GUIDELINE 3 (03/25/08)
USE OF COMPLETE FREUND'S ADJUVANT IN LABORATORY ANIMALS

BACKGROUND
The University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC) has adopted the following guidelines for the use of adjuvants in laboratory animals. These guidelines are based on the National Institutes of Health Intramural "Guidelines for the Use of Freund's Adjuvant in Laboratory Animals" [1]. The IACUC recognizes that the scientist is the best qualified person to select the appropriate adjuvant to be used. It will consider justified applications for use of adjuvants outside these guidelines. Use of Complete Freund's Adjuvant (CFA) must be specifically addressed in the protocol application, scientifically justified, and a comprehensive search for alternatives considered.

Complete Freund's Adjuvant is a water in oil emulsion containing killed, dried Mycobacterium butyricum which has been used to enhance antigenicity and stimulate an immune response greater than antigen alone. Incomplete Freund's Adjuvant (IFA), water in oil emulsion only, is used for similar reasons. The improper or unnecessary use of CFA may cause severe inflammation, indurations, and/or necrosis in laboratory animals [2]. The most severe inflammatory responses in animals are seen following multiple injections of CFA. The intention of the following guidelines is to minimize potential animal discomfort associated with the use of adjuvants in research.

REDUCTION, REPLACEMENT AND REFINEMENT
Alternatives which reduce the number of animals required (e.g. tissue culture, chicken eggs [4-7]) or utilize less traumatic adjuvants [8-12] must be considered. The USDA has declared that the use of CFA may cause more than momentary pain or distress [15]. Thus, a non-painful alternative must be considered. CFA may be used only with specific scientific justification as part of a written narrative describing a literature search for alternatives to use of CFA [16]. These refined adjuvants or antibody production alternatives specifically include use of:
- Incomplete Freund's Adjuvant
- RIBI®
- TiterMax®
- Specol®
- Montamides
- Syntex Adjuvant Formation (SAF)
- Aluminum compounds
- Subcutaneously implanted chambers
- SuperCarrier®
- Elvax®
- L-tyrosine
- AdjuPrime®
- Nitrocellulose-absorbed protein
- Gerbu adjuvant
- Immune-stimulating complexes (ISCOMS)
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Guidelines
1. Non-inflammatory adjuvants or adjuvants that produce a less intense inflammatory response should be strongly considered as an alternative to CFA [3]. CFA must be used only when absolutely necessary and its use must be justified in each protocol.

2. If CFA is used, it must be limited to the initial immunizing dose. If more than one dose is proposed, it must be strongly justified with objective data.

3. Typical routes of injected are listed in Table 1. IP and IM injections in rodents should be avoided. The IACUC prefers the use of subcutaneous injections in all species. Intravenous (IV) administration is prohibited. Even when given by the approved routes, CFA may cause severe local and systemic pathology if not properly used. The least invasive method should be applied.
   a. Much less pathology results from the IM injection in the lumbar muscles compared to the hindlimbs. Therefore, lumbar administration is encouraged.
   b. Intradermal injection, in particular, may result in skin necrosis and sloughing.
   c. If footpad injection is scientifically justified, then only one footpad may be injected in each experimental animal.

Regardless of the route of CFA administration (injection site, dose, or volume), NIH Guidelines must be followed [1].

<table>
<thead>
<tr>
<th>Species</th>
<th>Subcutaneous</th>
<th>Intradermal</th>
<th>Intraperitoneal</th>
<th>Footpad</th>
<th>Intramuscular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>≤0.1 ml</td>
<td>*</td>
<td>*</td>
<td>≤0.05 ml**</td>
<td>*</td>
</tr>
<tr>
<td>Rat</td>
<td>≤0.1 ml</td>
<td>≤0.05 ml**</td>
<td>*</td>
<td>≤0.1 ml**</td>
<td>*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>≤0.25 ml</td>
<td>≤0.05 ml**</td>
<td>*</td>
<td>*</td>
<td>≤0.25 ml***</td>
</tr>
<tr>
<td>Goat/Sheep</td>
<td>≤1.0 ml</td>
<td>≤0.1 ml**</td>
<td>*</td>
<td>NA</td>
<td>≤0.5 ml</td>
</tr>
</tbody>
</table>

* Not recommended
** Only When Justified
*** Only One Limb Recommended Without Justification
NA: Not applicable

4. All injection sites must be clipped and surgically scrubbed. Inoculum must be sterile, free of extraneous microbial or other particulate contamination.
5. The CFA:antigen emulsified mixture of 1:1 is commonly used. If the emulsion is complete, a 23 gauge needle on a 1/2 ml syringe will allow for accurate dosing. If the emulsion is not complete, dosing will be difficult.

6. Injection sites must be separated from each other widely enough to ensure continued blood supply to adjacent areas of skin and subcutis.

Post-inoculation Monitoring
Post-injection observations should be done daily for four weeks following injection, or until lesions have resolved. If pain, distress, or complications at the inoculation site occur (e.g. ulceration), the animal must be reported as a “sick animal” and ULAR veterinary services must be contacted and a treatment plan created for the animal.
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References


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15. USDA Policy #11, "Painful/Distressful Procedures, April 14, 1997

16. USDA Policy #12, "Written Narrative for Alternatives to Painful Procedures, April 14, 1997."