**ORT_01 - A high content image-based assay for detection and measurement of SARS-CoV-2 neutralizing antibodies**

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**Introduction:** The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has presented an urgency for neutralizing assays. The reference assay, plaque reduction neutralization test (PRNT), is not safe and requires a biosafety level-3 laboratory, since it demand for live viruses. Moreover, PRNT is laborious, time consuming and is not adapted to high throughput, a feature necessary to screen a high number of plasms, small molecules and antibodies.

**Objectives:** Our aim was to develop a safe, easy and faster neutralizing antibodies (nAbs) assay.

**Methodology:** Lentiviral and non-replicative particles (pseudovirus) were developed in HEK293T cells and used to transduce HEK293T-ACE2 overexpressing cells. The automated image analysis was standardized in a high content system, in which the average ZsGreen fluorescence intensity was used to select transduced and non- transduced cells, while the percentage of ZsGreen cells was the readout to calculate the potency of human plasmas (pNT50). Plasma samples from 29 healthy individuals were collected before and after vaccination.

**Results:** The pseudovirus produced was specific for ACE2 overexpressing cells. It was observed a linear correlation between the percentage of ZsGreen$^{+}$ cells and ZsGreen average fluorescence intensity with viral titer. Pearson correlation coefficient was 0.938 and 0.997, respectively. The cut-off was calculated from the mean of positive control for neutralization plus 3 times the standard deviation, resulting in 0.52% and 14.19, respectively. Before vaccination, individuals presented 60-70% of neutralizing capacity. Thirty days after the first dose, there was a significative increasing of neutralizing capacity in individuals vaccinated with BNT162b2 and ChAdOx1 (*$p$< 0.0284 and ****$p$<0.0001, respectively), but no difference with CoronaVac. Thirty days after the second dose, all vaccinated presented a high neutralizing capacity of 85-90%. These percentages comprising an increasing of approximately 20% of nAbs compared to before vaccination (***$p$<0.0009 for CoronaVac and ****$p$<0.0001 for BNT162b2 and ChAdOx1).

**Conclusion:** We developed and validated an image-based high content assay, which is safe, high-throughput compatible and able to determine the plasm neutralizing potency in two days.

**Keywords:** Covid-19, neutralizing assay, high content screening