A. Purpose

To provide guidelines for minimizing pain and distress in animals used in antibody production. The following are considered the recommended practices by the IACUC; deviation from this policy requires scientific justification in the Animal Subject Approval Form (ASAF) and must be approved by the IACUC before work can begin.

B. Definitions

- **Antibody** - a protein produced by the body’s immune system when it detects foreign substances or antigens.
- **Antigen** - any substance which provokes an adaptive immune response.
- **Adjuvant** - a substance that enhances the body’s immune response to an antigen.
- **Monoclonal Antibodies** - recognize only one epitope (molecular region) on an antigen.
- **Polyclonal Antibodies** - a mixture of immunoglobulins that recognize different epitopes on a specific antigen.
- **Ascites** - in this case, the term “ascites” refers to antibody-rich fluid produced in an animal injected (usually in the peritoneal cavity) with a specific type of cell. Excessive build-up of ascites is associated with considerable pain and distress for the animals. Please refer to IACUC Policy #23 for the policy covering the use of the mouse ascites method for producing of monoclonal antibodies.

C. Policy

Alternatives to the use of animals (in vitro methods) must be considered during the planning stages for any studies involving antibody production in accordance with the Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy, and the Animal Welfare Act and Regulations. Principal Investigators must justify the use of
Monoclonal Antibody Production: In vitro techniques are the default method for the production of monoclonal antibodies. The mouse ascites method may only be utilized after appropriate in vitro methods have been tried for that antibody and have failed. Please refer to IACUC Policy #23 covering the use of the mouse ascites method for producing monoclonal antibodies.

Custom Antibodies Produced by Vendor: If antibodies are produced by a company for general sale, then review and approval by the WSU IACUC is not necessary for their purchase. However, if a contract company produces custom antibodies using animals at the request of the WSU researcher and utilizes antigens provided by the researcher, then either review by the WSU IACUC or a MOU is necessary. See IACUC Policy #9 for additional information on MOUs and requirements for animal related work occurring at non-WSU facilities.

D. Procedure

1. Animal Selection:
   a) Careful consideration should be given to the selection of the species and/or strain to be used for antibody production. Commonly used animals are the BALB/c mouse strain for monoclonal antibody production and the New Zealand White rabbit breed for polyclonal antibody production. The Office of the Campus Veterinarian can provide guidance on appropriate species and/or strain selection.

2. Adjuvant Selection, Preparation and Use:
   a) The antigen and adjuvant must be prepared and stored in accordance with adjuvant manufacturer’s guidelines. Substances to be injected must be nontoxic, aseptically prepared, easily pass through a 25 gauge needle, and have a pH range of 6-8.

   b) All vehicles (PBS, saline) must be sterile. The presence of bacteria in the inoculate will produce infection.

   c) The use of Freund’s Complete Adjuvant (CFA) should be avoided where possible. Alternatives to CFA, such as Sigma Adjuvant System, Titer Max, etc., must be considered prior to using CFA. When necessary, CFA must only be used once for the primary immunization, and should contain less than 0.5mg/ml mycobacteria. Incomplete Freund’s adjuvant (IFA) may be
used for subsequent booster immunizations. The amount of adjuvant in the injection should be minimized.

3. Immunization & Sample Collection Guidelines:

a) Sterile syringes and needles are to be used for injections and collection procedures. A new needle and syringe are to be used for each animal.

b) To optimize visualization of the injection site, it is recommended that hair be clipped or removed prior to injection. The skin should be clean and free of dirt/debris.

c) Multiple injection sites should be spaced apart to avoid coalescence of inflammatory lesions.

d) Injection sites should be given in areas of the body that do not interfere with handling and restraint of the animal and in areas that are easy to monitor.

e) Animals must be restrained and handled by trained personnel for immunizations and blood collection procedures. If a restraint device is used, animals are to be acclimated to the device to reduce anxiety and stress.

f) Immunization protocols and procedures should be based on those recommended by the adjuvant manufacturer standard operating procedure (SOP). A copy of the manufactures SOP should be included with the ASAF. If the PI is not following a manufacturer’s SOP, a detailed description of injection timeline, volumes, routes and locations must be included in the ASAF. Deviation from manufacturer recommendations requires justification and approval by the IACUC.

- Table 1. Recommended maximum volume (ml) for injections of antigen/adjuvant mixtures by injection route and species. NR = not recommended; NA = Not applicable

<table>
<thead>
<tr>
<th>Species</th>
<th>Subcutaneous (ml) per site</th>
<th>Intradermal (ml) per site</th>
<th>Intramuscular (ml) per site</th>
<th>Intraperitoneal (ml) One site only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.1</td>
<td>NR</td>
<td>NR</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Max # of sites = 2-4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### 4. Other Immunization Guideline

a) Intravenous Injections cannot contain adjuvants

b) Foot pad and joint injections are strongly discouraged and require explicit approval by the IACUC.

c) For particle-mediated epidermal delivery (AKA “gene gun” or biolistic delivery) of DNA or RNA-encoded antigens on gold beads (0.5-5 µm in diameter), mice or rabbits should be anesthetized, the area of administration clipped and aseptically scrubbed (refer to WSU IACUC Policy #6), and multiple bullets (usually 3-10 for mice and 10-30 for rabbits) fired into non-overlapping sites on the shaved skin to deliver the proper amount of nucleic acid for each immunization. Helium pressures of 300-500 PSI are recommended, and gene gun operation should be carried out per the manufacturer’s protocol5-8.

### 5. Blood Collection:

a) The blood collection method, volume and frequency must be described in the ASAF. Please refer to Guidelines for Blood Collection.

### 6. Ascites Production:

a) Please refer to WSU IACUC Policy #23. In vitro techniques are the default method for the production of monoclonal antibodies. The failure of in vitro methods and the subsequent use of the ascites method must be documented in the investigator’s ASAF and approved by the IACUC
7. **Monitoring of Animals and Recordkeeping:**
   a) All procedures must be documented in the animal’s record. These records should be readily accessible by animal care, research, veterinary, IACUC and regulatory staff. Please refer to WSU IACUC Policy #4.
   b) Once animals are started on an antibody production study, animals must be monitored regularly for any abnormal signs. It is strongly recommended that animals be “flagged” with a procedure card for identification. Sterile granulomas are common at intradermal or subcutaneous injection sites.
   c) Any abnormal signs or animal welfare concerns must be reported promptly to the Office of the Campus Veterinarian.

8. **Exsanguination & Euthanasia**
   a) Exsanguination of an animal must be done under general anesthesia and described on the ASAF.
   b) Euthanasia procedure is to be performed in accordance with IACUC Policy #28 *Euthanasia for Research and Teaching Animals* unless scientifically justified and approved by the IACUC.

E. **References**

1) The Guide for the Care and Use of Animals, 8th Edition,  
2) NRC Monoclonal Antibody Production Report,  
3) Guidelines for the Use of Adjuvants in Research, NIH Animal Research Advisory Committee (ARAC)  
11-18 13 24.