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 with adequate veterinary care. They have adopted the following guidelines to clarify the regulatory responsibilities of the institution, and to outline the minimally acceptable standards for the production of monoclonal antibodies in mice, and polyclonal antibodies in select laboratory species.

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 (neoplastic plasma) cell

**General Considerations**
Tumor-producing and ascites-producing cell lines (parent hybridoma lines), especially those that have been passed in animals, should be tested and demonstrated free of murine viruses and other transmissible agents (mouse hepatitis virus, several mouse parvoviruses, Sendai virus, Theiler’s mouse encephalitis virus, rotavirus, pneumonia virus of mice, reovirus 3, lymphocytic choriomeningitis virus, and Mycoplasma pulmonis) that could contaminate an 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 “tissue culture 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.”

APPROVED
12/17/2015
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. 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
Penn Category C/Pain Category E (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. Signs of pain in mice may include decreased food and water consumption, loss of body condition, self-
mutilation, rapid breathing, unkempt appearance, vocalization, hypersalivation, or changes in posture (hunched,
head-pressing).

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.

Generation of Hybridoma Cell Lines
Immunization with the antigen is often performed concurrently with an adjuvant. Evidence suggests that use of
Complete Freund’s Adjuvant (CFA) is painful and alternative adjuvants should be used whenever possible. 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, CFA can only be used in the first (priming)
immunization, and Incomplete Freund’s Adjuvant (IFA) or other alternatives must be used for subsequent
booster antigen administration.

• Carefully select and prepare the immunization site to preclude unnecessary pain and distress during
  handling and restraint, as well as to minimize the chance of infection.
• Hair should be clipped or depilated 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 that are weight-bearing, used in restraint, or prone to self-mutilation.
• Subcutaneous (preferred) 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.
• The IACUC guideline on Blood Collection must be followed regarding sampling blood from animals for
  subsequent antibody detection.
IACUC Guideline
MONOCLONAL AND POLYCLONAL ANTIBODY PRODUCTION

Priming
Priming compounds and intraperitoneal injections may result in abdominal pain, potential for infection, and tissue damage. The primer most frequently used is pristane (2,6,10,14-tetramethylpentadecane). The maximum priming dose of pristane in mice is 0.2 ml administered intraperitoneally 10-14 days prior to hybridoma cell injection, as higher doses cause noticeable distress. Priming with agents other than pristane must be justified.

Inoculation with the hybridoma
• Just prior to injection of the mouse, its weight must be obtained and recorded as the “initial weight”.
• The mouse 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.

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 and death.

• Ascites production most commonly occurs between Day 7 and Day 14 after hybridoma cell injection.
• Once ascites development is first noted, animals should be observed daily (including weekends and holidays) to monitor the degree of abdominal distention and signs of illness.
• Ascites fluid must also be collected before body weight becomes 20% greater than the initial weight or abdominal distention leads to significant health problems.
• The collection site should be sterile prepped with either 70% isopropanol or Betadine, and allowed to dry completely prior to needle puncture.
• Fluid should be harvested following antiseptic preparation of the site. Each time the abdomen is tapped, a fresh sterile disposable 18-22g needle and syringe must be used.
• The number of abdominal taps is limited to three. Animals should be euthanized immediately following the third tap.
• Warm saline or lactated ringers solution (2-3 ml) may be given subcutaneously at the time the animal is tapped to avoid hypovolemic shock if large volumes (2-3 ml) of peritoneal fluid are removed.
• Mice must be observed for 30 minutes following a tap. 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.

Clinical End Points
• Mice must be euthanized if ascites fluid becomes blood-tinged or infected (thick, milky appearance).
• If the abdominal tap does not relieve abdominal distention, the mouse should be humanely euthanized.
• 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, loss of body condition, inactivity, difficulty ambulating, tachypnea or dyspnea).
• Any animal in moribund condition must be euthanized immediately.
• Mice should also be euthanized if they fail to produce ascites within 25 days of hybridoma injection.
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 (ex vivo techniques), as well as the commercial availability of select antibodies, must be considered prior to production.

Animal Selection
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.

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 that 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 injection sites used for any one administration event is allowed, although it is preferable to immunize at one site only. Each injection must be performed with a fresh disposable needle and syringe.
- Boosters should be administered when antibody titers plateau or begin to decline, and should be limited to a maximum of three injections with a minimum of two weeks between each injection.
- 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.
- Examples of commonly used adjuvants for PAb production include Freund’s complete adjuvant (FCA1), Freund’s incomplete adjuvant (FIA1), aluminum salts (e.g., Al(OH)3, AlPO4), Quil A, Iscoms, Montanide, TiterMax™, and RIBI™.

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.

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

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<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>
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<th>IM</th>
<th>IP</th>
<th>IV</th>
</tr>
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<td>NR</td>
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<td>Rat</td>
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<td>NR</td>
<td>NR</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Guinea Pig</td>
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<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>
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<tr>
<td>Sheep/goat</td>
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<td>0.05</td>
<td>2.0</td>
<td>NA</td>
<td>30</td>
</tr>
<tr>
<td>Cattle</td>
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<td>2.0</td>
<td>NA</td>
<td>NG</td>
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<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
References


