Antigens, Adjuvants, and Antibody Production

1. References:
   - *Guide for the Care and Use of Laboratory Animals*, NRC, Current version
   - *Title 9, Code of Federal Regulations*, Subchapter A – Animal Welfare, Parts 1 & 2

2. Recommendations and Policies:
   2.1. Commercial services are readily available for the production of both monoclonal and polyclonal antibodies. Companies providing these services are highly experienced with antibody production and are often more cost efficient at producing antibodies. Investigators needing assistance in identifying a commercial antibody production program may contact Animal Resources.

2.2. Antigens:
   2.2.1. Antigen preparations used for *in-vivo* antibody production shall meet the following criteria unless scientifically justified and approved by the IACUC on a case-by-case basis:
   - free of extraneous microbial contamination (*e.g.*, sterilized by filtration through a 0.22 micron filter)
   - free of inflammatory byproducts (*e.g.*, polyacrylamide gel)
   - free of particulate matter
   - uncontaminated with SDS, urea, acetic acid, other solvents, or other potentially toxic agents

   2.2.2. Use of “viable microbes” as antigens requires IBC approval prior to IACUC approval.

2.3. Adjuvants:
   The best adjuvant for producing a particular antibody varies from antigen to antigen. The following commercially available adjuvants are recommended for *in-vivo* antibody production:

   - **Freund’s Complete Adjuvant (FCA)** is a water-in-oil emulsion of mineral oil, mannide monooleate (a surfactant), and heat-killed *Mycobacterium tuberculosis* (or components of the *M. tuberculosis*). Immunization once with FCA followed by booster immunization(s) with Freund’s Incomplete Adjuvant have historically been the most widely utilized and effective adjuvants for experimental antibody production. One part or less of FCA to one part antigen [v/v] is recommended. Inflammation occurs because the mineral oil cannot be metabolized, and the mycobacterial elements elicit granulomatous reactions. **The use of FCA for booster immunizations is prohibited.**

   - **Freund’s Incomplete Adjuvant (FIA)** is a water-in-oil emulsion of mineral oil and mannide monooleate (a surfactant). FIA is most commonly used for booster immunizations after an initial immunization with FCA.

   - **Ribi Adjuvant System® (RAS®)** is an oil-in-water emulsion of water, squalene (metabolizable oil), polysorbate-80 (Tween 80® surfactant), and an immunostimulator refined mycobacteria derivative (*i.e.*, TDM {synthetic trehalose dicorynomycolate}, CWS {cell wall skeleton}, and/or MPL® {monophosphoryl lipid A}). When mixed, the antigen is blended.
with a minimal volume of oil, and these oil droplets are then emulsified in a saline solution containing Tween 80®.

- **TiterMax®** is a microparticulate water-in-oil emulsion of silica coated CRL-8941 (copolymer) and squalene (metabolizable oil). TiterMax® is compatible with a wide variety of antigens without the use of large amounts of emulsifying agents. When mixed, the antigen is bound by adjuvant copolymers on the surface of oil droplets which present the antigen to the cells of the immune system in a highly concentrated form.

Other adjuvants (e.g., alum, liposomes, etc.) may be used, provided that they have been shown to be safe and non-toxic when used in lab animals.

### 2.3. Monoclonal Antibody Production:

#### 2.3.1. General Policy: *In vivo* production of monoclonal antibodies using the mouse ascites model is acceptable only when tissue culture systems will not work. Once a hybridoma cell line is established, all production should occur in tissue culture unless scientific justification is provided to and approved by the IACUC.

#### 2.3.2. Animal Model: Mice are the recommended species for monoclonal antibody production (*e.g.*, BALB/c).

#### 2.3.3. Immunization Regimen:

- A recommended immunization regimen is:
  - Initial immunization with antigen-adjuvant.
  - 1<sup>st</sup> booster immunization with antigen-adjuvant 14-28 days after initial immunization.
  - Check antibody titer 10-24 days after 1<sup>st</sup> booster. If titers are sufficient give final booster. If titers are too low, repeat steps 2 & 3.
  - Final booster immunization with ≤0.2 ml of aqueous antigen IV or IP 21-28 days after verification of antibody titer; and
  - Euthanize 3 days after final booster and harvest spleenocytes for cell fusion with myeloma cell lines to produce hybridomas.

#### 2.3.4. Priming Agents:

- Pristane is the only IACUC recommended priming agent.
- The recommended pristane dose is ≤0.25 ml IP. Pristane should be injected 7-14 days (10 days is optimal) prior to inoculation of hybridoma cells.

#### 2.3.5. Hybridoma Inoculation:

- Mice must be observed at least daily (*i.e.*, 7 days a week including weekends and holidays).
- Mice must be weighed at the time of hybridoma cell injection to establish a baseline bodyweight, and then they must be weighed daily beginning when abdominal distention is evident.
- The recommended number of cells in the hybridoma inoculum is 5 x 10<sup>5</sup> (range of 10<sup>5</sup>- 10<sup>7</sup> cells) in basal cell culture media or PBS that is inoculated intraperitoneally (IP) in a total volume of 0.1 - 0.5 ml.
- Hybridomas shall be tested for the presence of adventitious viral and mycoplasma agents prior to induction into an animal host to prevent the potential transmission of infectious agents from contaminated cell lines into mouse colonies.
2.3.6. Abdominal Paracentesis:

- Abdominal paracentesis (i.e., taps) to remove accumulated ascites fluid shall be performed when a mouse’s bodyweight has increased 20% above baseline or when the mouse exhibits a markedly distended abdomen, whichever comes first.
- Aseptic technique and 18-20 gauge needles shall be used when performing abdominal paracentesis to collect ascites fluid.
- Abdominal paracentesis shall be performed on anesthetized mice (e.g., brief inhalant anesthesia with isoflurane) in order to minimize pain/distress, facilitate restraint, and maximize the amount of ascites fluid collected.
- Subcutaneous administration of 2 to 3 ml sterile physiologic fluids is recommended after each paracentesis to help prevent hypovolemic shock.
- Up to 3 taps may be performed per mouse provided that the 3rd (last) tap is collected immediately after the mouse is euthanized.
- Mice shall be euthanized if: (1) the ascites fluid collected from any tap is bloody or contains particulate matter, or (2) if the animal exhibits inactivity, hunched posture, roughened hair coat, anorexia, dehydration, difficulty in ambulation, or labored breathing.

2.4. Polyclonal Antibody Production:

2.4.1. Animal Model:

- Rabbits are the most commonly used species for polyclonal antibody production.
- SPF rabbits (i.e., free of Pasteurella multocida) shall be used for polyclonal antibody production.

2.4.2. Immunization Regimen:

- Booster injections should typically be administered after serum antibody titers have plateaued or begun to decline (often 2-4 weeks after initial immunization). Antibody titers typically peak 1-2 weeks after a booster immunization. Administering booster immunizations too soon or too frequently can suppress the immune response and reduce antibody yield.
- A recommended immunization regimen for polyclonal antibody production in rabbits is:
  - Initial immunization with antigen-adjuvant;
  - 1st booster immunization with antigen-adjuvant 2-4 weeks after initial immunization;
  - Collect blood and evaluate titers 2-4 weeks after 1st booster immunization. Administer a booster immunization if higher titers are needed.
  - Repeat blood collection for measuring titers and repeat booster immunizations until the desired titer is achieved or until the titer plateaus. Typically no more than 2-3 booster immunizations are useful for increasing initial titers. For long term production, subsequent boosters may be needed if/when titers begin to decline.

2.4.3. Blood Collection:

- No more than 10% of the animal’s blood volume may be collected at one time and not more than 15% of the total blood volume may be collected within a 2 week period without scientific justification, provisions for additional monitoring or fluid therapy, and IACUC approval.
- Nicking/transecting the marginal ear veins of rabbits with a razor to collect blood is prohibited.
The use of heat lamps, acepromazine, or xylazine are recommended methods at enhancing vasodilation of rabbit ear veins for blood collection.

Cardiac bleeding is limited to terminal procedures performed under general anesthesia.

2.5. Aseptic Injection Technique and Site Preparation:

2.5.1. Sterile needles/syringes and aseptic technique shall be used for all injections.

2.6. Recommended Injection Routes, Sites and Volumes:

2.6.1. Intradermal (ID) Route: Rabbit ID injections shall normally be administered using 25-30 gauge needles along the dorso-lateral thorax and/or lumbar area avoiding nape of the neck, rump, and/or other areas that are grasped when manually catching/restraining the animals. For most effective antibody production, multiple small inoculations are preferred over fewer, larger volume inoculations.

2.6.2. Subcutaneous (SC) Route: SC injections shall normally be administered using 21-25 gauge needles along the dorso-lateral thorax and/or lumbar area avoiding nape of neck and rump or other areas that are grasped when manually catching/restraining the animal. Injections sites should be well separated so that inflammatory reactions at each site can’t coalesce. For most effective antibody production, multiple small inoculations are preferred over fewer, larger volume inoculations.

3. Approval/Authentication:

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