

Institutional Animal Care and Use Committee

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IACUC GUIDELINE: ANTIBODY PRODUCTION

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Monoclonal and polyclonal antibodies are critical research reagents, which require immunization of a living animal host to be created. This immunization process has the potential to cause pain or distress to the animal being immunized; therefore, such procedures must be described in a protocol reviewed and approved by the IACUC. When a contract laboratory or vendor, and not the PI, performs the antibody production procedures, the PI needs to submit a Custom Antibody Production form to the UCSB IACUC in order to verify that the contract lab adheres to the PHS Policy. Further, the UCSB IACUC encourages the use of the following refinements or alternatives to those immunization procedures that have the greatest potential for causing animal pain and distress.

1. Procurement of Antibodies from a Contract Laboratory or Vendor

- “The generation of custom antibodies is an activity involving vertebrate animals and covered by PHS Policy. Antibodies are considered customized if produced using antigen(s) provided by or at the request of the investigator (i.e., not purchased off-the-shelf). An organization producing custom antibodies for an awardee must have or obtain an Assurance, or be included as a component of the awardee’s Assurance. In addition, the awardee must provide verification of project-specific IACUC approval for the production of the antibodies.”¹
 - o Custom antibody orders placed with Department Purchasing can be fulfilled through the UCSB Procurement Gateway. The PI will need to complete and submit a Custom Antibody Form and the vendor’s IACUC approval notice before the IACUC can approve the requisition.
- Not all organizations/vendors, especially those not based in the US, have a PHS Assurance or adhere to these animal welfare standards. PIs must use only those

vendors that adhere to these federal animal welfare standards. The OLAW maintains a list of contract laboratories and vendors that meet these standards.²

- The vendor must also be registered with the USDA, if the species used to produce the antibodies is regulated by the USDA-APHIS.

2. Use of Freund's Complete Adjuvant (FCA)

- Use of FCA is only recommended for the initial immunization, with the incomplete form (Freund's Incomplete Adjuvant, IFA), which lacks the mycobacterial components and is essentially a water-in-oil emulsion, recommended for subsequent immunizations (i.e., boosters).
- The intravenous (IV) or intraperitoneal (IP) administration of non-aqueous adjuvants (e.g., FCA or FIA) is contraindicated. The subcutaneous (SC) route is recommended, instead, for all species commonly used to produce antibodies. Intradermal injections can also be appropriate, but only for rabbits, sheep, and goats.

3. Preparation and Administration of Inoculum

- Antigen preparations for in vivo administration to animals should avoid physiologically incompatible vehicles and toxic purification byproducts such as polyacrylamide gel, and should have a physiologically compatible pH (4.5 - 8).
- The adjuvant/antigen mixture should be aseptically prepared and free of extraneous microbial contamination.
- Undesirable and painful side effects such as large inflammatory lesions or tissue necrosis can be effectively reduced or eliminated through the use of appropriate routes of administration, adequate preparation and separation of injection sites, and the use of a small volume of inoculum per site.²
 - The following routes and volumes of administration generally produce favorable results, while minimizing undesirable side effects: 0.05 ml intradermally per site in rabbits; 0.1 ml subcutaneously per site in rodents; and up to 0.5 ml intramuscularly per site in large farm animals.
 - In rabbits, the injection sites should be shaved and cleaned by alternating three times between Betadine® or Nolvasan® scrub and 70% alcohol. Also in rabbits, no injections should be made over bony prominences or in areas (i.e., scruff) that may lead to excoriation of the immunization sites.

4. Footpad Injection in Mice

- Footpad injections should not be performed in the front feet, because mice use these for handling food.

- Footpad injections in the hind feet should be limited to one leg, especially if adjuvant is co-administered with the antigen, as this may result in inflammation and swelling at the site of injection leading to progressive debilitation due to an inability to bear weight on the injected foot.
- Hock injections, which direct the immune response to the same draining lymph node (i.e., popliteal lymph node) without the incidental impairment of mobility, are recommended as an alternative to footpad injections.³

5. Ascites Production

According to the NIH Office of Laboratory Animal Welfare: "IACUCs are expected to critically evaluate the proposed uses of the mouse ascites method. Prior to approval of such protocols, IACUCs must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and (iii) the latter have been found unsuitable."⁴

The following refinements are recommended to minimize discomfort, distress, and pain in mice used for ascites production.²

- The volume of priming agent (i.e., pristaine) used to enhance ascitic fluid production should be <0.5 ml (0.1 – 0.2 ml is recommended).
- The cell suspensions (hybridoma cells) to be injected IP should be prepared under sterile conditions using sterile physiological solutions. The cell concentration in the inoculum needs to be determined empirically, but high concentrations (>5 x 10⁶ total number of cells) should be avoided due to the higher risk of mortality or severe pain.
- Abdominocentesis (peritoneal tap) should be performed before the abdominal distension causes discomfort or interferes with normal activity. This requires at least once a day observation of the mice by personnel familiar with the clinical signs associated with distress.
- Mice should be closely monitored for several hours following the peritoneal tap for signs of shock, including abnormal respiration, pale or shrunken eyes, decreased activity, or difficulty walking. Warmed saline (2 – 3 ml) may be administered subcutaneously to prevent or treat hypovolemia, but the mouse should be euthanized if its condition does not improve or deteriorates.

References:

1. Office of Laboratory Animal Welfare (OLAW), NIH.
http://grants.nih.gov/grants/olaw/faqs.htm#App_2
2. Office of Laboratory Animal Welfare (OLAW), NIH.
<https://olaw.nih.gov/guidance/obtaining-an-assurance.htm>

3. Leenaars M, Hendriksen CFM. 2005. Critical Steps in the Production of Polyclonal and Monoclonal Antibodies: Evaluation and Recommendations. *ILAR J.* 46(3):269-279.
4. Kamala T. 2007. Hock immunization: A humane alternative of mouse footpad injections. *J. Immunol. Method* 328:204-214.
5. Office of Laboratory Animal Welfare (OLAW), NIH.
http://grants.nih.gov/grants/olaw/faqs.htm#useandmgmt_8