Institutional Animal Care & Use Program - UTEP	
Title: Adjuvant Use and Monoclonal Antibody Production 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In Effect ☒ **Rescinded** ☐	**Date Rescinded**: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 the University of Texas at El Paso (UTEP) to abide by this policy. It is the responsibility of the Institutional Animal Care and Use Committee (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

The Guide for the Care and Use of Laboratory Animals and the PHS Policy on the Humane Care of Laboratory Animals requires that *in vitro* methods for antibody production be considered prior to the use of *in vivo* methods. The use of adjuvants for antibody production may promote robust immunity, however, the investigator needs to evaluate the effect of associated local and/or systemic pain and distress of the animal with the scientific benefit that may be gained. The use of inflammatory agents, particularly Complete Freund's Adjuvant (CFA), can result in severe side effects, including granuloma formation, tissue necrosis and sloughing, abscesses, and fever.[1-3] Alternatives to CFA should be used whenever possible and the use of CFA must be scientifically justified in the IACUC protocol.

Less problematic alternatives to Freund's adjuvant are available and should be considered.[4-5] RIBI Adjuvant System®, Specol®, TiterMax®, Montanide IAS50, and Montanide ISA70 are commonly used as appropriate alternatives. Noninflammatory adsorptive adjuvants such as alum and aluminum hydroxide gel may also be considered.

A commonly used method for in-vivo monoclonal antibody production is the mouse ascites model. This method involves injecting a priming agent followed by hybridoma

cells IP in mice. This generates ascites fluid, containing monoclonal antibodies, which is harvested from the mouse. When the ascites method is used for producing monoclonal antibodies, scientific justification is required and every reasonable effort should be made to minimize pain or distress, including frequent observation of the animal, limiting the number of survival taps, and prompt euthanasia if signs of distress appear.[6-7]

When utilizing adjuvants or using in-vivo methods to produce antibodies it is important to minimize the potential pain and distress associated with these procedures. The use of an analgesic is required unless robust scientific justification is provided and approved in the protocol.

D) PROCEDURES

1) Guidelines for the use of adjuvants
   a) The protocol must include:
      (1) identification of the antigen
      (2) the adjuvant or solution used for injections
      (3) the adjuvant source/manufacturer
      (4) the volume per injection site and total volume to be injected in ml or µl
      (5) the site and route of injection
      (6) boosting schedule
      (7) the 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
2) When possible adjuvants other than CFA should be used. These adjuvants will produce a less intense inflammatory response (e.g. Aluminum compounds, squalene-in-water emulsions, monophosphoryl lipid A, RIBI adjuvants).
3) If CFA is required, it must be scientifically justified in the protocol. The protocol must state route of administration, number of injections, volume to be administered, concentration of CFA to be used, source of CFA, preparation of antigen-adjuvant emulsion (sterility, vehicle, pH), preparation of injection site, and monitoring of the animal.

4) CFA is considered to a chemical hazard and appropriate areas of protocol must be completed.

5) Recommendations when working with CFA:

   a) CFA should only be administered once. Incomplete Freund's adjuvant should be used for successive immunizations.

   b) Sterile techniques must be used when preparing the antigen-adjuvant emulsions.

   c) Aseptic preparation of injection site.

   d) Adhering to recommended routes and volumes according to species (Table 1).

   e) Use of smallest possible volume for injections.

   f) Concentrations of CFA should be <0.1 mg/ml when possible.

   g) Whenever possible, the animal should be administered analgesics when injected with CFA or, at minimum, when signs of pain are observed.

   h) All species require daily observation.

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

Table 1. Recommended Volume (ml) of CFA-antigen Emulsion per Site and Route of Administration (NIH Guidelines)

Species	Subcutaneous	Intradermal	Intraperitoneal	Footpad
Mouse	<0.1	Not recommended	<0.2	<0.05*
Rat	<0.1	<0.05*	<0.5	<0.1*

6) <u>General Precautions/Suggestions</u>

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

   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 a higher concentration of the antigen. Appropriate precautions would then be needed for post-inoculation care and monitoring.

E) REFERENCES

1) Jackson LR, Fox JG. 1995. Institutional policies and guidelines on adjuvants and antibody production. ILAR Journal 37(3):141-150.

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3) Toth LA, Dunlap AW, Olson GA, Hessler JR. 1989. An evaluation of distress following intraperitoneal immunization with Freund's adjuvant in mice. Lab Anim Sci 39:122-126.

4) Lipman, N.S., Trudel, L.J., Murphy, J.C., and Sahali, Y. 1992. Comparison of immune response potentiation and in vivo inflammatory effects of Freund's and RIBI adjuvants in mice. Lab Animal Sci 42: 193-97.

5) Leenars, M., Koedam, M.A., Hendriksen, C.F.M., and Claassen, E. 1998. Immune responses and side effects of five different oil-based adjuvants in mice. Vet Immunol Immunopath 61: 291-304.

6) Jackson, L.R., Trudel, L.J., Fox, J.G., and Lipman, N.S. 1999. Monoclonal Antibody Production in Murine Ascites. I. Clinical and Pathologic Features. Lab Animal Sci 49:70-80.

7) Peterson, NC. 2000. Behavioral, clinical, and physiological analysis of mice used for ascites in monoclonal antibody production. Comp Med 50:516-526.

Review History	
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