IVD_03 - A flow cytometry-based assay to measure neutralizing antibodies against SARS-CoV-2 virus

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Introduction: Coronavirus disease (COVID-19) caused by severe acute respiratory syndrome 2 (SARS-CoV-2) virus was declared a pandemic in 2020. Serological assays can evaluate the neutralizing efficiency of antibodies. Here we developed a novel neutralizing assay based on antibody binding to S protein to measure antibodies against SARS-CoV-2 in plasma as a surrogate to the conventional plaque reduction neutralization test (PRNT).

Objectives: Developed and validated a flow cytometry assay to detect neutralizing antibodies against the S protein of SARS-CoV-2.

Methodology: Plasma samples of 15 adult patients previously tested for SARS-CoV-2 by RT-PCR hospitalized; 6 seronegative individuals and 9 adult patients SARS-CoV-2 vaccinated were collected. The neutralizing activity of antibodies against SARS-CoV-2 was analyzed by flow cytometry measuring the inhibition rate of interaction between the viral spike (S) protein, conjugated with the fluorochrome Alexa Fluor (AF) 488, and the angiotensin converting enzyme 2 (ACE2) receptor expressed on the surface of HEK 293 T cells. The percentage of ligation in wells incubated only with S AF 488 was considered the positive control to calculate the relative neutralization obtained in each well. In-house plaque reduction neutralization assay (PRNT) was kindly performed by Laboratory of Emerging Viruses (LEVE).

Results: Assay precision of plasma dilution 1:50 was good, with non-significant differences between 3 repetitions (CI 95% 0.790 - 0.967, $p = 0.1375$) and a good concordance (ICC = 0.910). The cut-off to detect neutralizing antibody positivity value 36.01%, sensitivity (100%) and specificity (100%) AUC 1.0, 95% CI 100% - 100%, $p = 0.0002$. In the intra-assay precision (n=20) for the positive sample, the mean of inhibition was 93.86% ±1.28 %CV was 1.36%. For the negative sample, the mean of inhibition was 0.00% ± 0.00 %CV was 0.00%. The flow cytometry assay showed significant correlation with PRNT assay $r = 0.88$, $p<0.0001$, ICC = 0.866. The results of performance obtained comparing the assays were sensitivity and specificity of 100%, positive predictive value (PPV) 100%, negative predictive value (NPV) 100%, accuracy and precision of 100%.

Conclusion: The assay was validated by comparing the data with PRNT results. The flow cytometry-based neutralization assay is reproducible and reliable.

Keywords: Neutralizing antibodies, SARS-CoV-2, Flow cytometry