Title: Immunization of Rodents and Rabbits in Antibody Production

Purpose:
- To provide guidelines for minimizing pain and distress to rodents and rabbits used in antibody production. The following are considered the recommended practices by the IACUC; deviations from these guidelines require a detailed description and scientific justification in the Animal Subject Approval Form (ASAF) and must be approved by the IACUC before work can begin. If your purposed research involves monoclonal antibody production, please refer to WSU-IACUC Policy #23 - Monoclonal Antibody Production at WSU.

Justification:
- Principal Investigators must justify the use of animals for the production of antibodies in their ASAF. They should also cite specific references in the ASAF to explain why in vitro methods of monoclonal antibody production are not appropriate for the aims and goals of the study.

Procedure:
- The veterinary staff of the Office of the Campus Veterinarian (OCV) can provide training to research staff in the proper restraint and immunization techniques listed in this SOP. OCV Veterinary technical staff is also available to perform all animal procedures for a nominal service fee.
- **Adjuvant Selection:** Alternatives to Complete Freund's Adjuvant (CFA), such as Sigma Adjuvant System (formally Ribi Adjuvant System), Titer Max. etc., must be considered. If Freund's Adjuvant is to be used, CFA can be injected only once per animal. Incomplete Freund’s Adjuvant (IFA) is to be used for subsequent booster immunizations.
- **Immunization Product Preparation:** The following guidelines have proven effective in significantly alleviating complications after immunization with adjuvants. Utilization of: a) sterile technique in the preparation of antigen-adjuvant emulsions; b) Use of sterile needles and syringes (and no reuse of needles); c) appropriate injection technique; d) appropriate routes and sites of administration; e) adequate separation of injection sites; and f) use of smaller volumes at each injection site have all proven efficacious in the elimination of post-immunization complications.
Antigen preparations should be sterile and, ideally, isotonic, pH neutral, and free of urea, acetic acid, and other toxic solvents.

Antigens separated using polyacrylamide gels should be further purified whenever possible in order to minimize the amount of secondary inflammation/irritation from gel fragments.

If further purification is not possible, then the amount of polyacrylamide contaminant should be minimized by careful trimming. Millipore ultrafiltration of the antigen, for example, prior to mixing it with the adjuvant, is recommended to remove extraneous microbial contamination.

**Injection Routes, Sites and Technique:** Some routes of injection may potentially be less disruptive to the animal than other routes (e.g., subcutaneous injection vs. footpad administration). Whenever possible, the least invasive methodology required to accomplish the experimental goal should be utilized. Footpad injections are not allowed unless scientifically justified. It is necessary to separate multiple injection sites by a distance sufficient to avoid coalescence of inflammatory lesions. The site of injection should be chosen with care in order to avoid areas that may compromise the normal movement or handling of the animal (e.g., intradermal injections in the neck scruff of a rabbit).

**Freund’s Complete Adjuvant (only allowed once) and IFA systems:** The following are the recommended injection sites and volumes:

- **Subcutaneous:** 0.05 ml in mice (2-4 sites), 0.10 ml in rats (2-4 sites), 0.1 to 0.25 ml in rabbits (3-5 sites)
- **Intradermal:** Must be justified. 0.05 ml in rabbits; use 25-27 gauge needle; no more than 10 sites.
- **Intraperitoneal:** 0.25 ml in mice (1 site); 0.5 ml in rats (1 site); not allowed in rabbits.
- **Intravenous:** Not allowed in any species
- **Intramuscular:** Not recommended – requires strong scientific justification.
- **Foot pad:** Not allowed without strong scientific justification and explicit approval of the IACUC

**Other Adjuvant Systems:** When using another type of commercially available adjuvant system, it is best to refer to the manufacturer’s recommendations for injection protocols. Here are two alternative adjuvant systems routinely used by WSU investigators:
o For an Adjuvant System not listed in this Standard Operating Procedure – please describe your injection protocol in your ASAF.

• **Post-injection Observations and Treatments**: Post-inoculation monitoring of animals for pain and distress or complications at the injection sites is essential, and should be done daily. If lesions, wounds or swellings are noted, the Office of the Campus Veterinarian is to be notified for evaluation and possible treatment.

• **Personnel Safety**: Adjuvants that contain mycobacterial products can be an occupational hazard to laboratory personnel and should be handled with extreme care. Reports of accidental needle punctures in humans have been associated with clinical pain, inflammatory lesions, and abscess formation. It is recommended that safety glasses be worn during sample preparation in order to avoid accidental splashing of CFA in the eyes.

**References:**

- Immunization Procedures and Adjuvant Products. ILAR 46(3), 2005
- ILAR Journal 37(3): 141-150.
- Guidelines for the Use of Adjuvants in Research - Special Emphasis on Freund's Adjuvant; Office of Animal Care and Use; National Institutes of Health; April 2013.