

## **Polyclonal Antibody Production**

## Choosing a Species

Rabbits are the species almost exclusively used at the Australian National University for polyclonal antibody production, principally because they are of a size capable of producing adequate amounts of antibodies, have a relatively long life span, and are relatively easy to handle. They are usually suitable for making antibodies for plant, rodent and human proteins and peptides.

Rats are used less frequently than rabbits, principally because of small blood volumes relative to the rabbit. They can sometimes produce antibodies to antigens that are not recognised by rabbits.

Mice are rarely used, because of their small size.

Chickens have been used on occasions with the chicken transferring the antibody to egg yolk. They can sometimes produce antibodies to antigens that are not recognised by rabbits.

## Training and Supervision

The procedures outlined below should only be carried out after appropriate training by animal house staff at JCSMR or RSBS. Assistance may be sought from animal house staff in these procedures. Veterinary advice should be sought in the event of sickness in the animals.

## Choice of Adjuvant

An adjuvant is any substance used to improve the immune response to an antigen. Antigens that are strongly immunogenic will not need an adjuvant. However many antigens used to produce antibodies are not very immunogenic and require an adjuvant to boost the desired response. As a general rule, antigens that are highly conserved over a wide range of species are weak immunogens while antigens that are highly specific to one species are strong antigens in another species. As well as considering its potential to enhance the production of antibodies it is essential that investigators also consider the potential of adjuvants to cause pain and distress to the animal

ANU has trialled a number of adjuvants over the last ten years. Assessing immune response while addressing animal welfare considerations, it has proved difficult to find an alternative to Freund's Complete Adjuvant (FCA) for initial immunisations and Freund's Incomplete Adjuvant (FIA) for booster immunisations. Nevertheless we continue to look for appropriate alternatives.

## Freunds Complete Adjuvant (FCA)

FCA is composed of 1mg/ml heat killed, dried Mycobacterium species added to mineral oil (Bayol F) at 85% and a detergent (Arlacel Ai mannide monooleate) at 10-15%. Freund's complete adjuvant is a potent adjuvant giving good antibody response. However severe hypersensitivity reactions may result in animals after the use of FCA. Therefore FCA should be used only for weakly immunogenic agents and only for initial immunisations with FCA.

## Freunds Incomplete Adjuvant (FIA)

Contains the mineral oil and detergent but does not have Mycobacterium spp included. It is generally used for booster immunisations following initial immunisation, but may be used for the initial immunisation.

## Adverse Effects of Freund's Adjuvants

FCA causes inflammation and local granuloma formation and local site lesions may follow injection of FCA or FIA. The severity of the reaction is dose dependent. Therefore multiple injections of small volumes are the key to avoiding inflammatory reactions.

## Route of Injection into Rabbits

In the rabbit the Freund's plus antigen must be injected subcutaneously in at least 4 sites with a maximum of 0.25 mls of the combination in each site and with the total not to exceed 1ml. It is beneficial to massage the area after injection to disperse the injected material over a wider area.

## Immunisation Schedule for Rabbits

At least 4 weeks is recommended between booster injections. A maximum of 6 immunizations is recommended, if the antibody response is not sufficient after 6 immunizations it is unlikely more immunizations will improve the response. A new animal may be used, as there is great variation in antibody response between individuals. Rabbits **must not** be bled at intervals of less than 2 weeks.

Animals are to be bled and killed once a sufficiently high titre of antibody is obtained. More immunizations after obtaining a high antibody titre can desensitize the animal and reduce the titre.

The recommended time animals are to be held is three months. The maximum holding time is 6 months.

Rabbits will be checked by animal house staff on a daily basis. Observation should include close inspection for injection site reaction.

### **Blood Sampling of Rabbits**

The recommended vessel for blood collection is the middle ear artery which is the vessel running down the centre of the external ear. Acetyl promazine injection (1mg/kg IM) 30 minutes before blood collection will cause peripheral vasodilation and make more accessible the entry of a 21 gauge needle.

If blood is collected 4 weekly or less, 10% of blood volume may be collected. If blood is collected at more than 4 weekly periods then 15% of blood volume may be collected. For the purpose of this exercise the blood volume of a rabbit averages 65 ml/kg, therefore 10% of the blood volume of a 3kg rabbit is 19.5mls. 15% of a 4 kg rabbit's blood volume is 39 mls. Bleeding must be done through the use of a hypodermic needle (Recommended gauge 21). A 21 gauge butterfly needle is readily used. **Cutting of blood vessels for blood collection purposes with a scalpel blade is not permitted.**

### **Terminal Bleeding**

Terminal bleeding of rabbits will be the responsibility of local animal house staff. Please arrange this through your local animal house supervisor. Terminal bleeding is done under general anaesthesia using approved technique, obtaining blood directly from the heart or by cannulation of larger vessels in proximity too the heart. Animals will be euthanized without regaining consciousness.