



Florida State University Immunization Guidelines for Research Animals

The following are generalized guidelines to adhere to when performing monoclonal or polyclonal antibody production. It has long been recognized that the procedures involved in antibody production for research may result in discomfort, distress or pain to the animal. In order to minimize such discomfort and pain, these guidelines and more detailed instructions on Antibody Production (Adjuvants, Monoclonal Antibody Production in the Mouse, Polyclonal Antibody Production and Immunization Routes for Antibody Production) are provided to FSU researchers.

Alternatives:

Consideration of the three R's (refinement, reduction and replacement) must be done during formulation of the research plan. Alternative procedures may be available to replace animals (e.g. *in vitro* cell culture for monoclonal antibody production) where viable. Selection of appropriate species and strains may result in improved antibody harvesting that should lead to a reduction in overall animal numbers. Consideration and selection of adjuvants may minimize discomfort or pain to the animal being immunized.

Adjuvants:

Some adjuvants cause severe inflammatory reactions after injection. Alternatives to these adjuvants should be evaluated and trial use of less inflammatory adjuvants attempted. The adjuvant most commonly associated with inflammatory reactions is complete Freund's adjuvant (CFA). Should CFA be the appropriate adjuvant, it may only be used for the first antigen injection. Subsequent boosters should use incomplete Freund's adjuvant or an alternative.

Antigens:

The antigen should be appropriately sterilized prior to injection. Filtration with a millipore filter is recommended (one with minimal protein adsorption and minimal disruption of protein conformation).

Antigens should be free of byproducts of purification (SDS, polyacrylamide gel, urea, other solvents) to avoid adding to the inflammatory reaction after injection. Excessive contamination of the antigen with byproducts may also reduce the stability of the adjuvantantigen preparation.

Extremes of pH should be avoided in order to reduce possible inflammation post-injection.

Injections:

Sites should be chosen carefully. Avoid injecting into areas where the animal is commonly handled (e.g., the back of the rabbit's neck).

Injection sites should be clean and free of debris.

Only sterile syringes and needles should be used for the injections. Preferably use luer-lock syringes to avoid the needle popping off the syringe.

Animals should be appropriately restrained or sedated for injections.

Total volume and route of administration should be considered. Refer to specific guidelines for Monoclonal Antibody Production in the Mouse, Polyclonal Antibody Production, and Immunization Routes for Antibody Production for further information.

Precautions should be taken to minimize inadvertent exposure of personnel to either adjuvant or antigen. Of particular importance is CFA which can lead to sensitization of lab personnel to tuberculin. Use of lab coats, gloves and safety glasses is recommended.

Adjuvant-antigen mixtures should be thoroughly homogenized prior to injection. Failure of the mixture to properly emulsify is indicative of contamination of the antigen with SDS or organic solvents. Such contamination may increase the risk of a severe inflammatory reaction.

Injection sites should be monitored for the development of abscesses, scabs, ulcers and tissue sloughs. Animals should be closely watched for abnormal behavior or inappetance which may be indicative of an infection or severe inflammatory reaction. Follow specific guidelines for Monoclonal Antibody Production in the Mouse and Polyclonal Antibody Production for monitoring recommendations.

Bleeding:

Various routes and methods of bleeding are published. The general rule of thumb is to harvest no more than 1% of the animal's body weight once every two weeks. More frequent harvesting may be done if approved by the ACUC and additional monitoring procedures implemented (routine measurement of hematocrit and total protein). Refer to the Blood Collection Guidelines for further information. Please consult with the LAR veterinarian for further information with regard to blood collection.