ORT_09 - Inactivated SARS-CoV-2 for safe use in analytical and non-clinical trials

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Introduction: Due to the limitation of BSL3 laboratories, successful of SARS-CoV-2 inactivation allows its safe use in a BSL2 environment, enabling applications such as standards to challenge diagnostic kits, assays validations, ELISA and development of monoclonal antibodies. Furthermore, working with the whole particle, rather than the isolated proteins, allows a correct molecular recognition and facilitates development of more accurate analytical methods, and a more reliable immune response and antibody production.

Objective: The purpose of this study is to characterize the inactivated SARS-CoV-2 and use it as an *in house* assays control, contributing to development and improvement of *in vitro* and *in vivo* assays.

Methodology: Production of SARS-CoV-2 occurred in stationary culture of Vero E6 cells grown in serum free media. After clarification, the virus suspension was treated with beta-propiolactone (βPL) and inactivation was confirmed by serial passages. Characterization of the inactivated material occurred by rapid antigen test, quantification by commercial ELISA for protein S and use as antigen in *in vivo* assays of active and passive immunity using transgenic K18-hACE2 mice.

Results: Serum free produced SARS-CoV-2 yielded 6.4 Log_{10} PFU/mL. After βPL treatment, inactivation was confirmed by absence of CPE after 5 passages along with reduction of genome quantification by qPCR analysis of passages supernatants. Commercial ELISA quantification indicates SARS-CoV-2 recognition by anti-S Ab as well as rapid antigen test for anti-N Ab. *In vivo* test showed that mice immunized with inactivated virus presented viremia with a peak within 3 dpi and survived longer than controls with no significant weight variation. After passive immunity testing, 4/5 animals, which received serum by intraperitoneal via from animals immunized with inactivated virus, survived until the end of the study with no or <25% pulmonary compromise, without symptoms.

Conclusion: The inactivation process developed at Bio-Manguinhos is effective in producing a promising inactivated antigen, which can be used in several fronts and contribute to the development and improvement of molecular and serological diagnostic kits; to aid in the validation of analytical methods; as antigen for antibody production and as a non-clinical trials control. However, other characterizations are necessary. We plan to develop an *in-house* ELISA, using the inactivated virus as a standard curve, instead of the recombinant protein S and to challenge the inactivated virus in a microarray assay with an anti-SARS-CoV-2 Ab library, to further assess the interaction with antibodies.

Keywords: SARS-CoV-2; COVID-19; Virus inactivation; Process improvement