

SOP 001: POLYCLONAL ANTIBODY PRODUCTION IN RABBITS

PURPOSE:

To describe the proper procedure to follow when injecting adjuvants into rabbits for the production of polyclonal antibodies.

POLICY:

The improper use of adjuvants can be detrimental to an animal's health and welfare, therefore it is important that the amount of adjuvant and its route of administration follow the guidelines set out. It is also the policy of this university to not allow adjuvants to be injected by intradermal, intrafootpad, or intraperitoneal routes.

RESPONSIBILITY:

Investigator, Graduate Students, Animal Care Centre Technical Personnel, Veterinarian.

PROCEDURE:

Animals

1. Female New Zealand White rabbits, obtained from Charles River Laboratories are housed in small groups of up to six animals.
2. The rabbits have access to commercial rabbit food and water ad libitum and the diet is supplemented with hay three times a week and carrots once each week.
3. The rabbits are sedated (see below) at time of first bleeding, the right ear is tattooed and a microchip bearing a unique number is implanted at the base of the neck.
4. Each pen is identified by a number, and the pen door is labeled with a cage card containing investigator name, protocol number, and the animals contained within.
5. All rabbits are acclimatized to their new surroundings for a minimum of 2 weeks prior to any procedures and must be at least 2 kg at the start of the immunization series.
6. For any procedures, animals are transported from their pen to a quiet room via a cart, occasionally covered with a towel.
7. Animals are weighed and examined monthly and also weighed at every bleeding interval.

Administration of antigens

1. Investigators provide the antigen in a stable emulsion ready for injection to a maximum volume of 1 ml.
2. The emulsion must pass easily through an 18 gauge needle.
3. The antigen is non-toxic and prepared aseptically, or otherwise rendered sterile and free of toxins and pyrogens.
4. Endotoxins and other toxic contaminants are minimized and the pH is adjusted within the physiological limits.
5. The investigators have ensured that an adjuvant is necessary for this particular program of polyclonal antibody production.
6. Initial immunizations consist of antigen in either Freund's Complete Adjuvant (CFA) or other adjuvant (for e.g., Ribi's, TiterMax) given subcutaneously (SC) in up to four sites (2 sites at the neck and 2 sites at the flank) with a maximum of 0.25 ml per site, since a maximum of 1 ml of antigen plus adjuvant may be injected, divided among 4 sites.
7. If CFA was used for initial immunization, subsequent immunizations consist of Freund's Incomplete Adjuvant (IFA). Otherwise, the same adjuvant or no adjuvant is used, and immunizations are given intramuscularly (IM) at intervals of 2 to 4 weeks.
8. The muscles of the hind legs are used since these sites of injection do not interfere with subsequent handling of the animals for blood sampling, etc.
9. All injections are made with 22 gauge needles and 3 cc glass syringes with luer-lock hubs, to reduce the risk of needle and syringe separation and the spraying of adjuvant.
10. Eye protection and gloves are also worn.

Blood collection

1. Rabbits undergo a pre-immunization bleed to ensure that antibodies to the antigens do not already exist.
2. Thereafter, blood samples are taken 7-10 days after an immunization.
3. Before blood samples are taken rabbits are given an analgesic/sedative combination (butorphanol, 0.2 mg/kg; acepromazine, 0.1 mg/kg), both mixed in a 1 cc syringe with a 25 gauge needle and given IM.
4. Emla analgesic cream is applied to both central ear veins on one ear.
5. Restrainers are not used.
6. When adequately sedated (after ~20 min.), 5-10 mls of blood is collected via a 22 gauge catheter and transferred into Falcon or Fisher tubes, or other tubes as specified by the researcher.
7. On occasion, larger samples are taken but they do not exceed 15% of the estimated total blood volume and the interval between larger bleeds is not less than 14 days.
8. After collection, animals are monitored for 30 minutes before being returned to their pens.

Record Keeping

1. A complete record is kept for each animal, containing identification information, details of the antigen used, the adjuvant, volume, routes and sites of administration, the dates of immunization and blood sampling, animal body weights and any other pertinent data.
2. A copy of this record is maintained in the animal unit.

Monitoring

1. Animals are monitored immediately following injection for anaphylactic reactions, both after the primary injection and after the subsequent booster injections for at least $\frac{1}{2}$ - 1 hour post-injection, and two more times that same day.
2. Thereafter animals are monitored daily for inflammatory responses, granulomas or ulcerative lesions at the injection site(s) and for food/water intake, activity level, alertness, responsiveness, pain and lameness.
3. If any of the above lesions are noted, the clinical veterinarian is consulted and either implements appropriate supportive treatment or recommends euthanasia.

Euthanasia

Experimental endpoints are determined by the individual study and generally average about 6 months. At the end of the study, the animals are exsanguinated under deep anesthesia through one common carotid artery while the circulation is maintained with the simultaneous administration of intravenous fluids. **(See below for full body bleed details.)**

Humane Endpoints

Animals will be euthanized as determined by a clinical veterinarian if:

- anaphylaxis is observed at any time and is unresponsive to medical management
- there is inappetance that results in weight loss exceeding 20% body weight
- pain at sites of injection lead to progressive lameness and are unresponsive to therapy beyond 7 d
- progressive ulcerations/inflammation at injection sites are unresponsive to therapy beyond 7 d
- there are other clinical signs to suggest a chronic progressive illness – for e.g., decreased activity, isolation from the group, self-mutilation, lack of grooming, recumbency, etc.

RABBIT CAROTID ARTERY BLOOD COLLECTION

PURPOSE:

The carotid artery can be used for removing large blood volumes as a terminal procedure (exsanguination). It can be used as a superior substitute for cardiac puncture.

POLICY:

This is performed on anaesthetized animals only.

RESPONSIBILITY:

Investigator, Veterinarian, Animal Care Centre Technical Personnel

MATERIALS:

- 16 gauge over the needle catheter (for the carotid artery)
- blood collection tubes (6 x 50ml)
- ketamine 100 mg/ml
- xylazine 20 mg/ml
- 1 ml syringe
- 25 or 23 gauge $\frac{3}{4}$ " needle
- 60 ml syringes (2)
- 500 ml bag 0.9% saline or Lactated Ringers Solution
- IV administration set (10 drops/ml)
- 20 or 22 gauge over the needle catheter (marginal ear vein)
- 4 x 4 gauze sponges
- alcohol
- silk ties (for carotid catheter)
- $\frac{1}{2}$ " tape
- IV pole
- carotid artery pack – scalpel handle, #10 scalpel blade, straight mayo scissors, 2 hemostats

PROCEDURE:

1. Confirm that the correct animal has been selected for bleeds.
2. Advance the IV administration set into the bag of fluids and allow the fluids to flow through the line until free of air bubbles. Hang the fluids from an IV pole.
3. Induce anaesthesia by giving the rabbit an intramuscular (IM) injection of ketamine (35 mg/kg) and xylazine (5 mg/kg), mixed in a 1 ml syringe and using a 25 gauge needle.

4. Wipe the dorsal surface of the ear with alcohol. Visualize the lateral ear vein, located on the lateral margin of the ear where it runs the length of the ear.
5. Hold off the vein close to the base of the ear and advance the catheter through the skin and into the vein at a 30 degree angle. A flow of blood will be present in the hub of the catheter when you are in the vein, at which point the catheter can be pushed over the needle into the vein. The catheter should be pointing towards the heart (base of ear) for the administration of fluids.
6. Secure ½" tape to the catheter and tape the catheter to the ear. Connect the administration set to the ear catheter and open it to check for patency. If the fluid flows easily the fluids are set to a very slow rate (1 drop/10 sec.) until later in the procedure.
7. Make a 5 cm incision over the left side of the trachea and bluntly dissect the fascia free over the left jugular furrow down to the level of the carotid artery. Take care to not damage the jugular vein or the carotid artery.
8. Dissect a 2 cm section of carotid artery free of its attachments and place 2 silk ties under the artery. Tie the distal suture (side closest to the head) on the artery as this will act as a handle for the advancement of the catheter. Loosely knot the other tie with one throw (do not tie the second silk suture around artery YET) as it will later be used to anchor the catheter.
9. Advance a 16 gauge catheter into the artery (with the catheter pointing towards the heart), and when blood appears in the hub of the catheter, advance the catheter over the needle. Tie the second suture around the artery and catheter.
10. Quickly attach a 60cc syringe and withdraw the required amount of blood. A 3-way stopcock may make changing syringes easier.
11. Remove the first 50 ml of blood without the administration of fluids, and clearly mark this on the first 50 ml collection tube. At this point fully open the administration set to allow a maximum flow of fluids into the rabbit as you continue to remove a further 200 ml of "blood."
12. After the required volume of blood has been removed, euthanize the rabbit by giving an injection of pentobarbital (120 mg/kg) intravenously (IV), through the ear vein catheter.
13. Record all drug volumes in the drug record, double bag the animal and place it in the freezer.
14. Securely seal and label the blood tubes and store them in a rack for delivery to the investigator.