NU–IACUC POLICY

Northeastern University Institutional Animal Care and Use Committee

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| Polyclonal Antibody Production |

*Re-Approved: 02/08/2022*

**General:**

The in-vivo production of antibodies using laboratory animals is considered to be an essential part of research. The intent is to obtain high-titer, high-affinity antisera generated by animals while causing only momentary pain or discomfort. Polyclonal antibody production is not considered painful or distressful to animals under normal conditions. Proper technique and monitoring must be incorporated into this procedure to assure the animal’s welfare.

A cocktail composed of an adjuvant and an antigen stimulates the production of polyclonal antibodies. Below is a short discussion of these components and their properties, as well as recommended methodologies.

**Definition:**

**Adjuvant** is described as any substance that improves the immune response to an antigen.

**Antigen** is the substance that you want an antibody produced against.

**Freund’s Adjuvants**: These are the most commonly used adjuvants for polyclonal antibody production. Freund’s Complete Adjuvant (FCA) is composed of heat-killed mycobacterium, mineral oil and an antigen. Freund’s Incomplete Adjuvant (FIA) is the same as FCA except that killed mycobacterial cells or cellular components, are absent. FIA is a water-in-oil emulsion.

FCA is used only for the initial immunization, while FIA is used for subsequent “booster” immunizations (usually 2-8 weeks apart). Re-injection of FCA can cause painful hypersensitivity reactions. Additional immunizations with FCA must be specifically justified by the investigator and approved by the NU-IACUC.

RIBI Adjuvant System: An oil-in-water emulsion in which the antigen is first blended with a minimal amount of metabolizable oil (squalene) and then emulsified in a saline solution and surfactant. The immuno-stimulant used in this system includes mycobacterial product (TDM) and/or endotoxin product. There are different species oriented formulations.

TiterMax: A water-in-oil emulsion with copolymer and squalene.

## Antigens

Characteristics of Antigens:

* Must be free of microbial contamination,
* The size of the antigen desired to be presented is a big factor. Typically, the larger the antigen, the better the presentation. Carrier molecules may be needed to present smaller antigens.

Quantity of Antigen:

The quantity of antigen needed varies widely and depends on the properties of the antigen and adjuvant selected. The amount is generally species-specific, not based on body weight. Too much or too little antigen will not give desired results. The optimal doses of antigen are listed below:

* Rabbit: 50 1000 ug
* Mouse: 10 200 ug
* Guinea Pig: 50 500 ug

Delivery Routes for the Antigen-Adjuvant Combinations:

The route of administration of antigen-adjuvant combinations has been a topic of discussion for the past years. The administration route should be chosen for the optimal delivery for antibody response from an antigen/adjutant composition that is widely distributed to the lymphoid tissue. NU-IACUC recommends the following routes for injection of antigen-adjuvant mixtures: subcutaneous (SC), intramuscular (IM), or intraperitoneal (IP). Intradermal, intravenous, and footpad injections are not allowed without strong scientific justification. Intradermal footpad injections can cause painful ulcers and skin necrosis. Intravenous injections can cause damage to lungs by lipid embolisms. Scientific justification must be given proving there are no alternatives to the specific route.

The subcutaneous (SC) route is the easiest route for personnel to use. This route allows for larger volumes of antigen/adjuvant to be administered followed by a slow absorption of antigen to the lymphatic system. The IM route is also a desired route due to its rapid absorption and distribution to the lymphatics and draining lymph nodes. Furthermore, the large muscles of certain species such as rabbits allow for large volumes of antigen/adjuvant. However with the use of FCA, there is the potential for increased pathology from granuloma formation and associated pain/distress with this method. The IP route is the most frequently used method in rodents, since IM and SC routes only allow for small volumes of inoculation.

Recommended Volumes for Injection of Antigen-Adjuvant Combinations:

* Rabbit:
* SC: 0.25-0.5 ml/site, divided into at least 4 sites.
* IM or IP: 0.5-0.75 ml/injection
* Total volume per injection: 1-2 ml
* Mouse:
* SC: 0.05-0.1 ml/site, divided into multiple sites
* IM or IP: 50-200 ul /injection
* Total volume per injection: 50-200 ul
* Rat:
* SC: 0.1-0.25 ml/site, divided into multiple sites
* IM or IP: 0.5-0.75 ml/injection
* Total volume per injection: 1-2 ml