

Guidelines for the Use of Adjuvants in Antibody Production
The University of Texas at Austin Institutional Animal Care and Use Committee

These guidelines have been written to assist faculty, staff, and students in performing vertebrate animal procedures in a humane manner and complying with pertinent regulatory requirements. Under some circumstances deviations from these procedures may be indicated but such variances must be approved in advance by the IACUC.

This document provides information to be used when planning and performing procedures utilizing the use of adjuvants in antibody production in vertebrate animals used for research, teaching, or other purposes at The University of Texas at Austin. It is organized into four sections:

Section A – Background

Section B – Suggested site and maximum volume for immunization using Freund’s Adjuvant

Section C – References

Section D – Acknowledgements

Section A – Background

Improper or unnecessary use of Freund’s complete adjuvant (CFA) has the potential to cause inflammation, induration, or necrosis in laboratory animals. Disseminated granulomas have been reported in lungs, liver, kidney, heart, lymph nodes and skeletal muscle after subcutaneous or intravenous injection in rabbits and rats, with similar results in hamsters, mice and guinea pigs. In some cases, humans accidentally injected have described the subsequent lesions as painful. The IACUC has adopted the following guidelines intended to minimize or eliminate animal discomfort associated with the use of this agent in research.

1. Before using CFA, consider the use of incomplete Freund’s adjuvant (IFA) or another adjuvant. The use of the refined adjuvants such as TiterMax or other commercially available immunoadjuvants may be acceptable alternatives. Although some studies have shown that CFA priming followed by IFA boosting results in titers that surpass the performance of alternatives, modern antibody concentration and purification techniques may allow somewhat lower titers to suffice. If using an alternative adjuvant, follow manufacturer’s instructions for its use. A description of the antibody production regime should be described in the animal utilization proposal (AUP).
2. If the use of CFA is indicated, the undesirable and painful side effects can be effectively reduced or eliminated by the use of appropriate routes of administration, adequate separation of injection sites, and the use of small amounts of inoculum per site.
3. CFA should be used only for the first (priming) antigenic dose. Booster injections should utilize IFA which lacks the hypersensitizing mycobacterial components of CFA. Use of two or more doses of CFA is not indicated for routine antibody production, and must be justified and approved in the IACUC protocol.
4. Suggested maximum immunization volumes at various injection sites for mice, rats, and rabbits, using CFA are provided in Section B, below. If an injection regimen that is not compatible with these guidelines is required, specific justification must be provided in the IACUC protocol.

5. Use of footpad injections is not recommended and will not be allowed unless sufficient documentation is provided which indicates that this route is specifically required for sufficient antibody production. If footpads must be used, specific justification must be provided in the IACUC protocol and special animal care and monitoring may be required.
6. The injection site must be carefully observed by trained personnel for one month after CFA administration. Written documentation of these observations must be maintained. Any abnormalities noted during the observations must be reported to the veterinary staff.
7. The inoculum should be free of extraneous microbial contamination. Microfiltration of the antigen before mixing with adjuvant is recommended whenever possible.
8. Injection sites should be clean and free of debris and contamination likely to result in infection, but need not be aseptically prepared. Clipping of the fur is recommended to allow for proper placement of the injection and to facilitate post-procedural observation of the injection site.

Section B – Suggested site and maximum volume for immunization using Freund’s Adjuvant

Note: Freund’s can only be used for the priming dose

Species	Injection Sites					Maximum blood withdrawal*
	Subcutaneous	Intramuscular	Intradermal	Intraperitoneal	Footpad	
Mouse	0.1 mL/site 4 sites max.	Not recommended	Not recommended	0.25 mL max.	Only when justified	0.1 mL/10 gm body weight
Rat	0.2 mL/site 2 sites max.	Not recommended	0.1 mL/site 8 sites max.	0.5 mL max.	Only when justified	0.1 mL/100 gm body weight
Rabbit	0.25 mL/site 8 sites max.	0.25 mL/site 2 sites max.	0.1 mL/site 12 sites max.	Not recommended	Not recommended	10 mL/1 kg body weight

*This is the maximum blood volume to be removed during a single survival bleed. It can be repeated at two-week intervals. If more frequent bleeding is needed, this volume should be proportionally reduced (i.e., only 50% of this volume taken if weekly bleeds are to be performed). If more blood than this recommended maximum volume is needed, the investigator should meet with the Attending Veterinarian to arrange for animal observation and frequent hematocrit determination.

Section C – References

Schiefer B, Stunzi H. 1979. Pulmonary lesions in guinea pigs and rats after subcutaneous injection of complete Freund's adjuvant or homologous pulmonary tissue. *Journal of Veterinary Medicine A* 26: 1-10.

Chapel HM, August PI. 1976. Report of nine cases of accidental injury to man with Freund's complete adjuvant. *Clinical & Experimental Immunology* 34: 358-541.

Leenaars PP, Hendriksen CF, Angulo AF, Koedam MA, Claassen E. 1994. Evaluation of several adjuvants as alternatives to the use of Freund's adjuvant in rabbits. *Veterinary Immunology and Immunopathology* 40(3): 225-41.

Johnston BA, Eisen H, Fry D. 1991. An evaluation of several adjuvant emulsion regimens for the production of polyclonal antisera in rabbits. *Laboratory Animal Science* 41(1): 15-21.

Section D – Acknowledgements

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