VAC_12 - Expression of the Rabies Virus Glycoprotein *in vitro* through three mRNA Delivery Systems

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Introduction: Rabies is an infectious viral disease transmitted through the saliva of infected animals causing severe encephalitis and death. Its control has been a global challenge given the cost of prevention and treatment, especially in underdeveloped and emerging countries, where cases are endemic. Available vaccines have proven efficacy, but they present high production costs and biosafety risks. New technologies have proven to be effective, safer, and less costly for production. One of them is the use of mRNA vaccines.

Objectives: This study aims to compare the expression of the rabies virus glycoprotein in three different mRNA delivery systems. For this study, a self-replicating mRNA will be used.

Methodology: Plasmids were obtained through transformation and DNA extraction, as well as confirmation by restriction patterns, and subsequently, *in vitro* transcription was performed. Adherent BHK-21 cells were transfected with lipofectamine, lipid nanoparticles containing mRNA-RVGP or viral pseudoparticles. After 24 or 48h, the cells were fixed and submitted to indirect immunofluorescence assays (IF) by using specifc anti-RVGP antibody and Alexa Fluor conjugate to verify protein expression.

Results: After preliminary tests, by immunofluorescence assays an expression of the rabies virus glycoprotein can be seen in cells treated with different delivery systems, at different intensities. The intensity also varies according to the incubation time in the analyzed period from 24h to 48h, however, in some delivery conditions fluorescence intensity is not dependent on the mRNA amount.

Conclusion: The three delivery systems of the same tested mRNA proved to be effective, however, complementary tests will be necessary to quantitatively analyze protein production. In addition, new kinetic tests will be performed to assess how long the protein expression.

Keywords: Rabies; mRNA; Delivery systems