



**CALIFORNIA STATE SCIENCE FAIR  
2013 PROJECT SUMMARY**

<b>Name(s)</b> Aryo Sorayya	<b>Project Number</b> <b>S0525</b>
<b>Project Title</b> <b>Designing a Novel Freeze-Stable Tetanus Vaccine</b>	
<p style="text-align: center;"><b>Abstract</b></p> <p><b>Objectives/Goals</b> The goal of this project is to design a novel Tetanus vaccine that does not lose its potency upon freezing as an alternative to freeze-sensitive aluminum-based vaccines. In this work, the applicability of a novel liposomal adjuvant was tested for developing freeze-stable Tetanus vaccines. Furthermore, the effect of particle charge on the efficacy of the adjuvant was explored.</p> <p><b>Methods/Materials</b> The immunogenicity of two liposomal vaccines was compared using Tetanus Light Chain (TLC) as an antigen. As control, a TLC solution without an immunostimulant (adjuvant) was used. The effects of multiple freeze-thaw cycles and lyophilization (freeze-drying) at -45 oC on the stability and immunogenicity of the liposomal vaccines were investigated. The immunogenicity of these vaccines were compared in immunized and non-immunized mice. Each formulation was injected into five mice intramuscularly on days 0 and 14, and blood was collected on Day 28. The mouse anti-Tetanus toxoid (IgG) was measured in diluted sera of immunized and non-immunized mice by an Indirect Enzyme-linked Immunosorbent assay (ELISA) method. The concentration of antibody in each mouse was measured twice, and the mean and standard deviation of the antibody response for each formulation was calculated. T-tests were applied to investigate if the difference in the immune response obtained for the liposomal vaccines before and after lyophilization was significant.</p> <p><b>Results</b> Both the liquid and lyophilized liposomal vaccines gave a significant immunogenic response in mice greater than that of the Tetanus solution without adjuvant. The positively charged liposomal Tetanus vaccine gave the strongest immune response. Tetanus without an adjuvant, as well as the naive mice did not induce a significant immune response. There was no significant difference in immune response of both positively and negatively charged liposomes before and after lyophilization.</p> <p><b>Conclusions/Discussion</b> It was possible to manufacture freeze-stable vaccines against the Tetanus toxoid, using specifically designed liposomes with entrapped Tetanus Light Chain. These vaccines did not lose their immunogenic activity despite multiple freeze-thaws and lyophilization at -45 oC and might thus be used as alternatives to the current freeze-sensitive Tetanus vaccines in the market.</p>	
<b>Summary Statement</b> A novel freeze-stable Tetanus vaccine with potent immunogenic IgG induction in mice was successfully designed and tested in vivo.	
<b>Help Received</b> Used lab equipment at HTD Biosystems under the supervision of Dr. Rajiv Nayar; Mice immunization was conducted at Pacific Biolabs	