The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) and the Attending Veterinarian (ULAR) are charged with ensuring that all research animals are provided adequate veterinary care.

Animal Welfare Regulations 2.33 (b)(5)
“Each research facility shall establish and maintain programs of adequate veterinary care that includes: Adequate pre-procedural and post procedural care in accordance with current established veterinary medical and nursing procedures.”

This guideline offers direction on the following topics:

Mouse ascites method of monoclonal antibody production
- Pre-protocol considerations
- Generation of hybridoma cell lines
- Priming
- Inoculation with the hybridoma
- Ascites production and monitoring
- Clinical endpoints

Polyclonal antibody production
- Animal selection
- Immunization
- Antigen-adjuvant emulsions
- Post-injection monitoring

Definitions
- **Adjuvant**: any substance that acts to accelerate, prolong, or enhance antigen-specific immune responses
- **Ascites**: accumulation of fluid in the peritoneal cavity
- **Hybridoma**: hybrid cell lines produced by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell

General Considerations
Tumor-producing and ascites-producing cell lines (parent hybridoma line), especially those that have been passed in animals, should be tested and demonstrated free of murine viruses and other transmissible agents that could contaminate animal colony, infect humans, and introduce unwanted experimental variables.

**MOUSE ASCITES METHOD OF MONOCLONAL ANTIBODY PRODUCTION**

In accordance with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Public Health Service Policy, alternatives to the use of animals (in vitro techniques) for the production of monoclonal antibodies (MAbs) must be considered in place of the ascites method. Furthermore the National Research Council’s (NRC) report on Monoclonal Antibody Production specifically states that “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.”
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MONOCLONAL AND POLYCLONAL ANTIBODY PRODUCTION

Pre-protocol Considerations
Experienced personnel may perform abdominal taps without anesthesia; however, personnel unfamiliar with the procedure must perform the abdominal tap for fluid removal only under anesthesia and with hands-on guidance from experienced personnel.

The IACUC will not approve protocols that do not provide scientific justification as to why in vitro techniques cannot be used. Due to the potential for unalleviated pain or discomfort from accumulation of fluid in the peritoneal cavity, the IACUC requires that animals used for ascites production must be listed under Pain Category C (defined as ‘pain or distress not relieved by appropriate anesthetics, analgesics or tranquilizing, or sedating drugs or other means’).

Every effort should be made to replace and refine the antibody production procedures to minimize pain and distress experienced by the animal. Before beginning the protocol, all personnel involved with the handling of the animals should be familiar with identifying signs of pain or distress in mice and endpoints should be clearly defined.

Generation of Hybridoma Cell Lines
Immunization of the antigen is often performed with an adjuvant. Evidence suggests that use of Complete Freunds Adjuvant (CFA) is painful and alternative adjuvants should be used whenever possible.\(^1\) If CFA is used, it must be justified and immunization procedures must comply with the IACUC guideline for the Use of Complete Freund’s Adjuvant in Laboratory Animals. Most importantly, Incomplete Freunds Adjuvant (IFA) must be used with booster antigen administrations if CFA was used in the initial injection.

- Carefully select and prepare the immunization site to preclude unnecessary pain and distress during handling and restraint, as well as to minimizing chances of infection.
- Hair should be clipped from the site of injection and the skin area prepared with appropriate antiseptics.
- The use of disposable sterile needles and syringes is mandatory to minimize microbial contamination. Avoid sites of injection which are weight bearing, used in restraint, or prone to self-mutilation.
- Subcutaneous or intraperitoneal routes of administration of antigen are recommended in mice.
- Please refer to Tables 1 and 2 for maximum injection volumes and administration routes.
- Boosters should be limited to a maximum of three injections with a minimum of two weeks between each injection.\(^6\)
- The IACUC guideline on Blood Collection must be followed regarding sampling blood from animals for subsequent antibody detection.

Priming
Priming compounds and intraperitoneal injections may result in abdominal pain, potential for infection, and tissue damage.\(^3,5\) The primer most frequently used is pristane (2,6,10,14-tetramethylpentadecane).\(^6\)

- The maximum priming dose of pristane is 0.2ml intraperitoneally, as higher doses cause noticeable distress.\(^10\)
- Priming with agents other than pristane must be justified.\(^8\)

Inoculation with the hybridoma
A decrease in weight or failure to gain weight in the later stages of intra-abdominal hybridoma cell growth may be indicative of an ill/painful mouse.\(^9\)

- On the day of and prior to cell inoculation, the mice must be weighed and this weight recorded as the “initial weight”.
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- The mice must be weighed at regular intervals; these intervals should be as described in the protocol and based on the expected rate of fluid accumulation. Clinical observations must be made for assessments related to posture, activity, food and water intake, respiratory pattern (labored, depressed or accelerated), body condition (e.g. rough hair coat, pale ears or eyes), and severe abdominal distention.\(^8\)

**Ascites Production**

If animals are not monitored appropriately, ascites production can be a life-threatening procedure due to tumor growth, metastatic spread, infiltrative growth, and, ultimately, respiratory distress.\(^6\)

- Ascites production most commonly occurs between Day 7 and 14 after the cells are injected.\(^9\)
- Once ascites development is first observed, animals should be observed daily (including weekends and holidays) to monitor the degree of abdominal distention and signs of illness.\(^6\)
- Ascites fluid must also be collected before body weight becomes 20% greater than the initial weight or abdominal distention leads to significant health problems.\(^10\)
- Fluid should be harvested following antiseptic preparation of the site using 18- to 22-gauge hypodermic needles.\(^6\) Each time the abdomen is tapped, a fresh disposable needle and syringe must be used.
- The number of abdominal taps is limited to three. The third tap should be performed after euthanasia.
- Warm saline or lactated ringsers solution (2-3 ml) may be give subcutaneously at the time the animal is tapped to avoid hypovolemic shock if large volumes (2-3 ml) of ascitic fluid are removed.
- Mice must be observed for 30 minutes following a tap.\(^8\) Clinical signs of hypovolemic shock include hunched posture, roughened haircoat, anorexia, dehydration, weight loss, loss of body condition, inactivity, difficulty in ambulation, pallor of the ears and eyes, tachypnea, and dyspnea. Persistence of these signs after treatment warrant immediate notification of the veterinary staff or euthanasia of the animal.\(^12\)

**Clinical End Points**

- Mice must be euthanized if ascites fluid becomes blood-tinged or infected (thick, milky appearance), or when the mice show signs of poor condition such as huddling, ruffled coat, or inability to reach food and water.\(^9\)
- If the abdominal tap does not relieve abdominal distention the abdomen of the mouse should be gently palpated to determine if distention is due to solid tumor growth.
- Animals must be euthanized promptly if they display severe signs of pain or distress or exhibit severe or persistent clinical abnormalities (ruffled coat, hunched posture, anorexia, dehydration, pallor, weight loss, inactivity, difficulty ambulating, tachypnea or dyspnea).
- Any animal in moribund condition must be euthanized immediately.\(^11\)

**Euthanasia**

Animals must be euthanized in accordance with the approved protocol, the AVMA Guidelines on Euthanasia, or as recommended by the ULAR veterinarian.

**POLYCLONAL ANTIBODY PRODUCTION**

*Alternative methods of polyclonal antibody (PAb) production, such as commercially available antibodies, must be considered.*

**Animal Selection**
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MONOCLONAL AND POLYCLONAL ANTIBODY PRODUCTION

When selecting and justifying the animal species for polyclonal antibody production, it is important to consider
(1) the amount of PAb needed, (2) the ease of obtaining blood samples, (3) the phylogenetic relationship
between the antigen and the animal species, and (4) the intended use of the PAb.6

Immunization
Carefully select and prepare the immunization site to preclude unnecessary pain and distress and to minimize
infection.

• The use of sterile needles and syringes is mandatory to minimize microbial contamination.
• Avoid sites of injection which are weight bearing, used in restraint, or prone to self-mutilation.
• The choice of injection route is dependent on the choice of the animal species and adjuvant, as well as
  by the character, quantity, and volume of the antigen.
• A maximum of four injections at any one time is allowed.6 Each injection must be performed with a
  fresh disposable needle and syringe.
• Boosters should be limited to a maximum of three injections with a minimum of two weeks between
  each injection.6
• Please refer to Tables 1 and 2 for suggested injection sites and maximum volumes to be administered.

Antigen-Adjuvant Emulsions
There are a variety of adjuvants in common use. Careful consideration should be given to selecting the one most
appropriate for the antigen being used. Alternatives to Complete Freund's Adjuvant (CFA) must be considered
because of the occasionally severe lesions which develop when using CFA. When using CFA, refer to the IACUC
guideline for the Use of Complete Freund's Adjuvant in Laboratory Animals.

Post-Injection Observation
All animal use protocols for antibody production should clearly state when and how the response will be
evaluated and how long the animals will be maintained.9

• After immunization, animals should be monitored at least three times a week and examined for specific
  side effects such as pain, swelling, abscess, fistula formation, infection or ulceration at or near the
  immunization site(s).6
• If any of these signs are noted the animal must be reported to the veterinary staff for treatment or be
  euthanized immediately.

Blood Collection
The IACUC guideline on Blood Collection must be followed regarding sampling blood from animals for
subsequent antibody detection.

Euthanasia
Animals must be euthanized in accordance with the approved protocol, the AVMA Guidelines on Euthanasia, or
as recommended by the ULAR veterinarian.
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MONOCLONAL AND POLYCLONAL ANTIBODY PRODUCTION

Table 1. Recommended maximum volume (ml) used for injection of oil and viscous gel adjuvants per injection route for different animal species.\(^6\)

<table>
<thead>
<tr>
<th>Animal</th>
<th>SC</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.1</td>
<td>NR</td>
</tr>
<tr>
<td>Rat</td>
<td>0.1-0.2</td>
<td>NR</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>0.2</td>
<td>NR</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.1-0.25</td>
<td>0.025-0.05</td>
</tr>
<tr>
<td>Sheep/goat</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Cattle</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Poultry</td>
<td>0.25</td>
<td>0.05</td>
</tr>
</tbody>
</table>

NR-not recommended
SC-subcutaneous
ID-intradermal

Table 2. Recommended maximum volume (ml) used for injection of aqueous antigen/adjuvant mixture per injection route for different animal species.\(^6\)

<table>
<thead>
<tr>
<th>Animal</th>
<th>SC</th>
<th>ID</th>
<th>IM</th>
<th>IP</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
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<td>NR</td>
<td>NR</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Rat</td>
<td>0.5-1.0</td>
<td>NR</td>
<td>NR</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>1</td>
<td>NR</td>
<td>NR</td>
<td>5.0-10.0</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.5</td>
<td>0.05</td>
<td>0.2-0.5</td>
<td>10.0-20.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Sheep/goat</td>
<td>2</td>
<td>0.05</td>
<td>2.0</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>Cattle</td>
<td>2</td>
<td>0.05</td>
<td>2.0</td>
<td>NA</td>
<td>NG</td>
</tr>
<tr>
<td>Poultry</td>
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<td>0.05</td>
<td>1.0</td>
<td>NA</td>
<td>0.5</td>
</tr>
</tbody>
</table>

NA-not applicable
NG-not given,
IM-intramuscular
IP-intraperitoneal
IV-intravenous
IACUC Guideline
MONOCLONAL AND POLYCLONAL ANTIBODY PRODUCTION

References


9. UC Berkeley Animal Care and Use Committee. 2009. Guidelines for use of Rodents in Experimental Neoplasia and Production of Polyclonal and Monoclonal Ascites

