1.0 Purpose:
The purpose of this procedure is to establish guidelines for the immunization of rabbits for the production of antibodies, as well as the proper techniques to perform blood collection in this animal species.

2.0 Scope:
This procedure applies to all CMLAF technicians and principal investigators involved with antibody production or blood collection in rabbits.

3.0 Procedure:
3.1 It is recommended whenever possible to use Specific Pathogen Free (SPF) rabbits to reduce morbidity and mortality, as well as using rabbits of a minimum of 2 kg of body weight.
3.2 Appropriate methods of restraint during immunization and blood collection must be used to avoid injuries to the animals and/or personnel. The use of rabbit restrainers is recommended. It is helpful to acclimate the animals to the restrainer prior to the initiation of the procedure.
3.3 CMLAF personnel are trained to perform manual restraint techniques in addition to the proper use of restrainers, and can assist with the procedure if requested.
3.4 Antigen Preparation
3.4.1 The preparation of the antigen solution for injection will be the responsibility of the PI and should be prepared in such a manner as to elicit an acceptable response without adversely affecting the well-being of the animals.
3.4.2 The antigen must be non-toxic and must be prepared aseptically, or otherwise rendered sterile and free of toxins and pyrogens. In particular, any chemical residue or contaminating endotoxins must be minimized. Urea, acetic acid, and polyacrylamide gel should be avoided as they have been associated with adverse reactions at the site of injection. The pH must be adjusted within physiological limits (7.2-7.5). Most protein antigens can be filter-sterilized through a microporous filter (0.22 μm pore size) of a type that has minimal adsorption of protein and disruption of protein conformation.
3.5 Adjuvants
3.5.1 An adjuvant can be broadly defined as any substance that improves the immune response to an antigen and when used should result in enhanced and sustained antibody (Ab) levels.
3.5.2 The most commonly used adjuvants include:
1. Freund’s Complete Adjuvant (FCA)
2. Freund’s Incomplete Adjuvant (FIA)
3. Ribi™
4. Titermax™
5. Mineral-based adjuvants

3.5.3 While FCA is one of the most effective adjuvants, it can cause a greater chronic inflammatory response and therefore should be used only where there is evidence that other adjuvants will not work.

3.5.3.1 FCA causes ulcerations when administered intradermally. It should only be administered via the subcutaneous route.

3.5.3.2 If FCA must be used, it should be used only for the initial subcutaneous immunization. Anaphylactic reactions that are potentially fatal can occur with repeated use of FCA.

3.5.3.3 FIA should be used for subsequent booster immunizations if an adjuvant is required.

3.5.3.4 A minimum of 2-3 weeks between initial immunization and booster is recommended.

3.5.3.5 The maximum recommended concentration of dry mycobacterium in the FCA preparation is less than 0.1 mg/ml resulting in a less severe inflammatory reaction. If the antigen is highly immunogenic, immunization without adjuvant or with other adjuvants are encouraged.

3.6 Rabbit Immunization

3.6.1 Injection Site Selection and Preparation

3.6.1.1 The selection of appropriate injection sites for rabbit immunization regardless of the adjuvant used is very important. Typically, the dorsal thorax and lumbar area are used.

3.6.1.2 Anatomic sites used for grasping, handling or restraint such as the cervical/scapular areas and rump should be avoided when possible. Also avoid sites that may be prone to self-mutilation and sites that may interfere with ambulation.

3.6.1.3 IV injections and footpad injections with FCA are not permitted.

3.6.1.4 Skin must be aseptically prepped to prevent infection.

3.6.2 Procedure

3.6.2.1 Fractious or excitable rabbits may need sedation with 1 mg/kg of Acepromazine IM or SC approximately 15 minutes prior to immunization.

3.6.2.2 Clip the fur from the area to be injected. Clip an area large enough to allow for disinfection and clear visualization of the intradermal or subcutaneous injection sites.

3.6.2.3 Skin must be aseptically prepped to prevent infection. First, clean the area with disinfectant scrub (chlorhexidine or betadine), followed by alcohol swab, and finally iodine paint.

3.6.2.4 Use sterile syringes and needles (25 or 27 gauge) to minimize microbial contamination of injected tissues. Use new needles for each rabbit.

3.6.2.5 To minimize painful inflammatory reactions, the injection of small volumes of inoculum (0.01-0.25 ml subcutaneous or
0.01-0.05 ml intradermal) per site is recommended, as well as the use of multiple injection sites (5-10 per rabbit).

3.6.2.6 Injection sites must be at least 2 cm apart to avoid overlapping of inflammatory lesions if they develop. Injection sites should be at least 3-4 cm from the spinal column to avoid accidental injection into the epaxial (paraspinal) muscles.

3.6.2.7 Booster injection sites should be distanced from previous injection sites.

3.6.2.8 Complete the Rabbit Use Record for immunization and turn the form in to the veterinary technicians in room 204 BEB, room 7071 CTRC, or room B2107 JSMBS. If sedation was provided for the injection procedure an “Anesthesia Monitoring/Surgery/Post-Operative Report” must also be completed and turned in to the appropriate location above.

3.7 Post-Injection Observation

3.7.1 Animals should be monitored 3 times per week following injection. Investigators should observe animals for evidence of pain or distress, and note the animal’s activity, food/water consumption, and body condition. Injection sites should be monitored for excessive swelling, abscess, tissue necrosis, or fistula formation. Any problems or concerns should be reported to the LAF veterinary staff for follow-up.

3.7.2 Avoid manipulation of the injection sites when handling the animals.

3.8 Blood Collection

3.8.1 Up to 10% of circulating blood volume can be collected every 2 weeks. Circulating blood volume (CBV) is equal to 60 mL/kg. For example, a 4 kg rabbit has a CBV of 240 mL, and 24 mL (10% of CBV) could be collected. See SOP 2.A.21 “Blood Collection Volumes in Laboratory Mammals.

3.8.2 Ear vessels in the rabbit (central ear artery or marginal ear veins) are readily accessible and can be used for collecting blood. However, in animals with small ears and very small veins, a possible thrombosis of the vessel with subsequent sloughing of the skin may occur. This risk is higher when the artery is used for collection.

3.8.3 Nervous or excitable rabbits may need to be sedated to reduce handling stress, enhance vasodilation, and prevent injury. The use of Acepromazine at a dose of 1 mg/kg IM or SC, approximately 15 minutes prior to collection is recommended. 5% lidocaine/prilocaine cream (e.g. ELA-MAX® or EMLA®) applied to the ear as a topical anesthetic 15 minutes prior to collection is also recommended.

3.8.4 Blood collection from rabbit ears by transecting the vein or the use of xylene or other irritants is not permitted. Never use a scalpel to cut the vessels. Always insert a needle into the vessel.

3.8.5 Procedure

3.8.5.1 Sedate rabbit as needed (see 3.8.3) and place in restrainer.
3.8.5.2 Clip, shave, or gently pluck the fur over the vessel. Apply topical anesthetic and allow at least 15 minutes for effect.
3.8.5.3 Clean the skin with alcohol.
3.8.5.4 Holding the needle with two fingers, penetrate the vein or artery with a 20 or 22 gauge needle or butterfly winged...
infusion set with the bevel up. Sampling of blood from the vein should be performed as close to the base of the ear as possible, whereas sampling from the artery should be performed nearer to the tip of the ear. Additional attempts can be made distally toward the ear tip for the vein and proximally toward the base for the artery. (See diagram.)

3.8.5.5 After collecting the desired volume, apply pressure to the site for at least 2 minutes to achieve hemostasis. A gauze sponge and clothes pin applied to the vessel provides good compression and helps to achieve hemostasis.

3.8.5.6 Flip the ear back and forth to assure that bleeding will not restart. Only release the rabbit from restrainer when bleeding has completely stopped and there is no evidence of hematoma formation.

3.8.5.7 Check blood collection site for bleeding immediately upon return to the cage, and again in 15-30 minutes.

3.8.5.8 Complete the Rabbit Use Record for blood collection and turn the form in to the veterinary technicians in room 204 BEB, room 7071 CTRC, or room B2107 JSMBS. If sedation was provided an “Anesthesia Monitoring/Surgery/Post-Operative Report” must also be completed and turned in to the appropriate location above.

3.9 Terminal Blood Collection

3.9.1 Intracardiac puncture for large volume blood collection is limited to **terminal procedures only** and is performed under general anesthesia. It is not an acceptable method for routine, survival blood sampling purposes.

3.9.2 Materials

3.9.2.1 5-60 mL syringes 18-22 gauge 2” hypodermic needles or 10 mL vacutainers with 18 gauge 2” vacutainer needles and vacutainer adapter +/- vacutainer collection set.

3.9.2.2 Anesthetic drugs: Ketamine HCl (100 mg/ml) and Xylazine (20 mg/ml)
3.9.2.3 Isopropyl alcohol and gauze
3.9.2.4 In some instances Fatal Plus (sodium pentobarbital, 390 mg/mL) may be required to ensure death.

3.9.3 Procedure
3.9.3.1 Anesthetize rabbit with 35 mg/kg ketamine and 5 mg/kg xylazine IM.
3.9.3.2 Once the animal is in surgical plane of anesthesia, lay animal on its back in dorsal recumbency.
3.9.3.3 Prep area with alcohol swab and insert needle at base of sternum (under the palpable xiphoid process) at a 30-45° angle just lateral to the midline (rabbit’s left side). See diagram.

3.9.3.4 An alternative approach is to insert the needle into the left lateral thoracic region. Palpate for maximal heart palpitation between ribs on the left side midway between sternum and spine (in the region of the left elbow).
3.9.3.5 When using a syringe, aspirate slowly until the desired volume is obtained. When using a vacutainer, insert the vacutainer needle or collection set into the vacutainer and insert the needle into the heart. Blood should flow freely into the vacutainer.
3.9.3.6 Verify that the heart has stopped beating, either through manual palpation or auscultation with a stethoscope. If the heartbeat has not ceased, euthanize with 100 mg/kg Fatal Plus IV or intracardiac. This will ensure death prior to disposal.
3.9.3.7 Submit completed Rabbit Use Record for exsanguination and turn the form in to the veterinary technicians in room 204 BEB, room 7071 CTRC, or room B2107 JSMBS.
3.9.3.8 Fill in the Removal Date in the lower right-hand corner of the cage card and complete the Animal Removal Card. Both cards should be turned into one of the locations listed above.
Comparative Medicine & Laboratory Animal Facilities  
Rabbit Use Record

Principal Investigator ___________________________ IACUC # ___________________________ Date ________
Phone # ___________________________ Cage Card ID# ___________________________ PI/Animal ID# ___________________________ Room # ________

ALL PROCEDURES MUST COMPLY WITH THE PROTOCOL AS APPROVED BY THE IACUC.
Fill out any applicable sections:

<table>
<thead>
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<th>Sedation for Reasons Other Than Those Indicated Below:</th>
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<tr>
<td>Current Weight ___________________________ Anesthetic used and dose: ___________________________</td>
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<td>Reason for sedation ___________________________ Performed by ___________________________</td>
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<th>Blood Collection:</th>
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<tr>
<td>Current Weight ___________________________ Topical anesthetic applied to ear ___________________________</td>
</tr>
<tr>
<td>Sedative Used? Yes or No Anesthetic used and dose ___________________________</td>
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<td>Site of Blood Collection (e.g. right ear artery) ___________________________ Amount of Blood Withdrawn ___________________________</td>
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<td>Bleeding Stopped? Yes or No, if No was LAF vet staff contacted? Yes or No</td>
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<td>Performed by ___________________________</td>
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<td>Site(s) Administered ___________________________ Amount Injected ___________________________ Route (sc/iv/im/ip) ___________________________</td>
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<td>Sedation Used? Yes or No Anesthetic used and dose: ___________________________</td>
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Please turn this sheet into room 204 BEB, room 7071 CTRC, or room B2107 JSMBS

LAF 24  8/3/09

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## COMPARATIVE MEDICINE & LABORATORY ANIMAL FACILITIES
### ANESTHESIA MONITORING/SURGERY/POST OPERATIVE REPORT

<table>
<thead>
<tr>
<th>After Hours Emergency Phone #:</th>
<th>Lab #:</th>
<th>Animal Holding</th>
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Principal Investigator: IACUC #

Procedure Performed

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<th>Induction &amp; Maintenance (Dose &amp; Route)</th>
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Ventilation (Spontaneous/Mechanical) (Circle one)

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Key: BT=Body Temperature; Reflexes=withdrawal, jaw tone, tail, palpebral; % gas= level of gas anesthetic; BP= Blood pressure; End CO₂=end tidal CO₂; HR=Heart Rate; CMM=Color of mucous membranes; RR=Respiratory Rate; CRT=Capillary refill time

page 1/2
Duration of Procedure
Total fluids given during procedure

Analgesics Given: (Dose and Route)
Time given:

Are any additional post-operative analgesics required in your IACUC protocol?  
☐ Yes  ☐ No

If you answered “Yes”, please identify what additional analgesics need to be administered (identify drug, dose, route, frequency, and duration):

Please identify who will give any additional analgesics:  
☐ PI staff  ☐ LAF staff

Please list any other treatments you would like the LAF staff to perform:

Condition post procedure:  ☐ Good  ☐ Fair  ☐ Poor  ☐ Euthanized
Drug (Dose & Route)

Surgeons and Assistants Names:

Post Operative Attendant

**POST-OP MONITORING**

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<th>CRT</th>
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