

THE UNIVERSITY OF ADELAIDE
STANDARD OPERATING PROCEDURE

RABBIT ANTIBODY PRODUCTION

- **Rabbits must not be held for longer than 6 months and weighed weekly.**
- Rabbits are acquired at approx 12 weeks of age, upon arrival rabbits are given a physical inspection, micro chipped and vaccinated against the Calici Virus.
- After the Calici vaccination, researchers should wait 2 weeks before commencing anti body production.
- Check microchip before commencing any procedure.
- A Technical Assistance Form **MUST** accompany all requests.
- A PRE- BLEED is usually preformed before any immunisations occur, using the Marginal Ear Vein Bleed technique.
- **MARGINAL EAR VEIN BLEED (PRE-BLEED AND TEST BLEED):** Gently restrain the rabbit and check the microchip identification number.
- Draw up 1.0 ml ACP 2 or 0.2 ml ACP 10 using a 25-gauge needle (16mm), expelling all of the air and inject intramuscularly.
- Shave the rabbits ear using a scalpel blade, smear some EMLA CREAM (Lignocaine) on the vein wait at least 5 mins.
- Place the rabbit on a towel and wrap the rabbit firmly to ensure it does not move, put the rabbit under a heat lamp. Flick the marginal ear vein as shown several times, this causes the vein to dilate.
- Hold the ear firmly with one hand, using a 23 gauge winged infusion needle and a 10 ml syringe insert into the vein away from the rabbit's head. The needle should be visible though the wall of the vein.
- Draw the plunger back slightly blood should appear in the tubing, continue slowly but don't pull back the plunger too quickly or the vein will collapse. If the vein does collapse, release the plunger and wait for the vein to refill with blood before recommencing.
- Once the desired amount of blood has been collected, remove the needle and apply pressure to the vein with a tissue until the bleeding stops. Gently squirt the blood into 10ml serum tubes.
- **BLOOD HANDLING:** All blood is put into 10 ml serum tubes; label both the lid and the tube with the rabbit number and researchers name.

- Gently invert tube several times, place in 37°C water bath for at least 30 minutes up to 1 hour allowing for blood to clot.
- Refrigerate blood over night and deliver to MSAH on ice. Label esky with rabbit numbers and researcher's name.
- **IMMUNISATIONS: Always wear eye protection, gloves and gown when handling the antigen and immunising the rabbit.**
- **Change gloves between rabbits.**
- Check rabbit's microchip identification number.
- Shave the rabbits back down to the skin using clippers to allow inspection of the sites, and spray with alcohol.
- Allow alcohol to evaporate; using a texta, mark the positions for the injection sites, ensure that sites are not in close proximity to each other or lesions.
- Using a 18 gauge 25mm needle if solution is thick, or 25gauge needle when protein adjuvant (clear solution) is used, draw up immunisation (if not prepared) and expel air bubbles.
- Inject no more than 1 ml TOTAL VOLUME, of Freund's complete and incomplete adjuvant and antigen mixture, subcutaneous injections should not exceed 0.1ml per site. **Freund's complete antigen must not be used more than once per rabbit.**
- Record all relevant details on "Rabbit Procedure Records" form, such as the amount, and name of antigen mixture to be injected, the type of adjuvant, and the number of sites.
- The number and position of sites must also be noted on the "Rabbit Antibody Production" diagram, plus any lumps and lesions, date and initials of the animal technician. These must be filed with the appropriate Rabbit Procedure Records.
- TEST BLEEDS (max blood vol 5ml/kg) are performed using the Marginal Ear Vein Bleed, Maximum blood volumes must not exceed the following:
MAXIMUM AMOUNT OF BLOOD ALLOWED:
 - 10 % total blood volume or 5.7 mls per kg fortnightly
 - 12.5% total blood volume or 7.1 mls per kg 3 weekly
 - 15 % total blood volume or 8.5 mls per kg monthly
- TERMINAL or CARDIAC BLEEDS are performed when an appropriate titre level has been achieved using the following technique.
- **CARDIAC / TERMINAL BLEED:** Restrain and weigh the rabbit, then check the microchip identification number.

- Draw up 0.25-ml/ kg of Xylazil 20 (pre- med) using a 25 gauge needle (16mm), expelling all of the air and inject intramuscularly.
- Draw up 0.45-ml/ kg of Ketamine using a 25-gauge needle (16mm), expelling all of the air and inject intramuscularly (opposite side to Xylazil).
- When the rabbit becomes unconscious place it on its back with its head facing the your left. Observing the loss of the corneal reflex and pedal reflex can check depth of anaesthesia
- Corneal reflex – gently touch the inner corner of the rabbit’s eye, when under anaesthesia, the rabbit should not blink.
- Pedal Reflex - Squeeze the toes on one of the front paws, observe any reaction. If the rabbit continues to withdraw its paw, wait for 5 minutes, if the rabbit still responds, inject a further 0.2ml of Ketamine (im), and wait for another 5 minutes, then squeeze the toes again until you see no further reaction.
- Feel for the rabbit’s heart beat with your left hand, using a 19-gauge needle slowly insert between the ribs where you can feel the heart beat is the strongest.
- When in the heart, the needle will pulse and blood will appear in the hub of the needle.
- While holding the needle attach a 30ml syringe, drawing back the plunger slowly as it fills with blood, if no blood appears readjust the needle until blood fills the syringe. **Do not create a vacuum in the syringe, as this will burst the red blood cells.**
- When the syringe is full, leave the needle in the heart and remove the syringe.
- Squirt the blood gently into the serum tube, and reattach to the needle and repeat.
- During a cardiac bleed it is important to massage the heart continuously to obtain maximum blood volume. If you cannot obtain any more blood from the rabbit and the heart is still beating, remove the needle and insert another, in the case of a blockage.
- The minimum acceptable amount of blood allowed for a researcher is 80 mls, however more is preferable (above 100 mls) depending on the size of the rabbit.
- When you have obtained the maximum amount of blood from the rabbit leave the needle in the heart (unless it is blocked), draw up 2 mls of Lethabarb and inject into the heart.
- Wait at least 15 mins then confirm that death has occurred before putting the carcass into a biohazard bag and placing into the freezer.
- Dispose of carcass in the medical waste bin.

- All rabbit records must be stapled and put into folder.

DRUGS

A.C.P.2 –	Acepromazine Maleate 2.7mg/ml Equivalent to Acepromazine 2mg/ml
EMLA cream -	Lignocaine 25mg/g Prilocaine 25mg/ml
Ilium Xylzail 20 -	Xylazine as hydrochlorine 20mg/ml
Ketamine -	Ketamine as Hydrochloride 100mg/ml
Lethabarb -	Pentobarbitone Sodium 325mg/ml