	UCSD INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE POLICY MANUAL	POLICY #3.03 Originally Issued: 7/12/02 Revised: 11/17/04 Revised: 9/17/14
	Policy on Use of Adjuvants	

I. Background and Purpose

The University of California is committed to the improvement of human and animal health and the advancement of science. When these endeavors involve the use of adjuvants in animals, UCSD is committed to their humane care and use as specified in the Guide for the Care and Use of Laboratory Animals, the PHS Policy and the Animal Welfare Act.

II. Who Should Read This Policy

All personnel engaged in or responsible for adjuvant administration in animals.

III. Definitions

Term	Definition
Adjuvant	A compound used to induce or enhance an immunological response such as CFA, IFA, RIBI Adjuvant System®, Specol®, TiterMax, etc.
Complete Freund's Adjuvant	A solution of antigen emulsified in mineral oil and used as an immunopotentiator (booster); composed of inactivated and dried mycobacteria (usually M. tuberculosis)
Incomplete Freund's Adjuvant	A solution of antigen emulsified in mineral oil and used as an immunopotentiator (booster); lacks the mycobacterial components (hence just the water in oil emulsion)

IV. Policy

1. 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.

2. When an adjuvant is necessary to accomplish experimental goals, the investigator must select the adjuvant that causes the least amount of pain and discomfort consistent with experimental goals. If using Complete Freund's Adjuvant (CFA), the Principal Investigator must explain why a less problematic adjuvant cannot be used.

3. The preferred route of adjuvant administration is subcutaneous. Alternate routes such as footpad, lymph node or intradermal inoculations with CFA or repeated inoculations of CFA are not acceptable unless scientifically justified and approved in advance by the IACUC. The P.I. must provide data showing that other routes of administration do not

give the result necessary for the experiments proposed. Note that quantity of antibody produced is not sufficient justification.

4. **Volume, Sites and Frequency -** Description and justification for the volume per injection site, site of administration, number of sites, and frequency of boosters must be provided in the animal use protocol.

5. **Monitoring –** Laboratory personnel must monitor animals daily for clinical signs (i.e. pain, distress, swelling, abscess, infection, ulceration, weight loss, etc.). If appropriate, an alternate and less frequent monitoring schedule may be permitted if justified and approved in the animal use protocol. The above monitoring requirements are not met by the routine daily health checks performed by animal technicians.

6. **Records -** Records of procedures and monitoring must be maintained (https://iacuc.ucsd.edu/policies/PIRecord-keeping.pdf).

V. Related Documents

Blood Collection Policy	http://blink.ucsd.edu/_files/sponsor-tab/iacuc/Policy%205%20Blood.pdf
PI Record Keeping Requirements	https://iacuc.ucsd.edu/policies/PIRecord-keeping.pdf
Animal Records Retention	http://ucop.edu/research-graduate-studies/_files/research/policies/documents/retention_disposition_reqs.pdf

VI. Additional information

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**
  IFA 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 splashes in the eyes 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 and Frequency of Administration Guidelines

- Injections in most species should be subcutaneous, or in rodents subcutaneous or 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 for subcutaneous 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 protuberances such as the spine. No injections should be done in the foot or footpad.

- Two to three weeks is generally considered the minimum time period between the initial and subsequent immunizations.

Blood Collection Guidelines

Blood collection guidelines can be found at http://blink.ucsd.edu/_files/sponsor-tab/iacuc/Policy%205%20Blood.pdf.