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# From a recombinant key antigen to an accurate, affordable serological test: Lessons learnt from COVID-19 for future pandemics

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ABSTRACT

Serological tests detect antibodies generated by infection or vaccination, and are indispensable tools along different phases of a pandemic, from early monitoring of pathogen spread up to seroepidemiological studies supporting immunization policies. This work discusses the development of an accurate and affordable COVID-19 antibody test, from production of a recombinant protein antigen up to test validation and economic analysis. We first developed a cost-effective, scalable technology to produce SARS-COV-2 spike protein and then used this antigen to develop an enzyme-linked immunosorbent assay (ELISA). A receiver operator characteristic (ROC) analysis allowed optimizing the cut-off and confirmed the high accuracy of the test: 98.6% specificity and 95% sensitivity for 11+ days after symptoms onset. We further showed that dried blood spots collected by finger pricking on simple test strips could replace conventional plasma/serum samples. A cost estimate was performed and revealed a final retail price in the range of one US dollar, reflecting the low cost of the ELISA test platform and the elimination of the need for venous blood sampling and refrigerated sample handling in clinical laboratories. The presented workflow can be completed in 4 months from first antigen expression to final test validation. It can be applied to other pathogens and in future pandemics, facilitating reliable and affordable seroepidemiological surveillance also in remote areas and in low-income countries.

## 1. Introduction

Several authors have been predicting that the occurrence of epidemics and the emergence of new infectious diseases will become more frequent and harder to control due to several reasons, such as urbanization, climate change, intense travelling, and deficient health systems [7,9,26].

In this context, lessons learnt from COVID-19 pandemic should be a starting point to contribute for a better preparedness for future pandemics. One of the challenges in early phases of a pandemic is related to the availability of reliable and cost-effective diagnostic tests, as it happened for COVID-19. The lack of diagnostic tools hinders

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epidemiological surveillance and understanding of pandemic dynamics, hampering decisions on public health policies for pandemic control. This holds more pronouncedly true in lower income countries.

Serological tests detect antibodies generated by infection and vaccination. The most popular tests detect immunoglobulins of the G class (IgG), which upon primary infection or primary vaccination take approximately 2 weeks since the contact with the pathogen (or with the vaccine antigen) to be produced by the organism. Early on in a pandemic, accurate antibody tests are essential to study prevalence, to understand the frequency of asymptomatic cases and to monitor pathogen spread. During vaccine development efforts, reliable serological assays are required for the analysis of samples from pre-clinical and clinical trials. Furthermore, they are important for investigation of humoral immune response in different segments of the population, evaluation of durability of immunity, investigation of different vaccination schedules in subjects with or without antibodies induced by previous infection, comparison of homologous and heterologous vaccination strategies, among other types of studies that are crucial to provide the seroepidemiological data needed to support public health decisions and immunization policies.

The COVID-19 pandemic brought an unprecedented global health crisis, causing approximately 500 million cases and 6 million deaths within approximately 2 years since the declaration of the Covid-19 pandemic by the World Health Organization (WHO) in March 2020 [20]. In most regions of the world, the number of infected individuals needing medical treatment exceeded hospitals capacity, and healthcare personnel became overwhelmed by the excess of working hours and by adverse mental health outcomes. In addition to this brutal scenario, the elevated human-to-human contagious rate obliged a severe restriction of social life including, in some cases, complete lockdown except for essential activities. As a result, this situation led to economic disruption in many countries experiencing several months of partial or complete lockdown. In this scenario, public health policies need to be guided by scientifically sound data, and accurate diagnostic testing is one important tool in this regard.

The structural spike (S) protein of coronaviruses is known to be a major target for neutralizing antibodies, thus making it a key antigen for the development of specific and sensitive sero-diagnostic tests. S protein contains the receptor binding domain in its S1 subunit and is also responsible for fusion to the cell membrane through its S2 subunit [35]. Enzyme-linked immunosorbent assays (ELISA) based on the S protein have been developed, showing minimal cross-reactivity with sera against circulating “common cold” coronaviruses (but some degree of cross reactivity with SARS and MERS-COV sera) [16] and providing correlation to virus neutralizing activity [4]. However, early in the COVID-19 pandemic the unavailability of low-cost and high-quality recombinant S protein showed to be a bottleneck for the development of affordable and reliable serological tests that were by then urgently needed by public health agencies to deal with the pandemic.

In the present work, we describe the development of an accurate, low-cost serological test based on S protein immunoreactivity, suited to be employed in epidemiological surveillance studies and for evaluation of vaccine immunogenicity and durability, with special relevance for remote regions and low-income countries with limited clinical laboratory network. Without compromising the performance of the assay, costs were cut mainly by optimizing the antigen production process and by simplifying sample collection and processing. Blood collection by finger prick, using dried blood spots (DBS), greatly facilitated collection, storage, shipping and processing of samples. The overall cost of the assay was estimated to be approximately one dollar per test. The workflow and rationale can be directly applied to other pathogens and contribute to a quick response in future pandemics.

## 2. Materials and methods

### 2.1. Stable recombinant cell line generation

HEK293–3F6 cells (NRC Canada) growing in suspension in the chemically-defined, animal component-free HEK-TF (Satorius Xell) culture medium were stably transfected by lipofection using a broad-spectrum reagent (Lipofectamine 3000, Thermofisher) as described previously [2]. A total DNA concentration of 0.9 μg/mL was used, combining two vectors: the pαH vector (at 0.75 μg/mL) containing the sequence encoding the ectodomain (aminoacids 1–1208) of the spike protein in the prefusion conformation [35], and an empty vector (pCI-neo, Promega) containing the neomycin phosphotransferase gene (at 0.15 μg/mL) for selection of stable transfectants. The pαH vector was kindly provided by B. Graham, VRC/NIH, and is also available from BEI Resources under #NR-52563. Cells were maintained under selection pressure with 100 μg/mL G-418 sulfate (Thermofisher) from 2 days post-transfection on. Twenty four days post-transfection cell viability had recovered to over 90% viability, and a cell bank was cryopreserved.

### 2.2. Cell cultivation

Stably transfected cells were transferred to chemically-defined, animal component-free culture media (HEK-TF or HEK-GM, Sartorius Xell, as described in the figures and text) and maintained at 37 °C and 5% $CO_2$ under orbital agitation (140–180 rpm, shaker with 2- to 5-cm stroke) in vented Erlenmeyer flasks containing up to 60% of their nominal volume. In the experiments carried out in fed-batch mode, the culture medium was supplemented with a concentrated solution of nutrients (HEK-FS, Sartorius Xell) according to the manufacturer’s instructions. Bioreactor runs were carried out in a 1.5-L stirred-tank bioreactor (EzControl, Applikon) at setpoints of pH, temperature and dissolved oxygen of 7.1, 37 °C and 40% of air saturation, respectively. Cell concentration and viability were determined by trypan blue exclusion using an automated cell counter (Vi-Cell, Beckman Coulter), whereas glucose and lactate concentrations were monitored using a metabolite analyzer (YSI 2700, Yellow Springs Instruments). Presence of S protein in the supernatants was determined by spot blots: 3 μL of each sample was applied to nitrocellulose membranes, then serum of SARS-COV-2 convalescent patients (1:1000) was used as primary antibody, followed by incubation with anti-human IgG(Fc) HRP-labeled antibody (Sigma, #SAB3701282) and finally by addition of chemiluminescent ECL reagent (BioRad). Images were captured using a FluorChem E system (ProteinSimple).

### 2.3. S protein concentration/purification

Cell suspension harvested from cell cultures was clarified by filtration using 0.45-μm PVDF membranes (Merck Millipore) and used to perform either ultrafiltration/diafiltration (UF/DF) or affinity chromatography (AC) experiments. Concentration/diafiltration (90-fold concentration by volume) was achieved by UF/DF using centrifugal devices based on cellulose membranes with 100-kDa cut-off (Merck Millipore). Affinity chromatography was carried out in an Äkta Purifier system (Cytiva) using a 5-mL StrepTrap XT affinity chromatography column (Cytiva) following manufacturer’s instructions. Protein concentration, purity and identity in the eluted fractions were confirmed by NanoDrop (Thermofisher), silver-stained SDS-PAGE and Western blot analyses, respectively. For Western blots, a pool of serum of SARS-COV-2 convalescent patients (1:1000) was used as primary antibody, followed by incubation with anti-human IgG (Fc) HRP-labeled antibody (Sigma, #SAB3701282) and then chemiluminescent ECL reagent (BioRad).

### 2.4. S-UFRJ ELISA for anti-S IgG detection

High binding ELISA 96-well microplates (Corning) were coated with 50 μL (all volumes are per well) of coating solution and incubated

overnight at room temperature (RT). The coating solution contained SARS-COV-2 S protein in PBS (Gibco) at 4 μg/mL concentration. One experiment was carried out to validate this concentration by comparing coating solutions at 0.3, 1, 3 and 10 μg/mL, and a minimum of 3 μg/mL was determined to be optimal, so coating solution was kept at 4 μg/mL. The coating solution was removed, and 150 μL of PBS-1% BSA (blocking solution) was added to the plate and incubated at RT for 1–2 h. The blocking solution was removed, and 50 μL of serum or plasma diluted 1:40 in PBS-1% BSA (or serially three-fold diluted), was added to the plate and incubated at RT for 2 h. For ELISA using dried blood spots, circles cut from filter paper using commercially available punching devices, or single filter paper pads cut from plastic strips were used to prepare eluates by incubating for 1 h at RT in 100 μL (filter paper circles) or 200 μL (pads) of PBS-1% BSA. In the same way as diluted serum or diluted plasma, 50 μL of eluate samples were added to the plate and incubated at RT for 2 h. The plate was then washed five times with 150 μL of PBS. Next, 50 μL of 1:8000 goat anti-human IgG (Fc) HRP-labeled antibody (Sigma, #SAB3701282) was added to the plate and incubated for 1.5 h at RT. The plate was then washed five times with 150 μL of PBS. At the end, the plate was developed with 50 μL of TMB (3,3',5,5-tetramethylbenzidine) (Thermofisher). The reaction was stopped with 50 μL of 1 N HCl, and the optical density (O.D.) was read at 450 nm with 655 nm background compensation in a microplate reader (BioRad). Results are expressed during test development either as O.D. or O.D. summation [18], and later during application of the final test protocol as the ratio of O.D. of the sample to the cut-off. After optimization by means of a receiver operator characteristic (ROC) analysis [28], the cut-off was defined as the sum of the O.D. mean of negative controls in the same plate plus 3 times the O.D. standard deviation determined when 90 negative controls were tested.

### 2.5. Commercial tests used for comparison to S-UFRJ test

A commercial ELISA to detect anti-SARS-COV-2 IgG produced by Euroimmun (#EI 2606–9601 G) and a commercial rapid diagnostic test (RDT) with separate bands for IgG and immunoglobulin M (IgM) detection were used following manufacters' instructions. The RDT is an immunochromatographic test manufactured by Hangzhou Biotest Biotech Co. and commercialized in several countries/regions, such as Brazil, Europe, USA and Australia. In Brazil, the brand name of the RDT is MedTeste (Medlevensohn).

### 2.6. Sample collection from human subjects

Samples collected at the State Hematology Institute Hemorio followed a protocol approved by the local ethics committee (CEP Hemorio; approval #4008095). Samples collected at UFRJ COVID Screening and Diagnostic Center followed a protocol approved by the national ethics committee (CONEP; protocol #30161620000005257; approval #3953368): subjects were initially interviewed and, if they accepted to participate, they signed the informed consent, answered a questionnaire (addressing demographic data, onset and type of symptoms, history of travel abroad, among other information) and had blood (venous blood and/or finger prick) and nasopharyngeal swab collected. Only symptomatic subjects who presented at least two of the following symptoms were included: loss of taste or smell, fever, shortness of breath, diarrhea, headache, extreme tiredness, dry cough, sore throat, runny or stuffy nose, or muscle aches. Dried blood spots (DBS) were obtained by finger pricking with commercially available sterile lancets and lancing devices. Either plastic strips containing pads of filter paper, or regular Whatman filter paper (2.5 ×7.5 cm) with three blood spots from the same volunteer, were used to collect whole blood from finger pricks.

### 2.7. Plaque reduction neutralization test (PRNT)

In order to determine the titers of neutralizing antibodies in serum samples, sera were first heat- inactivated at 56 °C for 30 min, and two-fold serial dilutions were incubated with 100 PFU of an ancestral SARS-COV-2 isolate (GenBank #MT126808.1) for 1 h at 37 °C to enable neutralization to occur. Virus-serum mixture was inoculated into confluent monolayers of Vero cells seeded in 12-well tissue culture plates. After 1 h, inoculum was removed and a semisolid medium (1.25% carboxymethylcellulose in alpha-MEM supplemented with 1% fetal bovine serum) was added. Cells were further incubated for 72 h and then fixed with 4% formaldehyde solution. Viral plaques were visualized after staining with crystal violet dye solution. PRNT end-point titers were expressed as the reciprocal of the highest serum dilution for which the virus infectivity is reduced by ≥90% ($PRNT_{90}$) when compared with the average plaque count of the virus control. All work involving infectious SARS-COV-2 was performed in a biosafety level 3 (BSL-3) containment laboratory.

## 3. Results

### 3.1. Recombinant antigen expression, production and purification

For recombinant production and straightforward purification of a heavily glycosylated protein such as the SARS-COV-2 spike protein, mammalian cell culture in serum-free media is the option of choice. We expressed the soluble ectodomain of the spike (S) protein in trimeric form stabilized in the prefusion conformation [35] in serum-free, suspension-adapted mammalian cell cultures. Initial transient transfections indicated that expression levels evaluated on days 2 and 4 post-transfection were higher in HEK293 cells than in CHO-K1 cells (Fig. 1 A, left panel). However, differently from previous studies that adopted transient protein expression techniques [4,13], we focused on stable and constitutive gene expression because transgene integration in the cell genome enhances scalability and significantly decreases costs of recombinant protein production in mammalian cells [3]. Due to the challenges posed in the early months of a pandemic, such as the urgency and the disruption of international supply chains for reagents and synthetic gene constructs, we decreased time and costs by using an old-fashioned technique of co-transfecting the plasmid containing the S gene with an intellectual property-free plasmid (pCIneo, Promega) containing an eukaryotic selection marker. A stable recombinant HEK293 cell line showing higher expression than transiently transfected HEK293 cells was generated (Fig. 1 A, center panel) and banked 24 days post-transfection. S protein expression by this cell line was shown to be stable for at least 100 days post-transfection (Fig. 1 A, right panel), confirming the suitability of this recombinant cell line for developing batch-refeed, fed-batch or perfusion cell culture processes, which have a longer duration, but provide higher yields.

In order to decrease costs and logistics burden, cell culture media available as dried powdered media were selected to be tested both in shake flasks and stirred-tank bioreactors at 300-mL and 1.5-L scale, respectively. The chosen medium (HEK-GM) was able to provide robust cell growth and efficient recombinant protein production (Fig. 1B). Carrying out cell culture in fed-batch mode by adding pulses of a concentrated nutrient solution over cell cultivation time avoided nutrient depletion and significantly increased viable cell density and secreted S protein levels, allowing enhanced production of the recombinant protein (Fig. 1B, right panel). Protein isolation from cell culture supernatant was investigated by ultrafiltration/diafiltration (UF/DF) and affinity chromatography (AC) techniques. In spite of the large S protein size (~170 kDa) and the use of a 100-kDa cut-off membrane device, UF/DF was not able to efficiently remove all smaller contaminating proteins, and an AC resin bearing a streptavidin mutein ligand was used to obtain the protein in high purity (Fig. 1 C). The affinity resin has shown a relatively high dynamic binding capacity for the S protein (Fig. 1D), and was used for up to 100 adsorption/elution/regeneration cycles with no detectable decrease in performance, reducing its impact on the final costs of the purified protein.

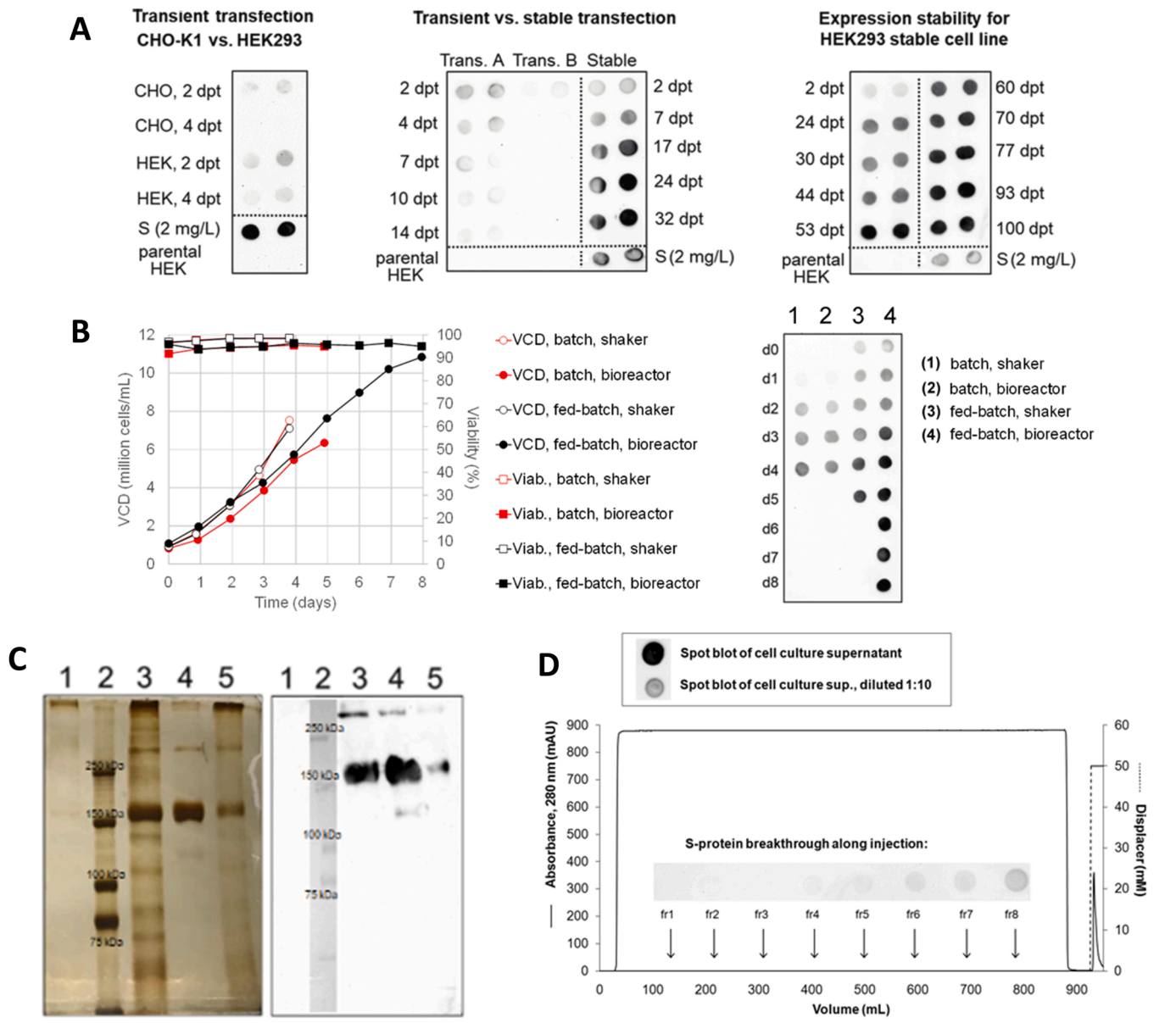


**Fig. 1.** S protein production and purification. (A) Left panel: CHO-K1 and HEK293 were transiently transfected with pαH plasmid. Center panel: HEK293 cells were transiently transfected at high (A, 2.0 μg/mL) and low (B, 0.75 μg/mL) pαH plasmid concentration, as well as stably transfected by co-transfecting the pαH plasmid (0.75 μg/mL) along with a second vector (0.15 μg/mL) containing the neo selection marker. Right panel: stability of expression of the secreted protein was confirmed for 100 days post-transfection (dpt). (B) Left panel: high cell viabilities and viable cell densities (VCD) were achieved for the stable cell line grown in shake flasks and 1.5-L stirred-tank bioreactors, in batch and fed-batch mode, using chemically-defined, animal component-free culture media. Right panel: spot blot for detection of S protein in the cell culture supernatant on different days. (C) S protein identity was confirmed by Western blot analysis at ~170 kDa. SDS-PAGE showed a lower purity for the concentrated/diafiltered (UF/DF) sample (lane 3), but a very high purity for the sample purified by affinity chromatography (AC, lane 4). 1: Cell culture supernatant from non-transfected parental HEK293 cells. 2: Molecular-mass standard (protein markers, from top to bottom, have a molecular mass of 250, 150, 100 and 75 kDa). 3: UF/DF sample after 90-fold volumetric concentration and buffer exchange into PBS. 4: Eluate from AC. 5: Supernatant from stable recombinant cell line. (D) A typical chromatogram of the AC purification. Fractions of the flow-through (fr1 to fr8) were collected during sample injection and analyzed by spot blot to evaluate progressive saturation of affinity ligands, showing a high binding capacity and a low degree of S protein leakage. For comparison, spot blots of the injected cell culture supernatant, or a 1:10 dilution, are shown in the box on top. In all immunoblots, presence of S protein was detected using a pooled serum of SARS-COV-2 convalescent patients (1:1000) as primary antibody, followed by incubation with anti-human IgG-HRP and chemiluminescent detection.

### *3.2. Early development of the serological test from a limited number of samples*

Early on in the COVID-19 pandemic in Brazil, March/April 2020, development of the test was started using a relatively limited number of samples that were available by then, in order to first compare S protein preparations of lower (UF/DF) or higher purity (AC) for the detection of anti-S antibodies in plasma samples. Nineteen positive or negative samples, collected from individuals scored as positive by PCR for SARS-COV-2 ($n = 16$), from a healthy non-infected individual ("post-pandemic negative", $n = 1$) or collected before SARS-COV-2 emergence ("pre-pandemic negative", $n = 2$), were tested for immunoreactivity against S protein preparations obtained either by UF/DF or AC. Considering the limited number of samples available at this stage, the threshold value to discriminate between negative and positive samples was considered as the mean plus 3 standard deviations of O.D. of the three negative controls added to each plate.

Comparison of the results of the ELISAs carried out with S protein of

different purities (UF/DF or AC) showed that the assay performance was greatly improved by the use of the highly purified AC antigen, showing much better discrimination between positive samples and negative controls (Fig. 2 A). For comparison, the samples were evaluated also by means of an imported rapid diagnostic test (RDT), which was licensed in many countries including Brazil. Interestingly, sample #1, which was collected from a patient that was PCR positive, scored negative for IgG by RDT, but clearly scored positive in the AC S-protein ELISA.

The better discrimination between positive and negative samples enabled by the AC antigen was also evident in titration curves (Fig. 2B), which were used to calculate the O.D. summation for each sample, evidencing again the superiority of the highly pure S protein as ELISA antigen (Fig. 2 C). Using plasma samples from negative controls and PCR-confirmed patients, we determined that a minimum of 150 ng of high-purity S protein per well (i.e. by coating wells with 50 μL of antigen solution at a minimum concentration of 3 μg/mL) was enough for clear discrimination of positive and negative samples (Fig. 2D). Additionally, we assessed assay robustness by evaluating representative data from experiments done by different operators and using different lots of the AC-purified S protein. The results are shown in Fig. 2E and corroborate the reliability of the recombinant antigen and of the test as a whole. The ELISA test with AC-purified S protein was named S-UFRJ test.

### 3.3. Late-stage assay development using a broader panel of samples

As broader sample panels became available, a final development of the test was performed. We applied the S-UFRJ test to evaluate the presence of anti-S IgG in 210 samples, including pre- pandemic negative controls and samples from symptomatic individuals diagnosed by PCR as positive for SARS-COV-2. The PCR-positive cohort consisted of 66 samples from 38 symptomatic individuals whose blood samples were obtained at different time points after symptoms onset. Negative control samples were collected either until 2018 from healthy individuals (pre-pandemic negative controls, n = 124) or in early 2020 from healthy individuals who tested negative for SARS-COV-2 by PCR (post-pandemic negative controls, n = 20), comprising a total of 144 negative control samples.

Results of serological tests are usually interpreted based on the ratio of the O.D. of the given sample to a pre-established cut-off. Since at this stage of development an optimized cut-off was not yet available, we adopted a conservative rationale of classifying as undetermined all samples having an O.D. value between one and two times the mean plus standard deviation (SD) of the O.D. of negative controls in the same plate. All samples showing an O.D. below the lower threshold value (mean + 1 SD) were considered negative, and all samples having an O.D. above the higher threshold value (2 *[mean + 1 SD]) were considered positive. Samples that presented an O.D. value inbetween were considered undetermined.

Out of 144 negative control samples, 142 scored as negative, revealing a very high specificity of the test of 98.6% (Fig. 3 A). Interestingly, we have observed that a large fraction of these pre-pandemic negative samples display immunoreactivity against spike proteins from other "common-cold" coronaviruses, highlighting the ability of the S-UFRJ assay to correctly discriminate immunity among different

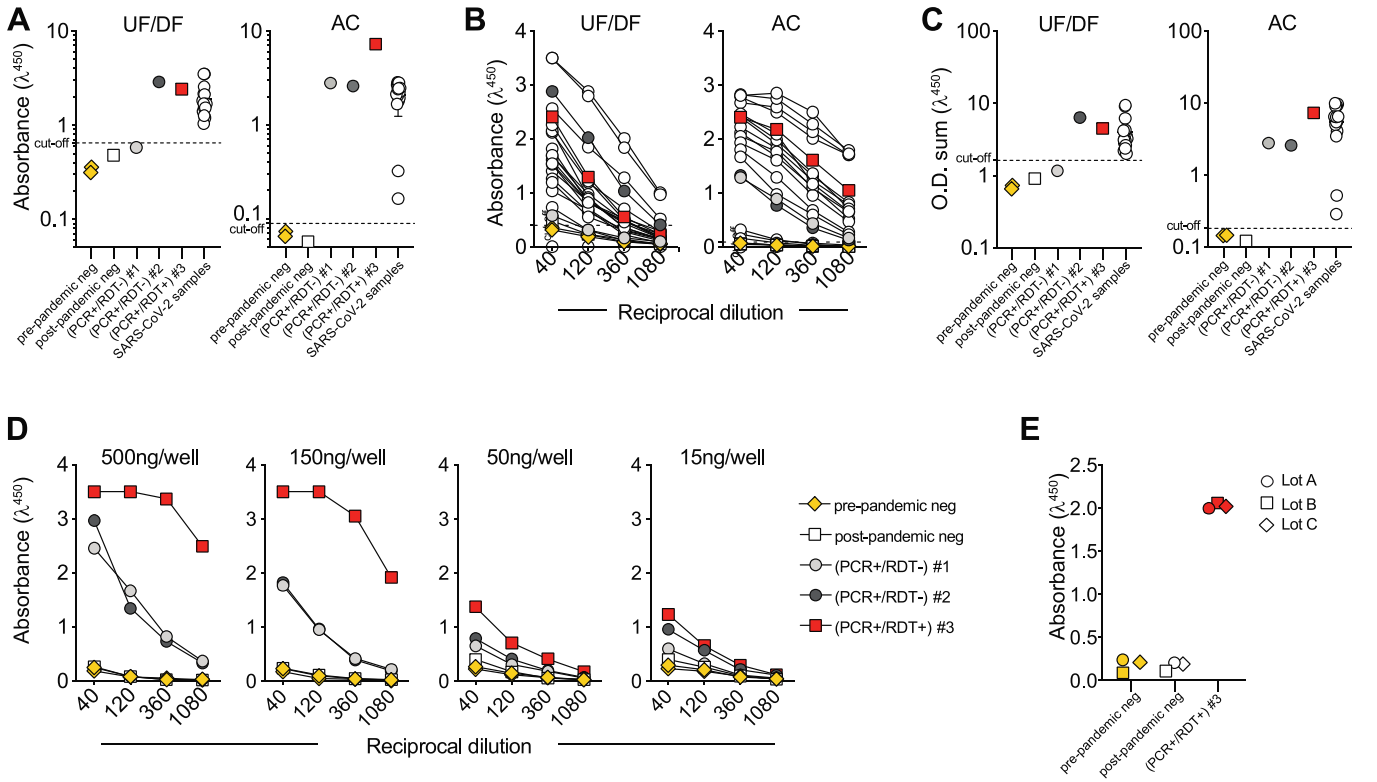


**Fig. 2.** Comparison of ELISAs with UF/DF- or AC-purified S protein. (A) ELISA performance using low-purity (UF/DF) or high-purity (AC) S protein antigen to coat plates. A total of 22 samples were used: 15 sera from COVID-19 convalescent patients (SARS-COV-2 samples, open circle), two control samples collected until 2018 (pre-COVID-19, yellow diamond), one post-COVID-19 control sample from a healthy individual, two samples from SARS-COV-2 infected individuals characterized as PCR+/RDT- (#1 light gray filled circle, #2 dark gray filled circle), and one sample from a SARS- COV-2 convalescent patient who had the severe form of the disease (#3, red square). The cut-off was defined as mean + 3 standard deviations (X + 3 SD) of the O.D. of negative controls. (B) Samples used in (A) were titrated in four serial dilutions (1:40, 1:120, 1:360 and 1:1080), both for UF/DF or AC ELISA. (C) O.D. summation of the titration curves shown in (B); symbols as in (A). (D) Samples used in (A) were titrated in four serial dilutions (1:40, 1:120, 1:360 and 1:1080) for AC ELISA using different S-protein coating concentrations (0.3 – 10 μg/mL, corresponding to 15 – 500 ng/well). (E) Three selected samples from (D) were diluted 1:40 to assess assay robustness by evaluating results from data obtained with different antigen lots and two different operators. Representative data from 3 experiments using 3 different lots of the AC-purified S protein (lots A to C) are shown. Experiment with lot A was performed by one operator, and a second operator performed experiments with lots B and C. RDT: rapid diagnostic test.

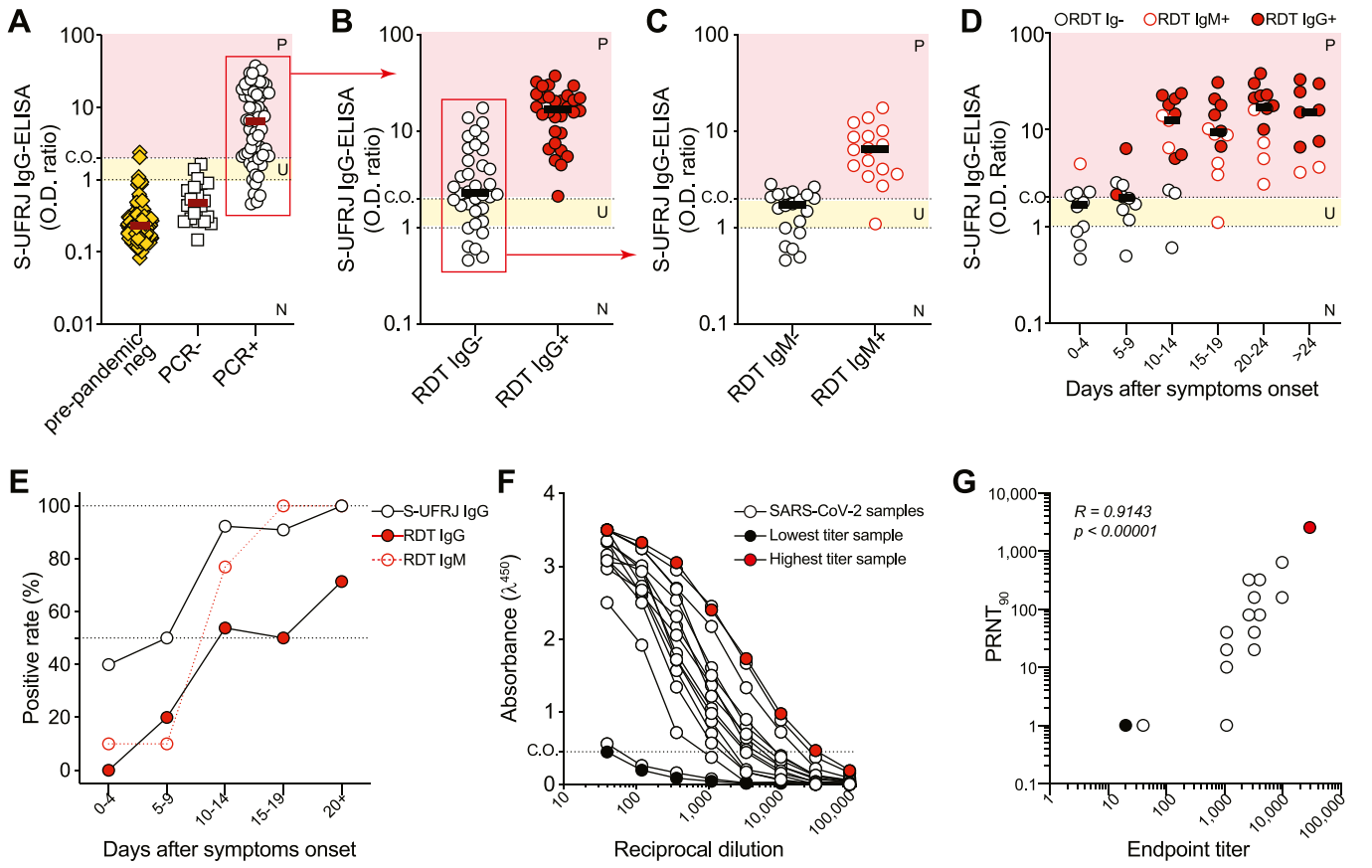


**Fig. 3.** Validation of S-UFRJ ELISA based on AC-purified antigen for early detection and quantification of anti-S IgG antibodies. (A) Anti-S IgG antibody detection in samples from healthy individuals, obtained as pre-pandemic controls (n = 124, yellow diamond) or post-pandemic controls from individuals who tested negative for the virus by PCR (n = 20, open square), and samples from COVID-19 patients who were PCR-positive (n = 66, open circle). Relative levels of antibodies are shown as O.D. ratio of values of individual samples to the [mean + 1 standard deviation (X + 1 SD)] of the O.D. of the negative controls in the same ELISA plate. Sera samples were diluted at 1:40. For this stage of development of the assay, it was conservatively established that an O.D. ratio below 1 indicates a negative result (N), an O.D. ratio above 2 indicates a positive result (P), and an O.D. ratio between 1 and 2 is considered undetermined (U). (B) The PCR-positive samples shown in (A) were tested for anti-SARS-COV-2 IgG by a commercial rapid diagnostic test (RDT) (MedTeste, Medlevensohn, Brazil, imported from Hangzhou Biotest Biotech Co.). Samples were then grouped by IgG reactivity according to the RDT result (RDT IgG-, open circle; RDT IgG+, red filled circle), and their anti-S IgG levels measured by the S-UFRJ ELISA are plotted. (C) Anti-S IgG levels measured by the S-UFRJ ELISA for the RDT IgG-negative samples shown in (B), grouped according to the RDT IgM result as RDT IgM- (open circle) or RDT IgM+ (open red circle). (D) Levels of anti-S IgG in samples grouped according to the timepoint of sample collection given in days after symptoms onset (DASO); symbol legends are indicated in the figure. (E) Positivity rates versus DASO for anti-S IgG measured by S-UFRJ ELISA (open circle), for IgM measured by RDT (open red circle) and for IgG measured by RDT (red filled circle). (F) S-UFRJ IgG titration of 16 samples from COVID-19 convalescent patients (SARS-COV-2 samples); samples with the highest and lowest endpoint titer of the group are indicated in solid red and solid black colors, respectively. (G) Correlation between S-UFRJ ELISA IgG endpoint titers and neutralization (PRNT90) for samples titrated in (F) (n=16). Three samples with PRNT90 values <10 are plotted as 1. Statistical analysis was performed using Pearson's test.

coronaviruses (data not shown).

Considering all samples from PCR-positive individuals, regardless of the time of collection after symptoms onset, 53 out of 66 samples (80.3%) were IgG positive (Fig. 3 A). For comparison, all samples were also tested for IgG using the RDT, and only 30 out of 66 samples scored positive for IgG (45.5%) (Fig. 3B). In order to gain insight into the samples from symptomatic PCR-positive individuals that scored negative for anti-S IgG in the S-UFRJ ELISA, samples were grouped as IgM- and IgM+ according to the RDT, since IgM detection is an indication of recent infection (Fig. 3 C). We found that all but one sample that scored negative for anti-S IgG in our ELISA, also scored as negative for IgM in the rapid test (Fig. 3 C). This result suggests that samples from PCR+ individuals that scored negative for anti-S IgG in our ELISA, may have been collected in the very beginning of the disease, only a few days after symptoms onset (DASO), thus before the time needed by the organism to seroconvert and start antibody production. For that reason, these samples also scored negative for IgM in the RDT. Indeed, when the results of S-UFRJ ELISA were charted against DASO, this was confirmed: samples were increasingly scored as positive for anti-S IgG according to the time point they had been collected after symptoms onset (Fig. 3D). Accordingly, PCR+ individuals that have been sequentially sampled on different DASO and scored negative in their first sampling, later converted to seropositivity for anti-S IgG. Anti-S IgG seroconversion rate, as scored by S-UFRJ ELISA, increased progressively from 41.66% (days 0–4) to 100% (20 + days) as a function of DASO, being above 90% for all samples collected 10 or more days after symptoms onset (Fig. 3E). Of note, even for DASO of 20 + days, the RDT reached a maximum positivity rate of 71.4%, whereas the S-UFRJ ELISA scored a rate of 100%. The results show the superiority of S-UFRJ ELISA when compared to the commercial RDT used for comparison, with higher sensitivity and earlier detection of seroconversion in PCR-positive symptomatic individuals.

A previous study using another S protein-based ELISA has observed a correlation between anti-S IgG or anti-RBD IgG (the receptor binding domain within the S protein) titers and virus neutralization [4]. To address whether the S-UFRJ ELISA could also bring information about the neutralizing capacity of positive samples, we compared anti-S IgG titers and virus neutralizing titers. We tested plasma samples of COVID-19 convalescent donors for their SARS-COV-2 in vitro

neutralization capacity as measured by classic plaque reduction neutralization test (PRNT), using a SARS-COV-2 strain isolated in Brazil. Importantly, analogously to what has been shown by Amanat et al. [4], we found that the higher the anti-S IgG ELISA endpoint titers (Fig. 3 F), also the higher the neutralization titers were (Fig. 3 G), resulting in a high correlation between those titers (Pearson's R=0.9143, $p < 0.00001$). Hence, S-UFRJ ELISA also provides an important functional correlation with SARS-COV-2 neutralization capacity.

### 3.4. ROC analysis and cut-off optimization

The receiver operator characteristic (ROC) analysis is a standard procedure for assessing diagnostic performance [28], since it allows both to evaluate accuracy with no need for a predetermined cut-off [24] and to determine an optimized cut-off value [34]. Using a large sample panel provided by the state blood bank of Rio de Janeiro (Hemorio) comprising of 420 positive and 68 pre-pandemic negative samples, a ROC analysis was performed. The ROC curve (Fig. 4) revealed a very high accuracy of the S-UFRJ test as given by an area under the curve (AUC) approaching 1. The curve was built by determining the sensitivity and specificity of the test for a wide range of cut-off values, and allowed establishing the optimized cut-off resulting in the best combination of specificity and sensitivity. The optimized cut-off was determined to be equal to the O.D. mean of the negative controls in the same plate (also usually known as calibrators) plus 3 times the standard deviation that was previously determined for a plate full of pre-pandemic negative controls (3 *0.016 = 0.048). Importantly, by establishing an optimized cut-off determined based on a large amount of samples, the definition of a realistic (instead of conservative) range of O.D. ratio for undetermined samples became possible. Thus, for final application of the S-UFRJ test using the optimized cut-off value determined herein, the recommended range for classification of samples as undetermined is a ratio of O.D. to cut-off between 0.9 and 1.1, which is also a range commonly adopted in commercial diagnostic ELISA tests.

### 3.5. Performance validation and comparison to a widely available commercial ELISA assay

To validate S-UFRJ test performance, an additional sample panel comprising of 437 positive samples from individuals who had tested positive by PCR and were followed over time, providing well-characterized samples with a wide range of days after symptoms onset (DASO of 0–98 days), was used. These samples were measured side-by-side by the S-UFRJ ELISA and by a high-reputation IgG ELISA based on the S1 subunit of the spike protein, which is commercialized worldwide by the company Euroimmun.

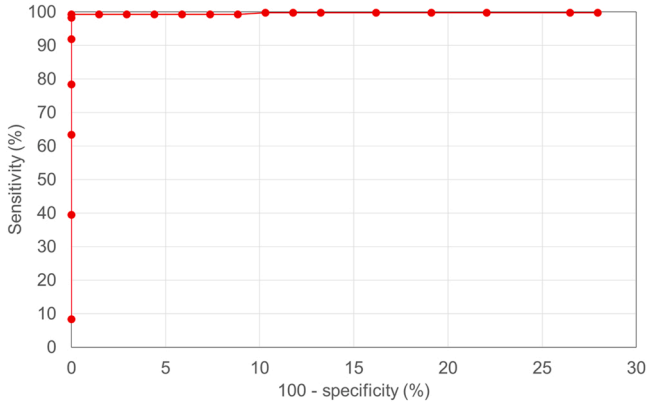


**Fig. 4.** Receiver operating characteristic (ROC) curve of S-UFRJ test. The curve is based on calculating the sensitivity and specificity as a function of varying cut-off values (each data point represents one cut-off value). This curve was prepared based on a panel comprising 420 positive and 68 negative samples, and allowed determining the sensitivity and specificity of the S-UFRJ test.

Side-by-side comparison of tests using the same sample panel is important, because sensitivity data can change a lot according to DASO of samples included in a given panel. We chose to use a sample panel having 17.2% of samples collected at early timepoints (DASO of 0–10 days), when seroconversion possibly had not yet occurred, in order to get a true insight into performance of both assays.

As shown in Table 1, the S-UFRJ ELISA allows earlier detection of seroconversion and presents a greater sensitivity than the Euroimmun S1-based ELISA. Considering samples with DASO of 11–98 days, sensitivity was 95.0% for S-UFRJ and 86.5% for Euroimmun. If all 437 samples of the panel are included in the computation (i.e. if early samples with DASO of 0–10 days are also included), then sensitivity was 82.4% for S-UFRJ and 73.7% for Euroimmun. Importantly, the higher sensitivity of the S-UFRJ assay is not achieved at the expense of specificity, since both assays present very similar specificities (98.6% for S-UFRJ as shown before, and 98.7% for the Euroimmun IgG ELISA according to the product package insert).

### 3.6. Simplification of sample collection by using eluates from dried blood spots (DBS) obtained by finger prick

The cost per sample of serological assays is an important aspect concerning large-scale public health actions, especially in low-income countries. ELISA samples are routinely based on venous blood collection followed by serum or plasma preparation. This requires clinical laboratory services, as well as refrigerated sample storage and transport, which would significantly increase the cost of the S-UFRJ ELISA. More relevant, it would severely limit the usage of the assay for epidemiological surveillance studies in world regions lacking an appropriate network of clinical laboratories. To overcome this critical limitation, we evaluated a simple means for storage and transport of blood samples, by collecting blood drops obtained by finger prick in filter paper. The resulting "dried blood spots" (DBS) represent a simple, low-cost and low-complexity method that enables sampling in remote regions, or regions lacking a laboratory network [23,27].

However, not all assays perform equally well with DBS samples [10], so further investigation on the suitability of the use eluates from DBS instead of serum or plasma was carried out (Fig. 5A). Titration curves of plasma samples and eluates from DBS collected in filter paper displayed comparable results (Fig. 5B and C). Additionally, we adapted low-cost plastic strips – resembling conventional pH measurement and water analysis strips –, having up to three square filter paper pads, since these allowed further precision in sampling and facilitated storing for eventual

**Table 1**
Evaluation of the S-UFRJ ELISA and of an IgG ELISA commercialized by the company Euroimmun. A panel of 437 samples from PCR-positive individuals who were followed along time was used. For S-UFRJ data analysis, the final optimized assay parameters were adopted (cut-off equal to mean + 3 *SD, and O.D. ratio interval of 0.9–1.1 for undetermined samples). For the Euroimmun ELISA, test was carried out according to manufacturer's instructions.

| Days after symptoms onset (DASO) | Number of samples | S-UFRJ ELISA | | Euroimmun ELISA (S1 protein) | |
|---|---|---|---|---|---|
| | | Number of positive samples | Sensitivity (%) | Number of positive samples | Sensitivity (%) |
| 00–05 | 33 | 3 | 9.1 | 1 | 3.0 |
| 06–10 | 42 | 13 | 31.0 | 8 | 19.0 |
| 11–15 | 83 | 70 | 84.3 | 53 | 63.9 |
| 16–20 | 62 | 59 | 95.2 | 53 | 85.5 |
| 21–25 | 56 | 54 | 96.4 | 50 | 89.3 |
| 26–30 | 54 | 54 | 100.0 | 53 | 98.1 |
| 31–98 | 107 | 107 | 100.0 | 104 | 97.2 |
| **11–98** | **362** | **344** | **95.0** | **313** | **86.5** |
| **All (0–98)** | **437** | **360** | **82.4** | **322** | **73.7** |

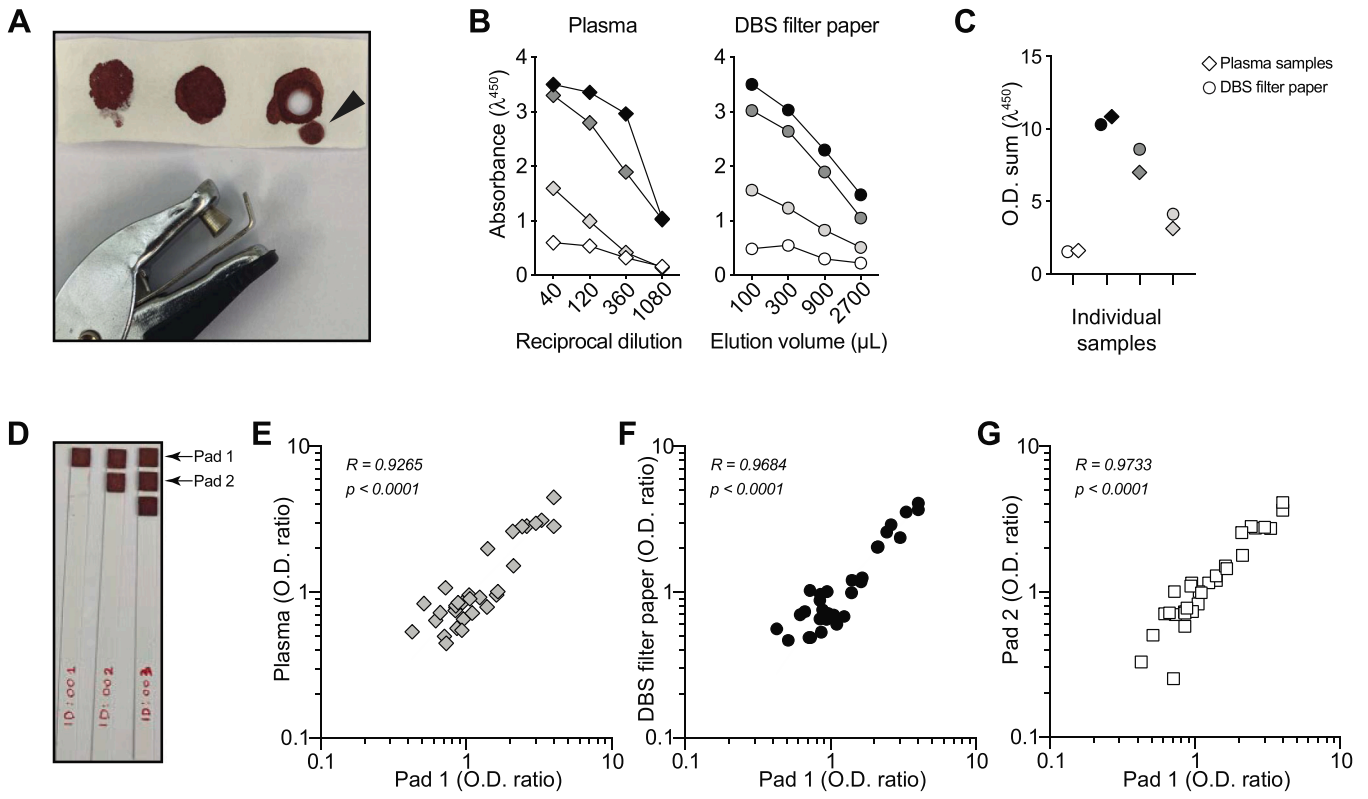


**Fig. 5.** S-UFRJ test optimization for sample collection by dried blood spots. (A) Dried blood spots (DBS) obtained by finger pricking with commercially available lancing devices: a 2.5 cm (W) x 7.5 cm (L) filter paper with three blood spots from the same volunteer and a commercially available paper hole punching device were used to prepare a DBS disk (arrowhead) from which blood was eluted for ELISA testing. (B) S-UFRJ ELISA comparing the O.D. values for plasma samples in increasing serial dilutions and for the corresponding eluates prepared by incubating the DBS disks in increasing volumes of buffer. (C) O.D. summation of the data shown in (B). (D) Dried blood spots collected in plastic strips containing 1, 2 or 3 pads of filter paper. (E-G) correlations of O.D. ratios between samples collected in the strip pad 1 and either the respective plasma sample (E), the respective DBS disk (F), or the second pad of the same strip (G). In E-G, plasma dilution was 1:40, DBS in filter paper disks were eluted in 100 μL of PBS-1% BSA, and DBS in single pads were eluted in 200 μL PBS-1% BSA. O.D. ratios were calculated as defined earlier, using data from negative control plasma samples (negative controls from DBS disks or pads can be equally used, if available). Statistical analysis was performed using Pearson's test.

retesting (Fig. 5D). Consistently, after determination of the adequate elution volumes, O.D. ratios obtained for eluates from DBS collected either in filter paper circles or in plastic strip pads showed very high correlation to the O.D. ratios obtained for plasma samples (Fig. 5E and F). Reproducibility between pads from a given strip was also warranted (Fig. 5G). Thus, by using dried blood spots, the low cost of the test was further warranted. We have additionally confirmed that blood samples collected in filter paper and kept in plastic bags at room temperature can be preserved for at least 2 months without altering their serological result (data not shown). Importantly, the optimal elution volume of 200 μL determined for DBS pads allows up to 4 experiment replicates per pad. Additionally, as each plastic strip can contain multiple pads, it also permits additional retesting or testing for immunoreactivity against other SARS-COV-2 antigens (e.g. RBD or nucleocapsid protein) or variants, for cross-reactivity evaluation against related viruses or for testing for antibodies for other non-related viruses.

### *3.7. Application of the S-UFRJ assay for evaluation of vaccine-induced immunity*

The first COVID-19 vaccines were approved for human use in December 2020, approximately one year after start of SARS-COV-2 spread in China. Their development was facilitated and accelerated due to long-lasting research on new vaccine platforms, including studies on vaccines for related viruses like SARS and MERS coronavirus [1,11, 14]. Serological assays were key tools enabling the pre-clinical and clinical trials of these vaccines. In early 2022, 14 months after the approval of the first COVID-19 vaccines, over 10.3 billion doses of vaccines had been administrated worldwide. Multiple vaccines were approved for human use, but Phase 3 clinical studies have reported variable effectiveness depending not only on the vaccine platform ([12], [21]), but also on circulating variants of SARS-COV-2 [8]. Although in high-income countries mRNA vaccines are widely used, inactivated and adenovirus-based COVID vaccines are widely used in lower income countries, including Brazil. All vaccines approved worldwide so far have in common that they generate antibodies against the spike protein, enabling tests like the S-UFRJ test as a valuable tool for seroepidemiological studies.

Among the different vaccine platforms in use for COVID-19, inactivated vaccines have shown to elicit lower levels of antibodies ([12], [21]). Thus, in order to check if the S-UFRJ test would be suitable for monitoring seroconversion elicited by vaccination, we collected DBS samples of a small cohort of 15 individuals, who had no history of SARS-COV-2 infection and were vaccinated with the inactivated vaccine Coronavac. We have tested these samples for IgG levels using the S-UFRJ ELISA. For each individual, we collected samples at different time points, before and after the first and the second doses of the vaccine (given 28 days apart), in order to monitor seroconversion. The results revealed that the S-UFRJ test was able to measure the increase in antibody level with time after the administration of the two vaccine doses, and that all individuals showed detectable anti-S IgG titers after the administration of the second dose (Fig. 6).

### *3.8. Economic analysis*

The use of the ELISA platform and the simplicity of the DBS method

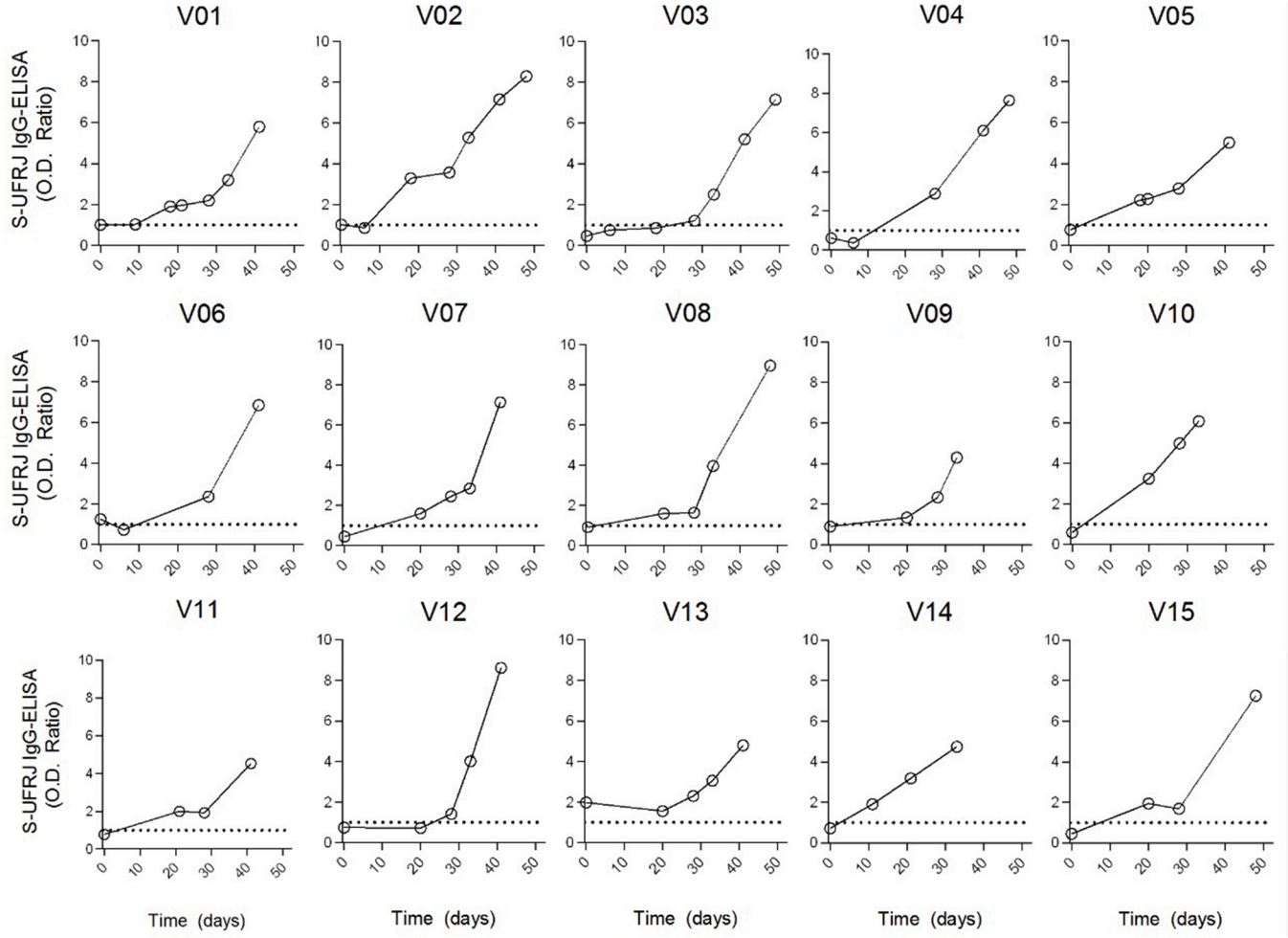


**Fig. 6.** S-UFRJ test applied to monitor anti-S IgG seroconversion following vaccination. Dried blood spots were collected in plastic strips containing pads of filter paper. For each of the 15 vaccinees (V01 to V15), DBS samples were collected at different time points, before and after the first and second doses of the inactivated vaccine Coronavac (Sinovac), which were administered on days 0 and 28.

allow a significant decrease of the overall cost of the S-UFRJ test. In terms of raw materials (consumables) needed for collecting samples and performing the assay, a detailed calculation revealed that consumables costs vary between two and four USD dimes per test. Two scenarios were considered (Table 2): one scenario considering items sold by international vendors in Brazil, and the second one considering items produced locally and adopting other saving measures that we confirmed not to compromise the performance of the test. Based on techniques routinely employed in economic feasibility studies [30], as shown in Table 3 the final price of the test was estimated by including in the computation other manufacturing costs (that include labor, utilities, laboratory charges, insurance, among others) and general expenses (which account for administrative, research, development, distribution and marketing costs). Costs can vary significantly from country to country, but we expect that the two scenarios in Tables 2 and 3 can represent most realities reasonably well. The final retail price that we estimated for the S-UFRJ test from sample collection to test result is approximately USD 1 per single-well test of the sample.

## 4. Discussion

Serological tests for the detection of antibodies are crucial tools for seroepidemiological surveillance in all phases of outbreaks, epidemics and pandemics, from monitoring of the spread of the pathogen in early phases to e.g. assessing the durability of vaccine-induced immunity in late phases. Investigating the early spread of the ethiological agent of an epidemic with the help of serological assays provides the data input needed for scientifically driven decision-making by government bodies, e.g. about social distancing and other preventative measures that can help limiting pathogen spread. Pollán et al. [31] discussed the importance of a population-based epidemiological study carried out in Spain during COVID-19 pandemic as a key element to inform authorities about the need for maintaining public health measures.

Reliable epidemiological data requires accurate diagnostic tests. Approximately 6 months after the identification of SARS-COV-2, [25] showed by means of a systematic review and meta-analysis on serological tests for COVID-19 that the evidence available by then on the accuracy of point-of-care lateral flow tests (rapid diagnostic tests - RDT) was particularly weak and did not support their continued use. On the other hand, these authors concluded that traditional ELISA tests and chemiluminescent assays showed good performance. However, these test types usually depend on collecting venous blood to obtain serum or plasma, thus requiring a clinical laboratory structure and being demanding in terms of sample collection, processing, storage and transport. Therefore, both these types of tests are expensive – in early phases of the COVID-19 pandemic retail prices in the range of USD 50 to USD 100 were common in Brazil.

In this context, we presented in this work a rationale and workflow for developing accurate and affordable antibody tests starting very early on with recombinant antigen development and then establishing the hallmarks of the test initially from a limited number of human samples. We showed that the combination of the ELISA platform with sample

**Table 2**
Cost estimate of raw materials needed for S-UFRJ test, for two scenarios: (1) purchase of materials sold in Brazil by international suppliers and with no saving efforts; (2) purchase of materials from domestic suppliers and making saving efforts that were validated as not interfering in test performance: use of skim milk (2% m/v) instead of BSA (1% m/v); in-house preparation of PBS; finger prick using just the sterile lancet, with no lancing device; and reuse of buffer reservoirs for an equivalent of 30 ELISA plates. As a conservative estimate to account for controls included in each plate and for any eventual repetition needs, a total of 80 tests were considered per ELISA plate. All quotes for consumables in this table were obtained for purchase in the local market in Brazilian reais (BRL). The conversion to USD was done considering the exchange rate by then (1 USD = 5.34 BRL).

| Raw materials (consumbles) | Supplier | Amount used (per 80 samples or per plate) | Unit cost (USD) | Package size | Cost per plate (USD) |
|---|---|---|---|---|---|
| **Scenario 1 (international suppliers)** | | | | | |
| Dilution plate | Sarstedt, #821581 (Germany) | 1 | 63.67 | per 100 | 0.64 |
| ELISA plate | Corning, #3590 (USA) | 1 | 146.07 | per 100 | 1.46 |
| S protein | Produced at UFRJ | 20 μg | 140.45 | per mg | 2.81 |
| PBS 10x | Sigma, #11666789001–4 L (USA) | 350 mL after 1:10 dilution | 166.67 | per 4000 mL | 1.46 |
| BSA (at 1% m/v) | Sigma, #A2153–100 G (USA) | 0.35 g BSA | 344.57 | per 100 g | 1.21 |
| HRP-conjugated antibody | Sigma, #SAB3701282–2MG (USA) | 5 mL after 1:8000 dilution | 261.61 | per mL @ 2 mg/mL | 0.16 |
| TMB | Thermo Fisher, #002023 (USA) | 5 mL | 186.30 | per 500 mL | 1.86 |
| HCl 37% | Sigma, #30721–2.5 L (USA) | 5 mL after 1:10 dilution | 32.58 | per 2500 mL | 0.01 |
| Pipette tips | Axygen (USA) | 400 yellow tips | 176.03 | per 20,000 | 3.52 |
| Reagent reservoir | Corning Costar (USA) | 6 reservoirs for 30 plates | 146.07 | per 1000 | 0.88 |
| Plastic strips w/ 3 pads | Organicoat (Brazil) | 80 | 243.45 | per 5000 | 3.90 |
| Lancet | G-Tech (Brazil) | 80 | 1.22 | per 100 | 0.97 |
| Lancing device | G-Tech (Brazil) | 1 | 2.79 | per 1 | 2.79 |
| Personal protective equipment (PPE) | Several suppliers | All PPEs (sample collection/analysis) | 2.50 | set of PPEs | 2.50 |
| Additional minor non- listed consumables | | 20% of all previous items together | 4.83 | | 4.83 |
| | | | **Total costs per plate:** | | **28.99** |
| | | | **Cost per test:** | | **0.36** |
| **Scenario 2 (domestic suppliers, skim milk, in-house PBS, reservoir reuse, no lanceting device)** | | | | | |
| Dilution plate | Alfa (Brazil) | 1 | 28.09 | per 50 | 0.56 |
| ELISA plate | Corning, #3590 (USA) | 1 | 146.07 | per 100 | 1.46 |
| S protein | Produced in-house | 20 μg | 140.45 | per mg | 2.81 |
| PBS (1x) (in-house prep) | Synth Chemicals (Brazil) | 350 mL | 0.09 | per liter | 0.03 |
| Skim milk (at 2% m/v) | Nestlé (Switzerland) | 0.7 g milk | 3.04 | per 280 g | 0.01 |
| HRP-conjugated polyclonal antibody | Rhea Biotech (Brazil) | 5 mL after 1:20000 dilution | 196.63 | per mL | 0.05 |
| TMB | Scienco Biotech (Brazil) | 5 mL | 209.74 | per 1000 mL | 1.05 |
| HCl 37% | Sigma, #30721–2.5 L (USA) | 5 mL after 1:10 dilution | 32.58 | per 2500 mL | 0.01 |
| Pipette tips | Olen-Econolab (Brazil) | 400 yellow tips | 3.75 | per 1000 | 1.50 |
| Reagent reservoir | Corning Costar (USA) | 6 reservoirs for 30 plates | 146.07 | per 1000 | 0.03 |
| Plastic strips w/ 3 pads | Organicoat (Brazil) | 80 per plate | 243.45 | per 5000 | 3.90 |
| Lancet | G-Tech (Brazil) | 80 per plate | 1.22 | per 100 | 0.97 |
| Personal protective equipment (PPE) | Several suppliers | All PPEs (sample collection/analysis) | 2.50 | set of PPEs | 2.50 |
| Additional minor non-listed consumables | Several suppliers | 20% of all previous items together | 2.97 | | 2.97 |
| | | | **Total cost per plate:** | | **17.85** |
| | | | **Cost per test:** | | **0.22** |

**Table 3**
**Estimate of the final S-UFRJ test price** taking 1 million tests as a calculation basis, including in the cost computation the raw materials shown in Table 2, other manufacturing costs (that include labor, utilities, laboratory charges, insurance, among others) and general expenses (which account for administrative, research, development, distribution and marketing costs) [30]. Profit margin and taxes were also accounted for to estimate the final S-UFRJ test price, since large-scale use of the test for epidemiological surveillance would probably require its industrial production.

| Type of cost | Calculation | Scenario 1 (USD) | Scenario 2 (USD) |
|---|---|---|---|
| *Raw materials (RM) for 1 million tests* | See Table 2 | *220,000* | *360,000* |
| *Other manufacturing costs for 1 million tests* | *145% of RM* | *319,000* | *522,000* |
| *General expenses for 1 million tests* | *20% of TPC* | *134,750* | *220,500* |
| Total product costs (TPC) for 1 million tests | | 673,750 | 1102,500 |
| Profit margin (PR) (10% of TPC) | | 67,375 | 110,250 |
| Taxes (35% of PR) | | 23,581 | 38,588 |
| Sum of TPC, PR and taxes | | 764,706 | 1251,338 |
| Estimated unit price per test | | 0.76 | 1.25 |
| **Final estimated price per test (average of the 2 scenarios)** | | **1.01** | |

collection as dried blood spots was key to combine low cost and simple sample collection by finger prick/DBS with high accuracy, which for the S-UFRJ COVID-19 antibody test was of 98.6% specificity and 95.0% sensitivity for samples collected 11 or more days after symptoms onset. The quality of the assay was enabled by the use of a high-purity conformationally sound recombinant antigen, in this case the trimeric spike protein of SARS-COV-2, which is less conserved in coronaviruses and thus less prone to induce cross-reactivity than other proteins of the virus [15,22]. Moreover, the recombinant, highly purified spike protein used herein consists of the full-length ectodomain and is produced in the prefusion trimeric conformation, thus maintaining most neutralization-sensitive epitopes [35] and reliably mimicking the antigenic properties of the virus upon infection and spread in the organism.

Serological studies are important in a pandemic also to establish priorities for vaccination policies and to evaluate vaccine immunogenicity and durability of immunity. In the example of COVID-19 pandemic, in spite of the rapid development of several vaccines, approximately 2 years after the identification of SARS-COV-2 and 14 months after approval of the first vaccines for human use, approximately 40% of the world population remained unvaccinated [29]. Furthermore, due to antibody waning and to the emergence of virus variants, vaccine studies remained necessary to evaluate the need for booster doses. Earle et al. [12] showed for COVID-19 vaccines of different platforms

(inactivated virus, adenoviral vector, mRNA and protein subunit) that there is a robust correlation between vaccine efficacy and antibody levels (normalized by antibody levels of convalescent patients), and that this correlation is higher for binding antibodies measured by ELISA than if considering only neutralizing antibodies. This might be related to the fact that non-neutralizing antibodies have been shown to also contribute to protection to SARS-CoV-2 infection, e.g. by mediating phagocytosis [5]. Other works have also shown that binding antibodies provide an adequate correlate of protection for COVID-19 vaccines [17,32]. Thus, monitoring binding antibodies by serological testing is a long-term need in public health.

The choice of the antigen to use in serological test development has many different implications. The primary goal is to select an antigen that allows high test accuracy, but additionally it can be useful if a serological test is developed using a recombinant antigen that is also the target of vaccines, in order to allow differentiation if antibodies were elicited by infection or by vaccination. In the example of COVID-19, the S-UFRJ test is based on the spike protein, which is also the target of most approved vaccines (used either as the purified protein or as a nucleic acid or viral vector encoding it for in vivo expression). With the exception of inactivated vaccines, which contain the whole virus, discrimination between immunity resulting from infection or vaccination can be assessed by using the DBS eluate volume to perform in parallel the S-UFRJ test and another serological assay based on a different viral antigen not present in the vaccines, such as the nucleocapsid (N) protein.

The rationale for serological test development proposed herein can be very useful in future pandemics, because usually the first tests that are made available in a global health emergency are very expensive, and additionally can have poor performance, as shown by [25] for COVID-19 rapid antibody tests. Inaccuracy leads to false positive and/or false negative results, which can have implications both at the individual level and at the level of public health decision-making. Furthermore, tests that present high accuracy allow deeper epidemiological analyses to be made. For example, in a population-based COVID-19 epidemiological study (EPICOVID-RS) carried out in 9 different cities in Rio Grande do Sul state (Brazil), the high accuracy of the S-UFRJ test (used from the 9th study round on) allowed the determination of a logit-linear relationship between the exposure level of study participants and the seroprevalence [6]. In the 8 first rounds of the study, a commercial rapid antibody test had been used, but it was replaced by the S-UFRJ test after a comparative evaluation indicated a clear superiority of the S-UFRJ ELISA [33].

The high cost of diagnostic tests, especially in early phases of a pandemic, poses an additional obstacle for governments to implement widespread testing, especially in less wealthy nations. However, as shown in this work, for the S-UFRJ ELISA for COVID-19 we determined an estimated cost of just one US dollar per single-well evaluation of samples. Thus, DBS-based ELISA tests can not only enable broad and reliable serological surveillance in populations, regardless of their geographical and socio-economic aspects, but can also allow government bodies to save very significant amounts of financial resources [19], which would then be available for investing in other types of countermeasures to fight the health and socio-economic consequences of a pandemic, with special relevance for low-income countries.

Scientists have been warning about the increasing probability of pandemics occurring [9,26]. So the need for preparedness should be a major lesson from COVID-19 pandemic. One important point to allow the workflow presented herein to be applied in the future is the need for enhancing local capability for production of reagents, especially of recombinant antigens. Thus, investments in biochemistry, bioprocessing and chemical engineering laboratories to make them able in the future to quickly develop and produce recombinant antigens is an important component in terms of ensuring availability of core reagents and thus enhancing diagnostic preparedness for future pandemics.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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