Jayydev Upponi

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San Diego, May 30th 2018

Dear Dr. Upponi,

Please find enclosed our manuscript entitled "Optimized Interferon-gamma ELISpot assay to measure T cell responses in the guinea pig model after vaccination*”* that we would like to be considered for publication in Journal of Visualized Experiments.

This paper highlights a protocol for an optimized ELISpot assay specific for the guinea pig model*.* Our protocol describes the processing of peripheral blood samples to obtain PBMCs. We give detailed instructions for preparing assay plates and testing the processed PBMCs. The manuscript provides representative results regarding number and viability of PBMCs and images of plate wells with Interferon-gamma positive spot to inform the reader about expected outcome when applying our protocol.

We consider of value publishing these data in Journal of Visualized Experiments, as they can be utilized to detect antigen-specific T-cell responses in the guinea pig animal model. Guinea pig is a highly relevant model for assessing many biologics and modeling disease, however it has been neglected due to the paucity of assay reagents and protocols available. For example, the physiology of the guinea pig skin is similar to that of humans, and thus is a highly relevant model for the development of therapeutics and vaccines which are delivered to the skin. The described IFN-g ELISpot is currently employed in our laboratory to successfully model vaccine T cell responses.

The techniques presented in this paper and demonstrated in video format will be highly useful for researchers working in the field of vaccine development and skin-delivery technologies.

Katherine Schultheis, Hubert Schaefer and Trevor R.F. Smithdesigned the procedures described in the manuscript. Katherine Schultheis, Holly M. Pugh, Bryan S. Yung, Janet Oh and Jacklyn Nguyenperformed the experiments and analyzed the data. Finally, Katherine Schultheis, Trevor R.F. Smith, Kate E. Broderick and Laurent Humeauwrote the manuscript.

Thank you for your consideration of this manuscript. We look forward to hearing from you.

Sincerely yours,

Katherine Schultheis

Katherine Schultheis

Scientist

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