range 0–30. The plasma spiked samples were mixed and aliquots of 0.04 mL were applied to different Whatman 903 cards in each corresponding concentration level. Dried blood samples were dried for 30 min and stored until analysis. Left overs in each level of spiked blood were centrifuged, and the separated plasma was directly analyzed for SARS-CoV-2 antibodies on the e601 module of a COBAS 6000 analytical platform according to standard manufacturer protocols.

2.4. ROC analysis

Sensitivity and specificity were determined with 94 cases where parallel COVID-19 serology results were available in both serum and dried blood samples collected at the same time. These studies consisted of measuring the instrument response with dried blood spots and correlating the results to a positive or negative paralleled serum result for each sample. The sensitivity and false positive probabilities were calculated at each COI level. Serum results were considered the gold standard when evaluating dried blood samples. The corresponding Receiver Operator data was calculated for determining the cut-off value with the highest true positive rate together with the lowest false positive rate for dried blood samples.

2.5. Precision and stability

Within-run precision was assessed by measuring ten positive, and negative dried blood spot replicates. The coefficient of variation (CV) was determined as CV (%) = (standard deviation x100)/mean, and results were considered acceptable at values of CV less than 5%. Inter-assay precision studies were not performed due to expected dried blood specimen decay over time. Instead, the stability of dried blood spots was determined at five different levels ranging from weakly to strongly positive samples. A sample was considered degraded when COI values were consistent with negative samples. Seven replicates were analyzed over a period of 30 days at intervals of 5 days to assess the stability of SARS-CoV-2 antibodies on this matrix. In each case, 0.04 mL of collected venous whole blood was spotted onto
Whatman 903 cards. Each dried blood sample was stored without desiccant in a regular mail envelope at oscillating ambient temperature (20–30 °C) using a standard laboratory incubator until analysis.

2.6. Correlation with diagnostic tests

Dried blood spot COVID-19 serology results were correlated with 30 cases where molecular (PCR) diagnostic data was available. This study was performed with dried blood samples collected at home by health awareness participants and sent to the laboratory by regular mail, courier service, or express service. Sensitivity and specificity data was obtained from laboratory records with PCR-confirmed SARS-CoV-2 infection or confirmed negative. The PCR method was considered the gold standard for comparison to COVID-19 serology.

Table 1

ROC Analysis of clinical sensitivity and specificity at variable COI levels.

| COI Result | Negatives | Positives | ^a True Positives | False Positives |
|------------|-----------|-----------|-------------------|-----------------|
| 0.069      | 1         | 1         | 1                 | 1               |
| 0.070      | 3         | 1         | 0.988             | 0.012           |
| 0.071      | 8         | 1         | 0.952             | 0.048           |
| 0.072      | 17        | 1         | 0.857             | 0.143           |
| 0.073      | 19        | 1         | 0.654             | 0.346           |
| 0.074      | 10        | 1         | 0.429             | 0.571           |
| 0.075      | 11        | 1         | 0.309             | 0.691           |
| 0.076      | 7         | 1         | 0.179             | 0.821           |
| 0.077      | 3         | 1         | 0.095             | 0.905           |
| 0.078      | 2         | 1         | 0.059             | 0.941           |
| 0.079      | 2         | 1         | 0.036             | 0.964           |
| 0.081      | 1         | 1         | 0.012             | 0.988           |
| 0.096      | 1         | 1         | 0                 | 0               |
| 0.118      | 1         | 1         | 0.9               | 0               |
| 0.126      | 1         | 1         | 0.8               | 0               |
| 0.226      | 1         | 1         | 0.7               | 0               |
| 0.251      | 1         | 1         | 0.6               | 0               |
| 0.276      | 1         | 1         | 0.5               | 0               |
| 0.362      | 1         | 1         | 0.4               | 0               |
| 0.464      | 1         | 1         | 0.3               | 0               |
| 0.786      | 1         | 1         | 0.2               | 0               |
| 2.450      | 1         | 1         | 0.1               | 0               |

^a The sensitivity (true positives) and false positive probabilities calculated at each COI level are tabulated side-by-side with the corresponding dried blood sample results for determining the most appropriate cut-off value.

Fig. 1. Anti-SARS-CoV-2 correlation of venous serum and capillary dried blood analyzed by the Elecsys-anti-SARS-CoV-2 method. Each data point represents the mean average of two sample replicates.

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2.7. Statistical analysis

Receiver Operating Characteristic (ROC) calculations were performed with Microsoft Excel Pivot Table Function. All other statistical analyses were performed using EP Evaluator (Data Innovations, LLC).

3. Results

3.1. Method comparison

A comparison between different levels of SARS-CoV-2 antibodies in plasma separated from antibody-spiked blood, and the corresponding dried blood spot samples revealed a proportional instrument response between each specific SARS-CoV-2 antibody level (Fig. 1). The responses were approximately linear up to a COI = 0.5 on dried blood samples or COI = 20 on the corresponding plasma ($R^2 = 0.9963$), and distinctive from samples not spiked with SARS-CoV-2 antibodies.

3.2. Sensitivity and specificity

On the basis of ROC curve analysis, the optimal signal-to-cutoff value was established at 0.100. This signal-to-cutoff value was thus used instead of the $>/= 1.0$ threshold recommended by the manufacturer to identify antibody-positive serum or plasma samples. Table 1 shows the frequency distribution of COI results from dried blood spots associated with positive and negative results using serum samples. It was associated with a sensitivity of 90.0% (95% confidence interval [CI], 59.6%–98.2%) and a specificity of 100.0% (95% CI, 95.6%–100.0%). The area under the ROC curve was 1.0000 ($P < 0.0001$; data not shown). In negative dried blood spot samples, the mean anti-SARS-CoV-2 antibody COI value ($/pm$SD) was 0.0741 $/pm$0.0053. Positive dried blood sample values were highly variable without a parametric distribution. The agreement between dried blood spots and serum results was 98.9% (95% CI, 94.2%–99.8%). Only one false negative result was found using the newly established signal-to-cutoff value.

3.3. Precision and stability

The dried blood spot method for SARS-CoV-2 antibody exhibited acceptable within-day precision of 1.5–2.4% for positive and negative samples, respectively (Table 2). The effect of long-term storage at ambient temperature (20–30 °C) on dried blood samples is shown in Table 3. SARS-CoV-2 antibodies remained stable in dried blood spots for at least 30 days. The observed changes in COI values did not change the qualitative outcome of the assay, except in one weakly positive sample (Sample #5) where the stability was only 10 days.

| Table 2 |
| Within-day precision studies. |
| Samples | Positive (COI Values) | Negative (COI Values) |
| 1 | 0.154 | 0.073 |
| 2 | 0.153 | 0.068 |
| 3 | 0.156 | 0.069 |
| 4 | 0.152 | 0.070 |
| 5 | 0.156 | 0.071 |
| 6 | 0.158 | 0.070 |
| 7 | 0.152 | 0.071 |
| 8 | 0.153 | 0.067 |
| 9 | 0.153 | 0.069 |
| 10 | 0.150 | 0.069 |
| Mean | 0.154 | 0.070 |
| CV (%) | 1.456 | 2.443 |

| Table 3 |
| Long-term stability (30 days) study for five dried blood spot samples stored at ambient temperature (20–30 °C). |
| Samples | Time (Days) |
| 1 | 1 | 2.790 |
| 2 | 1 | 0.437 |
| 3 | 1 | 0.170 |
| 4 | 1 | 0.389 |
| 5 | 1 | 0.105 |
| 1 | 5 | 2.240 |
| 2 | 5 | 0.364 |
| 3 | 5 | 0.181 |
| 4 | 5 | 0.275 |
| 5 | 5 | 0.104 |
| 1 | 10 | 2.200 |
| 2 | 10 | 0.410 |
| 3 | 10 | 0.192 |
| 4 | 10 | 0.221 |
| 5 | 10 | 0.109 |
| 1 | 15 | 1.940 |
| 2 | 15 | 0.313 |
| 3 | 15 | 0.150 |
| 4 | 15 | 0.195 |
| 5 | 15 | 0.097 |
| 1 | 20 | 1.720 |
| 2 | 20 | 0.322 |
| 3 | 20 | 0.174 |
| 4 | 20 | 0.191 |
| 5 | 20 | 0.108 |
| 1 | 25 | 1.620 |
| 2 | 25 | 0.274 |
| 3 | 25 | 0.157 |
| 4 | 25 | 0.167 |
| 5 | 25 | 0.102 |
| 1 | 30 | 1.480 |
| 2 | 30 | 0.245 |
| 3 | 30 | 0.159 |
| 4 | 30 | 0.153 |
| 5 | 30 | 0.098 |

COI values are shown in each column for the corresponding day and sample. A cut-off value of 0.100 is used to discriminate a positive (COI $/>= 0.100$) from a negative sample (COI $< 0.100$).
3.4. PCR correlation

Self-collected dried blood samples analyzed with the Roche Elecsys anti-SARS-CoV-2 method demonstrated acceptable accuracy of 86.7% (95% CI; 70.3–94.7%) when compared to PCR diagnostic assays. This qualitative method comparison was associated with a sensitivity of 71.4% (95% CI, 45.4% - 88.3%) and a specificity of 100.0% (95% CI, 80.6%–100.0%). The positive and negative predicted values of the dried blood method, calculated at a disease prevalence of 1%, was 100%, and 99.7%, respectively, support the method’s utility as a tool for identification of past SARS-CoV-2 infection in populations with low disease prevalence.

4. Discussion

Improving understanding of COVID-19 dynamics and evolution is necessary for successful future public health outcomes. Effective disease surveillance is critical, and also necessary in the context of massive vaccination programs in 2021, to differentiate the immune responses due to vaccine and natural SARS-CoV-2 infections. Since most vaccine technologies are based on the expression of the viral spike antigen, a nucleocapsid antibody assay can reveal true vaccine efficacy in asymptomatic individuals [8]. The challenge is to provide an easy-to-use blood collection system with simple logistics to expand seroprevalence studies to all sectors of the population. Today, more than 300 anti-SARS-CoV-2 immunoassays are commercially available worldwide [9]. However, the means to bring effective antibody testing directly and conveniently to people is limited. Previous reports have demonstrated the feasibility of using dried blood samples for serological testing of SARS-CoV-2 antibodies [3,4,6,10,11]. Our study was aimed at validating this strategy with Roche automated instruments for high volume analysis using a qualitative nucleocapsid assay. This approach has several advantages over ELISA methods, even when the sample preparation procedure is automated [12]. Moreover, Roche systems are available worldwide in many central laboratories, and dried blood spot analysis does not require any modification to instrument parameters or reagents. Our matrix equivalency studies included three sets of experiments including antibody-spiked samples, venous/dried blood parallels, and self-collected blood spot samples at home from patients who also provided PCR-based diagnostic data for correlation. At ambient temperature, antibodies degraded slowly, but qualitative outcomes remained constant. Dried blood spots decay has a rate proportional to the concentration of antibodies and detected for many days regardless of the antibody level. This property makes these samples suitable for regular mail transport and simplifies sample logistics since dried blood specimens are considered nonregulated, exempt materials by local and international regulations [13–15]. The main limitation of serological testing is the fact that after symptoms appear, analytical sensitivity directly depends on the day the sample is collected. However, at-home dried blood spot collections show that PCR-confirmed cases display an effective SARS-CoV-2 serology after 9–14 days after infection diagnostics (Table 4), with a positive

| Sample ID | PCR Result | Daysa | Dried Blood Serology Result |
|-----------|------------|-------|----------------------------|
| 1         | POS        | 73    | POS                        |
| 2         | POS        | 65    | POS                        |
| 3         | POS        | 14    | POS                        |
| 4         | POS        | 95    | POS                        |
| 5         | NEG        | 2     | NEG                        |
| 6         | NEG        | –2    | NEG                        |
| 7         | NEG        | 5     | NEG                        |
| 8         | NEG        | 1     | NEG                        |
| 9         | NEG        | –1    | NEG                        |
| 10        | POS        | 14    | POS                        |
| 11        | NEG        | 4     | NEG                        |
| 12        | POS        | 11    | NEG                        |
| 13        | NEG        | 11    | NEG                        |
| 14        | NEG        | –1    | NEG                        |
| 15        | NEG        | –1    | NEG                        |
| 16        | NEG        | 3     | NEG                        |
| 17        | NEG        | 10    | NEG                        |
| 18        | NEG        | –9    | NEG                        |
| 19        | NEG        | 15    | NEG                        |
| 20        | NEG        | 11    | NEG                        |
| 21        | NEG        | –2    | NEG                        |
| 22        | POS        | 9     | POS                        |
| 23        | POS        | 35    | POS                        |
| 24        | POS        | 34    | POS                        |
| 25        | POS        | 208   | POS                        |
| 26        | NEG        | 8     | NEG                        |
| 27        | POS        | 88    | NEG                        |
| 28        | POS        | 24    | NEG                        |
| 29        | POS        | 83    | POS                        |
| 30        | POS        | 33    | NEG                        |

a Indicates the number of days between the COVID-19 Diagnosis (PCR test) and the day the serology test was performed. Negative numbers indicate cases where a serology test was performed prior to a PCR test.
predictive value of 100% and a negative predictive value of 96.2%, calculated at 10% COVID-19 prevalence as reported in many jurisdictions [16]. Reliable, economic easily accessible ways to access the extent of pandemic effects in asymptomatic members of the population must also be advanced since subclinical patients usually do not contact healthcare professionals, resulting in misleading public health data [17]. In this context, the ability to provide reliable COVID-19 serology data from self-collected samples on filter paper combined with high throughput capability, not only makes epidemiology work more effective and easier by including a larger fraction of the population, but also is especially valuable in less developed parts of the world or in other situations where conventional blood collection is not an option.

5. Conclusions

We demonstrate that capillary blood specimens collected at home using the dried blood spot technique in combination with the Roche Elecsys Immunochemistry method is a useful tool for large-scale application to COVID-19 epidemiological monitoring. The method shows excellent agreement with serum samples obtained by conventional venipuncture, and provides a much faster and efficient workflow and analysis than ELISA. Additionally, the long-term temperature stability properties of the samples enable significant flexibility in storage and processing of dried blood specimens for SARS-CoV-2 antibody screening. A LOINC Code 95825-6 was generated to use this analytical approach with current Electronic Medical Record systems and to report results to public health authorities.

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Credit author contribution statement

Cristian Saez: Conceptualization, Investigation, Formal analysis, Visualization, Writing - original draft. Elizabeth Fontaine: Investigation, Writing - review and editing.

Declaration of competing interest

None of the authors have any conflict to report.

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