Acceptable Immunological Procedures

When beginning an immunization, choosing the correct adjuvant may be difficult. As a general suggestion, Freund’s Complete Adjuvant (FCA) may be used when only small amounts of soluble immunogens are available. FCA is considered to be an emulsion consisting of equal volumes of FCA to antigen (1 part FCA or less to 1 part antigen). If large amounts of particulate, or highly immunogenic immunogens are available, other adjuvants should be considered.

An important aspect in immunization procedures is the utilization of skilled, competent technical staff experienced in the handling of the species being used and in performing the technique. They must be knowledgeable and capable of recognizing signs of distress in all injected animals, and be responsible for taking action when necessary.

Freund’s Complete Adjuvant should be used only for the most problematic immunization situations. It must never be given either intravenously or in repeated doses. FCA must not be used in horses.

Intradermal Route

Sound scientific evidence and justification must be available if the intradermal route of injection of FCA is to be used, because of the frequent ulceration and infections that occur at the site of such injections. The use of the intradermal route may be justified only when the purpose is to induce cell-mediated response.

In rabbits, volumes of inoculum in excess of 0.05 mls (50 microliters) per site should not be used. The location of the site(s) should be carefully selected so as to prevent mutilation. A minimal number of sites should be selected, and the distance between each site be maximized.

The intradermal route is inappropriate in the mouse. Nor is it recommended in other rodents.

Subcutaneous Route

In guinea pigs, up to a total volume of 0.4 ml (400 microliters) of inoculum may be injected subcutaneously dorsally in the neck, in one or divided into several sites. In rabbits, the site of choice is the interscapular region (between the shoulder blades) on the dorsum (back), administering up to 0.25 ml of inoculum (250 microliters) per site, to a maximum of four sites. The distance between sites should be maximized. In the mouse, up to 0.1 ml (100 microliters) may be administered in the neck region.

Intramuscular Route

In rabbits, intramuscular injections of FCA may be administered in the thigh muscle; up to 0.5 ml (500 microliters), preferably in one site. Intramuscular injection of FCA is not recommended for small laboratory animals such as rats, mice, hamsters, gerbils, etc. For larger animals such as cats, dogs and poultry, up to 1 ml of FCA injected into the thigh muscles is acceptable. In livestock such as
pigs, cattle, sheep and goats, the intramuscular route is acceptable.

**Intraperitoneal Route**

The intraperitoneal route for injection of FCA is permitted in small rodents only. FCA should be administered only once, and be limited to minimal volumes of up to 0.1 ml (100 microliters).

**Intravenous Route**

FCA is not to be injected intravenously.

**Footpad Injection**

FCA should not be injected in the feet of rabbits. Footpad injection of FCA in rodents is not permissible unless there is scientific evidence indicating this route is essential as a specific requirement for the production of immune response. In rats and mice, only one footpad may be used. Animals should be maintained on soft bedding and not on wire-bottomed cages.

**Induction of Ascites Fluid in Animals**

Pristane or other recognized priming agent(s) (excluding FCA) may be used.

Ascites may be collected only for as long as the animal is not experiencing pain or distress, is in good body condition, and does not show signs of debilitation, dehydration or other complications from the procedure. Upon recognition of loss of condition, pain, or distress the animal must be euthanized according to a method approved by the Canadian Council on Animal Care.

**Observation of Injection Sites**

The injection site(s) must be observed by the investigator or his/her designate, a minimum of three times per week, for four weeks after each injection.

If a lesion(s) develops at any injection site, it must be reported through established channels, e.g., the animal resources supervisor or veterinarian, and must receive appropriate veterinary treatment. Such lesions should be inspected at least three times per week by the investigator or his/her designate, until all lesions are healed.

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