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## PRODUCTION AND PURIFICATION OF AN IMMUNOGENIC RECOMBINANT G PROTEIN OF RABIES VIRUS FROM S2 INSECT CELLS

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Rabies virus is still a major public health concern in under developed countries of Africa, Asia and Latin America. WHO estimates that almost 55.000 people die from rabies annually. Although Rabies virus strains from dogs still are the main responsible of causing human disease, the increasing case numbers of bat virus strains being transmitted to humans in America raises concerns. The called secondary transmission cycle of rabies involves cats or dogs which are exposed to contaminated bats and then transmit it to humans. Combating this type of transmission requires new strategies for disease control, including new vaccines. Particularly for cats, the current rabies vaccines have demonstrated to cause vaccine-associated sarcoma as a serious side effect. New rabies vaccines using recombinant technology have been proposed using the Rabies Glycoprotein (G) as antigen. The expression of this molecule in S2 insect cells has already proved the ability of this cell line to produce an immunogenic protein. Although this antigen is recognized as the main protein necessary for protection upon immunization, its utilization as a recombinant vaccine has been impaired by difficulties in its production and mainly purification steps. The G protein was expressed as a membrane bound protein in *Drosophila melanogaster* S2 cells. This membrane G protein was further solubilized using a standardized solution containing detergent and purified in a novel methodology using affinity chromatography resulting in protein recoveries up to 96 %. The membrane protein and the purified G were used for mice immunization, using or not ISCOM adjuvant resulting in good levels of specific antibodies and neutralizing antibodies. When immunized mice were submitted to lethal rabies virus challenge, the preparations showed to confer protection levels similar to that of commercially available veterinary rabies vaccine. A flow path from the production, purification, formulation and immunization against Rabies using a recombinant G protein is presented as a promisor veterinary vaccine candidate.