Institutional Animal Care Program		
Title: **Use of Freund's Complete Adjuvant Use in Laboratory Animals**		
Policy#: IACP 007	**Date in Effect**: 05/15/08	
Rev #: 01	**Rev Date**: 11/20/15	
	Re-reviewed: 01/11/19 – no changes	
In Effect ☒ **Rescinded** ☐	**Date Rescinded**:	

A) 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 is 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®, Freund's Incomplete Adjuvant [FIA], TiterMax®, muramyl dipeptide, ethylene-vinyl acetate copolymer). 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. Deviations from this policy must be scientifically justified in the protocol and approved by the IACUC.

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, or fistulous tracts.
3) Too many injection sites too close together, producing severe necrotizing dermatitis.
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 FCA and FIA, 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.

B) 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) the volume per injection site and total volume to be injected
        (4) site of injection
        (5) skin preparation of injection site(s)
        (6) boosting schedule
        (7) antibiotic and analgesic treatment plan should infection result unless the plan of treatment is euthanasia.
        (8) treatment plan for IP injections if excess ascites develop. Ascites excess must be clearly defined in the protocol.
        (9) humane as well as experimental endpoints.
    b) CFA use must be limited to the initial immunization only. Booster injections if needed shall use FIA.
    c) All species require daily observation and where infected abscesses develop, antibiotic therapy should be instituted.
    d) FIA & FCA should not be used intravenously in any species.
    e) Footpad injections should not be used in any species.
    f) When distress or pain becomes apparent from any immunization method, analgesics must be administered or the animal euthanized.

g) The 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/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.

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

i) In order to prevent infection at the site of the SC or ID injection and to facilitate the continued observation of 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. Clipping of fur helps in monitoring development of lesions.

j) Preferred injection sites are along the back and sides of the animal; these areas are generally not used in handling or restraining the animal, are easily visible for observation and allow the best lesion drainage should lesions occur.

k) Routes and doses:

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), back of neck or inguinal area.

(2) RATS

(a) Intramuscular. Maximum of 0.05 ml of total inoculum (adjuvant + antigen). A maximum of 2 sites.

(3) 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). 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.

(4) GUINEA PIGS

(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.