

COMPARATIVE MEDICINE
LABORATORY ANIMAL FACILITIES

STANDARD OPERATING PROCEDURE
for
RABBIT IMMUNIZATION AND BLOOD COLLECTION

- 1.0 Purpose:
The purpose of this procedure is to establish guidelines in the procedures of rabbit immunization 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 all principal investigators involved with antibody production in the rabbit.
- 3.0 Procedure:
- 3.1 It is recommended whenever possible to use Specific Pathogen Free (SPF) animals to reduce morbidity and mortality during use, as well as using animals of at least 2 kg of body weight.
- 3.2 Appropriate method 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 as well as in the proper use of restrainers, and will assist with the procedure if needed.
- 3.4 ANTIGEN PREPARATION
- A. The preparation of the antigen solution for injection will be the responsibility of the PI and it should be prepared in such a manner to elicit an acceptable response without adversely affecting the well-being of the animals.
- B. The antigen must be non-toxic and it must be prepared aseptically, or otherwise rendered sterile and free of toxins and pyrogens. In particular, any chemical residue, contaminating endotoxins, or other toxic contaminants 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. For example, most protein antigens can be filter-sterilized through a microporus filter (0.22 μm pore size) of a type that has minimal adsorption of protein and minimal disruption of protein conformation.
- 3.5 ADJUVANTS
- A. 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.
- B. The most commonly used adjuvants include:
1. Freund's Incomplete Adjuvant (FIA) or Freund's Complete Adjuvant (FCA)

2. RibiT[™]
 3. Titermax[™]
 4. Mineral-based adjuvants.
- C. 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.
1. If FCA must be used, it should be used only for the initial subcutaneous immunization.
 2. FIA should be used for subsequent booster immunizations if an adjuvant is required.
 3. A minimum of 2-3 weeks between initial immunization and booster is recommended.
- D. 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

A. INJECTION SITE SELECTION AND PREPARATION

1. The selection of appropriate injection sites for rabbit immunization regardless of the adjuvant used is very important.
2. Anatomic sites used for grasping, handling or restraint such as the cervical/scapular areas and rump should be avoided when possible.
3. Avoid sites that may be prone to self-mutilation and sites that may interfere with ambulation.
4. I.V. injections as well as footpad injections with Freund's are not permitted.
5. Skin must be properly disinfected to prevent infection.

B. PROCEDURE

1. Rabbits may need sedation with 1 mg/kg of Acepromazine IM or SC approximately 15 minutes prior to immunization.
2. Clip hair for the intradermal or subcutaneous injection sites.
3. Skin must be properly disinfected to prevent infection. Use 3-prep solution. First, disinfectant soap, followed by alcohol swab and finally iodine paint.
4. Use sterile syringes and needles (25 or 27 gauge) to minimize microbial contamination of injected tissues. Use new needles for each rabbit.
5. To minimize painful inflammatory reactions, the injection of small volumes of inoculum (0.01 ml to 0.25 ml subcutaneous) or (0.01-0.05 ml intra dermal) per site is recommended, as well as the use of multiple injection sites (from 5 to 10).
6. Injection sites must be sufficiently separated (at least an inch apart) to avoid overlapping of inflammatory lesions if they develop.
7. Booster injection sites should be distanced from previous injection sites.

3.7 POST-INJECTION OBSERVATIONS

- A. Investigators should observe animals for evidence of pain, distress or lesions such as swelling, abscess or fistula formation at least 3 times a week and report to LAF veterinary staff if problems are observed.

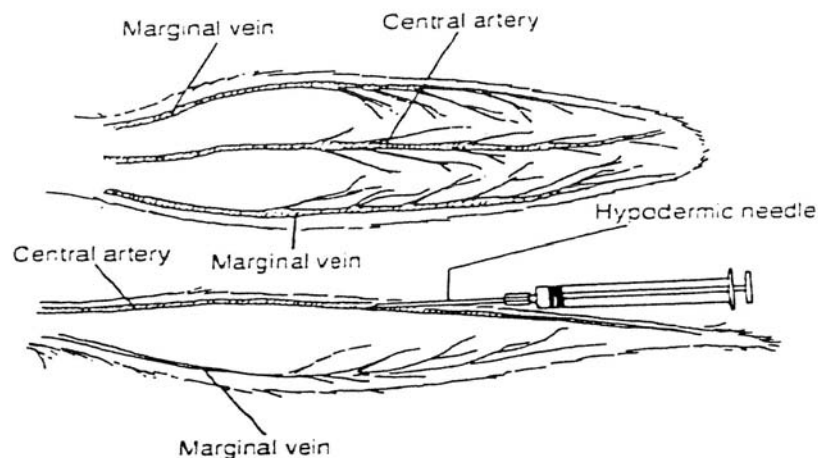
- B. Veterinary follow-up that will include clinical observation, palpation of injected sites and determination for treatment if required will take place.

3.8 BLOOD COLLECTION

- A. As a standard recommendation the amount of blood collected every 3 weeks should not exceed 15% of the total blood volume of the animal. Withdrawal of 15% of total blood volume equates to approximately 1% of body weight.
- B. 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. The most risk of sloughing occurs when the ear artery is used.
- C. Rabbits, particularly 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. The use of ELA-MAX® cream as a topical anesthetic applied to the ear 15 – 30 minutes prior is also recommended.
- D. 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.

E. PROCEDURE

1. If the rabbit is nervous or distressed then sedation with 1 mg/kg of Acepromazine IM or SC approximately 15 minutes prior to blood draw may be required.
2. Place the rabbit in the restrainer.
3. Shave or gently pluck the fur over the vessel.
4. Rub or tap the area with your finger to dilate the vessel.
5. Clean the skin with alcohol.
6. Holding the needle with two fingers, penetrate the vein or artery with a small 20 or 22 gauge needle 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.)



7. Allow the arterial blood to drip from the needle hub and free catch it in an appropriate collection tube.
8. After collecting the desired amount, apply pressure to the site to create hemostasis (clothes pin works well) for at least 2 minutes.
9. Flip the ear back and forth to assure that bleeding will not re-start.
10. Only release animal from restrainer when bleeding has completely stopped and no hematoma is formed.
11. Log necessary information in the Rabbit Use Record.
12. Complete Anesthesia Monitoring/Surgery/Post Operative Form if the rabbit was sedated.
13. Check rabbit immediately upon return and again 15 min. to ½ hour later after being returned to cage.
14. Submit form to area supervisor or veterinary technician office, room 204.

3.9 TERMINAL CARDIAC BLOOD COLLECTION

- A. 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 blood sampling purposes.

3.10 Materials:

- A. Syringes (5-60 ml) with hypodermic needles (20-25G)
- B. Evacuated containers with 16-18G needles
- C. Anesthetic drugs – Ketamine HCl (100 mg/ml) and Xylazine (20 mg/ml)
- D. Isopropyl Alcohol
- E. Gauze

3.11 Procedure.

- A. Anesthetize rabbit with Ketamine/Xylazine at 35/5 mg/kg IM respectively.
- B. Once the animal is in surgical plane of anesthesia, lay animal on its back using the restraining board.
- C. Prep area with alcohol swab and insert needle at base of sternum (under the Xiphoid) at a 30-45° angle just lateral to the midline (rabbit's left side). See diagram.

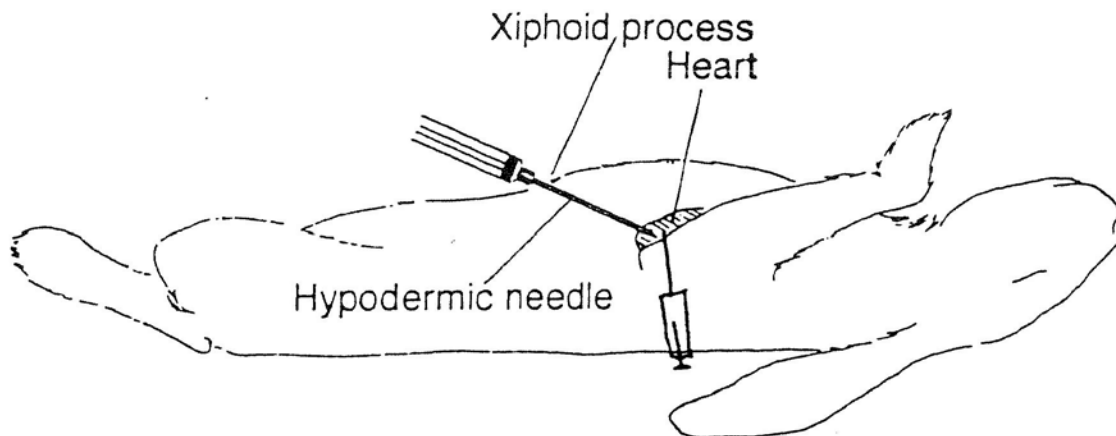


Figure 35. Cardiac puncture in the rabbit.

- D. Or, the needle can be inserted into the lateral thoracic region toward the area of maximal heart palpitation between ribs of rabbit's left side midway between sternum and back under left elbow.
- E. If using syringe, aspirate slowly the desired amount. If using evacuated container, open clamp on collection set, the blood should flow quickly into the container. The animal succumbs to exsanguination.
- F. Verify that heart has stopped. If it is still beating, euthanize with Fatal Plus IV or intracardiac (100 mg/kg). This will ensure that the heart has stopped prior to disposal.
- G. Submit completed Rabbit Use Record to the veterinary technicians' office, room 204, or area supervisor.
- H. Complete cage card by dating lower right corner.
- I. Complete Animal Removal Card.
- J. Submit completed cage card and animal removal card to area supervisor or leave them on the sink in the animal holding rooms in BEB for floor supervisor to collect.