

**TITLE: IACUC POLICY ON MONOCLONAL ANTIBODY PRODUCTION**

**PURPOSE:** Standardize acceptable means of producing antibodies.

**REVIEW/REVISIONS:** Permanent amendment/revisions to this policy must be presented to the IACUC for review before implementation and should be developed by the IACUC membership and/or UAC Veterinary personnel.

**EFFECTIVE DATE:** June 8, 2000; **Revisions/Re-approval:** 12/7/00, 11/2/06, November 1, 2007

**PERSON(S) RESPONSIBLE:** All University Animal Care or Research personnel charged with producing antibodies.

**POLICY/PROCEDURES:**

**Policy on Monoclonal Antibody (MAbs) Production:**

- 1) In vitro techniques shall be the default method for MAb production.
- 2) The proposed use of mouse model ascites is scientifically justified:

Recommendation 1: There is a need for the scientific community to avoid or minimize pain and suffering by the animals. Therefore, as in vitro systems are further developed and a production facility established at the institution, in vitro methods for the production of monoclonal antibodies should be adopted as the routine method unless there is a clear reason why they cannot be used or why their use would represent an unreasonable barrier to obtaining the product at a cost consistent with the realities of funding of biomedical research programs.

- \* When hybridomas fail to grow or fail to achieve a product consistent with scientific goals, the investigator is obliged to show that a good-faith effort was made to adapt the hybridoma to in vitro growth conditions before using the mouse ascites method.
- 3) When the mouse ascites method for Producing MAb is used, every reasonable effort should be made to avoid or minimize discomfort, distress, and pain (including *in vitro* methods), including frequent observation, limiting the numbers of taps, and prompt euthanasia if signs of distress appear.
    - \* Obtain a baseline weight of animals
    - \* Maximum Pristane to use: .2ml (0.1-0.2 ml has been found effective.)
    - \* Hybridomas should be tested before introduction into the animal host to prevent potential transmission of infectious agents.
    - \* After injection of hybridoma cells, mice should be evaluated at least daily, 7 days a week, after development of visible ascites.
    - \* Mice should be weighed daily and no more than 20% weight gain should be allowed.
    - \* The mice should be tapped, using aseptic technique, before fluid accumulation becomes distressful.
    - \* A maximum limit of 3 taps over a 4 day period is allowed. The 4<sup>th</sup> tap must be terminal. Multiple taps are only allowed if the animal does not exhibit signs of distress and must have prior IACUC approval.
    - \* If the exudate is bloody, cloudy or particulates are observed grossly, the animal is to be euthanized immediately.



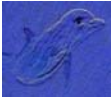
- \* Analgesics are NOT recommended due to CNS depression, but narcotic agonists, mixed agonist-antagonists or other species appropriate agents may be used to alleviate pain or distress.
  - \* All individuals involved in any phases of the procedure, including observation, handling, injection, and tapping of the animals must be well trained and experienced in all phases and wear appropriate personal protective equipment.
- 4) Prior to approval of the mouse ascites method, the IACUC must determine that:
- \* The proposed use is scientifically justified
  - \* Methods that avoid or minimize discomfort, distress, and pain have been considered (this includes in vitro methods)
  - \* And that the latter have been found unsuitable.

#### **Adjuvant Usage - background:**

Local irritation, pain, and distress are often associated with agents which are used in antibody production. These side effects should be viewed as unnecessary, and not accepted as normal events in the course of antibody production studies. It is possible to obtain high quality antibodies, while minimizing the deleterious effects to the host animal. Adjuvants other than Complete Freund's Adjuvant (CFA), which is a potent inflammatory agent, must be considered at the onset of a proposal. These systems include: TiterMax, RIBI Adjuvant System (RAS), Montanides, Syntex Adjuvant Formation (SAF), aluminum compounds, subcutaneously implanted chambers and others. The IACUC requires that investigators evaluate the use of an alternative adjuvant, and justify to the committee why an alternative method cannot be used in the place of CFA.

#### **Guidelines for Use of Freund's Adjuvant:**

- 1) In cases when CFA remains the adjuvant of choice, the following guidelines are to be followed:
- \* CFA should be used only in the primary injections, using sterile technique to prepare the CFA and aseptic injection site preparation.
  - \* A concentration of <0.1mg/ml antigen solution is recommended, and injected aseptically at scattered sites over the back and flank of the rabbit. Subcutaneous injections of 0.1 ml per site are preferred (see Handbook of Experimental Immunology in Four Volumes 4th ed. Edited by D.M. Weir - Chapter 8 Immunization of Experimental Animals); however, if intradermal injections must be used, the volume per site should be reduced to 0.05 ml to prevent necrosis or drainage. Scientific justification must be provided for intradermal injections. The uses of intramuscular injections of .5 - 1 ml are also effective. Monitoring of post-injection reactions by using this method is difficult; some animals may lose function of the limb. Therefore, intramuscular immunization is discouraged.
  - \* Reinjection of CFA into a sensitized animal causes hypersensitivity reactions that may be painful. Booster doses should be given as antigen in incomplete Freund's Adjuvant (IFA) or an aqueous vehicle such as saline after a suitable priming period (2 weeks minimum).
  - \* Laboratory personnel using CFA should be cautioned about inadvertent self-injection of CFA on needle tips. This has resulted in painful and long lasting inflammation in humans.
- 2) Footpad Injection
- \* Footpad injections must be approved by the IACUC. CFA inoculated into the footpad produced swelling, ulceration, and necrosis. Thus, it should be given by this route only when justified for scientific reasons. A maximum injection for mice is 0.01-0.05 ml and 0.10 ml for rats.



- \* Adjuvants should be inoculated into only one of the animal's feet and, for rodents, a hindfoot should be used. The animals must be housed on soft bedding not wire bottom cages.
- \* Rabbits do not have true footpads, are heavier than small rodents, place more weight on the feet, and are generally held in cages with wire bottoms. Use of CFA on the feet of rabbits is, therefore, inappropriate.

### 3) Post-injection Care

- \* Animals given aqueous solutions of antigens after sensitization should be observed for signs of anaphylactic shock and administered appropriate treatment if an acute reaction occurs.
- \* Severe inflammatory reactions at injection sites should be reported to the Staff Veterinarian for examination and treatment.

### JUSTIFICATION:

The NIH endorses the conclusions and recommendations of the National Research Council report, "Monoclonal Antibody Production". It reiterates the applicable federal animal welfare standards, affirms existing guidance from the Office for Protection from Research Risks on policy implementation at the institutional level, and lists important information resources and NIH-supported core facilities with tissue culture capabilities. Information was updated from the NIH ARAC guide on ascites production in mice (Revised 5/04).

From the 1997 OPRR Report, "there is evidence that the mouse ascites method of monoclonal antibody production causes discomfort, distress, or pain. Practical *in vitro* methods exist which can replace the ascites method in many experimental applications without compromising the aims of the study."

The Statutory and Policy Bases for Consideration of Alternatives, in the 1997 OPRR report, should be consulted. Because of the existence of alternatives to the mouse ascites method the NIH report raises the question: "Is there a scientific need for the mouse ascites method of producing MAb's?"

After reviewing comments received from intramural and extramural sources (see attachment), the following policy was developed for consideration by the IACUC.