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Abstract Supplement

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Pediatric Demyelination Disease study[1,3,4]. [1]McGill university, Montreal, QC, Canada; [2]Montreal Neurological Institute, Montreal, QC, Canada; [3]Hospital for Sick Children, Toronto, ON, Canada; [4]University of Toronto, Toronto, ON, Canada

Increasingly, clinical research initiatives aim to apply bio-assays to blood samples obtained from subjects recruited at multiple different sites. Often the decision is to ship samples to a centralized site for uniform processing, storage and assay implementation. While this 'centralized model' offers several advantages (dedicated team, SOPs applied to all samples, minimizing site-to-site and inter-assay variability), a critical issue relates to potential effects of variables encountered during shipping. We report on comprehensive assessment of the impact of temperature exposures on phenotypic and functional responses of immune cells, mimicking overnight sample shipment to a centralized facility. Healthy volunteer blood samples, placed in parallel in either of three different commonly used containers, were subjected to controlled temperature variation experiments (range: -20 to 37°C; different durations), with continuous ambient and internal temperature monitoring. Samples then underwent Ficoll isolation of PBMC, with a proportion cryopreserved using our established SOPs. Readouts - including cell yields; viability; cell subset analysis (FACS); and functional responses (proliferation; cytokine production (ELISA and ICS); differentiation (Th1, Th17) potential; and sensitive calcium signaling - were assessed in batch for both freshly isolated and cryopreserved PBMC. We define which readouts are compromised and which appear preserved under the range of pre-analytical temperature exposures, for each container. We underscore those conditions where different degrees of functional compromise are confirmed even when shipped samples appear perfectly intact. Our findings provide helpful guidelines for selecting the shipping container most appropriate for the particular study context and immune readouts of interest.

F.5. Determination of *in vivo* Toxicity, *in vitro* Cytotoxicity and Anti Venom Production of the Cypriotic Blunt-nosed Viper, Macrovipera Lebetina Lebetina Venom

Ayse Nalbantsoy[1], Ulku Karabay Yavasoglu[1], Ferah Sayım[1], Ismet Deliloglu-Gurhan[1], Bayram Gocmen[1], Huseyin Arikan[1], Mehmet Zulfu Yildiz[2]. [1]Ege University, Izmir, Turkey; [2]Harran University, Sanliurfa, Turkey

Snake envenoming is a major public health issue with large numbers of envenoming and deaths. There are nearly 3,000 different species of snakes found in the world. Approximately 300 of them are venomous. The Levantine viper, Macrovipera lebetina ssp. lebetina is endemic to Cyprus. In the present study, antivenom production was evaluated against M. l. lebetina venom followed by re-immunization schedule in mice including acute toxicity studies. The LD50 value was determined as 7.58 mg/kg for 24 h after intraperitoneal administration of venom at different doses. Approximately 1/5 of the estimated LD50 (1.5 mg/kg) dose of venom was used in the immunization of the mice using Freund's complete and incomplete adjuvants. The antigen mediated ELISA results exhibited immunoreactive properties with venom, as the IgG response varied between 2.336±0.042 - 2.535±0.031 A492 on day 32. A cell-based assay was administrated to determine the effects of venom and antivenom neutralizing potency on L929 cell viability by MTT assay. The IC50 value was found to be 0.62 µg/ml. Antivenom efficacy was assessed by mixing 1, 3 and 5 IC50 of the native venom diluted in physiologic saline with same amount of anti venom. Subsequent to the 48 h post-treatment of venom / anti-venom mixture, cell viability was found to be 65±1.1% for L929 cells. In conclusion, current study indicated that the M. l. lebetina antivenom production was achieved in a mouse model. A good *in vitro-in vivo* correlation was also obtained between the data of cytotoxicity and toxicity of venom.

F.44. Simple and Robust Whole Blood Staining Procedure Using PerFix-nc Reagents Generates