

**Institutional Animal Care and Use Committee  
Policy #6  
Use of Complete Freund's Adjuvant and other Adjuvants in Laboratory  
Animals**

In concurrence with  
TTUHSC El Paso Assurance #D19-01056  
and Federal Regulations and Guidelines

**Purpose**

Adjuvants are compounds that stimulate the immune response. Although adjuvants (particularly Freund's Complete Adjuvant (FCA)) are useful and sometimes essential for producing antibodies, they are capable of causing severe inflammation. FCA is a water-in-oil emulsion containing either killed *Mycobacterium butyricum* or *Mycobacterium tuberculosis*, and is used to enhance antigenicity and stimulate an immune response greater than the antigen alone.

In order to reduce the amount of inflammation-induced distress in research animals, Principal Investigators (PIs) must consider using alternative systems that reduce the number of animals used (e.g., tissue culture, chicken eggs, etc.), and should consider the use of alternative adjuvants instead of CFA. Alternative adjuvants include: aluminum sulfate (alum), Sigma Adjuvant System (SAS), Freund's Incomplete Adjuvant, TiterMax, muramyl dipeptide and ethylene-vinylacetate copolymer, among others.

This policy establishes reasonable guidelines for the use of FCA and other adjuvants which minimize the associated pain and distress due to undesirable side effects. Principal investigators (PIs) should be aware that animal welfare regulations and policies are constantly changing and this policy will be revised as needed. Deviations from the recommendations in this policy must be scientifically justified in the protocol and approved by the IACUC prior to their use.

**Guidelines for the Use of FCA and Other Adjuvants**

- A. The use of FCA is highly discouraged, as it is recognized to cause more than momentary or slight pain<sup>1</sup>. It is expected that the Investigators will try another adjuvant before using FCA. The use of FCA or other adjuvants must be scientifically justified in an IACUC protocol and approved by the IACUC prior to their use. The protocol must include:
  - 1) the identification of the antigen
  - 2) the adjuvant or solution used for injections
  - 3) the volume delivered per injection site
  - 4) the total volume to be injected
  - 5) the site of injection
  - 6) the boosting schedule
  - 7) the justification for the number of animals exposed to the adjuvant
  - 8) scientific justification for the use of FCA
- B. The investigator should strongly consider the administration of analgesics with use of either complete or incomplete Freund's adjuvant. Analgesics will provide relief from any pain or distress caused by the adjuvants.
- C. The use of FCA must be limited to the initial immunization. Animals will be monitored daily for adverse effects and consult with the Veterinarian should they occur. Booster injection solutions must contain incomplete Freund's adjuvant, saline solution, or other suitable booster reagent.



- D. Depending on the species being used (see the table below), FCA-containing injections may be given by either subcutaneous, intramuscular, or intraperitoneal routes. Intravenous injections can damage the lungs by creating a lipid embolism and shall not be used.
- 1) Footpad injections in rabbits are not allowed.
  - 2) Footpad injections in mice are strongly discouraged (exceptions may be made for mice if scientifically justified, and must fulfill the following criteria:
    - a) only one injection per mouse will be allowed.
    - b) they must be housed on soft bedding.
    - c) they must be monitored daily for potential health problems.
    - d) euthanasia must be performed for mice that exhibit signs of severe pain or distress, including (but not limited to) persistent inactivity, labored breathing, sunken eyes, hunched posture, piloerection/matted fur, unresolving skin ulcers, abnormal vocalizations when handled, anorexia, or excessive scratching.
- E. The number of injection sites and injection volumes will vary depending on the type and size of the animal used and the route of administration applied. Maximum allowed volumes per injection site and total volumes administered at each immunization are listed below for representative species. Smaller volumes may be equally effective. Multiple injection sites must be separated from each other by enough distance to maintain an adequate blood supply to the area. Deviation from these guidelines requires special justification in the protocol

<b>Routes of Administration (mL)</b>				
<u>Species</u>	<u>Subcutaneous</u>	<u>Intramuscular</u>	<u>Intraperitoneal</u>	<u>Footpad</u>
Mice	<0.1	NR*	<0.2	NR** <0.05
Rats & Hamsters	<0.1	NR*	<0.5	NR** <0.1
Rabbits	<0.25	NR*	NR*	Not allowed
Guinea Pigs	<0.2	NR*	NR*	Not allowed

\* Not recommended (NR)

\*\*Only when justified

In order to prevent infection at the site of the injection and to observe the injection site for complications, aseptic preparation of the site is required. This includes clipping of the fur and the use of a skin disinfectant.

- F. Some antigen-FCA combinations will result in open draining skin lesions even when used at recommended dosages. If this occurs, the lesions must be treated appropriately to prevent infection.
- G. Preferred injection sites are along the back and sides of the animal; these areas are generally not used in handling or restraining the animal and are easily visible for observation and allow the best lesion drainage should lesions occur.

### Related policies

Investigators must comply with all other institutional policies at TTUHSC El Paso and Federal Guidelines. This list includes, but is not limited to, the following:

IACUC Policy #2: Veterinary Care

IACUC Policy #4: Pain Categories for Experimental Protocols

IACUC Policy #19: Humane Endpoints Regarding Severe or Chronic Distress

IACUC Policy #21: Use of non-Pharmaceutical Grade Compounds

#### REFERENCES

1. USDA/APHIS *Animal Care Policy Manual* (2017, Oct 1) *Policy 11 Painful and Distressful Procedures* retrieved from ([https://www.aphis.usda.gov/animal\\_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf](https://www.aphis.usda.gov/animal_welfare/downloads/Animal%20Care%20Policy%20Manual.pdf))
2. NIH Guidelines for the Use of Adjuvants in Research. ([https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b8\\_adjuvants.pdf](https://oacu.oir.nih.gov/sites/default/files/uploads/arac-guidelines/b8_adjuvants.pdf))