

ORT_17 - Interference of EDTA on Flavivirus infectivity

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Introduction: EDTA (ethylenediaminetetraacetic acid) is an organic compound that chelates divalent cations such as Ca²⁺ and Mg²⁺ and is widely used in buffers and other lab preparations. It is known that its chelating effect can interfere with the activity of enzymes, modulating several processes dependent on divalent cations. It is largely used for example to stop endonucleases activity, dependent of Mg²⁺, in downstream processes for several virus vaccines.

Objectives: The objective of this work is to evaluate the effect of EDTA on zika (ZIKV) and yellow fever (YFV) virus infectivity, both used as antigens in vaccine development projects present in Bio-Manguinhos' portfolio.

Methodology: To investigate the effect of EDTA during the host cell DNA digestion, wild-type ZIKV and YFV 17DD, produced under similar conditions, were incubated at room temperature (RT) for 6 and 5 h, respectively, in the absence (control sample) and in the presence of 2 mM Mg²⁺, with or w/o benzonase. After incubation, EDTA was added, except in the control, and samples were frozen for further assessment of viral infectivity by plaque assays. To evaluate the influence of temperature, ZIKV was also subjected to the same conditions, but incubated at 37°C for 30 min-6 h.

Results: Preliminary results showed that the ZIKV submitted to EDTA at RT decreased the viral infectivity around 0.6 log PFU/mL in comparison with the control. In addition, ZIKV treated at 37°C reduced more than 1 log PFU/mL, demonstrating a lack viral stability at higher temperature. In contrast, EDTA does not seem to affect the stability and infectivity of YFV. However, new trials are being conducted to assess the time and dose response of effect of EDTA.

Conclusion: These results suggest that EDTA can irreversibly affect the viral particle, since, even after EDTA dilution for plaque assays, there was a reduction in the viral titer. Therefore, we believe that divalent cations like Ca²⁺ and/or Mg²⁺ could have an important role in the stabilization of the ZIKV particle and understanding the effects of EDTA on viral particles infectivity is of great importance for the development and improvement of processes that require its use. New trials are being conducted to assess the time and dose response of EDTA.

Keywords: EDTA, ZIKV, YFV