Supporting Information

Towards Control and Oversight of SARS-CoV-2 Diagnosis and Monitoring through Multiplexed Quantitative Electroanalytical Immune Response Biosensors

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Results and Discussion

Table S1. Optimization of the experimental conditions involved in the amperometric determination of N-Ms-IgG or S-Rb-IgG standard solutions with the developed bioplatforms.

| Variable                              | Tested range | Selected value | Tested range | Selected value |
|---------------------------------------|--------------|----------------|--------------|----------------|
| N/S antigen, µg mL\(^{-1}\)          | 0.0 – 10     | 5.0            | 0.0 – 100    | 25.0           |
| Incubation time N/S antigen, min     | 15 – 60      | 15             | 15 – 60      | 30             |
| Number of steps                       | 1 & 2        | 2              | 1 & 2        | 2              |
| Incubation time N-Ms-IgG/S-Rb-IgG, min | 15 – 60    | 30             | 15 – 120     | 60             |
| HRP-anti-Ms/Rb-IgG, dilution         | 1:500 – 1:5,000 | 1:2,500   | 1:100 – 1:500 | 1:500          |
| Incubation time HRP-anti- Ms/Rb-IgG, min | 15 – 60 | 15           | 15 – 60     | 15             |

Table S2. Slope values (in nA mL ng\(^{-1}\)) calculated from the calibration plots constructed with the developed bioplatforms for the amperometric determination of isotype (IgG, IgM and IgA) S- and N-specific anti-SARS-CoV-2 Igs standards prepared in the indicated matrices.

| S-specific | N-specific |
|------------|------------|
| IgG        | IgM        | IgA        | IgG        | IgM        | IgA        |
| Buffered solutions | (54 ± 2) | (20.4 ± 0.5) | (19 ± 1) | (449 ± 23) | (271 ± 9) | (155 ± 8) |
| 1,000 diluted serum sample | (56 ± 2) | (20.2 ± 0.8) | (20 ± 3) | (533 ± 151) | (193 ± 6) | (199 ± 15) |
| \(t_{exp}^{[a]}\) | 0.203 | 0.198 | 0.312 | 0.379 | 0.879 | 0.576 |

\(^{[a]}t_{tab} = 2.776, n = 4, \alpha = 0.05\)
Figure S1. Dependence of the amperometric responses measured with de developed bioplatforms in the absence (white bars, B) and in the presence (grey bars, S) of 50 ng mL$^{-1}$ N-Ms-IgG (A and C) or 250 ng mL$^{-1}$ Rb-S-IgG (B and D), as well as the corresponding S/B ratios (in red), with the N- (A) and S- (B) antigen concentration for their covalent immobilization on activated MBs, the steps involved in the immunoassay implementation for the determination of N-Ms-IgG (C), and the HRP-anti-Rb-IgG concentration for the enzymatic labelling of S-Rb-IgG captured on S-MBs (D).

As can be observed in Figures S1A and S1B, a progressive increase in the amperometric responses occurred with the increase in the concentration of both viral antigens. Importantly, the 0.0 bars in both figures confirmed that no discrimination between the presence and absence of the target Igs at the concentrations tested was possible without immobilized viral antigen (S/B 1.2 and 0.5 for N-Ms-IgG and S-Rb-IgG, respectively) and that the nonspecific adsorptions of both the IgGs used as standards and the secondary antibodies used for their enzymatic labelling with HRP were minimal in the absence of immobilized antigen. These results demonstrate both the effectiveness of the HOOC-MBs blocking step and the performance of the developed bioplatforms whose amperometric response is attributed solely to the specific recognition of each IgG type.
by its corresponding antigen and its enzymatic labelling with the appropriate secondary antibody. According to these results and due to the commitment of achieving high sensitivity at an affordable cost, N- and S-antigen concentrations of 5.0 and 25.0 µg mL\(^{-1}\), respectively, were selected for further work. The difference between the optimal immobilization concentrations for the two viral antigens can be attributed to several factors: N and S viral proteins are expressed in bacteria and mammalian cells, respectively, S is highly glycosylated, the non-oriented covalent immobilization may affect the capture of antigen specific Igs differently, and each viral antigen may have different affinities for the mammalian IgGs used as standards. On the other hand, the comparison of the results obtained by performing the capture and enzymatic labelling of N-Ms-IgG on N-MBs in the same step (1 step) or in successive steps (2 steps) shows that a higher degree of discrimination was attained in the latter case (S/B of 6.8 \(\text{vs.}\) 21.3; Figure S1C), which can be explained by a less efficient capture of N-Ms-IgGs on N-MBs when they are previously labelled with the secondary antibody. According to the results shown in Figure S1D, a more efficient enzymatic labelling of the S-Rb-IgG captured on S-MBs was achieved by incubation with a 1:500-diluted HRP-anti-Rb-IgG solution.
**Figure S2.** ROC curves obtained for the diagnostic ability of the developed bioplatforms based on the detection of total Igs and isotype-specific Igs (IgG, IgM and IgA) against COVID-19 S and N antigens. The detection of total Igs, IgGs, IgMs and IgAs against N and S proteins, except that of N-specific IgMs and IgAs, possessed an AUC, specificity, and sensitivity higher than 80% for the diagnosis of COVID-19 infection.
Figure S3. Diagnostic potential of the bioplatforms for the serological determination of total or isotype (IgG, IgM, and IgA) N- and S-specific anti-SARS-CoV-2 Igs when MBs were functionalized with N- and S- antigens (A) or with a mixture of both antigens (N+S) (B) in serum samples from non-infected (NC) and infected (PC) individuals.
Table S3. Ratios* of the IgG, IgM and IgA qualitative levels in non-infected and COVID-19 infected individuals obtained by the Luminex methodology. The mean signal obtained for IgG/IgM/IgA from non-infected individuals was used for normalization.

| Patient | Spike (S) | Nucleocapsid (N) |
|---------|-----------|------------------|
|         | IgG       | IgM   | IgG   | IgM   | IgG   | IgM   |
| NC-1    | 0.6       | 0.4   | 0.7   | 1.2   | 0.4   | 0.3   |
| NC-2    | 0.2       | 0.9   | 0.7   | 2.6   | 1.9   | 0.3   |
| NC-3    | 0.5       | 0.6   | 0.5   | 1.6   | 1.1   | 0.1   |
| NC-4    | 0.4       | 0.6   | 0.3   | 0.3   | 1.0   | 0.5   |
| NC-5    | 0.7       | 0.5   | 0.4   | 0.4   | 2.0   | 0.4   |
| NC-6    | 0.8       | 0.9   | 0.6   | 0.1   | 1.3   | 7.6   |
| NC-7    | 0.6       | 0.5   | 0.3   | 0.2   | 0.5   | 0.6   |
| NC-8    | 0.8       | 0.3   | 0.5   | 0.2   | 0.5   | 0.6   |
| NC-9    | 0.5       | 0.8   | 0.4   | 0.9   | 1.0   | 0.1   |
| NC-10   | 0.6       | 0.4   | 0.7   | 0.9   | 0.1   | 0.2   |
| NC-11   | 0.4       | 0.7   | 0.8   | 1.4   | 0.0   | 0.0   |
| NC-12   | 0.5       | 0.5   | 0.9   | 0.9   | 0.5   | 0.1   |
| PC-1    | 2.3       | 36.7  | 12.5  | 3.4   | 9.0   | 0.1   |
| PC-2    | 4.8       | 4.2   | 5.5   | 1.9   | 0.7   | 0.1   |
| PC-3    | 0.1       | 2.7   | 1.2   | 1.5   | 0.9   | 0.2   |
| PC-4    | 7.8       | 3.0   | 11.8  | 1.3   | 1.7   | 0.1   |
| PC-5    | 6.0       | 2.0   | 1.5   | 1.2   | 1.0   | 2.4   |
| PC-6    | 10.1      | 9.9   | 9.7   | 2.9   | 6.4   | 1.3   |
| PC-7    | 3.0       | 0.7   | 1.9   | 1.1   | 1.5   | 2.2   |
| PC-8    | 3.8       | 1.5   | 4.3   | 3.1   | 1.2   | 0.0   |
| PC-9    | 8.0       | 4.5   | 20.1  | 3.2   | 2.1   | 0.0   |
| PC-10   | 1.2       | 0.99  | ND    | 2.2   | 1.1   | 0.0   |
| PC-11   | 9.7       | ND    | 9.5   | 0.6   | 1.3   | 0.2   |
| PC-12   | 5.4       | 1.6   | 4.3   | 3.3   | 0.4   | 0.0   |

*, Positive = Ratio ≥1.0. Negative = Ratio <0.8 (IgG) or <0.9 (IgA or IgM). Uncertain = Ratio 1-0.8 IgG or 1-0.9 IgA or IgM. ND, not done.
Table S4. ROC Curves Data (in %) obtained for the Luminex diagnostic ability of the detection of isotype-specific Igs (IgG, IgM and IgA) against SARS-CoV-2 S and N antigens in non-infected and convalescent individuals.

|               | S-specific | N-specific |
|---------------|------------|------------|
|               | IgG        | IgM        | IgA        | IgG        | IgM        | IgA        |
| AUC           | 91.7       | 100        | 97.3       | 84.7       | 36.9       | 73.6       |
| Sensitivity   | 100        | 100        | 100        | 66.7       | 100        | 50.0       |
| Specificity   | 91.7       | 100        | 90.9       | 91.7       | 25.0       | 91.7       |
Figure S4. Correlation between the quantitative and qualitative results provided by the developed bioplatforms and Luminex methodology, respectively, for the detection of isotype-specific Igs (IgG, IgM, and IgA) against SARS-CoV-2 S (A) and N (B) antigens. R; Pearson correlation.
Experimental section

Apparatus and electrodes
Amperometric measurements were made with a CHI812B potentiostat (CH Instruments) and a portable potentiostat 3.4.1 μStat 200 (DRP-STAT200) STAT200 controlled by CHI812B and DropView, software, respectively. Screen-printed carbon electrodes (SPCE, DRP-110, 4 mm diameter) and screen-printed electrochemical arrays formed by eight 3-electrode electrochemical cells with carbon working electrodes (SP8CE, 2.56 mm diameter) and the appropriate specific cable connectors (DRP-CAC and CAC8X, respectively) were purchased from Metrohm-DropSens S.L. A DynaMag-2 Magnet from Invitrogen (Spain) and a MixMate microtube mixer from Eppendorf (Germany) were employed for the magnetic separation and incubation of the MBs, respectively. Capture of the modified MBs onto the working electrode/s surface/s of the SP8CE and SPCE was controlled by the neodymium magnets (AIMAN GZ) embedded in the home-made polymethacrylate (PMMA) or Teflon magnetic holders on which the disposable platforms were placed for amperometric measurement (see section 2.8).

Reagents and solutions
Carboxylic acid-functionalized magnetic beads (HOOC-MBs, 2.7 µm ∅ 10 mg mL⁻¹, Dynabeads® M-270 carboxylic acid, Cat. No: 14305D) were purchased from Invitrogen-Thermo Fisher. Goat anti-Mouse IgG Fc antibody HRP conjugated (ab97265, HRP-anti-Ms-IgG) and goat anti-Rabbit IgG (H + L) antibody HRP conjugated (Catalog # 170-6515, HRP-anti-Rb-IgG) were from Abcam and BioRad, respectively. Goat anti-Human IgG Fcγ antibody HRP conjugated (Catalog # 109-035-098, HRP-anti-h-IgG), anti-Human IgA antibody HRP conjugated (Catalog # 109-035-011, HRP-anti-h-IgA), anti-Human IgM antibody HRP conjugated (Catalog # 109-035-043, HRP-anti-h-IgM) were from Jackson Immunoresearch (Barcelona), and rabbit polyclonal cocktail of anti-Human IgG, IgA and IgM antibody HRP conjugated (Catalog # P0212, HRP-anti-h-IgG+IgA+IgM) was from Dako.
COVID 19 Nucleocapsid (N) Coronavirus Recombinant Protein (Catalog # MBS569934), in-house expressed recombinant Spike (S) protein (see protocol described in section 2.4) and Mouse Coronavirus (SARS-CoV N) Monoclonal Antibody (N-Ms-IgG, Catalog # MBS569903) and Rabbit COVID 19 Spike RBD Coronavirus
Polyclonal Antibody (S-Rb-IgG, Catalog # MBS154650) from MyBiosource Inc. were used as viral antigen and standards, respectively. S- and N- specific human IgG, IgM and IgA standards (Catalog # 2028, 2046, 2071, 2039, 2003 and 2090) from GenScript® were used as standards.

2-(N-morpholino)ethanesulfonic acid (MES) was purchased from Gerbu Biotechnik, GmbH; Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl, 99%), sodium dihydrogen phosphate dihydrate and di-sodium hydrogen phosphate anhydrous were purchased from Scharlab; 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was from Acros Organics, and hydroquinone (HQ), ethanolamine, N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS), and H$_2$O$_2$ (30% w/v) were purchased from Sigma-Aldrich. A blocker casein solution (BB, a ready-to-use, PBS solution of 1% w/v purified casein) from Thermo Scientific was also used. All other chemicals were of analytical grade, and ultrapure water (Millipore Milli-Q) was used throughout. A 0.05 M phosphate buffer (pH 6.0) solution was used to perform the electrochemical measurements.

**Patient sera**

The Institutional Ethical Review Board of the Hospital Clínic i Provincial de Barcelona (HCB/2022/0013) approved this study. Serum samples were obtained from the Hospital Clínic i Provincial de Barcelona. A total of 24 individuals (12 non-infected and 12 SARS-CoV-2 PCR-confirmed infected individuals in the first wave in Spain, whose blood was collected until June 2020) were recruited for the study (Table S5). Samples from infected patients were taken between 30 to 60 days after the onset of infection. Additionally, three vaccinated (two with Pfizer-BioNTech and one with Astra Zeneca) were included in the study, together with a pool of two serum samples from non-vaccinated and non-infected individuals as control (Table S5). Written informed consent was obtained from all individuals enrolled in the study. Serum samples were collected using a standardized sample collection protocol and stored at −80 °C until use.

Following the biocontainment guidelines established at the institutional level for safe handling, all the analyzed human serum samples were previously inactivated by treatment at high temperature (55 °C for 30 min) ensuring zero risk of infection and safe handling by the operator. After the inactivation process, the serum samples were stored
at −20 °C until analysis, which was performed in laboratories with biosafety level 2 (BSL-2).

Table S5. Characteristics of the non-infected (NC) and SARS-CoV-2 PCR-confirmed infected individuals (PC) and of the non-vaccinated (COVID-) and vaccinated (COVID+).

| Patient | Gender | Age | Disease               | Vaccinated    |
|---------|--------|-----|-----------------------|---------------|
| NC-1    | M      | 28  | Non-infected          | Non-vaccinated|
| NC-2    | M      | 32  | Non-infected          | Non-vaccinated|
| NC-3    | M      | 43  | Non-infected          | Non-vaccinated|
| NC-4    | F      | 25  | Non-infected          | Non-vaccinated|
| NC-5    | M      | 57  | Non-infected          | Non-vaccinated|
| NC-6    | M      | 51  | Non-infected          | Non-vaccinated|
| NC-7    | F      | 58  | Non-infected          | Non-vaccinated|
| NC-8    | M      | 33  | Non-infected          | Non-vaccinated|
| NC-9    | M      | 50  | Non-infected          | Non-vaccinated|
| NC-10   | M      | 36  | Non-infected          | Non-vaccinated|
| NC-11   | M      | 49  | Non-infected          | Non-vaccinated|
| NC-12   | F      | 51  | Non-infected          | Non-vaccinated|
| PC-1    | F      | 61  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-2    | M      | 31  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-3    | M      | 22  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-4    | M      | 23  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-5    | F      | 27  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-6    | F      | 62  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-7    | M      | 66  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-8    | M      | 48  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-9    | M      | 61  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-10   | M      | 28  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-11   | M      | 62  | SARS-CoV-2 infected   | Non-vaccinated|
| PC-12   | M      | 52  | SARS-CoV-2 infected   | Non-vaccinated|
| COVID-\_1| M    | 44  | Non-infected          | Non-vaccinated|
| COVID-\_2| F    | 44  | Non-infected          | Non-vaccinated|
| COVID- _3 | F | 92 | Non-infected | Pfizer-BioNTech |
| COVID+ _1 | F | 58 | SARS-CoV-2 infected | Pfizer-BioNTech |
| COVID+ _2 | M | 64 | SARS-CoV-2 infected | Astra Zeneca |

**Design, cloning, expression, and purification of Spike variants**

The plasmid pαH encoding the cDNA of S protein ectodomain (residues 1-1208) of the SARS-CoV-2 2019-nCOV (GenBank: MN908947) was kindly provided by Dr. Jason McLellan (the University of Texas at Austin, USA).\(^1\) Initially, a directed mutagenesis was carried out to obtain a HexaPro construct allowing for a high-yield production of a stabilized prefusion spike protein.\(^2\) The HexaPro ectodomain included the following substitutions: proline at residues 817, 892, 899, 942, 986, and 987, and "GSAS" substitution at the furin cleavage site (residues 682–685). For trimerization and purification, the C-terminal end of the S protein ectodomain was fused to the T4 fibrinogen trimerization motif (foldon), an HRV3C protease cleavage site, and an 8xHisTag. Finally, to prepare some of the spike like variants used in the study, directed mutagenesis was performed to generate the following constructs: Wuhan D614G (B.1) and Alpha-E484K (Δ69-70, Δ144, E484K, N501Y, A570D, D614G). On the other hand, to generate HexaPro derived constructs for Kappa (E154K, L452R, E484Q, D614G), Delta (T19R, E156G, Δ157-158, L452R, T478K, D614G, D950N) and Omicron (A67V, Δ69-70, T95I, G142D, Δ143-145, N211I, Δ212, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F) variants, the mammalian codon-optimized gene coding SARS-CoV-2 S was cloned into pcDNA 3.4 vector (Thermo Fisher Scientific, Waltham, MA, USA). In the Omicron construct the "GSAS" substitution was replaced by the natural furin cleavage site “RRAR” at residues 682-685. The expression vector coding for the SARS-CoV-2 S protein constructs was used to transiently transfect FreeStyle 293F cells (Thermo Fisher Scientific) using polyethylenimine. The S protein ectodomains were purified from clarified supernatants using His-Trap Excel columns (Cityva). Following a wash with 20 mM Na₂HPO₄, containing 200 mM NaCl and 20mM Imidazole pH 7.4, protein elution was performed using a gradient from 0 (wash buffer) to 100% 20 mM Na₂HPO₄, containing 200 mM NaCl and 300mM Imidazole pH 7.4. Fractions collected were concentrated with Amicon (Millipore) and buffer exchanged to 20 mM Na₂HPO₄.
containing 200 mM NaCl pH 7.4 without imidazole prior to load onto a Superose 6 10/300 gel filtration column (Cytyva), equilibrated and eluted with the same buffer.

**SDS-PAGE and Western Blot (WB) analysis**

SDS-PAGE and WB analysis were performed to assess protein quality (Figure S5). Briefly, 1 μg of HEK293 cell expressed Spike protein variants or *E. coli* expressed Nucleocapsid protein for Coomassie Blue staining or 500 ng for WB analysis were run in 10% SDS-PAGE under reducing conditions. For Coomassie blue staining analysis, gels were stained with Coomassie blue solution and destained with 7.5% acetic acid solution. For WB analysis, SDS-PAGE gels were transferred to nitrocellulose membranes (Hybond-C extra, GE Healthcare, Catalog # 10600041) at 100 V for 90 minutes. Then, membranes were blocked with 0.1% Tween PBS 1x supplemented with 3% skimmed milk (blocking buffer) during 1 h at room temperature (RT) and incubated with a polyclonal Anti-S (MyBiosource, MBS154650) and a monoclonal Anti-N (MyBiosource, MBS569903) antibody (Ab) at 1:1,000 dilution in blocking buffer, overnight (O/N) at 4 ºC and on shaking. Then, membranes were washed three times with 0.1% Tween PBS 1x and incubated with an HRP-conjugated goat polyclonal anti-rabbit secondary antibody (Sigma Aldrich, Catalog# A6154, 1:2,000) or an HRP-conjugated goat polyclonal anti-mouse (Sigma Aldrich, Catalog# A4416, 1:2,500) secondary antibody, respectively, 1 h at RT and on shaking. Finally, membranes were washed three times with 0.1% Tween PBS, and the signal was developed using the ECL Pico Plus chemiluminescent reagent (Thermo Fisher Scientific) and detected on an Amersham Imager 680 (GE Healthcare).
**Figure S5.** Quality control analysis of N- and S-SARS-CoV-2 proteins. Coomassie Blue staining -1 µg of protein- and western blotting analysis -500 ng of protein- of N and S proteins from Wuhan variant SARS-CoV-2 (A). Coomassie Blue staining of 1 µg of in-house produced indicated Spike variants (B).

**Mouse Wuhan D614G Spike variant immunization and mouse serum samples**
Animal studies were performed under the regulations of the Spanish and European legislation concerning vivisection and the use of genetically modified organisms. Protocols were approved by the “Comité de Ética de la Investigación y del Bienestar Animal” of “Instituto de Salud Carlos III” (CBA 17 208). Specific pathogen-free 6- to 8-week-old female C57BL/6J mice were obtained from Charles River Laboratories (France). Ten mice were immunized intraperitoneally (200µl) with two doses (4 weeks apart) with 2µg of Wuhan D614G (B.1) spike variant in PBS, mixed with equal volume of SAS adjuvant (Sigma Aldrich Cat#S6322). One week after the final dose, ~1 mL of blood was collected in a VACUETTE™ serum blood collection tubes from anesthetized mice by cardiac puncture. After centrifugation, serum samples were stored frozen at -20 ºC prior to use.

**Preparation of the magnetic bioconjugates**
The preparation of the magnetic bioconjugates was performed in microcentrifuge tubes and involved different incubation (at a controlled temperature of 25 ºC and under constant agitation at 950 rpm) and washing steps. After each washing step, the
microcentrifuge tubes were placed in a magnetic concentrator for 3 minutes to eliminate the supernatant.

In detail, a 3-µL aliquot of the commercial HOOC-MBs suspension was transferred to a 1.5 mL microcentrifuge tube and two successive washings were performed during 10 min with 50 µL of 0.025 M MES solution (pH 5.0). The carboxylic groups of the MBs were activated by incubation with 25 µL of an EDC and Sulfo-NHS (50 mg mL⁻¹ each) mixture solution prepared in 0.025 M MES (pH 5.0) for 35 min. Thereafter, the MBs were washed twice by manual shaking until complete resuspension with 50 µL of 0.025 M MES (pH 5.0) and incubated for 30 min with 25 µL of a 5.0 or 25.0 µg mL⁻¹ solution of the corresponding viral antigen (commercial N or in-house expressed S viral proteins, respectively), or a mixture of both types of antigens (N + S) (12.5 µg mL⁻¹ each), prepared in 0.025 M MES (pH 5.0). The resulting N-MBs, S-MBs or (N+S)-MBs were washed twice with 50 µL of 0.025 M MES (pH 5.0) and incubated for 60 min with 25 µL of a 1 M ethanolamine solution, prepared in 0.1 M phosphate buffer (pH 8.0), to block the remaining MBs active groups and minimize nonspecific adsorptions.

Thereafter, the N-, S- or (N+S)-MBs were washed twice with 50 µL of 0.1 M Tris-HCl (pH 7.2), once more with 50 µL of BB dilution, and incubated for 30 or 60 minutes with 25 µL of N-Ms-IgG or N-hIg, S-Rb-IgG or S-hIg standard solutions (prepared in BB solution), respectively, or for 60 minutes with the analyzed 1,000-fold diluted (in the same BB solution) human serum sample. Two washings with 50 µL of BB solution were carried out, and the N-Ms-IgG-N-MBs, N-hIg-MBs, S-Rb-IgG-S-MBs, or S-hIg-MBs were incubated for 15 minutes with 25 µL of HRP-anti-Ms-IgG (0.4 µg mL⁻¹), HRP-anti-Rb-IgG (1:500) (in the case of Ms and Rb standards) or HRP-anti-h-IgG, HRP-anti-h-IgA, HRP-anti-h-IgM or HRP-anti-h-IgG+IgA+IgM (1:1,000) (in the case of human standards and serum samples depending on the detection of total or isotype N- and S-specific anti-SARS-CoV-2 serum Igs) prepared in BB. After two additional washings with 50 µL of BB solution, the modified MBs were re-suspended in 50 or 20 µL of 0.05 M phosphate buffer (pH 6.0) or 0.05 M phosphate buffer (pH 6.0) containing 1.0 mM HQ to perform the single or the multiplexed amperometric transduction at SPCEs and SPsCEs, respectively.

For constructing calibration plots with N-hIg standards as well as for human serum analysis, N-MBs were prepared with 25.0 µg mL⁻¹ of N-antigen. To detect mouse serum IgGs against different S variants, the same protocol was used but varying the concentration of S antigen bound to activated MBs, dilution of mouse serum samples
and HRP-anti-Ms-IgG to 5.0 µg mL⁻¹, 100,000-fold and 1.0 µg mL⁻¹ HRP-anti-Ms-IgG, respectively.

**Amperometric measurements**

The appropriate electrode (SPCE or SP₃CE) was placed on the corresponding magnetic holder and 50 or 20 μL of the corresponding magnetic bioconjugate suspension was captured on each WE, respectively. The single amperometric determinations were carried out in stirred solutions (Figure S6A) while multiplexed measurements were made using drops of quiescent solution (Figure S6B). In both cases a potential of −0.20 V vs. an Ag pseudo-reference electrode was applied.

Single determinations were made by immersing the magnetic holder/SPCE assembly with the captured magnetic bioconjugates into an electrochemical cell containing 10 mL of phosphate buffer (0.05 M, pH 6.0) supplemented with 1.0 mM HQ (Figure S6A). Once the background current was stabilized, 50 µL of a freshly prepared 0.1 M H₂O₂ solution was added to the electrochemical cell and the current was recorded until the steady-state was reached.

Multiplexed determinations involved deposition of 20 μL of 0.05 M phosphate buffer pH 6.0 containing 1.0 mM HQ to cover the three electrodes of each of the 8 electrochemical cells (Figure S6B). Once the background current was stabilized, 2.5 μL of freshly prepared 0.1 M H₂O₂ solution in the same buffer were added to the quiescent solution drop and the current variation was monitored until stabilization.

The amperometric signals given throughout the manuscript were calculated as the difference between the steady-state currents measured after H₂O₂ addition and the background currents and correspond to the mean value of three replicates. In the case of serum samples measurements, the signals were subtracted from those measured under the same experimental conditions but using MBs without immobilized viral antigen. The error bars were estimated as three times the standard deviation of each set of replicates (α = 0.05).
Figure S6. Images of the electrode setups used for single (A) and multiplexed (B) amperometric determinations with the developed bioplatforms.

Luminex assays

Luminex beads (MagPlex microspheres, Luminex Corp.) were coupled to N- and S-SARS-CoV-2 proteins after activation with EDC and S-NHS. For coupling, 5x10⁵ beads were washed 3 times with 100 µL of activation buffer (0.1 M sodium phosphate, pH 6.1), prior to incubation with 2 mg of EDC (dissolved in DMSO) and 2 mg of S-NHS (dissolved in H₂Oṃq) in a final volume of 500 µL of activation buffer for 20 minutes at RT and 800 rpm. Then, beads were washed 3 times with 100 µL of coupling buffer (0.5 M 2-(N-Morpholino)ethanesulfonic acid hydrate (MES) pH 5.0, Sigma-Aldrich) and incubated with 2.5 µg of N- or S-SARS-CoV-2 proteins for 4 hours in 100 µL of coupling buffer at RT and 800 rpm. Beads were washed 3 times with 100 µL of coupling buffer and stored 48 h in 1000 µL of 1% BSA PBS 1x at 4°C in rotation. Then, beads were blocked in 500 µL of blocking buffer (3% BSA 0.1% Tween-20 PBS 1x) for 1 h at RT and transferred to 96-well plates (2500 beads per well) and incubated with 1:250 diluted in blocking buffer serum samples from 12 non-infected and 12 SARS-CoV-2 infected individuals. Beads were washed 3 times with 100 µL of washing buffer (0.1% Tween-20 PBS 1x) and incubated with 1:500 diluted in blocking buffer biotin-conjugated anti-human IgG, IgM or IgA secondary antibodies (Jackson Immunoresearch) for 1 h at RT in rotation. Each antibody isotype was analyzed in duplicate. Finally, beads were washed 3 times with 100 µL of washing buffer, incubated with 1:1000 diluted in blocking buffer streptavidin-PE (BioRad) for 1 h at RT in rotation, and washed 3 times with 100 µL of washing buffer and an additional washing
with 150 µL of PBS. Beads fluorescence signal was measured in the Bio-Plex 200 (Bio-Rad).

**Statistical analysis**
Sera analyses were made by duplicate, and positive sera were confirmed by three independent experiments. Values were plotted as mean values ± standard error of the mean (SEM). Plots, mean, and SEM were obtained with Microsoft Excel 2019 and GraphPad Prism 8 programs. Nonparametric Mann-Whitney U test values (p-values) were calculated using R (v3.6.2); p-values < 0.05 were considered statistically significant. ROC curves (Receiver Operating Characteristic Curve) of individual proteins and in combination were obtained with R (version 3.6.2), using the “ModelGood” and the “Epi” packages to determine the diagnostic ability of the test.

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