



INSTITUTIONAL ANIMAL CARE  
AND USE COMMITTEE

**IACUC POLICY # 16**  
**GUIDELINES FOR THE USE OF FREUND'S ADJUVANT**  
**IN LABORATORY ANIMALS**

Freund's complete adjuvant, a water in oil emulsion containing killed, dried Mycobacterium butyricum has been widely used in immunology to enhance antigenicity and stimulate an immune response greater than antigen alone. Freund's incomplete adjuvant (water in oil emulsion only) is also frequently used for similar reasons. The improper or unnecessary use of Freund's complete adjuvant may cause severe inflammation, induration and/or necrosis in laboratory animals. The most severe inflammatory responses in animals are seen following multiple injections of the complete adjuvant.

The IACUC has adopted the following guidelines intended to eliminate or minimize animal discomfort associated with the use of this agent in research:

1. Before using Freund's complete adjuvant, consider the use of the incomplete form or a different adjuvant. (RIBI, trehalose dimycolate)
2. **If Freund's complete adjuvant must be used, it should be limited to the initial immunizing dose. Use of two or more doses of the complete adjuvant is rarely warranted and must be strongly justified with objective data.**
3. The route of injection should be subcutaneous or deep intramuscular rather than intradermal. Intradermal injections may result in skin necrosis and sloughing. **The use of footpad injections is normally prohibited.**
4. For subcutaneous administration, the inoculum containing the adjuvant should be divided into fractions so that no more than 0.05 ml is injected per site. Injection sites should be separated from each other widely enough to ensure continued blood supply to adjacent areas of skin and subcutis.
5. Intramuscular inoculum should be of a volume no greater than 0.5 ml for rabbits (proportionally less for smaller species), administered by a single injection into a deep muscle mass. The deep lumbar muscles of the rabbit are recommended.

6. Injection sites should be clean and free of debris and contamination which could result in local infection.

7. The inoculum should be free of extraneous microbial or other particulate contamination. Millipore filtration of the antigen before mixing with the adjuvant is recommended when possible.

8. The oil and water phases should be thoroughly emulsified. Studies suggest that fully emulsified inoculum produces less undesirable reaction at the injection site.

9. Rabbits should be tranquilized or lightly anesthetized during inoculation to reduce distress. Acepromazine 1.0 mg/kg body weight, subcutaneously or intramuscularly may be used. If the animal vocalizes or struggles during administration of the immunogen, it should be anesthetized. Ketamine 44 mg/kg and xylazine 10 mg/kg im. can be used.