Institutional Animal Care & Use Program - UTEP	
Title: Adjuvant Use in Laboratory Animals	
Policy#: 007	**Date in Effect**: 11 December 2014
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In Effect ☒ **Rescinded** ☐	**Date Rescinded**:

A) RESPONSIBILITIES

It is the responsibility of all personnel using animals at UTEP to abide by this policy. It is the responsibility of the IACUC to review for approval, properly justified requests for an exception to this policy.

B) APPLICATION

This policy applies to all animals used in research at UTEP.

C) BACKGROUND

Although useful and sometimes essential for producing antibodies, adjuvants, particularly Complete Freund's Adjuvant (CFA), have the capability of causing severe inflammation. CFA is water in oil emulsion containing either killed *Mycobacterium butyricum* or *Mycobacterium tuberculosis*, which are used to enhance antigenicity and stimulate an immune response greater than the antigen alone. Principal Investigators (PIs) must consider using alternatives to adjuvants, which reduce the number of animals used (e.g., tissue culture, chicken eggs, etc.), or the use of non-inflammatory adjuvants (e.g., Ribi®, Incomplete Freund's Adjuvant (IFA), TiterMax® and others). This policy establishes reasonable guidelines for the use of CFA and other adjuvants, which minimize the associated pain and distress due to undesirable side effects.

Problems may arise for one or more of the following reasons, which should be taken into account when using adjuvants:

1) Failure to tap off excess ascites fluid from the abdomen resulting in over-distension and distress.
2) Excessive dose in a single subcutaneous or intramuscular site in any species, resulting in severe abscess formation, necrosis, and fistulous tracts.

3) Too many injection sites too close together, producing severe necrotizing dermatitis and potentially coalescing lesions.
4) Bilateral simultaneous intramuscular injections in thigh muscles, resulting in lameness of the animal.
5) Footpad injections in any species. These result in severe inflammation, lameness, and self-mutilation. It is universally held today that this practice has no basis in scientific necessity.
6) Hypersensitivity pneumonitis and embolic pneumonia associated with the use of CFA and IFA, which may occur even when these substances are not administered intravenously. These materials should never be deliberately administered intravenously.
7) Sterile peritonitis and abdominal adhesions associated with intraperitoneal administration of adjuvants.

D) PROCEDURES

1) Guidelines for the use of CFA and other adjuvants
   a) The protocol must include:
      (1) identification of the antigen
      (2) the adjuvant or solution used for injections
      (3) adjuvant source/manufacturer
      (4) the volume per injection site and total volume to be injected in ml or µl
      (5) site and route of injection
      (6) boosting schedule
      (7) antibiotic and analgesic treatment plan should infection result, unless the treatment plan is euthanasia
      (8) treatment plan for IP injections if excess ascites develops. Ascites excess must be clearly defined in the protocol
      (9) humane as well as experimental endpoints
   b) Whenever possible, the animal should be administered analgesics when injected with CFA or, at minimum, when signs of pain are observed.

c) CFA use must be limited to the initial immunization only. Booster injections if needed shall use Incomplete Freund's Adjuvant (IFA) unless it is scientifically justified.

d) All species require daily observation.

e) When pain becomes apparent from any immunization method, analgesics must be administered or the animal euthanized.

f) Depending on the species, CFA injections may be given by the intradermal (ID), subcutaneous (SC), or intramuscular (IM) route. Intraperitoneal (IP) injections can induce granulomas, fibrous adhesions and abdominal fluid distension and can only be used when justified in the protocol.

g) In order to prevent infection at the site and to facilitate the continued observation of the injection site for complications, aseptic preparation of the injection site is required. This includes clipping of the fur and the use of a skin disinfectant. Clipping of fur helps in monitoring development of lesions.

h) The protocol must describe how asepsis is maintained during antigen preparation.

i) Some antigen-CFA combinations will result in open draining skin lesions. If this occurs, the lesions must be treated appropriately to prevent infection.

j) Preferred injection sites are easily visible areas along the back and sides of the animal not typically used for handling or restraining the animal, and which would allow the best lesion drainage should lesions occur.

k) Routes and doses:

The permissible number of injection sites and injection volumes vary depending on the type and size of the animal and the route of administration. Maximum volumes per injection site and the maximum 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 ensure continued blood supply and avoid coalescing of lesions. In all instances, adjuvant injections may be repeated in 14-30 days unless complications develop. NOTE: Total injected volume should not exceed twice the recommended amount of

adjuvant. The following recommended total volume (adjuvant + antigen) must be considered:

(1) MICE

(a) Intraperitoneal. Maximum of 0.1 - 0.3 ml total inoculum (adjuvant + antigen). Mice must be observed daily and tapped whenever ascites is apparent, usually every two days. Whenever mice appear to be distressed or not eating they should be humanely euthanized. CFA IP injections must be justified.

(b) Subcutaneous. Maximum of 0.01 - 0.2 ml total inoculum (adjuvant + antigen), in the inguinal region or another area not utilized for scruffing the animal.

(2) RABBITS

(a) Rabbits must be carefully restrained or lightly anesthetized if multiple injections are to be administered.

(b) Intramuscular. Multiple sites, lateral to the vertebral column on the back; maximum of 0.3 ml of total inoculum (adjuvant + antigen) per site. Maximum of four (4) sites. If the thigh muscle is used, only one leg, maximum of 0.3 ml total inoculum (adjuvant + antigen) may be used.

(c) Subcutaneous. Multiple sites, maximum of 0.3 ml of total inoculum (adjuvant + antigen) per site. Maximum of six (6) sites. Usually administered lateral to the vertebral column.

(d) Intradermal. Multiple sites, usually lateral to the vertebral column; maximum of 0.05 ml of total inoculum (adjuvant + antigen) per site. Injection sites should be at least 1.0 cm apart. Maximum of ten (10) sites per challenge date. Use alternate route until epidermis is healed. Intradermal injections may be repeated once skin is healed.

(e) Intraperitoneal. Maximum of 0.5 ml of total inoculum (adjuvant + antigen) per injection interval. Rabbit must be observed daily; euthanize humanely if rabbit exhibits distress.

(3) RATS < 200 grams

(a) Intramuscular. Maximum of 0.05 ml of total inoculum (adjuvant + antigen). A maximum of two sites on opposing sides of the animal. Total dose should not exceed 0.1mL of inoculum (adjuvant +antigen) \

(b) Subcutaneous. Multiple sites along the back not used for restraint or in the inguinal region. Maximum of 0.2 ml total inoculum (adjuvant + antigen)/site and 4 sites.

(c) Subcutaneous.

(d) Intradermal. Multiple sites along the back not used for restraint or in the inguinal region. Maximum of 0.02 ml inoculum per site (adjuvant + antigen)/site and 6 sites.

(4) GUINEA PIGS AND RATS >200g

(a) Intramuscular. Only a single site (thigh) is recommended. Dose not to exceed 0.2 ml total inoculum (adjuvant + antigen).

(b) Subcutaneous. Multiple sites along the back. Maximum of 0.2 ml total inoculum (adjuvant + antigen)/site and 4 sites.

(c) Intradermal. Multiple sites along the back. Maximum of 0.03 ml total inoculum (adjuvant + antigen)/site and 6 sites.

2) General Precautions/Suggestions

a) It is critical to adjust the concentration of the antigen to levels that will facilitate mixing with equal volume of adjuvant and stay within the recommended volumes per injection site. This will minimize the total volume injected in all species.

b) If the concentration of the antigen cannot be obtained for use within the suggested volumes there needs to be justification and evidence for the inability to obtain higher concentration of the antigen. Appropriate precautions would then be needed for post-inoculation care and monitoring.