



Dartmouth College

Animal Care and Use Program

Institutional Animal Care and Use Committee

### IACUC Policies and Procedures

**Title:** Guidelines for Polyclonal Antibody Production

**Purpose:** The use of immunologic adjuvants in laboratory animals has the potential for causing pain. Refinement of the techniques of immunization including: preparation of antigen; injection techniques, sites and volumes; and choice of adjuvant can all contribute to minimization of pain. These Guidelines have been created to help research staff design immunization protocols which will be both ethically and scientifically sound and meet regulatory requirements.

**Animal Subjects Review Form (ASRF) Protocol Preparation and Review:** In question H of the ASRF, "Reduction, Replacement, Refinement, the investigator must describe the methods and sources used to determine that no alternatives exist which would further minimize animal distress, reduce animal usage, or replace whole animal tests altogether.<sup>1</sup> The response to this question most often takes the form of a literature search. In the case of polyclonal antibody production, the investigator should consider the use of adjuvants and techniques which may cause less distress. It is recognized that no adjuvant is the best for all situations and antigens, therefore, investigators should review the literature for methods which would be expected to provide maximal results with minimal discomfort. It is further recognized that such literature may not exist for all types of antigens. Although not an exhaustive list, several references are included at the end of these Guidelines which may be helpful when choosing an adjuvant.

Details of the procedures to be used must be outlined in Appendix A of the ASRF, "Antibody Production Description". Details should include: adjuvant to be used, location and number of injections and volumes per site for both the initial immunization and booster(s). Number of boosts and intervals between boosts should also be included. Please note the use of Complete Freund's Adjuvant more than once is seldom warranted and must be justified.<sup>2-5</sup> Details of the blood collection should include: method of collection; volumes to be collected and intervals between collections.

In question F1, "Details of post-procedural care", the investigator must describe possible complications as well as potential for pain and discomfort. In this section, please state that 'animals with ulcerated or painful lesions may be treated with topical wound care, antibiotics, or analgesics as prescribed by the veterinary staff'. If your experiment precludes the use of these agents (as outlined below), you must provide the scientific justification for withholding treatment. If the antigen you use is known to cause excessive reaction, this must be noted. In cases where excessive reaction has been seen in the past, reduced volumes or concentrations per injection should be considered. If the veterinary staff does identify a lesion which they feel requires treatment, the situation will be reviewed with the PI prior to initiation of therapy whenever possible.

A completed sample ASRF describing the use of animals for polyclonal antibody production is available in the ARC office as a general guide for successful completion of this form.

**Procedure:** To assure post immunization clinical evaluation, the veterinary staff must be notified when immunization procedures (initial injections and boosters) are to be performed.

#### **Initial Immunization:**

Since infection of the immunization site greatly increases the pain associated with a granuloma, all immunization materials should be free of all extraneous contamination, especially of microbial origin. In addition, the injection site should be appropriately shaved and aseptically scrubbed to remove contamination which may result in infection. Materials should be prepared to minimize adverse reactions.

Although the immunization injection itself is rarely painful, sedation or anesthesia is often recommended, especially in rabbits.<sup>2,4</sup> Anesthesia/sedation is not required, however, it reduces the chance of unexpected or rapid movement by the animal which could result in improper injection technique or needle stick to personnel.

The optimal site and volume of injection will vary with different studies and different antigens. Current literature suggests that the intradermal site usually provides the highest quantity of antibody and the least tissue destruction. For intradermal injections, injections can be made more easily and with less personnel risk by placing the needle into the subcutaneous tissues and advancing the needle point (bevel up) into the overlying dermis. Other sites of injection, subcutaneous and intramuscular may not exhibit visible lesions but usually cause more tissue damage. The following serve as guidelines for maximal volumes per injection for each site.<sup>2-4, 6</sup> Investigators are urged to use the smallest volume necessary within each dosage range. If volumes larger than the maximum of the range are to be used, it must be justified in your ASRF.

- 0.05 ml intradermally in rabbits (maximum total volume of 0.5 ml)
- 0.1-0.5 ml subcutaneously in rabbits or rodents (maximum total volume of 1.0 ml)
- 0.25-0.5 ml intramuscularly in one site in rabbits (one site)
- 0.05 ml intramuscularly in rodents (one site)
- 0.2 ml intraperitoneally in mice (obvious abdominal distention should be relieved or the procedure terminated)

\*Footpad injection may only be done in rodents. The use of footpad injection must be scientifically justified and is only allowed if the antigen is a poor immunogen by other routes or if a draining lymph node is to be examined. Only one footpad (hind) may be injected and animals must be housed on soft bedding. The volume may not exceed 0.05 ml for mice and 0.1 ml for rats.

When multiple sites are injected, the distance between sites should be maximized to prevent sites from becoming confluent. The site(s) should not be located in areas which are commonly used in handling such as the neck.

When techniques such as whiffle ball implantation or solid supports such as nitrocellulose are used, the concentration and volume of adjuvant per site should be based on the subcutaneous guidelines. As with any procedure, the IACUC will rely upon the investigator's experience with the specific immunization procedure when reviewing and approving the protocol.

#### **Boosts:**

Peak serum antibody titers are usually reached about 10 days after initial injection, however, since efficient priming can take place even in the absence of detectable antibodies, initial antibody response is often not monitored. A minimal interval of 2-3 weeks is recommended prior to the first booster (although rabbits often remained primed over a year). Peak levels of serum antibodies are usually seen 10-14 days after the booster. If additional boosters are to be given, the interval between injections must be long enough to allow the circulating levels of antibody to drop enough to prevent rapid clearance of injected antigen. For rabbits, this is normally 4-6 weeks, for mice, 3 weeks.

The use of Complete Freund's Adjuvant for booster doses is seldom warranted and must be justified.<sup>2-5</sup>

#### **Blood Collection:**

Test bleeds are usually taken about 10-14 days after injection. Blood is normally collected from the marginal ear vein in rabbits and tail vein or orbital sinus of rats and mice. For rabbits, 5-10 ml of blood is usually collected, for rodents, 200-400 ul. Once a good titer has developed, blood may be withdrawn as needed using the following guidelines:

- Up to 10% of intravascular blood volume\* may be collected weekly

- Up to 20% of intravascular blood volume\* may be collected monthly
- Over 20% of intravascular blood volume\* should only be collected, under anesthesia, as a terminal procedure. This is often done via cardiac puncture.

\*A rabbit has approximately 56 ml of blood/kg of body weight. Therefore, in an average antibody rabbit weighing 3 kg, one could be safely collect 16 ml weekly or 32 ml monthly.

#### **Help with Immunization or Bleeding Procedures:**

The ARC staff can provide assistance with immunizations and/or blood collection. If you would like the ARC staff to provide training or perform any of these procedures for you, please call the the ARC at 650-7592, or blitz the ARC blitzmail account. Training is provided at no charge. If procedures are to be performed by ARC personnel, the investigator will be billed for time and materials.

#### **Clinical Follow-up:**

After immunization, the research staff is responsible for completing the top portion of an Immunization Clinical Incidence Form (CIF). This form, along with notification of the veterinary staff, will assure clinical evaluations WHICH ARE ESSENTIAL FOR REGULATORY COMPLIANCE. This form must include the routes and volumes of injection and the weight of the rabbit on the day of immunization.

Veterinary follow-up will include clinical observation, palpation and grading of tissue reaction. This follow-up is documented on the Immunization CIF. When wounds are ulcerated, topical wound care with or without antibiotics will be instituted. Topical treatment would typically include one or more of the following: cleansing; nitrofurazone powder or ointment; and Panalog®. If an animal is determined to be in pain (as determined by palpation score or behavior) analgesics will be given. If guidelines are followed, painful sequelae should be rare and thus, it is not anticipated that the use of analgesics or other treatments will become "routine". When analgesics are used, it is anticipated that they will only need to be given for a few days. The analgesic most commonly used is Buprenorphine, 0.1 mg/kg SC bid. If an alternative analgesic is desired or if analgesics are scientifically contraindicated, it must be noted in the ASRF and in the case of withholding analgesics, be justified in the protocol and reviewed and approved by the IACUC. All observations and treatments will be recorded. There is no charge for veterinary follow-up, medications used, or technician time for treatment. All charges will be absorbed by the ARC. If they prefer, investigators may provide medication and perform and document treatments themselves. Regardless of who performs treatments, treatments must be documented on the Immunization CIF.

Since adverse reactions may signify technical problems with the procedures, it is also imperative that the animal is observed by the investigator or his/her designate. Observations should occur at least 3 times a week for 4-6 weeks after immunization. If adverse reactions are seen, modifications in the techniques should be discussed with the veterinary staff.

## References

1. The Animal Welfare Act, 7 U.S.C. Sec 2131 et. seq. Title 9, Code of Federal Regulations, Parts 1, 2 & 3, December 1986.
2. Harlow, E. and Lane, D. Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988.
3. Grumstrup-Scott, J and Greenhouse D.D. eds. NIH intramural recommendations for the research use of complete Freund's adjuvant. ILAR News,; Vol XXX, Number 2, Spring 1988:9.
4. Canadian Council on Animal Care, CCAC Guidelines on Acceptable Immunological Procedures, July 5, 1989. CCAC, 1000-151 Slater, Ottawa, Ontario, Canada, K1P 5H3.
5. Johnston, B. A., Eisen, J. and Fry, D. An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits. Laboratory Animal Science, 41:15-21, 1991.
6. Stills, H.F. and Bailey, M.Q, The Use of Freund's Complete Adjuvant, Laboratory Animal, Volume 20, Number 4, p25-30.
7. Amyx, H.L., Control of Animal pain and Distress in Antibody Production and Infectious Disease Studies. JAVMA, Volume Number 191, pg 1287-1289, 1987.
8. Kelley, M., et al. Clinical Assessment of an Adjuvant Comparison Study in Rabbits for Polyclonal Antibody Production, Laboratory Animal Science, Volume 42, Number 4, pg 419.
9. Stills, J.F., Bailey, M.Q., and VanEpps, C.L., The Use of Freund's Complete Adjuvant in Polyclonal Antibody Production, Laboratory Animal Science, volume 42, Number 4, Pg 419.
10. Mallon, F.M. et al, Comparison of Antibody Response by the use of Synthetic Adjuvant System and Freund Complete Adjuvant in Rabbits, JAVMA, Vol. 52, Number 9, September 1991.
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12. Toth, L.A. and Olson, G.A., Strategies for Minimizing Pain and Distress in Laboratory Animals, Laboratory Animal, Volume 20, Number 4, pg 33-39.
13. Spinelli, J.S., Reducing Pain in Laboratory Animals, Laboratory Animal Science, Special Issue, January 1987.