

Stony Brook University
Institutional Animal Care and Use Committee (IACUC)

POLYCLONAL ANTIBODY PRODUCTION IN LABORATORY ANIMALS
GUIDELINES FOR THE USE OF FREUND'S ADJUVANT

Improper or unnecessary use of Freund's adjuvant may cause excessive inflammation and skin necrosis in laboratory animals. The IACUC has developed the following guidelines intended to eliminate, or reduce to a minimum, animal discomfort associated with the use of this agent. Any deviation from these guidelines requires specific justification in the IACUC protocol.

- 1) Before using Freund's complete adjuvant, consider the use of incomplete Freund's or another adjuvant (e.g. RIBI)
- 2) Complete Freund's adjuvant should be used only for the first (priming) antigenic dose. Use of two or more doses of complete adjuvant must be scientifically justified in the IACUC application. If more than one dose must be used, an interval of at least 3 weeks should be allowed between doses. If incomplete Freund's adjuvant is used for the second and subsequent doses, an interval of at least 1 week should be allowed between doses.
- 3) Injection sites should be free of hair and cleansed with an appropriate antiseptic agent prior to injection. The inoculum containing the adjuvant should be divided into fractions so that no more than 0.1 ml is injected per site for rabbits and 0.05 ml for mice.
- 4) Whenever possible, the injections should be subcutaneous. Investigators must scientifically justify immunization by any other route.
 - a) Intradermal injections may result in skin necrosis
 - b) Intramuscular injections may result in temporary or permanent lameness and significant muscle necrosis
 - c) Footpad injections are highly discouraged and requires strong scientific justification
 - d) Peritoneal injections are only acceptable in mice
- 5) The inoculum should be free of extraneous microbial contamination. This can be accomplished by sterilization of the antigen preparation by filtration through a membrane (0.22 micron) before mixing with the adjuvant.
- 6) Acrylamide gels can cause animals additional pain. It is recommended that acrylamide gels not be included in the adjuvant preparation (e.g. elute the protein antigen from the acrylamide whenever possible).
- 7) Animals may be sedated for antigen injections and for blood withdrawal to check for antibody titer using acepromazine 1-2 mg, IV. These procedures should always be performed by a trained individual, with an assistant, or by a trained individual using a rabbit restraint device.
- 8) The maximum volume of blood withdrawal should be 10% of their blood volume (7% of their body weight) per week. Rabbits should be bled from the marginal ear vein and mice should be bled either from the tail vein or from the retroorbital sinus, depending on volume needed.

Final blood collection (exsanguinations) may be collected from the heart via intra-cardiac puncture. This procedure must be done under anesthesia and is a non-survival, terminal procedure.

- 9) Animals should be monitored at least twice weekly after inoculation. The inoculation site should be checked for signs of infection, erythema (redness) and swelling. The rabbit should also be checked for signs of pain and distress (tenderness of the injection sites, anorexia, lethargy, weight loss, etc.). If any of these signs are seen, the investigator should contact the DLAR Veterinary staff so that appropriate medical treatment can be initiated.