Mouse polyclonal (hybridoma)

Our overriding consideration is to minimize pain and distress to the animals used. Therefore the following guidelines will be adhered to:

- 1) . Immunogen Preparation : The immunogen will be prepared in such a manner to elicit an acceptable response without adversely affecting the well-being of the animals.
- 2) Choice of Adjuvant: The advice of Orith Leitner, Head of the Antibody Unit will be obtained for each protocol involving a new adjuvant or novel immunogen. Freund's Complete Adjuvant will only be used for the primary immunization, and should contain less than 0.5mg/ml mycobacteria.
- 3) Route of Injection: The route of injection will be selected with the objective of causing the least possible distress for the animal. The preferred route of injection is subcutaneous (SC). Footpad (one hind limb only) is strongly discouraged and if required must be justified on a case by case basis and only after demonstrating failure using the preferred route. Injection into two footpads is not permitted. Subcutaneous injections should be used for oil or viscous gel adjuvants, in particular for FCA, in order to minimize the formation of sterile abscesses.
- 4) The injection volume will be kept as small as possible.
- 5. Blood Collection: Maximum blood volume removed should not exceed 10% of the total blood volume of the animal (approximately 1%body weight), if collected every two weeks, and not more than 15%
- of the total blood volume if collected every four weeks (Morton, et al.,1993;McGuill & Rowan,1989;Diehl,et
- al.,2001). The selection of bloodcollection procedure should aim to minimize stress for the animal. Method of

collection will be explained and will be done under the supervision of Dr. Shelly Zinamon of the veterinary resources department. Animals will be euthanized by CO2. Disposal of animals must be carried out in accordance with the Weizmann Institute regulations. It is the investigator's responsibility to inform the animal care staff when the collection of antibody has been completed, so that animals can be disposed of as previously agreed in the protocol.

Animal will be monitored on a daily basis. If animal exhibit signs of severe stress (hunched appearance, piloerection, respiratory distress, reduction in eating and drinking), or sever ulcerated wounds, animals will be euthanize.