IVD_16 - Standardization and automated analysis of the SARS-CoV-2 Focus Reduction Neutralization Test (FRNT)

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Introduction: The Plaque Reduction Neutralization Test (PRNT) is the gold standard to study Neutralizing Antibodies (NAbs), but other approaches have shown more scalability and suitability to automate data analysis, improving data integrity. One of them is the Focus Reduction Neutralization Test (FRNT), performed on 96 well- plates and generates antibody-specific cytopathic effect. This assay allows a higher throughput and the development of automated methods to acquire images and quantify Focus Forming Units (FFUs).

Objectives: We aim to use SARS-CoV-2 as a model to develop and standardize FRNT, as well as to establish reliable procedures for image acquisition and virus FFU count automation.

Methodology: To standardize FRNT, experimental parameters were selected to generate homogeneous cell monolayers and to optimize FFUs number and shape. Samples from donors previously screened by PRNT were used to assemble a panel (arranged by titer ranges) and examined by FRNT, with the defined parameters to monitor Nab titers, set control sera and compare to PRNT-generated titers. To improve image acquisition and analysis of FRNT plates, automated equipment and software were adopted. Images generated from the settings were used to teach the software morphological patterns which must be recognized to classify the observed objects as distinct FFUs. To verify the teaching effectiveness, images were run during the reading step, and the data were compared to the manual counting.

Results: The FRNT experimental parameters for 96 well-plates were standardized like 200.000 cell/well for density, 70-100 FFU/well for viral input, 1.5% CMC for semi-solid overlay medium, 15 min with 4% PFA 24h post-infection for cell fixation and 1:1000 as antibody dilution, resulting in unambiguous FFU identification. Results from control sera were qualitatively equivalent to those observed by PRNT with the same samples. The image acquisition methods produced high-resolution pictures with a proper signal-noise ratio. The comparison between FFU automated and manual counting showed the count method is numerically equivalent to the human task, but mitigates inherent operator biases, provides data traceability, and enables faster release of results.

Conclusion: The standardized FRNT has shown to be a trustful assay to quantify NAbs in serum samples, and its associated methods for image acquisition and analysis have improved data generation. This way, our work has developed a high-performance tool whose analysis are in line with the GMP data integrity, capable to support studies that monitor Nab titers from vaccine responses.

Keywords: Neutralizing antibodies; FRNT; Automation