

Multiplex IgG Reactivity to SARS-CoV-2 Antigens in Senegal

Moussa Ndour^{1*}, Moustapha Mbow^{1,2}, Biscotine F. Makoutchouang Nzone²,
Joséphine K. Badiane², Marouba Cisse¹, Matar Gningue¹, Ibrahima Diallo¹, Abdou Aziz Diallo²,
Mady Ndiaye³, Massamba Sylla³, Babacar Mbengue¹, Souleymane Mboup², Tandakha N. Dieye^{1,2}

¹Department of Immunology, Faculty of Medicine, Pharmacy and Odontology, Cheikh Anta Diop University, Dakar, Senegal

²Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formations (IRESSEF), Diamniadio, Sénégal

³Biology Laboratory, Faculty of Science and Technology, Cheikh Anta Diop University, Dakar, Senegal

Email: *drmoussandour@gmail.com

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Abstract

Background: Multiplex serology can distinguish antibody reactivity to several viral targets, but its interpretation in African populations requires attention to the timing of sampling and pre-pandemic cross-reactivity. This study aimed to describe IgG reactivity to five SARS-CoV-2 antigens in three Senegalese groups. **Methodology:** In this retrospective study, 380 archived plasma samples were tested using the CoViDiag[®] multiplex immunoassay: 162 collected before the pandemic, 162 from participants with RT-PCR-confirmed SARS-CoV-2 infection, and 56 from occupationally exposed healthcare workers. The assay simultaneously assessed IgG antibodies against nucleocapsid (N), spike S1, spike S2, the receptor-binding domain (RBD), and the N-terminal domain (NTD). Proportions are presented with Wilson 95% confidence intervals (CIs). **Results:** Overall CoViDiag positivity differed among groups ($P < 0.001$): 4/162 (2.5%; 95% CI, 1.0% - 6.2%) in pre-pandemic samples, 68/162 (42.0%; 95% CI, 34.6% - 49.7%) in the RT-PCR-confirmed group, and 3/56 (5.4%; 95% CI, 1.8% - 14.6%) in exposed healthcare workers. Among RT-PCR-confirmed participants, antigen-specific positivity was most frequent for N (35.8%), followed by S2 (21.6%), S1 (16.7%), and RBD (12.3%); no NTD reactivity was detected. Pre-pandemic reactivity was predominantly directed against N. **Conclusion:** Multiplex testing revealed heterogeneous IgG profiles dominated by N reactivity. The observed positive agreement with RT-PCR cannot be interpreted as an estimate of the assay's intrinsic sensitivity because the interval between infection and blood collection was unavailable. Pre-pandemic signals support cautious interpretation of serosurveys and do not demonstrate protective immunity.

Keywords

SARS-CoV-2, COVID-19, Multiplex Serology, IgG, Nucleocapsid, Spike, Senegal, Pre-Pandemic Cross-Reactivity

1. Introduction

Serological testing complements molecular diagnosis by documenting the humoral response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] [2]. IgG generally becomes more reliably detectable after the early phase of infection, and its magnitude and persistence vary with disease severity, sampling time, host factors, and the antigen used [3]-[5]. Serology is therefore most informative for retrospective exposure assessment and population surveillance rather than for diagnosing acute infection [2] [6].

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA betacoronavirus. Its virion contains four principal structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N). N associates with the viral RNA, whereas M and E contribute to virion assembly and envelope organisation. The trimeric S glycoprotein projects from the viral surface and is divided functionally into S1 and S2. S1 contains the N-terminal domain (NTD) and receptor-binding domain (RBD), including the site that engages angiotensin-converting enzyme 2; S2 contains the more conserved membrane-fusion machinery (Figure 1) [7] [8].

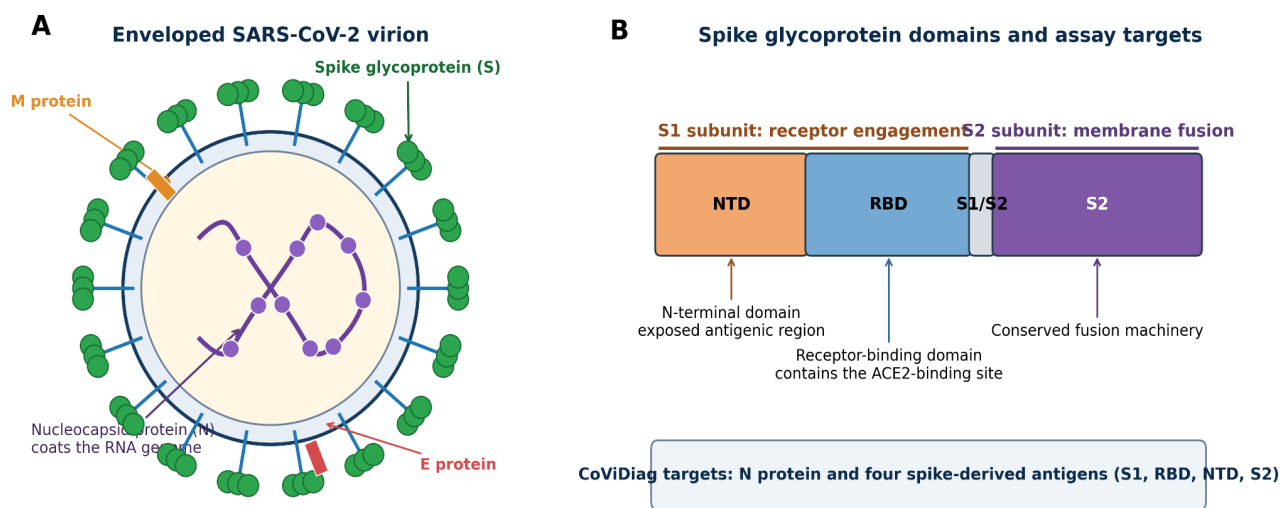


Figure 1. Structural organisation of SARS-CoV-2 and location of the antigens assessed by CoViDiag®. (A) Enveloped virion showing S, M, E, N, and the RNA genome. (B) Functional organisation of spike into S1, including NTD and RBD, and the fusion-mediating S2 subunit. Original synthesis based on recent structural reviews and intact-virion imaging [7] [8].

Most assays target the nucleocapsid (N) protein or regions of the spike glycoprotein. The receptor-binding domain (RBD) lies within S1 and contains major neutralising epitopes, whereas N is abundant and immunogenic but is not itself a neutralisation

target. Multiplex platforms can assess multiple antigens in a single well, potentially increasing information content and revealing antibody signatures that single-antigen assays cannot capture [9] [10]. However, analytical thresholds, antigen composition, and the interval since infection strongly influence performance [8]-[11].

Interpretation is particularly important in sub-Saharan Africa. Early in the pandemic, Mbow and colleagues proposed that demographic structure, previous microbial exposures, immune background, and public-health responses might contribute to the discrepancy between projected and reported COVID-19 burden in Africa; these were hypotheses requiring direct epidemiological and immunological evaluation rather than evidence of protection [12]. In Senegal, a national survey conducted in October-November 2020 estimated anti-SARS-CoV-2 seroprevalence at 28.4%, showing that reported cases substantially underestimated exposure [13]. Studies also identified pre-pandemic reactivity to SARS-CoV-2 antigens in African samples [11] [14]. Such signals may reflect cross-reactive antibodies induced by endemic pathogens or seasonal coronaviruses, assay-specific effects, or non-neutralising responses and should not be equated with protection [15]-[18]. We therefore compared CoViDiag[®] IgG reactivity to N, S1, S2, RBD, and NTD in pre-pandemic controls, RT-PCR-confirmed cases, and occupationally exposed healthcare workers in Senegal [18]-[21].

2. Materials and Methods

2.1. Study Design and Setting

This is a retrospective, descriptive, and analytical laboratory study conducted at the Institut de Recherche en Santé, de Surveillance Épidémiologique et de Formations (IRESSEF), Senegal. Archived pre-pandemic specimens were obtained from the institutional biobank. Specimens from participants with COVID-19 were collected in epidemic treatment centers or during home follow-up. Immunological testing was performed at the IRESSEF immunology platform.

2.2. Study Population and Sampling

The study population comprised 380 subjects divided into three groups: 1) 162 pre-pandemic controls, recruited prior to the confirmed circulation of SARS-CoV-2, with sampling dates ranging from November 2, 2018, to July 9, 2019; 2) 162 participants with RT-PCR-confirmed SARS-CoV-2 infection, with sampling dates ranging from January 17 to September 21, 2020; and 3) 56 healthcare workers considered occupationally exposed due to their work in healthcare facilities during the pandemic, with sampling dates ranging from July 21 to October 9, 2020. Plasma samples were analysed as they became available; study documents did not specify a fixed interval between RT-PCR testing and serological sampling, nor between RT-PCR testing and symptom onset.

2.3. CoViDiag Multiplex IgG Assay

Plasma was tested using the CoViDiag[®] multiplex immunoassay (Innobiochips;

lot CVD-2009301), an ELISA-derived, miniaturised solid-phase assay. Five SARS-CoV-2 antigens were printed as spatially distinct replicate spots in each microplate well: nucleocapsid (N), spike subunit 1 (S1), receptor-binding domain (RBD), N-terminal domain (NTD), and spike subunit 2 (S2) (**Figure 2(A)**). Seven positive-control spots were distributed near the corners of each well. The spatial arrangement of the antigen and control spots is shown in **Figure 2(B)** and permits simultaneous qualitative assessment of several antigen-specific IgG responses in a single reaction [9] [10] [22] [23].

Specimens and reagents were handled according to the manufacturer's instructions [22]. The plasma samples were tested within 5 days of collection or stored at 2°C - 8°C; longer-term storage was at -20°C. Prior to testing, frozen specimens were thawed and mixed, and reagents were brought to 20°C - 25°C for 30 min

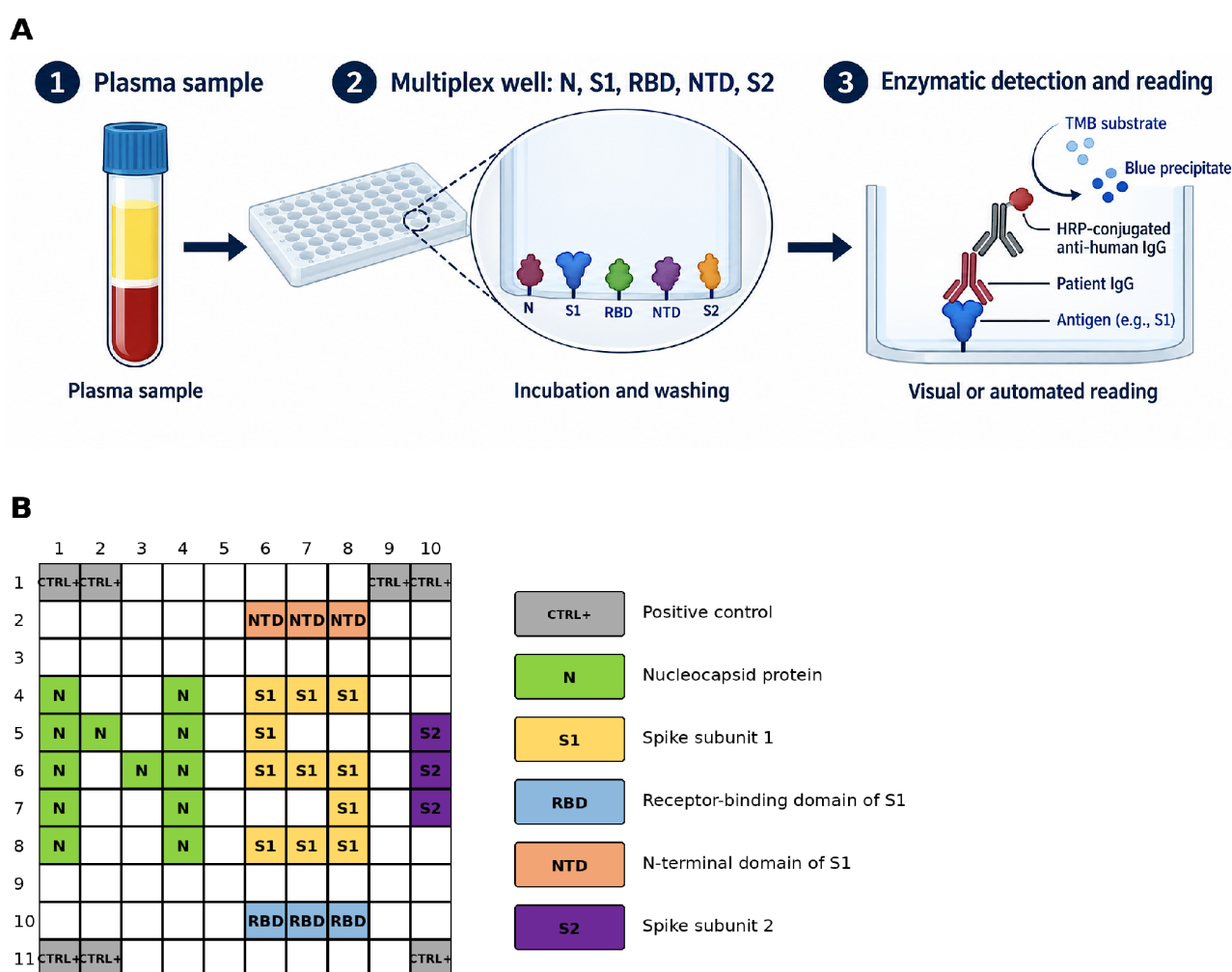


Figure 2. CoViDiag[®] assay principle and spot layout. (A) Patient IgG binds printed SARS-CoV-2 antigens and is detected with HRP-labelled anti-human IgG and precipitating TMB. (B) Spatial distribution of the positive-control and replicate N, S1, RBD, NTD, and S2 spots within one well. The diagram was redrawn, and the supplied layout was adapted from the manufacturer's instructions for use [22] [23].

and then mixed. Each plasma specimen was diluted by adding 10 μ L of plasma to 1 mL of sample-dilution buffer (1 + 100; nominal 1:100 dilution) [22] [23].

A 100- μ L aliquot of diluted plasma was dispensed into each assigned antigen-printed well; the plate was covered, and incubation proceeded for 1 h at 37°C $\pm$ 1°C, with shaking at 300 rpm when a plate shaker was used. Wells were emptied, and the plate was washed three times with 200 μ L of wash buffer each, with more than 5 s between buffer addition and aspiration; residual liquid was removed by tapping the plate on absorbent paper. A fresh 6:1000 dilution of HRP-conjugated anti-human IgG was prepared in sample-dilution buffer (60 μ L of conjugate in 10 mL of buffer), and 100 μ L was added to each well. After incubation for 1 h at 37°C in direct sunlight, the three-cycle wash was repeated [22] [23].

Next, 50 μ L of precipitating TMB substrate was added to each well, and the plate was incubated for exactly 15 min at 20°C - 25°C in the dark. After incubation, the substrate was removed, and the wells were rinsed three times with 200 μ L of de-ionised water each time. Plates were tapped, dried, and allowed to dry for 15 min at 37°C away from light before reading. Except for this final drying step, wells were not allowed to dry between procedural steps. HRP activity generated a localised insoluble blue precipitate at reactive spots, with color intensity proportional to antigen-bound IgG (**Figure 2(A)**) [22] [23].

2.4. Visual Quality Control and Instrumental Reading

Each well was first inspected macroscopically. Seven control spots positioned near the corners were used to assess technical validity; the test was considered invalid when more than two control spots were absent. The printed N- and S-shaped spot patterns permitted rapid visual screening for anti-N and anti-S1 reactivity. Under the manufacturer's visual rule, a visible N and/or S pattern indicated seroconversion. Representative visual and automated appearances are shown in **Figure 3**. Visual inspection served as quality control and rapid screening; it did not, by itself, resolve the full five-antigen profile, which required instrumental image analysis [22] [23].

Acquisition was performed on the IRESSEF immunology platform using an

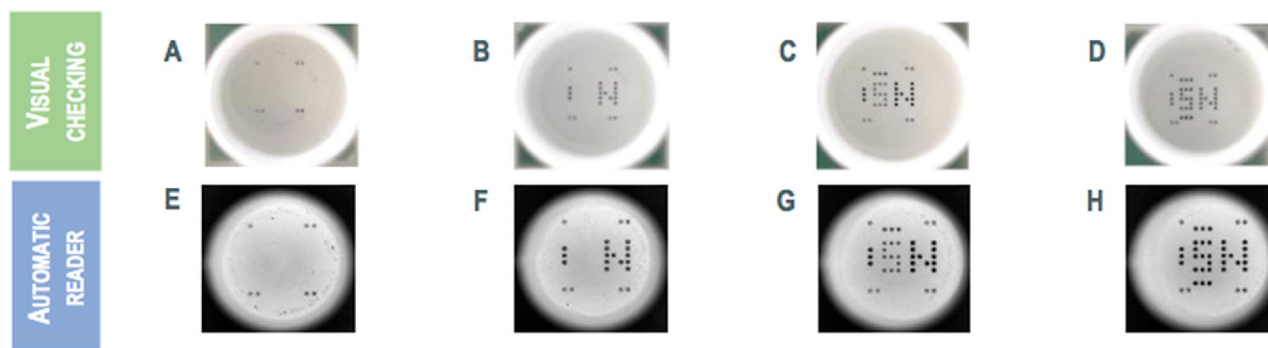


Figure 3. Representative CoViDiag® well appearances. (A)-(D) Direct visual inspection; (E)-(H) Corresponding automated-reader images. Control spots frame the well, while N- and S-shaped patterns indicate antigen-specific color development. Adapted from the manufacturer's application note [23].

AID ELISPOT Reader, which captured high-resolution images. Applying user-defined analysis parameters, data were then exported as numerical results. **Figure 4** shows a processed plate, the reader workstation, and a representative automated plate map. The manufacturer's IFU and application note describe assay evaluation with the SciReader colorimetric biochip reader (Sciension, Berlin, Germany) [22] [23]. The study records did not include the AID software version, exposure and segmentation settings, calibration procedure, or an AID-SciReader bridging study.

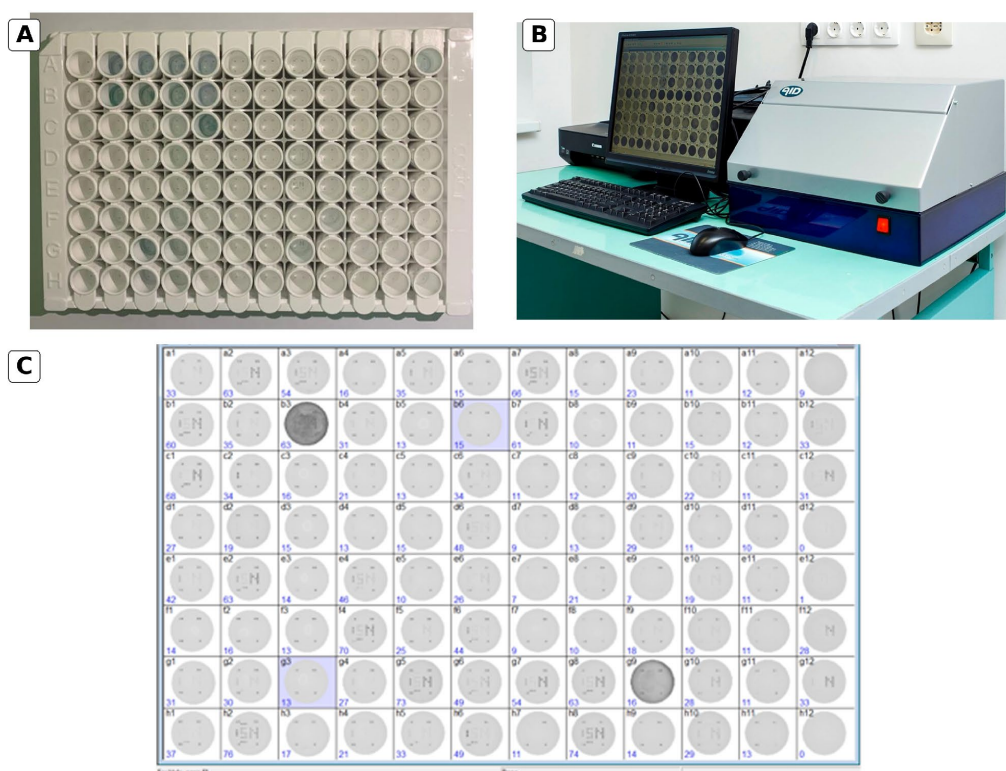


Figure 4. Plate processing and automated acquisition. (A) Representative CoViDiag[®] plate after color development; (B) AID ELISPOT Reader and workstation used for plate imaging; (C) Representative automated plate map with well-level images and exported signal values. Images supplied in the study records.

2.5. Signal Quantification and Classification

For each antigen, the mean net signal of replicate spots was calculated after subtraction of the local background surrounding each spot. Instrumental validity required a mean net control signal greater than 10 arbitrary units (AU). Antigen-specific results were categorised as negative, borderline, or positive using the predefined thresholds in **Table 1**. These thresholds had been established during assay development using 200 serum or plasma samples collected before June 2019 [22].

Overall sample status was assigned hierarchically using the manufacturer-defined combination rule. A valid sample was positive when S1, RBD, or NTD was positive; when N or S2 exceeded 40 AU; or when N/S2 positivity or borderline reactivity was accompanied by an additional borderline antigen signal. Isolated N

or S2 positivity, or isolated borderline S1, RBD, or NTD reactivity, was classified as equivocal. All remaining valid patterns were classified as negative [22]. The manufacturer's application note treated borderline results as negative for its diagnostic-performance calculations [23]. For the present analysis, the final positive/negative status recorded in the study database was used; no equivocal category was present in the validated summary table.

Table 1. CoViDiag[®] antigen-specific signal thresholds.

Printed antigen	Negative (AU)	Borderline (AU)	Positive (AU)
Nucleocapsid (N)	0 - 15	15 - 30	>30
Spike S1	0 - 10	10 - 20	>20
Spike S1-RBD	0 - 10	10 - 20	>20
Spike S1-NTD	0 - 10	10 - 20	>20
Spike S2	0 - 15	15 - 30	>30

AU, arbitrary units; RBD, receptor-binding domain; NTD, N-terminal domain. The IFU prints shared endpoints between adjacent categories (10, 15, 20, and 30 AU); categorical calls exported in the study data were retained rather than reassigning observations exactly on those boundaries.

2.6. Statistical Analysis

Counts and percentages were calculated for categorical variables. Wilson 95% confidence intervals (CIs) were computed for positivity proportions. Overall CoViDiag positivity was compared across the three groups using Pearson's chi-square test. All tests were two-sided with a significance threshold of 0.05. Summary counts were re-analysed for this manuscript using Python 3.12.14 and SciPy 1.17.0. Because individual-level timing and clinical data were incomplete, no adjusted model was fitted.

2.7. Ethical Considerations

This study was conducted in line with the Declaration of Helsinki and has been approved by the "Comité National pour l'Ethique et la Recherche en Santé" of the Senegalese Ministry of Health (reference No. 000159/MSAS/CNERS/Sec). Written informed consent was obtained from all participants before enrolment and sample collection.

3. Results

3.1. Participant Characteristics

The 380 participants had a mean age of 34.8 years and a median age of 32 years (range 0.1 - 88 years). Men represented 60.8% of the study population. The RT-PCR-confirmed group had the highest proportion of males (76.5%), whereas women predominated in the pre-pandemic group (56.8%) (Table 2).

Table 2. Demographic characteristics of the analytical population.

Group	n	Mean age, years	Median [range], years	Male, n (%)	Female, n (%)
All participants	380	34.8	32 [0.1 - 88]	231 (60.8)	149 (39.2)
Pre-pandemic	162	37.1	32 [0.1 - 88]	70 (43.2)	92 (56.8)
RT-PCR-confirmed	162	34.5	32 [13 - 88]	124 (76.5)	38 (23.5)
Exposed healthcare workers	56	29.1	32 [24 - 55]	37 (66.1)	19 (33.9)

3.2. Overall Multiplex IgG Positivity

Overall, 75 of 380 samples (19.7%) were classified as positive by the CoViDiag algorithm. Positivity was 2.5% in pre-pandemic samples, 42.0% in the RT-PCR-confirmed group, and 5.4% in exposed healthcare workers (**Table 3**). The difference among groups was significant ($\chi^2 = 88.38$, 2 degrees of freedom; $P < 0.001$). In descriptive terms, the corresponding positive agreement with RT-PCR was 42.0%, whereas negative agreement in pre-pandemic samples was 97.5%. These quantities are not presented as intrinsic diagnostic sensitivity or specificity because sampling time and participant selection were not standardised for an accuracy study.

Table 3. Overall CoViDiag IgG positivity by study group.

Group	Positive, n	Negative, n	Positive, % (95% CI)
Pre-pandemic	4	158	2.5 (1.0 - 6.2)
RT-PCR-confirmed	68	94	42.0 (34.6 - 49.7)
Exposed healthcare workers	3	53	5.4 (1.8 - 14.6)
Total	75	305	19.7 (16.0 - 24.0)

CI, confidence interval. Confidence intervals were calculated using the Wilson method.

3.3. Antigen-Specific IgG Profiles

N was the most frequently reactive antigen across all groups. Among RT-PCR-confirmed participants, 58/162 (35.8%) were positive to N, 35/162 (21.6%) to S2, 27/162 (16.7%) to S1, and 20/162 (12.3%) to RBD (**Table 4**). No NTD-positive sample was identified. All four reactive pre-pandemic samples recognised N, and only one also recognised S1. In the healthcare-worker group, three samples recognised N and two each recognised S1, S2, and RBD; co-reactivity means that antigen-specific counts are not mutually exclusive.

4. Discussion

In this study, we compared multiplex IgG reactivity against five SARS-CoV-2 antigens in three groups of participants with different epidemiological profiles in Senegal. CoViDiag[®] positivity was significantly higher among participants with

Table 4. Antigen-specific IgG positivity by study group.

Group	N, n (%)	S1, n (%)	S2, n (%)	RBD, n (%)	NTD, n (%)
Pre-pandemic (n = 162)	4 (2.5)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
RT-PCR-confirmed (n = 162)	58 (35.8)	27 (16.7)	35 (21.6)	20 (12.3)	0 (0.0)
Exposed healthcare workers (n = 56)	3 (5.4)	2 (3.6)	2 (3.6)	2 (3.6)	0 (0.0)
Total (n = 380)	65 (17.1)	30 (7.9)	37 (9.7)	22 (5.8)	0 (0.0)

N, nucleocapsid; S1/S2, spike subunits 1/2; RBD, receptor-binding domain; NTD, N-terminal domain. Antigen categories are not mutually exclusive.

RT-PCR-confirmed infection (42.0%) than among pre-pandemic controls (2.5%) and occupationally exposed healthcare workers (5.4%). The antibody response was heterogeneous and strongly dominated by N: among RT-PCR-confirmed participants, 35.8% showed reactivity to N, compared with 21.6% to S2, 16.7% to S1, 12.3% to RBD, and 0% to NTD. These results indicate that the multiplex panel provides information not only through an overall positive or negative classification but also through antigen-specific IgG reactivity profiles. The observed positive agreement with RT-PCR was 42.0%; however, because the interval between infection and blood collection was unknown, this agreement should not be interpreted as an estimate of the assay's intrinsic sensitivity. In addition, N-directed reactivity observed in pre-pandemic samples should be interpreted cautiously, as it may reflect cross-reactivity or nonspecific binding [24].

The five antigens are not interchangeable diagnostic markers. Each contributes differently to the serological profile [8] [9]. N is highly abundant and strongly immunogenic and, in this study, was the most frequently reactive individual antigen. However, N is an internal viral protein, and anti-N binding antibodies are generally not expected to neutralise intact virions [25]. In addition, N contains conserved epitopes that may promote cross-reactivity with other coronaviruses [15] [16] [24], and anti-N antibodies may decline over time [2] [6]. Thus, an isolated anti-N result should be considered evidence of antibody recognition of N, of epidemiological interest and potentially complementary to clinical data, but not definitive evidence of SARS-CoV-2 infection [2] [16] [25]. S1 contains both RBD and NTD and is generally more immunogenic than the more conserved regions of the spike protein. RBD is biologically important because it mediates ACE2 binding and contains major neutralising epitopes. However, CoViDiag[®] measures IgG binding only and does not measure neutralising activity; therefore, isolated RBD positivity cannot establish the presence of protective immunity. S1 and RBD are therefore mainly useful as relatively specific components of a multi-antigen signature. NTD may provide complementary information on the breadth of the response directed against spike, but the absence of reactivity across the different groups indicates that, under the conditions of this study, it was neither sensitive nor discriminative. This observation could be explained by the antigen design, the

assay threshold, the viral strain, the timing of sampling, or simply by a weak or waning anti-NTD response. It should not be interpreted as evidence that anti-NTD antibodies are absent in the Senegalese population [24]. S2 contains the most conserved fusion machinery of the spike protein. Its reactivity rate of 21.6% among RT-PCR-confirmed participants is potentially of interest as a complementary marker of positivity, but the risk of cross-reactivity between coronaviruses is high. Isolated S2 reactivity should therefore be interpreted more cautiously than a concordant response directed against S1 and RBD. N and S2 may contribute to detecting broader or waning responses, whereas S1 and RBD may contribute more to specificity. The value of multiplexing lies precisely in combining these signals rather than selecting the antigen with the highest positivity rate [9] [10].

Based on the available data, no antigen in this panel can be considered a diagnostic reference standard. The independent reference for infection was RT-PCR confirmation, whereas N, S1, S2, RBD, and NTD were components of the index test. Each antigen should therefore be evaluated against the independent reference standard in a direct comparison at standardized time points after infection, using a prespecified threshold, receiver operating characteristic analysis, and an independent validation set [26].

In this study, RBD or S1 could constitute the most appropriate antigenic anchor for supporting recognition of a SARS-CoV-2-specific response, but not on its own. The strongest evidence of previous natural infection is a concordant profile combining several complementary viral targets, particularly RBD or S1 together with N. Serological profiles limited to N or S2 are more difficult to interpret because they may reflect cross-reactivity, nonspecific binding, or different antibody kinetics. This approach is broadly consistent with the manufacturer's hierarchical algorithm, which assigns direct positivity to S1, RBD, or NTD and requires multiple or combined signals for N and S2 [22] [23]. However, the algorithm cannot be considered independent of the classification rule because it is precisely the rule being applied.

The 42.0% positive agreement with RT-PCR was lower than the performance reported for CoViDiag® in studies conducted during the late convalescent phase [9] [10] [22]. It should not be described as intrinsic diagnostic sensitivity because the interval between infection and blood collection was unavailable and the study was not designed as a prospective diagnostic-accuracy evaluation. Early sampling before IgG seroconversion, mild or asymptomatic infection, waning antibody levels, host-related variation, and specimen- or reader-related factors may have reduced detection. Likewise, the 97.5% negative agreement observed in pre-pandemic samples is more descriptive than definitive, as the controls were selected retrospectively and reactive specimens were not assessed with a second serological method [11] [27].

The pre-pandemic samples provide a local estimate of background seroreactivity that can be used to assess the panel in Senegal. All four reactive pre-pandemic samples recognised N; only one also recognised S1, and none showed reactivity to

RBD, S2, or NTD. Because these samples were collected before SARS-CoV-2 circulation was well established, their signals cannot be attributed to infection with the pandemic virus. The predominance of N-directed reactivity suggests the presence of antibodies against conserved coronavirus epitopes, antibodies induced by other endemic infectious agents, polyreactive antibodies, or nonspecific binding to the assay.

African studies of pre-pandemic samples have also reported appreciable and heterogeneous responses to N and, in some cases, to spike antigens [15]-[21]. Malaria-associated binding to carbohydrate epitopes on spike further demonstrates that apparent SARS-CoV-2 reactivity may occur without previous infection with this virus [19]. These controls therefore have a dual value: they challenge the specificity of isolated antigen signals, particularly those directed against N, and show that thresholds developed exclusively in other populations may not be directly applicable in West Africa. The four reactive controls do not constitute evidence of previous SARS-CoV-2 circulation, prior protection, or reduced susceptibility to COVID-19. No neutralisation assay, antibody-avidity assessment, epitope mapping, seasonal-coronavirus serology, or absorption experiment was performed to establish the origin or function of these antibodies. These findings are therefore interpreted as pre-pandemic cross-reactivity or nonspecific binding, requiring further mechanistic investigation [15] [21].

Only 3 of the 56 healthcare workers (5.4%) met the overall positivity rule. At the antigen level, three participants showed reactivity to N, and two showed reactivity to S1, S2, and RBD, indicating that some positive samples had multi-antigen profiles. Concordant reactivity directed against spike and N is more compatible with previous natural infection than an isolated signal directed against N or S2. However, occupational exposure was defined by the work context and not by documented infection, and the low positivity rate does not constitute a measure of the occupational attack rate or evidence of protection. Concordant reactivity directed against spike and N may be more suggestive of previous natural infection than an isolated N or S2 signal, although this pattern is not definitive and should be interpreted in clinical context [2] [16] [28].

Serology cannot determine where or when infection occurred, and the absence of IgG does not exclude previous exposure, particularly if sampling occurred before seroconversion or after antibody levels had declined. Individual sampling dates and vaccination status were not available. If the samples were collected before vaccination began, profiles combining spike and N would be compatible with previous natural infection. If collection occurred after vaccination began, spike-only profiles could suggest vaccination, whereas N reactivity would be more compatible with natural infection, although waning anti-N antibodies and cross-reactivity would remain potential confounding factors. The exposed group should therefore not be considered an infected group, but rather a comparison group defined by epidemiological criteria [2] [6] [16].

The difference between RT-PCR-confirmed participants and pre-pandemic

participants is compatible with infection-related seroreactivity, although the number of confirmed cases was limited. At the population level, Senegalese serosurveillance showed that cumulative exposure was more than 10 times the number of reported cases at the time of the study [13]. Mbow and colleagues previously suggested that demographic composition, microbial exposures, immune background, and public-health responses may have contributed to the initially lower-than-expected number of cases in Africa [12]. The results presented here neither confirm nor refute these hypotheses. They only show that background immune reactivity exists and can substantially influence the interpretation of SARS-CoV-2 serology, and that the presence of antibodies should not be equated with neutralising activity or protection against infection.

In sub-Saharan Africa, producing a single composite positive or negative result may conceal biologically and analytically significant profiles. Reporting N, S1, S2, and RBD signals separately, together with a predefined multi-antigen classification rule, could help distinguish strong concordant responses from potentially cross-reactive signals directed against a single antigen. Serology should be used primarily for retrospective exposure assessment and population surveillance; it should not replace molecular or antigen detection for the diagnosis of acute infection [2].

The main strengths of this study are the simultaneous assessment of five antigens and the comparison of a large number of pre-pandemic samples with RT-PCR-confirmed participants. Antigen-specific analysis highlights features that would be difficult to observe with a binary single-antigen assay, particularly the absence of NTD reactivity. Several limitations restrict the interpretation of the findings. The retrospective, convenience-based design and the absence of standardised intervals between infection and sampling make it difficult to estimate sensitivity [2] [6] [11].

Disease severity, clinical history, vaccination status, comorbidities, and antibody kinetics were not available. The healthcare-worker group was small, and exposure was not biologically confirmed. Reactive pre-pandemic samples and samples from exposed participants were not evaluated using an independent serological method or a neutralisation assay. Available individual-level data were insufficient for a more detailed analysis [2] [16] [28].

In addition, the AID ELISPOT Reader settings, software version, calibration procedure, and any use of an AID reader comparable to the instrument used during the manufacturer's evaluation were not sufficiently documented. This uncertainty regarding the reader is important; instrument-comparison and repeatability studies would be required before drawing conclusions about diagnostic performance from numerical values obtained with the AID reader [22] [23] [29].

Future studies should use prospectively collected samples with documentation of symptom-onset dates, RT-PCR dates, vaccination status, disease severity, and repeated sampling. Antigen-specific thresholds should be reassessed using local pre-pandemic controls and confirmed cases, followed by blinded validation in an

independent Senegalese sample. Samples showing discordant reactivity limited to N or S2 should undergo independent serology, testing for antibodies against seasonal coronaviruses, and neutralisation or inhibition assays. These studies would help determine whether a locally optimised multi-antigen rule can improve specificity without compromising sensitivity and, if not, whether these profiles reflect SARS-CoV-2-specific antibody responses, cross-reactivity, or non-functional antibody responses.

5. Conclusion

The CoViDiag multiplex assay revealed heterogeneous IgG recognition of SARS-CoV-2 antigens in Senegal, with N as the dominant target and lower reactivity to S2, S1, and RBD. Overall positivity was substantially higher in RT-PCR-confirmed participants than in pre-pandemic controls or exposed healthcare workers. Because sampling time was unknown, the 42.0% positive agreement with RT-PCR should not be treated as an intrinsic sensitivity estimate. The predominantly N-directed pre-pandemic signals reinforce the need for local validation, orthogonal confirmation, and cautious separation of antibody binding from protective immunity.

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Author Contributions

Conceptualization: Moussa Ndour, Moustapha Mbow, and Tandakha N. Dieye. Investigation: Moussa Ndour, Biscotine F. Makoutchouang Nzone, and Joséphine K. Badiane. Sample collection: M. Cisse, M. Gningue, I. Diallo and A.A. Diallo. Formal analysis and interpretation: Moussa Ndour and Moustapha Mbow. Resources, materials, and analytical tools: Mady Ndiaye, Massamba Sylla, Tandakha N. Dieye, and Souleymane Mboup. Project administration and coordination: Babacar Mbengue and Tandakha N. Dieye. Writing-original draft: Moussa Ndour and Moustapha Mbow. Writing-review and editing: Babacar Mbengue, Moustapha Mbow, and Tandakha N. Dieye. All authors read and approved the final version of the manuscript.

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Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request, subject to institutional and ethical restrictions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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