



## A. PURPOSE

To standardize the acceptable uses of Complete Freund's Adjuvant (CFA).

## B. GUIDELINES

### Use of Complete Freund's Adjuvant as an adjuvant

- Local irritation, pain, and distress are often associated with adjuvants used in antibody production. These side effects should be viewed as unnecessary, and not as normal events. High quality antibodies can be obtained while minimizing the deleterious effects of adjuvants on the animal. Complete Freund's Adjuvant (CFA) is a potent inflammatory agent, and the IACUC requires that investigators scientifically justify why an alternative adjuvant cannot be used instead of CFA. These systems include, but are not limited to, TiterMax, RIBI Adjuvant System, Montamides, Syntex Adjuvant Formation, Incomplete Freund's Adjuvant (IFA), aluminum compounds, subcutaneously implanted chambers and others.

### In cases where CFA is scientifically justified, the following guidelines must be followed:

- CFA must only be used in the primary injections, using sterile technique to prepare the CFA-antigen emulsion.
- Before injection, the skin must be swabbed with antiseptic and a sterile needle must be used for injection.

### Injection sites:

- Subcutaneous injections are preferred. Injection of <0.1ml volumes of <0.1mg/ml antigen solution is recommended, which must be injected at no more than four scattered sites over the back and flanks. Multiple injection sites should be separated by a distance sufficient to avoid coalescence of inflammatory lesions. See *Handbook of Experimental Immunology in Four Volumes*, edited by D.M. Weir.
- Additional scientific justification must be provided for intradermal injections. If intradermal injections are approved, the volume per site should be reduced to 0.05ml to prevent necrosis or drainage.
- Intramuscular immunization is discouraged, as monitoring of post-injection reactions is difficult and some animals may lose function of the limb.
- Sites of injection which are weight bearing should be avoided. See below.
- Personnel must be cautioned about self-injection of CFA, which can result in painful, long lasting inflammation.

### Footpad injection in mice and rats:

- Footpad injections must be scientifically justified before IACUC approval, as CFA inoculated into the footpad can produce swelling, ulceration, and necrosis. The maximum volume that can be



injected is 0.05ml for mice and 0.1 ml for rats. Adjuvants should be inoculated into only one foot and a hind foot should be used. The animals must be housed on soft bedding, not wire bottom cages.

- Injection of CFA into the feet of rabbits is inappropriate as rabbits do not have true footpads, are heavier than small rodents, place more weight on the feet, and are generally housed in wire bottom cages.
- Booster immunizations must be given as antigen in Incomplete Freund's Adjuvant (IFA) or an aqueous vehicle such as saline, as reinjection of CFA into a sensitized animal may cause painful hypersensitivity reactions. Booster immunizations should be limited to a maximum of three injections with a minimum of two weeks between each injection.

**Post-injection care:**

- Animals must be observed for signs of anaphylactic shock and administered appropriate treatment if an acute reaction occurs.
- After immunization, animals must be monitored at least three times a week and examined for clinical signs such as pain, or infection, or swelling, inflammation, abscess formation or ulceration at the site of injection.
- Severe reactions at injection sites must be reported to a UAC Veterinarian for examination and treatment.
- If any signs of pain or distress are observed, approved palliative therapy must be administered, or the animal must be euthanized if the pain and distress are so severe that palliative therapy is ineffective.

**Non-adjuvant use of Complete Freund's Adjuvant**

- The IACUC recognizes that the use of CFA to induce inflammation is a scientifically valid method for development of models of autoimmune diseases such as arthritis. As above, the IACUC requires that investigators scientifically justify why an alternative method cannot be used instead of CFA. Administration and post-injection care must follow the guidelines above, although the site of CFA injection may vary with different disease models.

**C. REFERENCES, MATERIALS, AND/OR ADDITIONAL INFORMATION**

- Researchers must follow the guidelines established by the NIH Office of Animal Care and Use for the research use of adjuvants. The guidelines can be viewed on their webpage at:

[https://oacu.oir.nih.gov/system/files/media/file/2022-04/b8\\_adjuvants.pdf](https://oacu.oir.nih.gov/system/files/media/file/2022-04/b8_adjuvants.pdf)