IVD_06 - New approaches in the detection of neutralizing antibodies using SARS-CoV-2 as a model

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Introduction: The Plaque Reduction Neutralization Test (PRNT) is considered the gold standard to study Neutralizing Antibodies (NAbs), but due to its limitations, more scalable approaches are required. Focus Reduction Neutralization Test (FRNT) is suitable for automation which allows large-scale testing and data integrity. SARS-CoV-2 PRNT and FRNT must be held in Biosafety Level 3 (BSL-3) labs. To circumvent this issue, the use of SARS-CoV-2 Pseudovirus (PV) has enabled the manipulation in BSL-2 areas, increasing throughput and reducing costs.

Objectives: We aim to develop and standardize FRNT - using automation for data analysis - as well as designing and setting up the Pseudovirus-Based Neutralization Test (PBN), as a new strategy for the NAbs quantification, using SARS-CoV-2 as a model.

Methodology: To standardize the assays, a panel of serum samples from donors was organized by titer ranges, previously obtained by PRNT. Different systems for *in-house* PV generation are being tested, as well as commercial kits. For FRNT, experimental parameters were set to optimize *Focus* Forming Units (FFUs). To improve the image analysis of FRNT plates, automation is being used.

Results: We developed an *in-house* anti-spike protein monoclonal antibody conjugated to HRP (horseradish peroxidase) for FRNT, which showed to be specific and detectable. Moreover, experimental parameters for 96 well-plates were standardized like 200.000 cell/well for density, 70-100 FFU/well for viral input, 1.8% [CMC] for semi-solid overlay medium, 15 min with 4% PFA for cell fixation and 1:500 as antibody dilution resulting in countable FFU. Cell fixation 48h post-infection generated heterogeneous *foci* morphology, thus 24h with other CMC concentrations is currently being tested. For the PBN with kits, NAbs titer from sera positive (low, medium and high) and negative controls were qualitatively equivalent to those observed by PRNT.

Conclusion: The FRNT and PBN tests are promising for the quantification of NAbs in human samples. PBN results from sera panel correlated with PRNT data. Through the milestones reached so far, we are close to develop accurate and high-performance tools, with significantly lower costs, to support studies that monitor vaccine responses.

Keywords: Neutralizing antibodies, pseudovirus, FRNT