

3. FCA is considered a human biohazard since accidental self-inoculation or splashing in the eye have been shown to cause painful inflammatory lesions and abscesses, hypersensitivity reactions, as well as sensitization to tuberculin which negates future skin testing. **Always** use gloves, gowns, and protective eyewear when handling FCA. **Always** use Luer-lock syringes when working with FCA. **Avoid** needle sticks: **Do Not** Re-Cap Needles!

4. Alternative adjuvants are now available that produce high antibody titers, in some cases exceeding those obtained with FCA, and with less severe inflammatory or toxic side effects. Many of these new adjuvants are suitable and highly effective for research use. A few examples include Hunter's TiterMax[®], the RIBI adjuvant system[®] (RAS[®]), Monophosphoryl Lipid A[®], and Adjuvax[®]. Hunter's TiterMax[®] and RIBI adjuvant system[®] are two adjuvants in common use. The Ribi adjuvant system[®] consists of monophosphoryl lipid A, synthetic trehalose dicorynomycolate, and cell wall skeleton, and recommendations of the manufacturer are followed (Ribi Immunochem Research, Inc., 406-363-6214). The Titermax[®] adjuvant system consists of a block copolymer CRL-8941, and recommendations of the manufacturer are followed (Hunter's Titermax 800-345-2987). Although Titermax[®] and RAS[®] are less toxic than FCA, similar precautions should be used

self mutilation lesions of the rear legs and feet as well as an evaluation of the animal's activity, food/water consumption and body condition. The attending veterinary staff should be notified immediately of any health concerns.

VI. BLOOD COLLECTION PROCEDURES

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VII. EXSANGUINATION/EUTHANASIA

1. To anesthetize the rabbit administer 40-45 mg/kg Ketamine + 5-8 mg/kg Xylazine IM in the caudal (quadriceps) muscles of the hind leg. Allow approximately 15 minutes for the drugs to take effect. Alternatively, isoflurane (1-5%) may be administered by via an induction chamber and anesthesia is maintained with a mask/nose cone throughout the blood collection process.
2. Place the rabbit in dorsal recumbency.
 - a. Attach a vacutainer adaptor needle to a vacutainer tube holder. Attach an 18 gauge X 2 inch needle to the vacutainer adaptor needle. Insert a red top or serum separator vacutainer tube into the holder and onto to the adaptor needle.
 - b. Insert the needle directly into the heart (IC) after verifying the position of the heart by palpation and stethoscope.
 - c. Replace and fill as many tubes as necessary to collect the blood.
3. When blood ceases to flow the rabbit is euthanized by injecting 1 ml/4.5 kg (1 ml/10 lbs) of Euthasol directly into the heart (IC) through the 18-20 gauge 1 ½ inch long needle using a 3 cc syringe, or alternatively by isoflurane overdose followed by bilateral thoracotomy.
4. Verify that the heart and respirations have stopped with a stethoscope.
 - a. Make appropriate entries in the rabbit's medical record and on the **Antibody Production Schedule** (CMDC #011).
 - b. Place the body in a red biohazard bag and store carcass in the freezer.
5. Label each tube of blood collected with the date, animal ID number, and PI.
 - a. Allow the tubes to sit until the blood has clotted.
 - b. After a clot has formed centrifuge the tubes for ten minutes at 2700 RPM.
 - c. Put the tubes of blood in the refrigerator in Room 1301.
 - d. Notify the PI that the samples are ready to pick-up.

VIII. REFERENCES:

1. Harlow, E. and D. Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1988.
2. Hanly, W.C., J. E. Artwohl, and B.T. Bennett. "Review of Polyclonal Antibody Production Procedures in Mammals and Poultry", *ILAR Journal*, 37(3), pages 93-124, 1995.
3. Schunk, M.K., Macallum, G.E. "Applications and Optimization of Immune Procedures", *ILAR Journal*, 46(3), pages 241-257, 2005.
4. Leenaars, M., Hendriksen, C.F.M. "Critical Steps in the Production of Polyclonal and Monoclonal Antibodies: Evaluation and Recommendations", *ILAR Journal*, 46(3), pages 269-279, 2005.

Approved:

Date: