Arizona State University
Institutional Animal Care and Use Committee
STANDARD INSTITUTIONAL GUIDELINE

ANTIBODY PRODUCTION IN RODENTS

Antibody production in rodents, whether performed by DACT personnel or investigators, will follow the protocol described here unless alternate methods are described in an IACUC-approved protocol.

Adjuvant use

Adjuvants greatly enhance the magnitude and duration of antibody response and promote the more desirable secondary (IgG) response. The use of Freund's complete adjuvant (FCA), the traditional adjuvant, has been associated with pain and distress in rodents. Therefore, the use of FCA is discouraged. The use of alternative adjuvants (e.g., Alum, Titermax or agonists such as TLR-7 or CD40) is highly encouraged. If FCA is used, its use must be restricted to the primary injection with subsequent booster injections using no adjuvant or Freund's incomplete adjuvant (FCA but without *Mycobacteria*).

Antigen delivery

To minimize negative side effects, investigators should use aseptic conditions, select the proper site of injection, and inject the smallest volume possible. Regardless of route of delivery, the inoculum should be as contaminant free as possible (i.e., filter through a 0.2 micron filter) and adjusted to a biologic pH to reduce the likelihood of an excessive inflammatory response. Maximum injection volume per injection site and maximum total volume are as follows:

Species	**subcutaneous (ml)***	**intradermal (ml)**	**intramuscular (ml)**	**intraperitoneal (ml)**	**Intranasal (ml)****
mouse	0.1 / 0.2	NR	NR	0.20	0.010
rat	0.25 / 0.5	NR	NR	2.0	0.020
Syrian hamster	0.25 / 0.5	NR	NR	1.0	----
guinea pig	0.5 / 1.0	NR	NR	2.0	----

* maximum volume per site / maximum total volume
** maximum volume per nostril
NR = Not Recommended

Injections are usually given in three doses - the initial dose on day 1, then booster doses at approximately day 7 to 10 and day 21 to 30. Subsequent boosters are occasionally necessary based on the animal's titer. Alternative injection schedules may be desired or necessary depending on the adjuvant chosen, and such details should be provided in the IACUC protocol.

Blood collection

Sites for non-terminal blood collection should be disinfected prior to collection. The volume of blood removed at any one bleeding must not exceed 1% of the animal's body weight (e.g., a maximum of 0.3

ml can be drawn from a 30 g mouse and 10 ml from a 1.0 kg guinea pig). When collecting serial samples, total blood volume collected over 30 days must not exceed 1.5% of the animal's body weight. Test bleeds for antibody monitoring must be included when determining the total blood volume collected.

Rodents can be non-terminally bled from the tail vein, lateral saphenous vein, or submandibular vein using a syringe needle (or a lancet for submandibular) and microhematocrit or microcentrifuge tubes. Vasodilation of the tail vein can be accomplished by immersing the tail in warm (not hot) water for a couple minutes and drying prior to blood collection. For more details on the recommended routes of blood collection, see the separate SIGs for "Blood Collection in Rodents Using the Lateral Saphenous Vein" and "Blood Collection in Mice Using the Submandibular Vein." Retro-orbital bleeding is greatly discouraged and is only permissible with strong justification and must be done under anesthesia. Due to the risk of cardiac tamponade or pulmonary hemorrhage, cardiocentesis (e.g., cardiac puncture) in mammals is only permissible for terminal bleeding.