

Guidelines on Acceptable Immunological Procedures

The University of Florida has adopted the Canadian Council on Animal Care Guidelines on Acceptable Immunological Procedures.

General comments:

Many adjuvants are non-pharmaceutical grade agents, however the IACUC requires that pharmaceutical-grade components be used when available (e.g., saline) and that methods to preclude bacterial contamination be employed according to current IACUC Guidelines.

The use of sterile needles and syringes is required.

Freund's adjuvant results in significant pain or distress, investigators must consider alternatives. When using Freund's Complete Adjuvant (FCA) the host response to the oil and the mycobacterium can result in both local and disseminated intense granulomatous (inflammatory) reactions.

General guidelines

1. Non-inflammatory adjuvants or adjuvant that produce less intense responses but still produce a humoral antibody response should be used if possible.
2. The concentration of Mycobacterium in the CFA antigen-adjuvant emulsion should be 0.5 mg/ml or less. CFA is available in different strengths. The most common is 1 mg/ml, which when mixed 1:1 with the antigen preparation yields the maximum concentration of 0.5 mg/ml of Mycobacterium.
3. CFA is only necessary for the initial immunization; Incomplete Freund's Adjuvant (IFA) can be used in subsequent immunizations -boosters.
4. The priming dose with CFA should be followed by boosters given either (1) as a response to confirmed waning antibody titers or (2) empirically once between 4-8 weeks after priming and once again 2-3 weeks after the first booster. If additional boosters are necessary, IACUC approval with justification is required
5. The investigator should know the characteristics of the antigen and avoid factors that will excessively stimulate or inhibit the inflammatory effect. Extraneous microbial and protein contaminants, pH extremes, and chromatographic by-products, may lead to a low titer.
6. Filtration of the antigen preparation through a low binding 0.22 micron filter (i.e., cellulose acetate) should be done whenever possible.
7. Antigen prepared by gel electrophoresis should be eluted, lyophilized, ground to a fine powder, and resuspended in sterile saline or transferred to nitrocellulose paper, trimmed and cut into fine pieces.
8. The adjuvant should be injected subcutaneously in small doses (see Table 1). For optimal immune-stimulation it is recommended that the injections to non-rodents be distributed over the 4 quadrants of the animal (i.e., bilaterally, on the side behind the shoulder and in front of the hind leg).
9. Bilateral injections at the base of the tail in rats and mice often give optimal immunological response.

Foot Pad Injections

4/28/2008

Guidelines on Acceptable Immunological Procedures

10. Intradermal and intramuscular injections are discouraged and must be specifically justified.
11. The recommended maximum total injection dose and amount at each site is shown in Table 1.
12. Animals with lesions resulting from previous immunizations must not be boosted by the same route prior to resolution of pre-existing lesions.

FCA must never be given either intravenously or in repeated doses and never placed into the foot pads of rabbits.

ROUTES OF INJECTION:

Intradermal Route:

Sound scientific evidence and justification must be available if the intradermal route of injection of FCA is to be used, because of the frequent ulceration that occur at the sites. The use of the intradermal route is generally only justified when the purpose is to induce cell-mediated response.

In rabbits, volumes of inoculum must not exceed 0.05 mls (50 microliters) per site. The location should be carefully selected so as to prevent mutilation. A minimal number of sites should be selected, and the distance between each site should be maximized.

The intradermal route is inappropriate in rodents.

Subcutaneous Route: See Table 1 for guidelines

Table 1. The Maximum Total Volume/Animal and Maximum Amount of Adjuvant-Antigen Emulsion at Each Subcutaneous Injection Site.

SPECIES	MAXIMUM TOTAL INJECTION (ML)	MAXIMUM AMOUNT (ML)/SUBCUTANEOUS SITE
Mice	0.3	0.05
Rat	0.5	0.1
Chicken	0.5	0.1
Guinea Pig	1.0	0.1
Rabbit	1.0	0.1
Goat/Sheep*	2.0	0.2
Primates **		

Guidelines on Acceptable Immunological Procedures

* Deep intramuscular injections at a maximal volume of 0.5 ml antigen-adjuvant emulsion per site are permitted in large domestic animals.

** Freund's Complete Adjuvant is not recommended for use in primates. It causes an excessive inflammatory response and negates TB testing in treated animals.

Intramuscular Route:

In rabbits, intramuscular injections of FCA may be administered in the thigh muscle; up to .5 ml (500 microliters), preferably only one site being used site.

Intramuscular injection of FCA is not recommended for small laboratory animals such as rats, mice, hamsters, gerbils, etc.

For larger animals, such as cats, dogs and poultry, up to 1 ml of FCA injected into the thigh muscle is acceptable. In livestock such as pigs, cattle, sheep and goats, the intramuscular route is acceptable.

In avian species, the common routes of injection of FCA are intramuscular and subcutaneous.

Intraperitoneal:

Peritoneal Exudates

Intraperitoneal administration of antigen and adjuvant is often used in rodents to obtain high titered reagent or monoclonal antibodies. The undesirable side effects of painful abdominal distention associated with development of the peritoneal exudates can be readily avoided by daily monitoring and relieving ascites pressure as appropriate. Please refer to the *IACUC Guidelines on Monoclonal Antibody Production* for methodology for this procedure

The intra-peritoneal route for injection of FCA is permitted on small rodents only. FCA should be administered only once and be limited to minimal volumes up to a maximum of 0.1 ml (100 microliters).

Intravenous Route:

Freund's Complete Adjuvant is **not** to be injected intravenously.

Footpad Injection:

Investigators proposing to use footpad and/or IP injections in any species must provide scientific rationale to the IACUC.

Footpad injection is not permissible in the rabbit.

Guidelines on Acceptable Immunological Procedures

Footpad injection of CFA in rodents is not permissible. Subcutaneous injection near the footpad in the lateral tarsal region is considered to be as effective as footpad injections and is allowed.

In rats and mice, only one foot may be used. Animals should be maintained on soft bedding.

Front feet should not be used for footpad immunization.

If scientific justification is provided, the recommended maximum injection volumes are 0.01 ml in mice and 0.10 ml for rats.

ALTERNATIVE TECHNIQUES

Antibody production in chickens is an alternative to the use of other animals for polyclonal antibody production. Antibody production in chickens offers the advantage of obtaining antibody through a non-invasive method (egg yolk).

Another alternative method in rabbits consists of placing a subcutaneous whiffle ball chamber. Immunizations are made directly into the chamber and antibody-rich fluid is harvested from the chamber. This procedure does require surgical placement of the chamber.

Observation of Injection Sites:

Investigators or their staff must observe animals daily, including weekends and holidays, for the following: pain, swelling, abscessation, fistula formation, infection, and/or ulceration at or near the immunization site(s) lameness or loss of body condition, additionally in rodents non-specific observations should include the common signs of pain and distress in rodents include: 1) ruffled or “spikey” fur (mouse looks unkempt); 2) weight loss which may be mild to severe (cachexia), anorexia, dehydration; 3) ocular discharge; 4) lethargy, depression, or reluctance to move; 5) sitting with the back in a hunched position result in ACS veterinary evaluation . Investigators identifying animals with the above signs must contact a member of ACS’s veterinary staff immediately

Since rabbits are the most commonly used species for polyclonal antibody production and are a USDA covered species records must be kept. Information that must be recorded in the animal’s record includes, but is not limited to, sedation/anesthetic events, blood collection, compound administration, and euthanasia. When administering any compound, the compound’s name, volume administered, and the site(s) where administered must be recorded. Rabbits maintained as part of long-term (>3 months) projects are expected to have periodic health examinations.

Guidelines on Acceptable Immunological Procedures

References:

Allison, AC; and Byars, NE. 1991. Immunologic adjuvants: Desirable properties and side-effects. *Mol. Immunol.* 28(3):279-284.

Bennett, B.; Check, IJ; Olsen MR; and Hunter, RJ. 1992. A comparison of commercially available adjuvants for use in research. *J. Immunol.Methods* 153(1-2):31-40.

Broderson, JR. 1989. A retrospective review of lesions associated with the use of Freund's adjuvant. *Lab. Anim. Sci.* 39:400-405.

Canadian Council on Animal Care (CCAC). 1993. CCAC Guidelines on acceptable immunological procedures. Ottawa, Ontario, Canada:

Hanly, WC; Artwohol, JE; Bennett. 1995. Review of Polyclonal Antibody Production Procedures in Mammals and Poultry. *ILAR Journal* 37(3): 93-118.

Lipman, NS; Trudel, LJ; Murphy, JC; and Sahali, Y. 1992. Comparison of immune response potentiation and in vivo inflammatory effects of Freund's and Ribi adjuvants in mice. *Lab. Anim. Sci.* 42:193-197.

Smith, DE; O'Brien, ME; Palmer, VJ; and Sadowski, JA. 1992. The selection of an adjuvant emulsion for polyclonal antibody production using a low-molecular weight antigen in rabbits. *Lab. Anim. Sci.* 42(6):599-601.

Stills, HF; and Bailey, MQ. 1991. Use of Freund's complete adjuvant. *Lab. Anim.* 20(4):25-30.