**Purpose of Policy:** This policy is intended to reduce the number of animals used in research by requiring investigators to consider commercial vendors as a source of polyclonal antibodies (pAbs) and, if the desired pAbs are not commercially available or suitable, to provide guidance in designing the most efficient methods for their production while minimizing potential pain and distress to the animals.

**Policy Information:** As stated in the current edition of the National Research Council’s *Guide for the Care and Use of Laboratory Animals*, the 3Rs “have become an internationally accepted approach...to apply when deciding to use animals in research.” The third R is Reduction, i.e. development of a strategy that will permit investigators to obtain the same scientific information with the use of fewer animals. The use of commercial vendors as a source of pAbs meets this objective and, therefore, investigators must first consider the use of commercial vendors as a source of pAbs before the IACUC will approve the use of animals for their production.

Whenever possible, pharmaceutical-grade adjuvants and components, such as normal saline, must be used. To preclude microbial contamination, it is recommended that the antigen be passed through a 0.22 micron filter before mixing with the adjuvant.

The presence of byproducts e.g. polyacrylamide gel, solvents e.g. sodium dodecyl sulfate (SDS), urea and acetic acid and particulate matter should be avoided as they may be inflammatory or toxic. Extremes in pH should also be avoided.

**Special precautions may be necessary if the antigen itself is a viable microbe.** If so, consultation with the Institutional Biosafety Committee may be required to determine if a biosafety protocol must first be submitted and approved before the microbe is approved for use in animals.

Freund’s complete adjuvant (FCA) is a commonly used booster for the production of pAbs. It contains mineral oil, the surfactant mannide monooleate, and inactivated mycobacteria whereas incomplete Freund’s adjuvant (IFA) contains only mineral oil and surfactant. Although both products can cause adverse reactions, FCA is more offensive because the host may mount an antigenic response to the mineral oil and mycobacteria which can result in granulomatous inflammatory reactions, focal
necrosis, skin ulceration, fistulous tracts, muscle atrophy, self-induced trauma and weight loss. For these reasons, IFA is preferred over FCA.

Scientific justification must be given for the use of priming agents other than incomplete Freund’s adjuvant or pristane, although Ribi Adjuvant System®, TiterMax® and non-inflammatory adsorptive adjuvants such as alum and aluminum hydroxide gel might also be considered.

If FCA is approved for use, it is to be used only for weakly immunogenic antigens and only for initial immunizations. IFA must be used for booster immunizations. Subcutaneous injections of FCA and IFA may be performed as recommended in the following table.

<table>
<thead>
<tr>
<th>Species</th>
<th>Injection volume (ml)</th>
<th>Max. injection sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.05</td>
<td>10</td>
</tr>
<tr>
<td>Rat/Other rodents</td>
<td>0.10</td>
<td>10</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.10</td>
<td>10</td>
</tr>
</tbody>
</table>

Other commonly used routes of administration include intradermal, intramuscular (IM) and intraperitoneal. Intradermal administration of priming doses might be preferable due to efficient lymphatic delivery. Subcutaneous injection will accommodate larger volumes but absorption is slower. Intramuscular injection of FCA is not recommended as it can cause severe muscle necrosis. Intraperitoneal injections are more common in rodents as their limited muscle mass may not be suitable for IM injections. Injections sites should be adequately separated to avoid coalescing of inflammatory lesions that can disrupt blood supply and lead to the formation of abscesses and tissue sloughs.

Animal restraint appropriate for the species must be used. Animals should first be acclimated to handling and any restraint device to avoid stress or injury to the animal or the handler. Handlers must be properly trained in restraint techniques.

Rabbits are the most commonly used laboratory animal for the production of pAbs because they are easy to handle, specific pathogen free rabbits are available and they can produce reasonable amounts of high titer antibodies using a wide variety of antigenic material. However, mice, hamsters, rats, guinea pigs, goats, sheep and chickens have also been used for this purpose.

Rabbits are covered under the Animal Welfare Act and, therefore, individual medical records must be kept documenting all procedures performed on each animal. When recording the administration of any drug or compound, the name of the drug or compound, the volume in which it is given and the route or site of administration must be included.

Footpad injections in rabbits are not recommended because footpads are not anatomically well defined. Such injections can also interfere with the weight-bearing function of their feet. Footpad injections might be permitted in rodents but only in one hind foot and with the animals housed on soft bedding.

Sterile needles and syringes must be used to minimize the risk of infection or abscess formation. Each injection site should be shaved and prepped with three alternating scrubs of betadine and alcohol. Proper preparation of the injection site will facilitate visualization of the site and proper treatment if necessary. A new needle must be used for each injection site.

Staff performing injections must wear proper personal protection equipment that includes eye protection and sterile gloves. Inadvertent needle sticks or other exposure can cause painful cutaneous lesions and false positive PPD tests. Do not recap needles!
A needle with a sufficiently wide bore to permit smooth passage of an emulsion must be used. Otherwise, attempting to force an emulsion through a small bore needle will cause the needle to separate from the syringe causing spattering of the emulsion that could harm the animal and staff. A sterile glass syringe should be used as the rubber in disposable syringes can interfere with adjuvants.

Post-injection, the animals must be observed daily, including weekends and holidays, for signs of pain, swelling, abscesses, fistulas, infection or ulceration at or near the injection site(s). Should any of these signs or symptoms appear, the Attending Veterinarian must be contacted immediately (Office phone: 310-794-0286; Cell phone: 310-980-7888) to determine the proper treatment or other course of action.

For Ribi Adjuvant System® and TiterMax®, follow the manufacturer’s recommendations regarding product preparation, routes and sites of injection, total volume and volume per site to be injected appropriate to the species.

Any blood collection must follow the IACUC’s policy on Blood Collection (Policy No. 2014-004).