

## **ANTIBODY PRODUCTION: PRINCIPLES FOR PROTOCOLS OF MINIMAL SEVERITY**

*These notes provide information appropriate to protocols of minimal severity for raising antibodies using living animals. Where protocols of greater severity can be justified in a project licence application, consideration will be given on a case by case basis for licence authority to cover such procedures.*

The following points should be noted:-

### **Primary immunisation**

1. It may be necessary to combine the antigen with an adjuvant in order to enhance the antibody response. A range of adjuvants are available; one should be chosen which will stimulate antibodies of the desired affinity, avidity, titre and class, with minimal local tissue damage. Some adjuvants, such as Freund's Complete Adjuvant (FCA), cause significant local reactions and should be used as set out below. No adjuvants should be used via the intravenous route, although antigen in PBS may be given intravenously, usually as the final booster inoculation. The intradermal route should be avoided when adjuvants are used.

2. When a significant local reaction is expected, such as with FCA, the antigen/adjuvant mixture should be given subcutaneously in areas of loose skin and doses should not exceed 0.1 ml at each of 2 sites in mice, 0.2 ml at each of 2 sites in rats and 0.25 ml at each of 4 sites in guinea-pigs, rabbits, sheep, goats and equids. FCA should never be used on more than one occasion in the same animal. Stable emulsions should be used with no more than 50% FCA mixed with antigen in aqueous solution. FCA should not be used in horses or other equids. The intramuscular route may be used in chickens and should not exceed 0.1 ml in each of four sites.

### **Boosting**

3. In order to raise or maintain the antibody titre it may be necessary to administer the antigen on one or more further occasions. These 'boosters' should conform to the principles set out for primary immunisation, but must not include FCA, and should be no more in number than required to achieve and maintain the required titre. Animals that fail to respond within four 'boosts' should be withdrawn from the protocol.

### **Sampling**

4. Superficial blood vessels are usually adequate for blood collection during monitoring of antibody titre in all species. No more than 15% of total blood volume (TBV) should be taken over any four-week period and usually no more than 10% TBV should be removed as a single collection. The TBV of laboratory animal species averages 65 ml/kg. Consideration should be given to the use of local or general anaesthesia as appropriate for the species.

### **Harvesting**

It is recommended that when the antibody titre has reached a plateau, rodents and rabbits should be bled out under terminal general anaesthesia. The serum can then be separated, divided into aliquots and deep frozen. Eggs should be collected during a laying season, but moulting must not be induced. Where serial harvesting is appropriate, the volume of blood collected should be limited as set out above, unless special arrangements such as haematological monitoring can be included in the licence authority.