IVD_07 - Quantitative IgG elisa of SARS-COV-2 spike-protein: analysis of blood samples from vaccinated individuals

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Introduction: Serological tests can complement molecular diagnosis, confirming the antibody response of vaccinated individuals either in the presence or absence of a natural Covid-19 infection history.

Objective: In this article, we present the development of a quantitative IgG ELISA of S protein from blood samples of individuals vaccinated against Covid-19.

Methodology: In the standardization of quantitative methods, some parameters are important to obtain feasible results, such as the calculation of the confidence interval of the controls, as well as incubation temperature, conjugated batch, operator, and quantification and detection limits.

Results: Negative and positive controls, as well as the background, were analyzed in replicates and the 95% of confidence interval was calculated from the arithmetic mean with two errors below and above. The negative control was set to 0.115 (± 0.0407), positive control to 1.007 (±0.125), and the background to 0.058 (±0.008). The robustness of the ELISA was evaluated. Eighteen standard curves of the positive control were analyzed and no statistically significant difference was observed between the Optical Densities (OD) against variations in the incubation temperature (36 - 38ºC) ($p=0.0590$), conjugated lots ($p = 0.2495$) and operator ($p = 0.9426$). The quantification limit was calculated from the analysis of the average of four standard curves of the positive control, with the detection limit from an OD of 0.2 where the analyte produces a signal three times higher than the noise signal (0,06) and quantification limit of 0.6, as long as the signal-to-noise ratio is greater than 6 (9.316 EU/mL). Blood samples from 33 volunteers vaccinated against Covid-19 were analyzed. IgG antibodies concentration were calculated using the 4-logistic parameter. A statistically significant increase in antibody titers ($p<0,001$) was observed after second dose, and the agreement of the results with the liquid microarray platform will be evaluated.

Conclusion: The test should be revalidated if there is a change in the final product. However, our findings suggest that a feasible, useful quantitative ELISA assay was obtained, with the potential of helping to elucidate the antibody response dynamics after Covid-19 natural infection and/or vaccination.

Keywords: SARS-CoV-2; Quantitative IgG ELISA; S protein