

**UNIVERSITY OF CALIFORNIA SAN DIEGO  
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

**Policy and Guidelines on Adjuvants  
and Polyclonal Antibody Production**

The Principal Investigator must provide a specific rationale for selection of species, adjuvant, route, sites and handling of antigens when completing the Animal Use Protocol.

When an adjuvant is necessary to accomplish experimental goals, the investigator should select the adjuvant that causes the least amount of associated pain and discomfort. Therefore, the use of other adjuvants causing less inflammation than complete Freund's adjuvant (CFA) is desirable.

Repeated inoculations of CFA, OR footpad, lymph node or intradermal inoculations with CFA are not acceptable unless scientifically justified and approved in advance by the IACUC. The P.I. must provide data showing that usual methods do not give the result necessary for the experiment(s) proposed and that the requested method would give the desired result. Note that quantity of antibody is not sufficient justification.

**Adjuvants**

Freund's Complete Adjuvant (CFA) can cause severe inflammation and ulceration at the site of injection if used improperly. CFA should be used only for the initial immunization, with Freund's Incomplete Adjuvant (IFA) used for subsequent booster injections. Other adjuvants should be considered before CFA and IFA. CFA should only be used if no appropriate alternatives are available.

- **Freund's adjuvant**

FIA consists of 85% mineral oil or paraffin oil and 15% mannide monooleate (Arlacel A) as emulsifier. With the addition of heat-killed mycobacteria (*M. butyricum* or *M. tuberculosis*) the mixture is termed CFA. CFA is known to commonly produce undesirable side effects including granuloma formation, tissue necrosis and sloughing, abscesses, and fever. Other deleterious systemic effects, such as polyarthritis, have been reported. CFA is considered a human biohazard, such that accidental self inoculation, or splash in the eye have been shown to cause painful sequelae not readily amenable to treatment.

- **Other Adjuvants**

Less problematic alternatives to Freund's adjuvant are available and should be considered. RIBI Adjuvant System®, Specol®, TiterMax®, Montanide IAS50, and Montanide ISA70 are commonly used as appropriate alternatives. Noninflammatory adsorptive adjuvants such as alum and aluminum hydroxide gel may also be considered.

**Routes of Administration, Volume, Sites**

Consideration and justification must be given in the animal use protocol for selection of the laboratory animal species, adjuvant, volume per injection site, site of administration, number of sites, and response required. Particularly with the use of Freund's adjuvant, it is important to note that the severity of potentially painful inflammatory reactions may be minimized by injection of a small volume of inoculum per site and the use of multiple, sufficiently separated, injection sites when appropriate.

- **Routes of Administration**

Injections should be subcutaneous or, in rodents, intraperitoneal. Choice of other routes, such as intradermal are discouraged and must be scientifically justified by the investigator.

For multiple subcutaneous sites, not more than 0.25 ml per SC site should be used for rabbits, 0.5 ml SC for sheep and goats, and 0.1 ml SC or 0.2 ml IP for mice. It is recommended that no more than five sites are used.

If intradermal injections are scientifically justified by the P.I. and approved by the IACUC, no more than 0.05 ml may be injected at a site. Sites should be well separated to prevent consolidation of inflammatory responses.

Subcutaneous inoculations should not be done in areas over bony protruberances such as the spine. No injections should be done in the foot or footpad.

- **Frequency of Boosters**

The frequency of boosters must be addressed in the animal use protocol. Two to three weeks is generally considered the minimum time period between the initial and subsequent immunizations. Booster immunizations cannot use CFA.

- **Monitoring**

It is the Principal Investigator's responsibility to ensure the animals are regularly checked. This is in addition to the daily checking done by animal technicians. Investigators should observe the animals for evidence of pain or distress, and for evidence of lesions such as swelling, abscess or fistula formation, and infection or ulceration at the immunization sites and the ACP veterinary staff should be notified if these clinical problems are found. The animal weight should periodically be compared to initial animal weights and this should be indicated in the protocol and documented. Sites of inoculation must be examined daily or an alternate schedule must be described in the Animal Use Protocol and approved by the IACUC.

- **Records**

The date of each injection and any adjuvant used must be recorded on the cage card, in a chart in the animal room, or on procedure stickers.

## Blood collection Guidelines

- **Sampling routes**

In rabbits, large blood samples may be collected via the central auricular artery. Small blood samples of 5 ml or less may be obtained from the marginal ear vein. In rodents the tail veins or lateral saphenous vein are most easily accessed. Retro-orbital bleeding must be done under general anesthetic unless P.I. describes and gives scientific justification within the protocol. Cardiac puncture may only be done as a terminal procedure under general anesthetic.

- **Vasodilation**

Do not use xylene or other inflammatory agents as vasodilating agents. Radiant heat or warm water is the recommended alternative. Anesthesia is not required for titer sampling using recommended routes.

- **Blood collection in Rabbits:**

Total blood volume = 6% of lean body weight

Maximum blood collection = 20% of total blood volume every two weeks

4 lb rabbit	=	1.80 kg x 0.06	=	108 ml x 0.20	=	21.6 ml
6 lb rabbit	=	2.72 kg x 0.06	=	163 ml x 0.20	=	32.6 ml
8 lb rabbit	=	3.60 kg x 0.06	=	216 ml x 0.20	=	43.2 ml
10 lb rabbit	=	4.50 kg x 0.06	=	270 ml x 0.20	=	54.0 ml

Rabbits may follow the above bleeding schedule as long as packed cell volume (PCV) and total plasma proteins (TPP) are monitored every 2 months. The occurrence of anemia, hypoproteinemia, or unthriftiness require appropriate supplementation and a rest from further bleeds. The duration of this rest will be determined by the attending veterinarian. Animals should be weighed monthly if on the above bleeding schedule and their weights recorded appropriately.

- **Blood Collection in Mice**

Total blood volume = 6% of lean body weight.

Maximum blood collection = 10% of total blood volume every two (2) weeks.

Example: 30 gm mouse = 0.03 kg x 0.06 = 1.8 ml x 0.10 = 0.18 ml

- **Blood Collection in Rats**

Total blood volume = 6% of lean body weight.

Maximum blood collection = 10% of total blood volume every 2 weeks.

Example: 250 gm rat = 0.25 kg x 0.06 = 15 ml x 0.10 = 1.5 ml