

FLSC Standard Operating Procedure for Antibody Production in Rabbits

- Type Used:** New Zealand White (Specific Pathogen Free)
- Purchased From:** Vendors approved by Freimann Life Science Center
- Quarantined:** 7-10 days, checked over by Veterinary Technicians or Attending Veterinarian.
- Available for Use:** One week after arrival, having fully adjusted to the facility (i.e. eating and drinking, etc.)

Antigen Preparation:

1. Antigen used to inject into the rabbits is prepared by the individual labs that are using the rabbits. The following guidelines are to be used:
 - a. The antigen must be filter sterilized to reduce the amount of inflammation at the site.
 - b. The antigen must be given to the FLSC staff in vials that facilitate sterile removal of the antigen (i.e. rubber-capped serum bottles).
 - c. Purification of the protein is vital for both optimal antibody production and for the reduction of inflammation at injection sites.
 - d. The needed volume of antigen each time is 0.5 cc. This is then combined with an equal volume of adjuvant for a total of 1.0 cc.
2. Antigen preparations include the use of adjuvants - Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (ICFA), and Alum - to aid in the stimulation of the immune response. Use of either the Ribi or Titermax adjuvant systems are encouraged as a means of possibly minimizing the inflammatory response. Complete Freund's Adjuvant is used in the first injection only. The CFA and ICFA are matched in volume to the antigen, making a 1:1 mix. This mix must be thoroughly emulsified. Only ICFA can be used for booster immunizations if CFA was used for the initial immunization.
3. All antigen preparations must be labeled with the complete name of the antigen and the number of the rabbit that is to be injected.

Injection Procedures:

1. Fractious rabbits may be tranquilized with IM acepromazine (0.1-2.0 mg/kg). Rabbits are placed in a cat-type restraint bag and transported to the procedure area.
2. Shave the area to be injected (6-8" long strip along the dorsal midline extending 3" on each side of the spine).
3. Wet the area of injection with water, alcohol or disinfectant solution and wipe with a clean paper towel to remove hair and debris.
4. Injections of the antigen are given in multiple sites to stimulate the best immune response. A 22g x 1" needle is used for injections.
5. The following sites are routinely used:
 - a. SQ - multiple sites along one side of the back (0.1-0.2ml/injection) - a maximum of five sites are used, spaced an inch apart; thus a maximum volume of 1.0 cc is allowed per rabbit.
 - b. IM - used for initial immunizing with CFA only. The maximum amount to inject into one site is 0.5ml. The *biceps femoris* muscle should be used.
6. The rabbits are re-immunized (boosted) at 21 day intervals until peak antibody titers are reached. ICFA is used, at 1:1 ratio with antigen. Re-immunization injection sites should be on the opposite side of the back from the initial immunizations. Use alternate sides for additional immunizations.
7. Alternative method is to inject in popliteal lymph node only. Ideal to use when only a very small amount of antigen is available. This procedure requires following aseptic technique (sterile gloves and surgical scrub preparation of the injection site).
 - a. Shave area behind the rabbit's knee. Remove excess hair with an alcohol wipe.
 - b. Perform a surgical scrub using alcohol followed by iodine or chlorhexidine scrub).
 - c. Apply 1% iodine solution to site prior to injection.

- d. Inject with 25g 5/8" needle, maximum amount of 0.25ml. (0.125cc antigen: 0.125cc adjuvant).
 - e. The lymph node must be closely monitored for excessive inflammation. Do not inject into the same lymph node on subsequent injections.
8. The animal must be monitored closely for signs of pain, limping, anorexia, vocalization, etc. All injection sites must be closely watched for signs of inflammation and/or infection. Supportive therapy (clipping, cleaning wounds, etc.) should be done by a competent animal/veterinary technician. All lesions must be documented in the animal's individual health record. Also, report all lesions to the facility's Attending Veterinarian by using the Veterinary Log form (pink sheet). Analgesics (1.0 - 5.0 mg/kg butorphanol IM or SC or 2-5 mg/kg Ketoprofen SC) will be used if the lesions are deemed to cause the animal significant discomfort.
 9. An animal may be euthanized at the request of the Attending Veterinarian if it meets the IACUC's Humane Endpoints in Animal Experimentation guidelines. Harvest of blood will be performed prior to euthanasia at the request of the PI.

Blood Sampling Procedures:

1. Ear Bleeds - Medial Artery
 - a. Administer Acepromazine (10 mg/ml)^{1&2} at a dose of 0.1 – 0.2 ml SC or 1-5 mg/kg IM⁴.
 - b. Place the rabbit in a restraint bag and shave both ears using clippers with a #40 blade.
 - c. Insert a 20gx 1" short beveled needle with an appropriate size syringe (10cc usually) into the artery. Pull back on the plunger slowly until desired amount of blood is obtained. Remove needle.
 - d. Apply direct pressure to the entry site until bleeding has completely stopped.
 - e. Always recheck the rabbit in 10-15 minutes to assure complete hemostasis. If the ear continues to bleed, apply digital pressure with a clean cotton ball until hemostasis is achieved.
2. Large Volume Ear Blood Samples

A maximum of 30-40ml of blood can be obtained by this method. No more than 30-40cc of blood should be drawn from an average 8-10 lb. rabbit. A safe figure is 3.5-4cc/lb maximum to be drawn at one time. If the maximum is drawn, that rabbit cannot be bled again for at least 3 weeks.

 - a. Administer anesthetic as listed above.
 - b. Place rabbit in a restraint bag.
 - c. Shave both ears with clippers.
 - d. Break the hub off of an 18g x 1 ½" needle.
 - e. Place the needle into the median artery of the ear. Blood will pulse out of the needle when placed properly.
 - f. Collect the blood in an appropriate tube, usually a 50 ml centrifuge tube.
 - g. Apply direct pressure to the entry site until bleeding has completely stopped.
 - h. Always recheck the rabbit in 10-15 minutes to assure complete hemostasis.
3. Cardiac Blood Sampling Procedure
 - a. Use ketamine (100mg/ml), a dissociative drug and Acepromazine (10mg/ml) a tranquilizer at a ratio of 10 parts Ketamine:1 part Acepromazine. The dosage is 0.35cc/10 lb. rabbit² IM. Allow approximately 5 minutes for the drug to take effect.
 - b. Place the rabbit on its back on a rabbit V-trough restraint board.
 - c. Secure all limbs using cotton ties.
 - d. Insert an 18g x 1 ½" needle attached to 30-60ml syringe between the 4th and 5th rib on the rabbit's left side at the point where the heart beat is the strongest.
 - e. Advance the needle slowly until a flash of blood is observed in the hub, then draw back on the plunger.
 - f. Alternatively, the needle may be inserted to the rabbit's left of the xiphoid in the notch of the xiphoid and the ribs. Direct the needle cranially and toward the rabbit's right at a 30 to 45° angle into the heart.
 - g. After filling the syringe, remove it from the needle, leaving the needle in place in the heart.
 - h. Empty the blood into a tube and reattached syringe to needle.

- i. During exsanguination the rabbit will slip deeper and deeper into an anesthetic state resulting in respiratory arrest followed by cardiac arrest after about 90-100cc of blood have been drawn. This results in death. A total of 120-150cc can usually be obtained.
- j. If after exsanguination the rabbit has a heart beat but blood can no longer be obtained, the Attending Veterinarian will require euthanasia with 3-5ml of euthanasia solution³ given by the intracardiac route by trained technicians only.
- k. When the heart has stopped after exsanguination, death is ensured by creation of a bilateral pneumothorax and severing of the aorta.

NOTES:

1. Acepromazine maleate is a tranquilizer that also causes vasodilation.
2. Anesthesia is administered by trained technicians only.
3. Euthanasia solution is a controlled drug comprised of 26% sodium pentobarbital, 10% Isopropyl alcohol, and 20% propylene glycol.
4. Hawk, C.T., Leary, S.L., Formulary for Laboratory Animals, Iowa State Press, 1995, p.3.