REA_06 - Assessment of humoral response to SARS-CoV-2 using an ELISA kit, developed and validated at references laboratories in Brazil

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Introduction: SARS-CoV-2, a member of the *Coronaviridae* family and the causative agent of COVID-19, was first isolated in December 2019, in Wuhan, China. Since then, it has spread quickly, and as of February 2021, the disease reached the mark of 100 million people affected worldwide and has caused more than 2.3 million deaths. Several countries, including Brazil, are import-dependent of currently available commercial kits with significant impact on population tests and costs for public health system. This study describes the development of an ELISA to detect antibodies against SARS-CoV-2 using a recombinant viral nucleocapsid (N).

Objective: ELISA kit development, validation and use for the detection of antibodies against SARS-CoV-2.

Methodology: *Antigen production*: nucleocapsid (N) protein of SARS-CoV-2 was expressed in *E.coli* BL21(DE3) strain under IPTG induction and purified by affinity chromatography. The antigen seroreactivity was evaluated in ELISA (EIE COVID19). *Validation*: In all, 894 samples were tested, 362 from SARS-CoV-2-positive patients after positive qRT-PCR (nasal swab) and/or rapid test-dual path platform (DPP) COVID-19 IgM/IgG (Bio-Manguinhos-Fiocruz), 407 from SARS-CoV-2-negative donors (before 2020 and sera obtained after 2020 from qRT-PCR individuals) and 125 samples from other viruses and interference study. *Longitudinal study*: COVID-19 patients (non-hospitalized, n=62) were followed up after positive PCR confirmation. Serum samples were collected at week 1 to three months and tested by DPP (IgM and IgG) rapid test and EIE COVID-19 IgG kit. This study was approved by the UFMG's Ethics Committee and by the National Research Ethics Committee (CAAE: 1686320.0.0000.5149).

Results: The EIE COVID19 kit was able to detect IgG antibodies against SARS-CoV-2 with high sensitivity (93%) and specificity (100%) when compared to DPP. The repeatability assessments indicated high precision parameters (CV<10%), with no significant difference among different batches of the developed prototype (p=0,9700 by Kruskal-Wallis test), and shelf-stability of at least nine months. Three external validation studies were performed at reference laboratories using panels of pre-characterized sera (Laboratory of Virology -USP, Laboratory of Respiratory Viruses and Measles, Fiocruz-RJ, Laboratory of Diagnostic Technology -Fiocruz-RJ) and showed an accuracy of >90%. Three distinct patterns were observed: IgM seroconversion earlier than that of IgG (17%), IgG seroconversion earlier than that of IgM (39%) and synchronous seroconversion of IgG and IgM (43%). The median day of seroconversion for both EIE COVID-19 IgG kit and DPP (IgG) was 14 days post PCR confirmation. The IgM against SARS-COV-2 has reached its peak level after 15 days post qPCR and the IgG after 20 days. At the end of the evaluated period (85 ± 4 days post qPCR), 87% and 34% of patients no longer presented antibodies IgM and IgG against SARS-COV-2, respectively.

Conclusion: The EIE COVID-19 kit represents an important and a national addition to the currently available immunological tests for diagnosis and epidemiological studies on SARS-CoV-2.

Keywords: SARS-COV-2 serodiagnosis ; COVID-19 diagnostic assays ; ELISA prototyping