PRODUCTION AND PURIFICATION OF Zika VIRUS FOR AN INACTIVATED VIRUS VACCINE CANDIDATE

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Key Words: Inactivated Zika vaccine

Zika arbovirus is the most recent causative agent of an unattended emerging viral disease. Previously restricted to the African continent, Zika has spread rapidly during the last five years, reaching Asia and America. The emergence of Zika in Brazil revealed that pregnant women is a particular at-risk population due to the possibility of the infection during pregnancy causing congenital Zika syndrome, which in the worst cases is evidenced by severe microcephaly in neonates. Instituto Butantan as a public vaccine producer started studies for the development of an inactivated Zika vaccine as soon as the first birth defects cases came to knowledge. The first strategy chosen for Zika production was based on the production process already established for dengue vaccine. However, in opposition of what was believed at the beginning of the Zika outbreak, this virus has some differential characteristics when compared to Dengue viruses. Mainly due to the lytic behavior of Zika infection, which is not present in Dengue infection, a new process was developed to propagate and purify Zika virions. In order to establish the best culture conditions, Vero cells were seeded in different cell concentrations and culture media, in several flask sizes and types, infected with a range of Zika virus comprising MOI from 0.01 to 0.11, in kinetic studies with or without medium exchange. These studies were responsible for reaching PFU titers above 1E+07 PFU/mL in just 72 h of process with consistent reproducibility in production levels. For purification, harvested Zika was submitted to sucrose gradient ultracentrifugation or to two chromatography steps, reaching the required level of purity regarding host cell protein (< 100 ng/mg) and residual DNA (< 100 pg/dose). Zika vaccine was finally established in more than one formulation, after efficient inactivation with betapropiolactone. Inactivation was carefully evaluated by performing multiple passages of the inactivated material in C636 cells followed by a plaque assay. This work focused not only on generating a proof-of-concept of the immunization with inactivated Zika, but also on the development of scalable process aiming the establishment of a technology ready to enter the next phases of the vaccine development. This project has been funded in part with Federal funds from the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Grant No. IDSEP130015. Supported by WHO, Butantan Institute and BARDA.

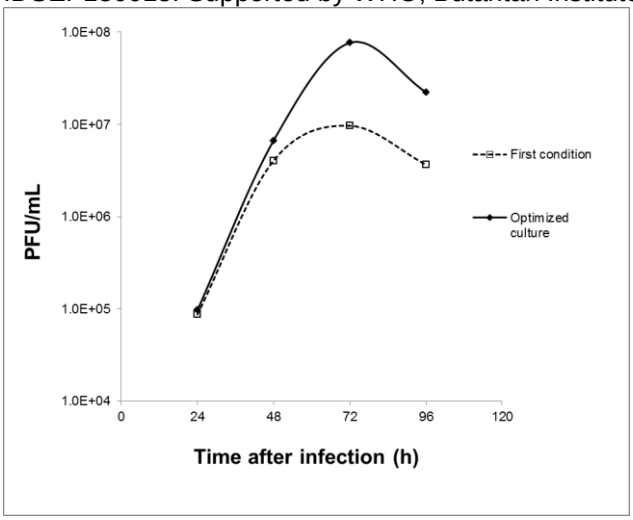

Figure: Zika production kinetic profile. *Zika virus (Strain BeH815744) was inoculated in Vero cells at several conditions in order to establish the best production parameters. The optimization studies resulted in an increase of the mean virus titer from 9.7 E+06 PFU/mL to 7.7 E+07 PFU/mL.*