GUIDELINES FOR THE PRODUCTION OF ANTIBODIES IN LABORATORY ANIMALS

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Purpose

Rodents and rabbits are often used to produce antibodies for a variety of research objectives. The following guidelines are intended to eliminate or reduce to a minimum, animal discomfort associated with the use of antibody production in laboratory animals.

Choice of Species and Strain

The choice of species and strain must be justified by the investigator in the Animal Use Protocol (AUP). *Ex vivo* methods of antibody production or off-the-shelf commercially available antibodies should always be considered before *in vivo* antibody production. When commercial sources are used, refer to the ACUC guidelines for custom antibody production. For further information on required justification for monoclonal antibody production, please refer to the NIH report on this subject: http://grants.nih.gov/grants/policy/antibodies.pdf.

Immunizing Antigen

The purity and method of preparation of the immunizing antigen is extremely important. The immunizing material must be virtually free of toxic substances (e.g., urea, acetic acid). It should present no risk of pathogenicity or toxicity to the host animal, other animals in the colony, or personnel. If toxic or pathogenic immunogens must be used, they must be justified in the AUP and approved by the Animal Care and Use Committee (ACUC). Proposed arrangements for animal housing, monitoring, and antibody procedures must be fully documented. Investigators must notify the Office of Laboratory Animal Care (OLAC) before animals are inoculated with cells that may harbor transmissible pathogens (human or murine). Please refer to OLAC's policy "Testing Biologicals Used in Laboratory Rodents" for additional information.

Procedures for Polyclonal Antibody Production

1. Injections
    a. Injections for routine antibody production should be administered subcutaneously in two to four sites per animal, generally on the back, away from the spine. Other routes, such as intradermal, intramuscular, intraperitoneal may also be used; however, the intradermal route should not be used in mice and intramuscular injections should not be used in small rodents. Recommended injection volumes and amounts are listed in Table 1; maximum injected volumes are listed in Table 2. The inoculum should be free of extraneous microbial contamination. Always use the smallest inoculum and total volume possible. Filtration (0.2 micron) of the antigen before it is mixed with adjuvant is recommended. Injection sites should be free of debris and disinfected with alcohol, Betadine or chlorhexidine.
    b. Intravenous (antigen only), footpad, and popliteal lymph node injections are allowable, but not necessary. Footpad injections in rabbits are not acceptable due to the lack of anatomical structure. Protocols for these types of injections must be justified and approved by the ACUC. The description should clearly describe the experimental objective, details of the antigen and the entire procedure, including monitoring of the animals after injection. No adjuvant should ever be administered intravenously (IV).

Table 1. *Recommended immunization sites and injection volumes for injection of immunogen/depot forming adjuvant.*

Route of Administration	**Species**				
	Mice	**Rats**	**Hamsters**	**Guinea pigs**	**Rabbits**
Subcutaneous (SC)	0.1 ml/site 4 sites max	0.1-0.2 ml/site 4 sites max	0.1 ml/site 4 sites max	0.1-0.2 ml/site 4-6 sites max	0.1-0.25 ml/site 8-10 sites max
Intramuscular (IM)	0.05 ml if required: Not recommended	Not recommended	0.05 ml if required: Not recommended	Not recommended	0.25ml/site 2 sites max
Intraperitoneal (IP)	0.1 ml/site maximum	0.25 ml/site maximum	0.25 ml/site maximum	0.25 ml/site Maximum	Not recommended
Intradermal (ID)	Not recommended	Not recommended	Not recommended	Not recommended	0.025 ml/site 5-8 sites

Table 2. *Recommended maximum volume of injection used for antigen without adjuvant*

Route of Administration	Species				
	Mice	**Rats**	**Hamsters**	**Guinea Pigs**	**Rabbits**
Subcutaneous (SC)	0.5 ml	1.0 ml	1.0 ml	1.0	1.5 ml
Intramuscular (IM)	0.5ml	0.1 ml	0.1 ml	0.1 ml	0.5 ml
Intraperitoneal (IP)	1 ml	5 ml	2-3 ml	10 ml	5 ml
Intradermal (ID)	Not Recommended	Not Recommended	Not Recommended	Not Recommended	0.05 ml
Intravenous (IV)	0.2 ml	0.5 ml	0.3 ml	0.5 ml	0.5 ml

2. Adjuvants

   The proven safety and efficacy of other adjuvants makes it difficult to justify use of Freund adjuvants. Because of the potential adverse effects of Freund's adjuvants, and the availability of other, potentially less harmful adjuvants, the justification for use of Freund's adjuvant must be included in the AUP and approved by the ACUC. If Freund adjuvants must be used, the "complete" adjuvant can be used only for the first (priming) immunization. Personnel using complete Freund adjuvant should be particularly careful to avoid accidental self-injection with needle tips, which causes a protracted, painful inflammation at the injection site.

3. Bleeding

   10% of blood volume can be removed without replacement at one time and repeated every 2 weeks. For recommendations regarding bleeding volumes and methods, please refer to OLAC's guidelines, "Blood Collection Techniques and Limits".

4. Duration of Experiment

   All animal use protocols for antibody production should clearly state when and how the response will be evaluated (e.g., immunoassay, western blot, immunofluorescence, etc.), and how long the animals will be maintained. Instead of housing rabbits for months or years with an occasional boost and bleeding, it is preferable to raise a good response and perform a terminal bleed if an ongoing need for the antibody is required. Therefore, rabbits should not be maintained longer than 18 months for antibody production when adjuvants are utilized.

5. Alternative Techniques

   Antibody production in chickens is an alternative in vivo technique for polyclonal antibody production. Antibody production in chickens offers the advantage of providing a non-invasive means to obtain antibody that is recovered from the egg yolk.

   Another alternative method in rabbits consists of placing a subcutaneous whiffle ball chamber. Immunizations are made directly into the surgically implanted chamber and antibody-rich fluid is harvested from the chamber. This procedure has been shown to provide an efficient alternative method to ear bleedings for antibody collection. Advantages cited for this technique include greater flexibility in immunogen preparation, minimal discomfort and minimal tissue reaction, ease of immunization and fluid collection, and recovery of large volumes of antibody-rich fluid with low cellularity and absence of lipids. Investigators should contact OLAC Veterinary Staff and submit a Research Service Request for this procedure.

6. Note about Species Used

   It is also possible to produce substantial amounts of polyclonal antibodies by inducing ascites in mice that have raised antibodies to a particular immunogen. Mice cost much less than rabbits to purchase and maintain, they require much less space, are easier to handle, generally respond to less antigen, and their genetics of immunoresponsiveness offers more options. A high-titer ascites from two to four mice may give the user about as much antibody as all of the serum from a rabbit.

   In this case, the ascites is induced by intraperitoneal injection of a sarcoma cell line, and the desired antibodies are secreted into the ascitic fluid by the host's B-lymphocytes. The sarcoma cells used to generate polyclonal ascites can be stored indefinitely in liquid nitrogen.

Procedures for Monoclonal Antibody Production

The most common reason for ascites production is the growth of hybridoma lines as ascites to obtain large amounts of monoclonal antibodies.

1. Priming for Monoclonal Ascites Production

   Rodents should be primed once intraperitoneally with 0.2 ml of Pristane 10 to 14 days before hybridoma cells are injected. Use of a larger volume or other adjuvant requires scientific justification and must be approved by the ACUC.

2. Monitoring

   After inoculation with an ascites-producing tumor line, mice should be observed at least three times per day for the first week, and then daily thereafter including weekends and holidays. The amount of abdominal distention should be monitored, as well as signs of illness and distress. Mice should be weighed daily

and should not gain more than 20% of their baseline body weight before harvesting ascites.

3. Harvesting Ascites
    a. The ability to judge when and how to harvest ascites and when to euthanize the mice should be learned from experienced personnel. New personnel and students should be trained using anesthetized mice.
    b. Ascites fluid should be removed by peritoneal tap before abdominal distention is great enough to cause obvious discomfort or interfere with normal activity. Unanesthetized mice may be held by properly trained personnel during the procedure or the animal may be anesthetized. The abdominal area should be disinfected with 70% ethanol or Betadine and gently dried before puncture with the needle. Shock due to hypovolemia may be prevented by subcutaneous injection of 2-3mls of warm saline or lactated Ringer's solution. When collection is complete, the puncture site should be disinfected again before the animal is returned to its cage. A maximum of three taps per mouse may be performed. Animals should be euthanized immediately following the third tap.
    c. Mice that fail to produce ascites within 25 days after hybridoma injection should be euthanized. Mice that form solid tumors should be euthanized if the tumor mass exceeds 10% of the average body weight. Mice that show signs of distress, cachexia (loss of weight), failure to eat and drink, abnormal respiration, or any signs of abdominal hemorrhaging or infection (bloody or cloudy ascites) should be euthanized.

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