 doi:10.20944/preprints202203.0375.v1

Article

Rapid detection of anti-SARS-CoV-2 antibodies with a screen-printed electrode modified with a spike glycoprotein epitope

Willson A. Ameku[1], David W. Provance, Jr.[1,2,] Carlos M. Morel[1] and Salvatore G. De-Simone[1,2,3,*]

[1] FIOCRUZ, Center for Technological Development in Health (CDTS)/National Institute of Science and Technology for Innovation in Neglected Populations Diseases (INCT-IDPN), Rio de Janeiro 21040-900, RJ, Brazil; akira.ameku@gmail.com (W.A.A.); bill.provance@fiocruz.br (D.W.P.J.); carlos.morel.@fiocruz.br (C.M.M.); salvatore.simone@fiocruz.br (S. G. D-S.)

[2] Laboratory of Epidemiology and Molecualr Systematics (LESM), Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro 21040-900, RJ, Brazil

[3] Cellular and Molecular Department, Biology Institute, Federal Fluminense University, Niterói 24020-141, RJ, Brazil.

* Correspondence: salvatore.simmone@.fiocruz.br; Tel.: +55-21386-58183

Abstract: Background: The coronavirus disease of 2019, COVID-19, is caused by an infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was recognized in late 2019 and has since spread worldwide leading to a pandemic with unprecedented health and financial consequences. There remains an enormous demand for new diagnostic methods that can deliver fast, low-cost, and easy-to-use confirmation of a SARS-CoV-2 infection. We have developed an affordable electrochemical biosensor for the rapid detection of serological immunoglobulin (Ig) G antibody in sera against the Spike protein. **Materials and Methods**: A previously identified linear B-cell epitope (EP) specific to SARS-CoV-2 spike glycoprotein and recognized by IgG in patient sera was selected for the target molecule. After synthesis, the EP was immobilized onto the surface of the working electrode of a commercially available screen-printed electrode (SPE). The capture of SARS-CoV-2 specific IgGs allowed the formation of an immunocomples that was measured by square wave voltammetry from its generation of hydroquinone (HQ). **Results**: An evaluation of the performance of the EP-based biosensor presented a selectivity and specificity for COVID-19 of 93% and 100%, respectively. No cross-reaction was observed to antibodies against other diseases that included Chagas disease, Chikungunya, Leishmaniosis, and Dengue. Differentiation of infected and non-infected individuals was possible even at high dilution factor that decreased the required sample volumes to a few microliters. **Conclusion:** The final device proved suitable for diagnosing COVID-19 assaying actual serum samples and the results displayed good agreement with the molecular biology diagnoses. The flexibility to conjugate other EPs to SPEs suggests that this technology could be rapidly adapted to diagnose new variants of SARS-CoV-2 or other pathogens.

Keywords: SARS-CoV-2; COVID-19; spike glycoprotein; epitope; electrochemical biosensor; point of care; immunological diagnostic.

 doi:10.20944/preprints202203.0375.v1

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a global pandemic of coronavirus disease 2019 (COVID-19) [1]. Citizens of many countries were compelled to stay under partial or complete lockdown for months due to high transmissibility and disease severity [2]. Between the uncertainty about the effectiveness of vaccines aga inst severe disease and transmission to new variants along with an absence of adequate therapies [1], diagnosis has played an important role in decision making. Case detection, monitoring, infection prevention, and supportive care are all tools for fighting the SARS-CoV-2 pandemic [1,3].

To keep up with our healthcare needs, it is crucial to develop a rapid point-of-care test to detect potential carriers of COVID-19 and those who have recovered as disease dynamics change at the speed of its spread [1]. While the gold standard technique for the diagnosis of the initial phase of an infection is the detection of viral genomic nucleic acid through reverse transcription-polymerase chain reactions (RT-PCR) from nasal and mouth swabs, its implementation in resource-limited settings is restricted due to infrastructure, skilled personnel and time restrictions [1]. Serological assays for the presence of COVID19 related immunoglobulin (Ig) M or IgG antibodies has provided a cost-effective and accurate tracking of virus transmission to implement socio-political strategies against the spread of the contagion [1,4,5].

As a platform, electrochemical biosensors stand out to meet the demands for serological diagnostics through their characteristics; rapid, simple, portable, sensitive, easy-to-use, miniaturize, and compatibility with portable instruments [6–9]. They can be combined with a wide range of biological recognition elements to detect clinically relevant compounds such as enzymes, nucleic acid, antibodies, epitopes, and others [10–15]. Amongst these choices, epitopes (Eps) hold a high interest since they represent the minimum amino acid sequences in a pathogens proteome that are bound by antibodies generated in a patient in response to an infection [7]. Their use can improve selectivity by the elimination of cross-reactivity based on sequence similarity to other pathogen, which a major concern when whole antigens are used due to the presence of non-specific epitopes that can react with antibodies [16].

Here, the sensitivity of electrochemical measurements was combined with the specificity of EPs to develop an affordable biosensor for a serological assay to detect the presence of anti-SARS-CoV-2 antibodies as a diagnosis of COVID-19. A commercially available screen-printed electrode (SPE) was employed while an epitope in SARS-CoV-2 spike glycoprotein (S) was employed as a binding target for antibody capture. The high performance in identifying infected patients and the absence of cross-reactivity against sera from patients with other illnesses such as Chagas, Chikungunya, Leishmaniasis, and Dengue suggests that his platform could be a viable solution to screening large number of people. The flexibility of the proposed technology is rapidly adaptable to variants to a wide range of diseases by altering only the binding target.

 doi:10.20944/preprints202203.0375.v1

2. Materials and Methods

2.1. Patient samples

Three serum samples confirmed positive for a SARS-CoV-2 infection by an RT-PCR diagnostic test on nasopharyngeal or oropharyngeal swabs were used as control. Additional negative controls were collected before the pandemic from healthy individuals and patients diagnosed with Chagas disease, Dengue, Leshmaniose, and Chikungunya. To ensure patient privacy, all samples were provided without identifying information. For experiments, a panel of 14 sera collected from individuals with suspected contact with individuals with COVID-19 was used. RT-PCR on nasopharyngeal or oropharyngeal swabs was used to confirm cases of COVID-19.

2.2. Chemicals and Reagents

Glutaraldehyde (50% w/w, GA), bovine serum albumin (BSA), tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), NaH_2PO_4, Na_2HPO_4, $MgCl_2$, and NaCl were purchased from Sigma-Merck (St Louis, MO, USA). Goat anti-human IgG conjugated with alkaline phosphatase (secondary IgG antibody, sec-IgG) was purchased from ThermoFisher (Waltham, MA, USA). Hydroquinone diphosphate (diPho-HQ) salt was purchased from Methron Dropsens (Astúrias, Astúrias, Spain). Deionized water with a resistivity >18.1 MΩ cm was obtained from a Nanopure Diamond system (Barnstead, Dubuque, IA, USA) and used to prepare all solutions.

2.3. Solid-phase peptide synthesis

The epitope in SARS-CoV-2 spike protein (LPPLLTDEMIAQYTS) was synthesized as an amidated peptide by solid-phase chemistry using the 9-fluorenylmethoxy carbonyl (F-moc) strategy on an automated synthesizer (Multipep-1, CEM Corp. Charlotte, NC, USA) as described previously [17] Briefly, benzotriazole-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was added to the F-moc amino acid. The reaction was run in the reactor with a sintered glass filter containing the Rink amide AM resin (Sigma-Merck, Saint Louis, MO, USA). The F-moc moiety was removed with 25% 4-methylpyridine. The F-moc amino acid coupling reagents were 0.1 mmol L^{-1} oximes (Sigma-Merk, St Louis, MO, USA) in dimethylformamide and 8% N-methyl morpholine. The resin-bound peptide was deprotected and cleaved using trifluoroacetic acid and triisopropylsilane (Sigma-Aldrich, St Louis, MO, USA). The peptides were precipitated with diethyl ether and lyophilized. The concentration of the peptides was determined by measuring the optical density using the molar extinction coefficient generated by the PROTPARAM software package [http://www.expasy.ch]. The peptide sequence was confirmed by mass spectrometry (MALDI-TOF MS; Matrix-Assisted Laser Desorption Ionization Time-of-Flight).

2.4 Modification of the SPE's working electrode

 doi:10.20944/preprints202203.0375.v1

Carbon SPE working electrodes (DRP-110, Metrohm DropSens, Oviedo, Spain) were modified by drop-casting a synthetic peptide (epitope, EP) containing the following amino acid sequence: LPPLLTDEMIAQYTS. First, the SPE was electrochemically treated in 0.1 mol L^{-1} phosphate buffer solution (PBS), pH 7.4, applying +2 V (vs. Ag) for 60 s using a CompactState portable potentiostat (Ivium Technologies B.V., Eindhoven, Netherlands), rinsed with PBS and allowed to dry at room temperature. Next, 2 µl of EP (100 µg mL^{-1} in PBS) was placed onto the surface of the SPE followed by 10 µl 2.5% (w/w) glutaraldehyde. The solution was allowed it dry at room temperature and after 30 min appeared gel-like. The peptide modified SPE was blocked overnight at 4 °C with 1% (w/w) BSA prepared in 0.1 mol L^{-1} PBS (pH 7.4).

2.5. Electrochemical Assay to Detect Antibodies COVID-19 antibody IgG

The detection of anti-SARS-CoV-2 IgG antibody was based on an indirect immunoassay wherein the subsequent binding of anti-human IgG conjugated with alkaline phosphatase (AP) hydrolyzed diPho-HQ to hydroquinone (HQ) that could measured as electrochemical signals, shown schematically in **Figure 1**. Briefly, EP-specific IgG in 4 µl of dilute patient serum (1:100 in PBS with 1% BSA) was captured onto the sensitized working electrode surface of the SPE. After a minimum 10 min incubation at 37 °C for complete drying, SPEs were rinsed in PBS and 4 µL of anti-human IgG secondary antibody solution conjugated with AP was added for another incubation to dry at 37 °C before rinsing in reaction buffer (0.1 mol L^{-1} Tris-HCl and 20 mmol L^{-1} $MgCl_2$, pH 9.8) Next, the reaction buffer with 5 mM diPho-HQ was placed onto the SPE. After bried incubation (2 min), the presence of HQ was measured by square wave voltammetry (SWV) with the following parameters: amplitude,10 mV; frequency, 6.3 Hz; step, 10 mV; applied potential window, −0.5 to 0.2 V vs. Ag. Each cycle required 11 s, and a stable measurement was observed after the twentieth cycle (3.7 min total time).

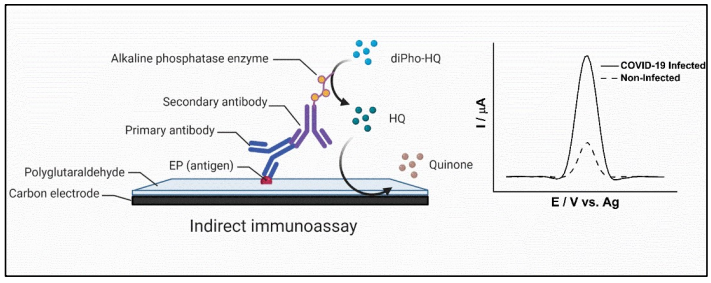

Figure 1: Schematic representation of a positive indirect immunoassay to detect COVID19. Anti-SARS-CoV-2 IgG antibody (Primary antibody) in patient serum samples is captured onto the surface of a peptide modified SPE. Retained human antibodies are bound by AP conjugated anti-human antibodies (Secondary antibody).Enzymatic activity converts diPho-HQ into HQ that can be measured by squarewave voltometry (solid line).

 doi:10.20944/preprints202203.0375.v1

2.6. Analytical Curve and Analysis of Blood Serum Samples

Electrochemical signals from positive and negative serum control samples were recorded to assess the analytical performance. From equation 1, described in item 3.3, and taking to account all negative signals, the cut-off value was determined that was used to normalize all data as a reactivity index. A gray zone was defined as 1.0 ± 0.1. Next, sera from whom was suspected of carrying COVID-19 were analyzed. The indirect immunoassay was accomplished to detect IgG as described. The sera were diluted at a ratio of 1:100 with PBS and used 1:50,000 diluted sec-IgG solution. In both cases, the incubation time was 8 min at 37 °C. Then, they were analyzed by indirect immunoassay. The current intensity was high when the person was infected with SARS-CoV 2.

2.7. Statistical Analysis

First, the one-way ANOVA test was performed with a significance of 95% to check whether the device-to-device surface variation during the optimization of preparation or analytical parameters — e.g., sequence of EP, sec-IgG and EP concentrations, and serum dilution ratio — was significant, as done in studies found in the literature [7,17,18]. Next, a two-tailed Students t-test with a confidence level of 95% was performed for pairwise comparisons [7,17,18].

3. Results

3.1. Development of electrochemical immunosensor

Previously, IgG linear B-cell epitopes (EPs) in the Spike protein of SARS-CoV-2 was mapped by spot synthesis analysis (Gomes et al 2020). Four of these were chosen to serve as antibody capture molecules on the surface of screen printed electrodes. The intention was to develop an immunosensor utilizing a drop-casting approach with glutaraldehyde (GA) to sensitize the electrode surface. GA has been widely used to modify electrode surfaces due to its introduction of aldehyde functional groups that allow the covalent bonding of compounds containing terminal amino moieties such as EPs [19]. Single-use SPEs were fabricated by mixing PBS solutions containing a prospective EP and GA onto the surface of the electrode. After drying and washing, its ability to differentially detect anti-SARS-CoV-2 antibodies as a diagnosis for COVID-19 was evaluated. The formation of immunocomplexes with AP-conjugated sec-IgG antibodies allowed enzymatic reduction of diPho-HQ to generate HQ, a redox molecule measurable by square wave voltammetry (SWV) [7].

Initially, the performance of SPEs conjugated to peptides EP1 (GPLQSYGFQPTG), EP2 (LPPLLTDEMIAQYTS), EP3 (GLDSKVGGNYNYG) and EP4 (RSYTPGDSSSGWTAG), which represent different EPs in the spike protein, were evaluated. The peak currents were recorded from measurements of a SWV while exposed to diluted serum samples from patients positive and negative for COVID19 (**Figure 2A**). From the ratio of the positive to

 doi:10.20944/preprints202203.0375.v1

negative peak currents (**Figure 2A,** inset), the most robust measurement was obtained with EP2 as it demonstrated a significantly higher positive/negative signal ratio (p-value < 0.001) than the others. Therefore, EP2 was chosen for additional optimization.

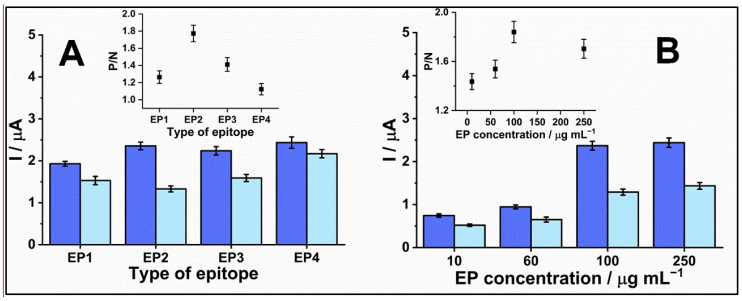

Figure 2: Target choie for conjugation to SPE and measurement optimization. SPEs were sensitized peptides that represent four EPs from SARS-CoV-2 spike protein and incubated with patient sera (diluted 1:100 in PBS). After rinsing, electrodes were incubated with an AP-labeled anti-human secondary (1:30,000), rinsed and incubated with 5 mM diPho-HQ in 100 mM Tris-HCl and 20 mM $MgCl_2$ (pH 9.8). (A) Peak current measured by SWV from positive (blue) or negative (light blue) sera. (B) The peak currents from SPEs sensitized over a range of peptide EP2 concentrations (10–250 µg mL^{-1}). All experiments were performed in duplicate. Solution volumes were 2 µL for antibody solutions and 50 µl for washes. Incubations lasted 8 min at 37 °C (time to evaporation). The parameters of SWVs for amplitude, frequency, step, and applied potential window were 10 mV, 6.3 Hz, 10 mV, −0.6–0.6 V (vs. Ag), respectively. Inset graphs represent the ratio between positive and negative signals (P/N).

Next, production of the SPE was optimized. Figure 2B show that the EP concentration on the SPE surface affected the current measured from the immunoreaction (**Figure 2B**). The ratio between SWV signals obtained after incubation in positive and negative samples significantly rose as increasing concentrations of EP were used (p < 0.02, **Figure 2B inset**), reaching a plateau at 100 µg mL^{-1} (p > 0.08). This suggested that increasingly antibodies were captured until reaching electrode surface saturation. Therefore, the most suitable EP concentration was 100 µg mL^{-1} and was used to produce all subsequent SPEs.

3.2. - Optimization of Experimental Parameters, Reproducibility, and Stability

To optimize the analytical signal, the level of dilution for patient serum and secondary antibody was evaluated. A fixed time of 16 min for the incubation times was chosen for antibodies. As the dilution of the positive serum sample was increased, there was a decrease in the signal from the positive samples (**Figure 3A**). Similarly, the non-specific binding of antibodies to the surface of the EP sensitized SPE, represented by the the negative controls, showed decreasing signals with higher dilutions. The ratio between

Commented [billp1]: Split this into two figures with A and B in figure 2. Make C and B into Figures 3A and 3B
.

Commented [billp2]: What was the concentration of peptide for the initial comparison between peptide performance?

 doi:10.20944/preprints202203.0375.v1

positive and negative reached a maximum difference at a dilution of 1:100 (**Figure 3A, inset**) and was chosen as the optimal dilution factor for patient samples. Differential signals were seen up to a dilution factor of 1,000 ($p < 0.02$), which suggests a dynamic range greater than10-fold that could allow detection of antibodies at low titers.

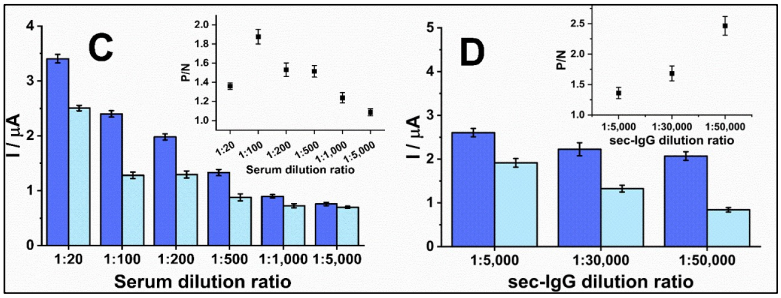

Figure 3: Optimization of conditions to perform measurements. All SPEs were sensitized with EP2 (100 µg/mL). (A) The peak currents from sensitized SPEs incubated to a range of sample dilutions of positive (blue) and negative (light blue) patient sera. (B) The peak currents from SPEs sensitized with 100 µg/mL EP2, incubated with positive (blue) and negative (light blue) patient sera dilute 1:100 and incubated over a range of secondary antibody dilutions.

Another critical factor for detecting EP/IgG immunocomplexes was the concentration of the secondary antibodies. Small decreases in the positive signals over the range of secondary antibody concentrations suggested that its presence is not a limiting factor to the measurement (**Figure 3B**). However, a large difference in the measurement of negative sera suggests there is a potential for non-specific background signals at higher concentrations. The background signal significantly decreased to the lowest levels at a dilution of 1:50,000 ($p < 0.02$), which did not impact meaningfully the signal from the positive control (comparison among 1:5,000 and 1:30,000 with 1:50,000 provided $p < 0.02$, and > 0.34, respectively) and provided the most prominent sign-to-noise ratio (**Figure 3B, insert**).

*3.3 - **Biosensor Performance***

The reproducibility of the SPEs was analyzed by evaluating electrodes prepared on different days using the same protocol. A relative standard deviation (RSD) of 5% in the SWV HQ response was calculated from 3 measurements of a 1:100 diluted positive serum on the electrodes prepared on different days, which demonstrated the practical reproducibility of the method (**Figure 4A inset**). SPEs prepared on the same day were stored at 4 °C in PBS to test stability. After 14 days of storage, the signal obtained from a positive sample diluted at 1:100 showed an RSD of 7% (n = 3) and decay in the average

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 29 March 2022 doi:10.20944/preprints202203.0375.v1

response of 10% compared to using a SPE prepared on the same day. The measurements showed that the response was preserved statistically (p = 0.1) (**Figure 4A inset**). However, the SWV current decreased by 20% and presented an RSD of 8% (n = 3) after 30 days of storage. These levels suggested that the performance of the SPE was significantly decreased compared to the same day as prepared. Thus, these biosensors had only a 14 day shelf life at 4 °C.

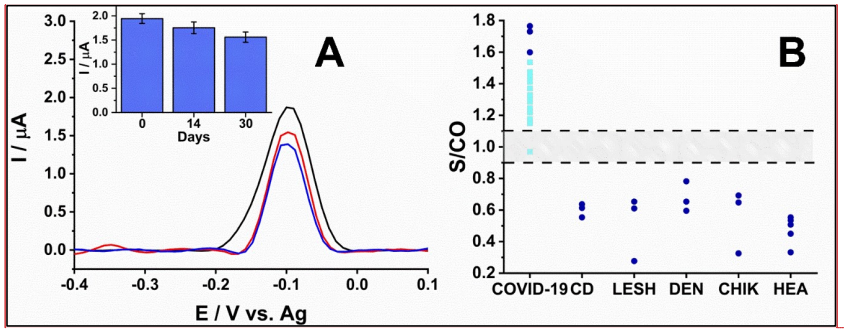

Figure 4: Stability, performance and cross-reactivity of EP2 conjugated SPEs. (A) SPE reproducibility was evaluated by fabricating multiple SPEs according to the optimized protocol and performaing SWVs in positive serum (1:100) on the day of fabrication (black line, n = 3), after 14 days at 4 °C (red line, n = 3) and 30 days at 4 °C (blue line, n = 3). The inset shows the variations in recordings. (B) For real world applications, the cutoff was determined using positive and negative controls (dark blue). Multiple serum samples from persons suspected with COVID-19 (light blue) as well as from individuals with Chagas disease (CD; Trypanosoma cruzi), Leishmaniosis (LESH), Dengue (DEN), Chikungunya (CHIK) and heathy (HEA) patients were assayed. The gray region represents ±10%.

To evaluate the performance of the EP2 sensitized SPEs, a previsouly confirmed panel of positive (N = ?) and negative (N = 17) patient samples (1:100 in PBS) were measured (**Figure 4B**). Using equation 1, a cut-off was calculated as 1.4 µA for detecting COVID-19.

$$Cut\text{-}off = a.X + f.\mathrm{SD} \quad \text{(Eq. 1)}$$

Where X is the mean and SD is the standard deviation of independent negative control readings, and a and f are two arbitrary multipliers which in the present case were 1 and 3, respectively [20]. The final results are normalized as the ratio of the sample signal to the cut-off value (S/CO). A a gray zone was defined between ±10% of 1.0 wherein results between 0.9-1.1 were considered indeterminate, >1.1 positive and <0.9 as negative. Cross reactivity was evaluated with sera collected from patients diagnosed with Chagas disease, Dengue, Leshmaniose, and Chikungunya. Each presented a S/CO value less than 0.9 that corresponds to a specificity of 100%. Positive controls collected from those confirmed with COVID-19 analyzed previously by RT-PCR demonstrated a median S/CO ratio near 1.7 (**Figure 4B, dark blue**). To simulate a real-world application for the diagnosis of COVID-

Commented [billp3]: The teal color is hard to see in panel B, please change to a darker color.

Commented [billp4]: You need to improve the organizaiton of the figures. Placement of the "A" & "B" as well as the inset.

Commented [billp5]: What is this number?

 doi:10.20944/preprints202203.0375.v1

19, 14 samples from persons with suspected contacts were assayed with the SPE. Of them, 13 volunteers were considered positive and 1 was in the gray zone for a selectivity of 93%.

4. Discussion

Serological diagnostic tests that utilize the presence of antibody as the definition for if an individual was infected by a pathogen detects antibodies in their serum or blood. This can be conducted as a lab-based assay such as an enzyme-linked immunosorbent assay (ELISA) [7] or chemiluminescent immunoassay [22], which are time-consuming and expensive, or a point-of-care test based on lateral flow technology that can show limited sensitivity [23, 24]. We propose to implement an electrochemical assay based on commercially available SPEs that can be easily sensitized to capture diagnostic antibodies. We began with the diagnosis of COVID-19 by chosing to sensitize the SPE with a peptide that represents an epitope in the Spike protein to capture anti-SARS-CoV-2 IgG antibodies. S protein is a highly exposed part of viral structure [3] and contains a number of immunodominant epitopes [1,23]. The use of a single epitope allows for the development of highly specific serological assays. Further, a focus on IgG antibodies, which are more prevalent than IgM antibodies [1,3], enabled improvements in sensitivity and specificity [1]. Ultimately, sensitized SPEs can be easily fabricated at a low cost that is compatible with portable equipment to provide rapid results in non-laboratory settings.

Generally, an electrochemical immunosensor consists of an electron-conducting solid surface where the molecule of interest (antibody or antigen) can be selectively captured, its presence detectd by tthe amplification [7] or suppression [25] of an electrochemical signal. A key element of the biosensor is the biological component that can be immobilized onto its surface such as antigen [25] or EP [7]. Here, the SPE was modified with a peptide representing an EP specific to the S protein of SARS-CoV-2. No cross-reactivity was displayed against serum from patients with Chagas, Chikungunya, Leishmaniosis, and Dengue (**Figure 3B**), all pathogens endemic to Rio de Janeiro, Brazil [26]. The single sample that presented an S:CO ratio in the gray zone was from an individul whose blood sample was collected soon after presenting symptons that may not have seroconverted. Overall, within the electrochemical platform, the biosensor showed high specificity and sensitivity of 100% and 93%, respectively.

The requirement of a small patient sample combined with a low cost could make it economically viable to perform multiple assays ensure a confident result [1,3]. The diagnostic accuracy of different assays has been shown to be variable [27-30] and a recent work compared the performance of ten assays. Sensitivities of 40-77% (65-81% considering IgG plus IgM) were found [31], making our electrochemical platform a competitive methodology for the diagnosis of COVID-19. In addition, the proposed device provided a reliable method for detecting COVID-19 IgG in serum and was rapid, 22 min compared to >90 min required for an ELISA. Notably, the measurements can be performed in volumes lesser than 100 μL, the required volume for ELISA assay that translates to the

 doi:10.20944/preprints202203.0375.v1

need for 2 nL of serum easily aquired from a finger prick sample of blood. Furthermore, the ease of electrode preparation and use, accuracy, and low cost suggest a high possibility for its use for helping track the spread of disease and highlight potential donors for convalescent plasma therapy or as a point of care diagnostic assay to detect a high concentration of antibody as a result of recent pathogen exposure [32,33]. Furthermore, considering that the measurements performed were defined by the EP peptide, the surface of the electrode can be sensitized by peptides that represent other pathogens or diseases.

5. Conclusions

We have developed a portable and affordable biosensor to detect rapidly COVID-19 infection by detecting anti-SARS-CoV-2 specific IgG antibody in patient serum. Based on an electrochemical reactivity, it showed a sensitivity of 93% and a specificity of 100%. Single use electrodes were fabricated by the surface modification of commercially available SPE with a peptide that represents an epitope specific SARS-CoV-2 Spike glycoprotein. The immobilized peptide could capture COVID-19 specific IgG antibodies for measurement by an indirect immunoassay using an enzyme-conjugated secondary IgG that hydrolyzed diPho-HQ into HQ, a redox molecule detectable by SWV. Under optimized conditions, it differentiate infected and non-infected individuals that correlated with RT-PCR diagnosis. No cross-reactivity was displayed to other pathogens such as *Trypanosoma cruzi* (Chagas disease), Chikungunya, Leishmaniosis, and Dengue. The biosensor platform has the flexibility to meet the demands for other pathogens and their respective diseases

6. Patents

The peptide described in this study is protected under Brazilian and US provisional patent applications BR 10.2019.017792.6 and PCT/BR2020/ 050341, respectively, filed by FIOCRUZ and may serve as a future source of funding.

Author Contributions: Conceptualization, W.A.A.; methodology, W.A.A..; software, W.A.A..; formal analysis and data curation, W.A.A. and S.G.D-S..; writing-original draft preparation, W.A.A.; writing—review and editing, D.W.P-Jr and S.G.D-S.; project administration, C.M.M.; S.G.D-S.; funding acquisition, C.M.M., and S.G.D-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro/FAPERJ (#110.198-13) and the Brazilian Council for Scientific Research (CNPq, #467.488.2014-2 and 301744/2019-0). Funding was also provided by FAPERJ (#210.003/2018) through the National Institutes of Science and Technology Program (INCT) to Carlos M. Morel (INCT-IDPN).

Institutional Review Board Statement: The study was approved by the University of Estacio de Sá (CAAE: 33090820.8.0000.5284) study center ethics committee and conducted under good clinical practice and applicable regulatory requirements, including the Declaration of Helsinki

Informed Consent Statement: This information is contained in the review board statement.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We are grateful to National Institute of Quality Control, FIOCRUZ, RJ, Brazil for the MALDI-TOF analysis.

Conflicts of Interest: The authors declare no conflict of interest involving the research reported. The funding agencies had no role in study design, data collection, data analysis, decision to publish, or preparation of the manuscript.

References

1. Ejazi, S.A.; Ghosh, S.; Ali, N. Antibody detection assays for COVID-19 diagnosis: an early overview. *Immunol Cell Biol* **2021**, *99*, 21–33. doi:10.1111/imcb.12397
2. Karim, S.S.A.; Karim, Q.A. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *Lancet* **2021**, *398*, 2126–2128. doi:10.1016/s0140-6736(21)02758-6
3. Kudr, J.; Michalek, P.; Ilieva, L.; Adam, V.; Zitka, O. COVID-19: A challenge for electrochemical biosensors. *Trends Anal Chem* **2021**, *136*, 116192. doi:10.1016/j.trac.2021.116192
4. Noce, A.; Santoro, M.L.; Marrone, G.; D'Agostini, C.; Amelio, I.; Duggento, A.; Tesauro, M.; Di Daniele, N. Serological determinants of COVID-19. *Biol Direct* **2020**, *15*, 1–9. doi:10.1186/s13062-020-00276-1
5. Harvala, H.; Mehew, J.; Robb, M.L.; Ijaz, S.; Dicks, S.; Patel, M.; Watkins, N.; Simmonds, P.; Brooks, T.; Johnson, R.; Gopal, R.; Roberts, D.J.; Zambon, M.N.H.S. Blood and transplant convalescent plasma testing group. convalescent plasma treatment for SARS-CoV-2 infection: analysis of the first 436 donors in England, 22 April to 12 May 2020. *Euro Surveill.* **2020,** *25*, 2001260. doi: 10.2807/1560-7917.ES.2020.25.28.2001260. Erratum in: *Euro Surveill.* **2021**, 26.
6. Romanholo, P.V.V.; Razzino, C.A.; Raymundo-Pereira, P.A.; Prado, T.M.; Machado, S.A.S.; Sgobbi, L.F. Biomimetic electrochemical sensors: New horizons and challenges in biosensing applications. *Biosens Bioelectron* **2021**, *185*, 113242. doi:10.1016/j.bios.2021.113242
7. Ameku, W.A.; Ataide, V.N.; Costa, E.T.; Gomes, L.R.; Napoleão-Pêgo, P.; William Provance, D.; Paixão, T.R.L.C.; Salles, M.O.; De-Simone, S.G. A pencil-lead immunosensor for the rapid electrochemical measurement of anti-diphtheria toxin antibodies. *Biosensors (Basel)* **2021**, *11*, 489. doi:10.3390/bios11120489
8. Hughes, G.; Westmacott, K.; Honeychurch, K.C.; Crew, A.; Pemberton, R.M.; Hart, J.P. Recent advances in the fabrication and application of screen-printed electrochemical (bio)sensors based on carbon materials for biomedical, agri-food, and environmental analyses. *Biosensors (Basel)* **2016**, *6*, 50. doi:10.3390/bios6040050
9. Ameku, W.A.; De Araujo, W.R.; Rangel, C.J.; Ando, R.A.; Paixão, T.R.L.C. Gold nanoparticle paper-based dual-detection device for forensics applications. *ACS Appl Nano Mater* **2019**, *9*, 5460-5468. doi:10.1021/acsanm.9b01057
10. Orzari, L.O.; Cristina de Freitas, R.; Aparecida de Araujo Andreotti, I.; Gatti, A.; Janegitz, B.C. A novel disposable self-adhesive inked paper device for electrochemical sensing of dopamine and serotonin neurotransmitters and glucose biosensing. *Biosens Bioelectron* **2019**, *138*, 111310. doi:10.1016/j.bios.2019.05.015
11. Núnez-Bajo, E.; Carmen Blanco-López, M.; Costa-García, A.; Teresa Fernández-Abedul, M. Integration of gold-sputtered electrofluidic paper on wire-included analytical platforms for glucose biosensing. *Biosens Bioelectron* **2017**, *91*, 824–832. doi:10.1016/j.bios.2017.01.029
12. Torres, M.D.T.; Araujo, W.R. de; Lima, L.F. de; Ferreira, A.L.; de la Fuente-Nunez, C. Low-cost biosensor for rapid detection of SARS-CoV-2 at the point-of-care. *Matter* **2021**, *4*, 2403-2416. doi:10.1016/j.matt.2021.05.003
13. Yakoh, A.; Pimpitak, U.; Rengpipat, S.; Hirankarn, N.; Chailapakul, O.; Chaiyo, S. Paper-based electrochemical biosensor for diagnosing COVID-19: Detection of SARS-CoV-2 antibodies and antigen. *Biosens Bioelectron* **2021**, *176*, 112912. doi:10.1016/ j.bios. 2020.112912
14. Ataide, V.N.; Ameku, W.A.; Bacil, R.P.; Angnes, L.; De Araujo, W.R.; Paixão, T.R.L.C. Enhanced performance of pencil-drawn paper-based electrodes by laser-scribing treatment. *RSC Adv* **2021**, *11*, 1644–1653. doi:10.1039/d0ra08874a.
15. de Araujo, W.R.; Frasson, C.M.R.; Ameku, W.A.; Silva, J.R.; Angnes, L.; Paixão, T.R. Single-step reagentless laser scribing fabrication of electrochemical paper-based analytical devices. *Angew Chemie Int* **2017,** 129, 15113-15117. doi:10.1002/anie.201708527
16. Bottino, C.G.; Gomes, L.P.; Pereira, J.B.; Coura, J.R.; Provance, D.W.; De-Simone, S.G. Chagas disease-specific antigens:

Characterization of epitopes in CRA/FRA by synthetic peptide mapping and evaluation by ELISA-peptide assay. *BMC Infect Dis* **2013**, *13*, doi:10.1186/1471-2334-13-568

17. Souza, A.L.; Faria, R.X.; Calabrese, K.S.; Hardoim, D.J.; Taniwaki, N.; Alves, L.A.; De-Simone, S.G. Temporizin and temporizin-1 peptides as novel candidates for eliminating *Trypanosoma cruzi*. *PLoS One* **2016**, *11*, e0157673. doi: 10.1371/journal.pone.0157673
18. Doughty, P.T.; Hossain, I.; Gong, C.; Ponder, K.A.; Pati, S.; Arumugam, P.U.; Murray, T.A. Novel microwire-based biosensor probe for simultaneous real-time measurement of glutamate and GABA dynamics *in vitro* and *in vivo*. *Sci Rep* **2020**, *10*. 12777. doi: 10.1038/s41598-020-69636-1
19. Ahmad, H.M.N.; Dutta, G.; Csoros, J.; Si, B.; Yang, R.; Halpern, J.M.; Seitz, W.R.; Song, E. Stimuli-responsive templated polymer as a target receptor for a conformation-based electrochemical sensing platform. *ACS Appl Polym Mater* **2021**, *3*, 329–341, doi:10.1021/acsapm.0c01120.
20. Ternynck, T..; Avrameas, S. Polymerization and immobilization of prteins using ethylchloroformate and glutaraldehyde. *Scand J Immunol* **1976**, *5*, 29–35. doi:10.1111/j.1365-3083.1976.tb03834.x
21. Lardeux, F.; Torrico, G.; Aliaga, C. Calculation of the ELISA's cut-off based on the change-point analysis method for detection of *Trypanosoma cruzi* infection in Bolivian dogs in the absence of controls. *Mem Inst Oswaldo Cruz* **2016**, *111*, 501–504. doi:10.1590/0074-02760160119
22. Dopico, E.; Del-Rei, R.P.; Espinoza, B.; Ubillos, I .; Zanchin, N.I.T.; Sulleiro, E.; Moure, Z.; Celedon, P.A.F.; Souza, W.V.; Da Silva, E.D.; Gomes, Y.M.; Santos, F.L.N. Immune reactivity to *Trypanosoma cruzi* chimeric proteins for Chagas disease diagnosis in immigrants living in a non-endemic setting. *BMC Infect Dis* **2019**, *19*, 1–7. doi:10.1186/s12879-019-3872-z
23. Fan, F.; Shen, H.; Zhang, G.; Jiang, X.; Kang, X. Chemiluminescence immunoassay based on microfluidic chips for α-fetoprotein. *Clin Chim Acta* **2014**, *431*, 113–117. doi:10.1016/j.cca.2014.02.003.
24. Michel, M.; Bouam, A.; Edouard, S.; Fenollar, F.; Di Pinto, F.; Mège, J.L.; Drancourt, M.; Vitte, J. Evaluating ELISA, immunofluorescence, and lateral flow assay for SARS-CoV-2 serologic assays. *Front Microbiol* **2020**, *11*, 1–8. doi:10.3389/fmicb.2020.597529
25. Dowlatshahi, S.; Shabani, E.; Abdekhodaie, M.J. Serological assays and host antibody detection in coronavirus-related disease diagnosis. *Arch Virol* **2021**, *166*, 715-731. doi: 10.1007/s00705-020-04874-2
26. Lima, L.F. De; Ferreira, A.L.; Torres, M.D.T.; Araujo, W.R. Minute-scale detection of SARS-CoV-2 using a low-cost biosensor composed of pencil graphite electrodes. *Proc Natl Acad Sci U S A*. **2021**, *118*, e2106724118. doi: 10.1073/pnas.2106724118
27. Nicol, T.; Lefeuvre, C.; Serri, O.; Pivert, A.; Joubaud, F.; Dubée, V.; Kouatchet, A.; Ducancelle, A.; Lunel-Fabiani, F.; Le Guillou-Guillemette, H. Assessment of SARS-CoV-2 serological tests for the diagnosis of COVID-19 through the evaluation of three immunoassays: Two automated immunoassays (Euroimmun and Abbott) and one rapid lateral flow immunoassay (NG Biotech). *J Clin Virol* **2020**, *129*, 104511. doi: 10.1016/j.jcv.2020.104511
28. Beavis, K.G.; Matushek, S.M.; Abeleda, A.P.F.; Bethel, C.; Hunt, C.; Gillen, S.; Moran, A.; Tesic, V. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. *J Clin Virol* **2020**, *129*, 104468. doi: 10.1016/j.jcv.2020.104468
29. Guevara-Hoyer, K.; Fuentes-Antrás, J.; De la Fuente-Muñoz, E.; Rodríguez de la Peña, A.; Viñuela, M.; Cabello-Clotet, N.; Estrada, V.; Culebras, E.; Delgado-Iribarren, A.; Martínez-Novillo, M.; Torrejón, M.J.; Pérez de Diego, R.; Fernández-Arquero, M.; Ocaña, A.; Pérez-Segura, P.; Sánchez-Ramón, S. Serological tests in the detection of SARS-CoV-2 Antibodies. *Diagnostics* (Basel) **2021,** *11*, 678. doi: 10.3390/diagnostics11040678
30. Mekonnen, D.; Mengist, H.M.; Derbie, A.; Nibret, E.; Munshea, A.; He, H.; Li, B.; Jin, T. Diagnostic accuracy of serological tests and kinetics of severe acute respiratory syndrome coronavirus 2 antibody: A systematic review and meta-analysis. *Rev Med Virol* **2021**, *31*, e2181. doi: 10.1002/rmv.2181
31. Gutiérrez-Cobos, A.; Gómez de Frutos, S.; Domingo García, D.; Navarro Lara, E.; Yarci Carrión, A.; Fontán García-Rodrigo, L.;

 doi:10.20944/preprints202203.0375.v1

Fraile Torres, A.M.; Cardeñoso Domingo, L. Evaluation of diagnostic accuracy of 10 serological assays for detection of SARS-CoV-2 antibodies. Eur J Clin Microbiol Infect Dis. 2021 May;40(5):955-961. doi: 10.1007/s10096-020-04092-3

32. Gomes, L.R.; Durans, A.M.; Napole, P.; Waterman, J.A.; Freitas, M.S.; S, N.B.R. De; Pereira, L. V; Furtado, J.S.; Aquino, G.; Machado, M.C.R.; Fintelman-Rodrigues, N.; Souza, T.M.L.; Morel, C.M.; Provance, D.W.; De-Simone S.G. Multiepitope proteins for the differential detection of IgG antibodies against RBD of the spike protein and non-RBD regions of SARS-CoV-2. *Vaccines (Basel)* **2021**, *9*, 986. doi: 10.3390/vaccines9090986
33. Riffelmann, M.; Thiel, K.; Schmetz, J.; Wirsing Von Koenig, C.H. Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *J Clin Microbiol* **2010**, *48*, 4459–4463. doi:10.1128/JCM.01371-10