

**THE UNIVERSITY OF MELBOURNE  
ANIMAL WELFARE COMMITTEE**

**GUIDELINES ON POLYCLONAL ANTIBODY PRODUCTION**

**Purpose**

The purpose of this document is to provide guidelines for the production of polyclonal antibodies in animals at The University of Melbourne. They are designed to assist researchers, animal technicians and Animal Ethics Committees (AECs) to ensure that the scientific aims of a proposal to produce polyclonal antibodies are achieved with minimal discomfort for the animals involved. Appendix A provides the background information on which the guidelines are based. When Freund's adjuvant is to be used, the Guidelines on the use of Freund's Adjuvant should also be read.

**Policy**

All scientific procedures carried out on animals must comply with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (2004).

**Guidelines**

***General***

When producing polyclonal antibodies, the overriding consideration must be to minimise the pain and distress experienced by the animals.

***Training***

Investigators and animal technicians involved in polyclonal antibody production must have appropriate training in all the techniques they are required to perform.

***Animal Selection***

Careful consideration should be given to the selection of the species to be used.

The species of animals selected should be based on the amount of antibody required, the intended use of the antibodies to be produced, and the characteristics of the antigen concerned.

***Immunisation Protocol***

The AEC must evaluate immunisation protocols with respect to animal welfare outcomes and the 3Rs (replacement, reduction, refinement). The committee should be satisfied that reasonable attempts have been made to ensure that the antibody is not available commercially or from another research group. Standard operating procedures for routine production of polyclonal antibody should be established and presented to the AEC for approval.

***Antigen Production***

The antigen should be prepared so that a suitable antibody response can be obtained without adversely affecting the well-being of the animals.

***Choice of Adjuvant***

The decision to use adjuvants should be carefully considered and justified. The most appropriate adjuvant for the antigen of interest should be sought.

***Route of Injection***

The route of injection must be selected with the aim of causing the least pain and distress for the animal.

***Volume and Number of Injections***

The injection volume should be as small as possible and divided over multiple, suitably spaced sites.

***Blood Collection***

The blood collection procedure should be selected with the aim of minimising stress for the animals.

***Monitoring of Animals***

Animals must be monitored daily and records of observations and interventions must be maintained.

## APPENDIX A

### **Background to Guidelines**

#### ***Training***

Guideline: Investigators and animal technicians involved in polyclonal antibody production must have appropriate training.

All personnel involved in the immunisation protocol should have a basic understanding of immunological principles and be experienced in the handling, injecting, anaesthetising and bleeding of the animal species used for antibody production. Investigators and animal technicians should be capable of recognising signs of pain and distress in the animals, and must have a management plan for dealing with adverse effects when necessary.

#### ***Animal Selection***

Guideline: Careful consideration should be given to the selection of the species to be used.

The species of animals selected should be based on the amount of antibody required, the intended use of the antibodies to be produced, and the characteristics of the antigen concerned.

The rabbit is the most commonly used species for the production of polyclonal antibodies. In general, rodent species are used less frequently than rabbits but they are a suitable choice when only small quantities of antibody are required. Larger species (eg sheep, goats, horses) may be used for the production of large volumes of antibody. Chickens may also be a suitable choice in some circumstances and their use represents both a refinement of the technique, since blood collection to obtain antibodies is not required, and a reduction in animal use as chickens produce larger quantities of antibodies than rodents.

Where species-specific considerations are not an issue, then species selection should be driven by the need to minimise pain and distress in the animals. Factors to consider are the nature of the antigen/adjuvant formulation, the species, strain, health and genetic status of the animals, availability of suitable housing, the training and level of competence of the personnel, the ease of handling of the animals and the method of blood collection.

#### ***Immunisation Protocol***

General Guideline: The AEC must evaluate immunisation protocols with respect to animal welfare outcomes and the 3Rs. The committee should be satisfied that reasonable attempts have been made to ensure that the antibody is not available commercially or from another research group. Standard Operating Procedures for routine production of polyclonal antibody should be established and presented to the AEC for approval.

Factors which must be addressed in the standard operating procedure are

- The method of antigen preparation
- Use of adjuvant – whether to use and which one to use
- Route of injection and number of sites used
- Volume to be injected
- Schedule of injections and blood collections
- Length of time an individual animal will be held

### ***Antigen Production***

Guideline: The antigen should be prepared so that a suitable antibody response can be obtained without adversely affecting the well-being of the animals.

The antigen should be sterile, contain minimal toxic contaminants and of a pH adjusted to within physiological limits. The quantity of antigen to be injected must be determined on the basis of the species and strain of animal to be immunised, the adjuvant used, the route and frequency of injection and the immunogenicity of the antigen itself.

### ***Choice of Adjuvant***

Guideline: The decision to use adjuvants should be carefully considered and justified. The most appropriate adjuvant for the antigen of interest should be sought.

Adjuvants have the potential to cause considerable pain and/or distress to animals. Therefore, it should be first determined whether an adjuvant is required.

When adjuvants are used, the antigen/adjuvant mixture should be easily injectable in small volumes and should have low toxicity. In general, an antigen should be mixed with an equal volume of adjuvant. The broad categories of adjuvants, based on the components used in their formulation, are shown in Table I.

Table I: Overview of Categories of Adjuvant that May Be Used for Routine Polyclonal Antibody Production

Category	Examples
Oil-based (water-in-oil, oil-in-water, water-in-oil-in-water)	Complete Freund's adjuvant, incomplete Freund's adjuvant, TiterMax™, RIBI™
Mineral-based	Al(OH) <sub>3</sub> , AlPO <sub>4</sub>
Microbial products	LPS, MDP, MPL, TDM
Saponins and ISCOMs	Quil A

*LPS = lipopolysaccharide; MDP = muramyl dipeptide; MPL = monophosphoryl lipid A ; TDM = trehalose dimycolate; DDA = dimethyldioctadecylammonium bromide; NBP = non-ionic block polymer; ISCOMs = immune stimulating complexes.*

While CFA is one of the most effective adjuvants, it has the potential to cause significant undesirable side effects and therefore should only be used where there is evidence that other adjuvants will not work. When the use of CFA is unavoidable, it must only be for the primary immunisation, and in accordance with the University of Melbourne *Guidelines on the use of Freund's adjuvant*.

### ***Route of Injection***

Guideline: The route of injection must be selected with the objective of causing the least pain and distress for the animal.

The most suitable route of injection will be influenced by the species selected, the nature of the antigen and whether or not an adjuvant is required. Generally, the most appropriate routes of injection will be subcutaneous, intravenous or intraperitoneal. Injection into any closed space can be painful and may induce unwanted sequelae, so the intramuscular route should be questioned as a route of choice. Intradermal injections are not permitted.

Table II: Suggested Routes of Injection

Primary Injection		Booster Injection(s)	
With Adjuvant	Without Adjuvant	With Adjuvant	Without Adjuvant
s.c.	s.c.	s.c.	s.c.
i.m.	i.m.	i.m.	i.m.
	i.v.		i.v. <sup>a</sup>
	i.p.		i.p. <sup>a</sup>

s.c. = subcutaneous, i.m. = intramuscular, i.p. = intraperitoneal, i.v. = intravenous.

<sup>a</sup>With i.v. and i.p. booster injections, there is a risk of inducing anaphylactic shock in the animals.

#### *Subcutaneous injection*

The subcutaneous route is preferred for the injection of most adjuvant formulations.

#### *Intramuscular injection*

The intramuscular route should not be the first choice for injection of depot-forming adjuvants in small laboratory animals.

#### *Intraperitoneal injection*

The intraperitoneal route is not recommended when depot-forming adjuvants are used. Use of this route should be scientifically justified to the AEC on a case-by-case basis.

#### *Intravenous injection*

The intravenous route is not recommended when depot forming-adjuvants are used, because there is a high risk of lethal complications due to embolism.

#### *Footpad, intra-lymph node or intrasplenic injection*

These routes of injection are not necessary for routine polyclonal antibody production.

### ***Volume and Number of Injections***

Guideline: The injection volume should be as small as possible and divided over multiple, suitably spaced sites.

In all cases the sites for injection should be aseptically prepared and sufficiently separated so that blood supply to the area is not disrupted due to overlapping inflammatory lesions.

The time between immunisations should be at least 4 weeks (when a depot-forming adjuvant is used for the primary immunisation) or at least 2 weeks (when a non-depot-forming adjuvant in the primary immunisation). An adjuvant is not always needed for booster immunisations.

Animals can be rested between boosting but they must not be kept in an antibody production program unnecessarily.

The volume of injection should be as small as possible.

Table III: Maximum Volumes for Injection of Antigen/Depot-Forming Adjuvant Mixtures per Site of Injection for Different Animal Species

Species	Maximum Volume per Site	Maximum Total Volume	Primary Injection	Subsequent Injections
Mice	50 µL	200 µL s.c. 100 µL i.p.	s.c., i.p.	s.c., i.p.
Rats	100 µL	400 µL s.c.	s.c.	s.c.
Guinea-pigs	200 µL	400 µL s.c.	s.c.	s.c.
Rabbits	250 µL	1 mL	s.c.	s.c.
Sheep, goats, pigs	500 µL		s.c., i.m.	s.c., i.m.
Chickens	250 µL	1 mL	s.c.	s.c.

s.c. = subcutaneous, i.m. = intramuscular, i.p. = intraperitoneal

### ***Blood Collection***

Guideline: The blood collection procedure should be selected with the aim of minimising stress for the animals.

The blood collection procedures selected should reflect the training and expertise of the personnel carrying out the procedure and the species of animal being bled. The maximum volume of blood collected from a conscious animal should not exceed 1% of the animal's bodyweight. Exsanguination must be performed under non-recovery general anaesthesia after which the animal must be euthanased according to UMAWC guidelines.

### ***Monitoring of Animals***

Guideline: Animals must be monitored daily and records of observations and interventions must be maintained.

An immunisation record and a daily checklist for monitoring animals should be incorporated into the standard operating procedure for polyclonal antibody production. Food and water intake, activity, general appearance and responses at the injection sites should be monitored. The SOP should include procedures for intervention in the event of adverse reactions.

### **References**

Canadian Council on Animal Care (2002). Guidelines on: antibody production

Hanley, W.C., Artwohl, J.E. & Bennett, B.T. (1995). Review of Polyclonal Antibody Production Procedures in Mammals and Poultry, Institute for Laboratory Animal Research Journal 37:93-118

Leenars, M.P.P.A., Hendrickson, C.F.M., De Leeuw, W.A., et al (1999). The production of polyclonal antibodies in laboratory animals: The report and recommendations of ECVAM Workshop 35. Alternatives to Laboratory Animals 27(1):79-102  
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