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Challenges and Methodological Aspects of Detecting Anti-RBD Antibodies by ELISA in SARS-CoV-2 Research

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Data Note

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Abstract

The detection of IgE antibodies specific to the receptor-binding domain (RBD) of SARS-CoV-2 has been associated with aspects of COVID-19 severity, including potential mast cell–mediated mechanisms. Recent studies analyzed IgE anti-RBD across different COVID-19 severity groups using ELISA. The coating-antigen concentration influences the detection capacity of the immunoassay; thus, low-abundance antibodies, such as IgE, may require higher antigen concentrations to achieve optimal experimental conditions. Here, we present the effect of different coating concentrations for anti-RBD IgE and IgG4 detection in samples of vaccinated individuals. Our results suggested that intermediate antigen concentrations provide a better balance between sensitivity and specificity, highlighting the importance of methodological standardization.

Background

The recent study of De la Poza et al. (2025), published in the Virology Journal, investigated the presence of IgE antibodies directed against the Receptor binding domain (RBD) of SARS-CoV-2 in patients with different clinical presentations of COVID-19, according to disease severity, proposing an association between IgE responses, mast-cell activation, and the hyperinflammatory response observed in later stages of the disease. The identification of IgE antibodies in this context represents an important contribution, as antibody-mediated mechanisms extend beyond viral neutralization and involve complex immunoregulatory functions [1].

Antibodies exert multiple biological functions, including pathogen neutralization, complement activation, and promotion of phagocytosis, with these effects being modulated by antibody class, subclass, and interactions with Fc receptors [2,3]. Importantly, different immunoglobulin isotypes circulate in serum at markedly distinct concentrations. While IgG and IgM are present at high levels, being detectable even at limited antigen coating concentrations in immunoassays, low-abundance immunoglobulins such as IgE and IgG4 are present at much lower concentrations, rendering their detection particularly sensitive to experimental conditions in immunoenzymatic assays [4,5].

Previous studies have demonstrated that the magnitude and profile of humoral immune responses to SARS-CoV-2 vary according to disease severity, including differences in antibody kinetics and intensity, further emphasizing the need for sensitive and carefully optimized methodologies when assessing specific antibody classes and subclasses [6].

Critical assessment and methodological considerations

One methodological aspect of the study by de la Poza et al. that warrants discussion is the antigen concentration used for IgE detection by ELISA. In that work, recombinant SARS-CoV-2 RBD was employed at a coating concentration of 10 µg/mL**.** High antigen density may increase binding capacity, but assays targeting low-abundance immunoglobulins are particularly susceptible to changes in signal-to-noise ratio and to increased nonspecific binding under conditions of antigen excess.

The use of lower RBD concentrations for IgE and IgG4 detection has been described for the first time in studies conducted by our group. Previous work standardized coating concentration of 1 µg/mL of recombinant RBD, enabling sensitive detection of antigen-specific IgE and IgG4 with reduced nonspecific background signals [7]. The parallel evaluation of the IgG4 subclass was used as a complementary methodological reference for low-abundance immunoglobulins.

Widely adopted SARS-CoV-2 ELISA protocols were standardized before employing different RBD concentrations for different isotypes. While our investigation of IgE and IgG4 used 1 µg/mL [7], Stadlbauer et al. (2020) used 2 µg/mL in a broadly validated serological screening protocol for IgG [8]. Similarly, Moura et al. (2021) and Costa et al. (2024) employed 2.5 µg/mL of RBD for the detection of IgG and its subclasses, achieving appropriate analytical performance [5,9]. Together, these examples indicate that intermediate RBD concentrations are often sufficient, underscoring the need for standardized antigen-coating conditions for each antibody class. In addition, differences in ELISA plate surface chemistry and binding capacity may significantly influence antigen immobilization and antibody detection, thereby affecting assay sensitivity and specificity [10].

Exploratory evidence and practical implications

In addition to the conceptual considerations described above, comparative experimental analyses were performed to assess the impact of antigen density on the detection of IgE and IgG4. 12 individuals, known to have anti-RBD IgE, were selected within a larger COVID-19 cohort (CAAE 31924420.8.0000.0059). Each individual provided 3 samples: before the vaccine, after two and after three vaccine doses. 6 of those were vaccinated with a CoronaVac (Instituto Butantan)+Comirnaty (Pfizer-BioNTech) schedule, and the other 6 received a Vaxzevria (AstraZeneca)+Comirnaty schedule. 12 pre-pandemic samples were used as controls [7].

Briefly, high-binding plates were coated with different RBD concentrations: 1, 2, 5, and 10 µg/mL. Sera samples were incubated at a 1:5 dilution, followed by anti-IgE-ε chain or anti-IgG4 (Sigma-Aldrich) at a 1:1000 dilution, and the reactions were revealed with TMB substrate (Sigma-Aldrich). More details about the cohort, RBD production and ELISA protocols are described in Costa et al. (2024) and Portilho et al. (2024).

Within this exploratory context, increasing RBD coating concentrations did not affect reactivity in pre-pandemic control samples. For IgE, antibody reactivity remained low in pre-vaccination samples and increased after the second and, more markedly, after the third vaccine dose, with a clear improvement in signal observed when moving from lower coating concentrations (1–2 µg/mL) to an intermediate concentration. Notably, IgE reactivity showed minimal differences between 5 and 10 µg/mL, supporting the selection of 5 µg/mL as an optimal coating concentration that balances assay performance and reagent consumption (Figure 1A–D). A similar pattern was observed for IgG4, a low-abundance serum immunoglobulin that is generally present at higher concentrations than IgE, with minimal reactivity before vaccination and a modest increase predominantly after the third dose, while control samples

remained unaffected regardless of the antigen concentration used (Figure 1E–H). Noteworthy, anti-SARS-CoV-2 IgG4 is mostly induced after mRNA vaccination schedules or when multiple booster doses are administered [11,12]. Finally, although no increase in background was observed in this analysis, optimal antigen concentrations and relative assay sensitivity may vary across laboratories depending on protocol design, reagents, and cohort characteristics.

Beyond antigen concentration, the physicochemical nature of the antigen itself represents a critical factor in defining immunoenzymatic assay conditions, influencing adsorption to the solid phase and epitope accessibility. These variables directly affect assay reliability and reproducibility, particularly when evaluating immunoglobulins present at low concentrations.

Conclusion

The study by de la Poza et al. provides valuable insights into IgE responses associated with COVID-19. However, given the intrinsic sensitivity of these assays to experimental conditions, careful selection of antigen concentration and coating parameters is essential for robust data interpretation. Regarding the role of low immunoglobulin levels in the immunopathogenesis of COVID-19, the adoption of standardized and empirically validated conditions, including the use of 5 µg/mL RBD concentrations for IgE and IgG4, may improve assay reproducibility and evidence-based conclusions.

Abbreviations

Ig - Immunoglobulin

RBD - Receptor-binding domain

SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2

COVID-19 - Coronavirus disease 2019

ELISA - Enzyme-linked immunosorbent assay

Declarations

Ethics approval and consent to participate

This study was registered and approved by the institutional ethics committee of Adolfo Lutz Institute (CAAE 31924420.8.0000.0059), and written informed consent was provided by all participants. Additionally, all experiments were performed following relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization and study design were performed by GSO, AIP, and EDG. Volunteer recruitment and data collection were performed by VOS and LFMB. Antigen production was carried out by HHMC and CRP. Laboratory experiments were performed by GSO, AIP, AFS, NSM, and EDG. Sample management was conducted by VOS and LFMB. Statistical analysis was performed by GSO and AIP. The manuscript was written by GSO, AIP, AFS, and NSM, and EDG revised the original draft. All authors contributed to the study, commented on previous versions of the manuscript, and approved the final manuscript.

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Figures

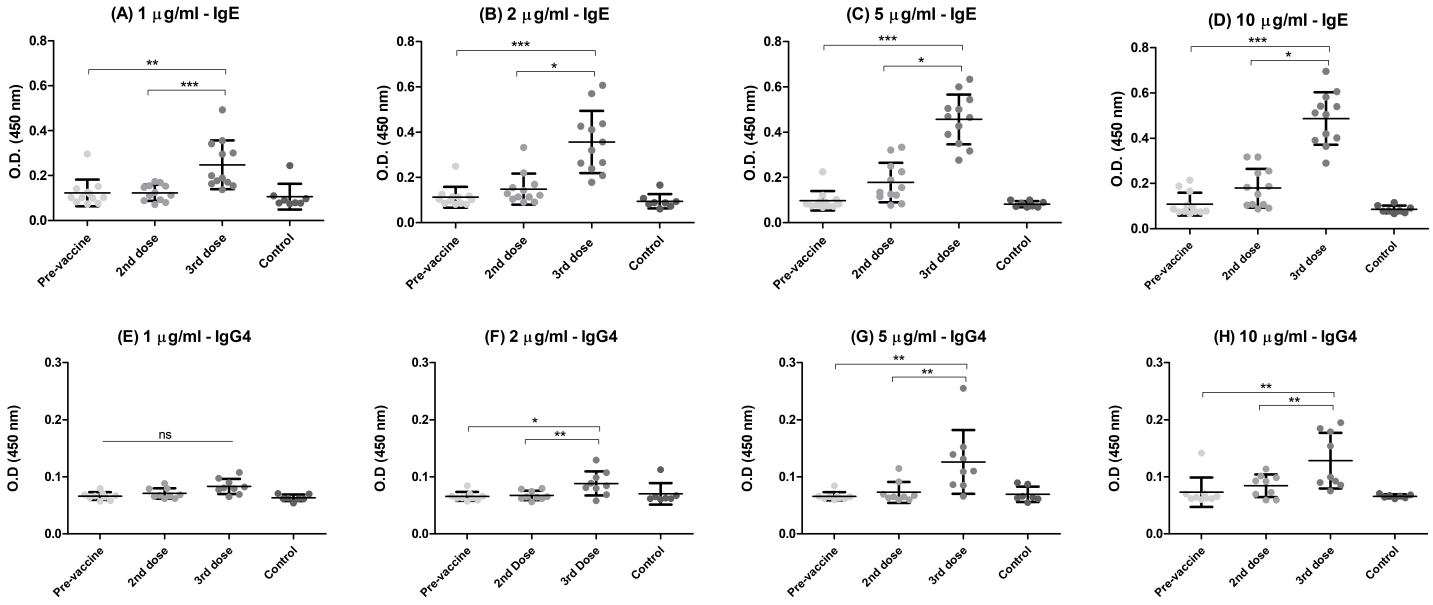


Figure 1

Effect of RBD coating concentration on IgE and IgG4 detection by ELISA on high-binding plates. Exploratory analysis of anti–SARS-CoV-2 RBD IgE and IgG4 reactivity assessed across vaccination stages. Recombinant RBD was immobilized at 1, 2, 5, or 10 μg/mL, and antibody levels are shown as optical density at 450 nm (OD_{450}). (A–D) IgE reactivity measured at 1 μg/mL (A), 2 μg/mL (B), 5 μg/mL (C), and 10 μg/mL (D). (E–H) IgG4 reactivity measured at 1 μg/mL (E), 2 μg/mL (F), 5 μg/mL (G), and 10 μg/mL (H). Statistical comparisons among vaccination stages were performed using the Friedman test followed by Dunn's multiple comparisons post hoc test. Asterisks indicate levels of statistical significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$); ns - not significant.