VAC_02 - Chikungunya virus replicative profile in Vero cells for immunobiological development purposes

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Introduction: Chikungunya virus (CHIKV) is the etiological agent of arthropod-borne disease Chikungunya that causes acute and chronic diseases characterized by fever, rash and arthralgia in infected individuals. It has been considered a public health problem due to the lack of efficient treatment or licensed vaccine able to prevent CHIKV infection. Studies related to viral replication will contribute to the development of immunobiologicals, as a vaccine or a monoclonal antibody.

Objective: The aim of this study is to follow up CHIKV cellular infection and evaluate the replicative profile in Vero cells to determine optimal conditions of viral production.

Methodology: The viral kinectics were obtained by infecting Vero cells monolayers with CHIKV at the multiplicity of infection 0.01 as previously reported by our group. Supernatant cultures were collected at seven time points during 76 hours post infection (h.p.i.) and stored at -80º C, until processing. CHIKV quantification in the supernatant samples were performed by TaqMan quantitative real-time polymerase chain reaction (RT-qPCR) and by plaque assay. Cultures cytopathic effect (CPE) was monitored by optical microscope to demonstrate the extension of infection.

Results: Preliminary results reveled the beginning of CPE at 21 h.p.i. and at 45 h.p.i. there was almost none adhered cells in the culture flask. Plaque assay titration from CHIKV-infected supernatants demonstrated a significant increase of PFU and viral copies, suggesting an intense viral replication between 4 to 21 h.p.i. At 28 and 45 h.p.i. we observed an optimal virus replication, resulting in a maximum titer of 8.48 log10 PFU/mL. The viral titer was decreasing at 52 h.p.i. Data obtained by RT-qPCR revealed similar replication profile to that obtained in plaque assay, although titers was higher using molecular biology technique. When compared to plaque assay, RT-qPCR reached a titer increase of 0.98 and 1.35 $\log_{10}$ viral copies/mL at 28 and 45 h.p.i., respectively, with a major difference viral titer of 1.80 $\log_{10}$ viral copies/mL at 64 h.p.i.

Conclusion: These data indicated that Vero cells are susceptible to CHIKV infection and preliminary results showed high titers during viral production, which is suitable for downstream processing during vaccine development, manufacturing and for diagnostic purposes.

Keywords: Chikungunya virus; Plaque assay; Real Time PCR