



CARE 404.03 Polyclonal Antibody Production

The intent of this standard operating procedure (SOP) is to describe the procedures to follow for producing polyclonal antibodies. This SOP is intended for use by researchers and CARE staff involved in producing polyclonal antibodies. This SOP is approved by the Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemptions must be approved by the IACUC prior to its application.

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1. Introduction

CARE produces polyclonal antibodies as a service offered primarily to Cornell research staff, although the service may also be used by persons outside of the Cornell community. Polyclonal antibodies can be produced in a variety of mammals as well as chickens but the focus of this SOP is antibodies that are produced in rabbits.

2. Materials

- antigen
- Freund's Complete Adjuvant (FCA)
- Freund's Incomplete Adjuvant (FIA)
- Vortex mixer
- Acepromazine
- Buprenorphine
- Ketamine
- Topical anesthetic (e.g. lidocaine & prilocaine)
- Needles (20g, 22g, 23g)
- Syringes (1cc, 3cc, 12cc)
- Luer-lock Syringes (1cc) if using FCA
- 23g butterfly infusion sets
- 21g vacutainer needles
- 10 ml red top vacutainer blood collection tubes
- Nolvasan
- 3x3 gauze
- small hair clippers

3. Procedures

- a. Researchers
 - i. Prepare immunogen that is non-toxic, sterile and free of pyrogens.
 - ii. Adjust the pH of the immunogen to within physiological limits.

- iii. Prepare antigen in such a way that it will easily pass through a 20g needle.
NOTE: Proteins in polyacrylamide gel may cause adverse reaction at the site of injection. Use another method of purification or a dilution when possible.
 - iv. Provide CARE with 3–4 samples of antigen, each consisting of 200–500 micrograms of antigen in sterile PBS to a volume of 0.5 mL per sample.
- b. CARE Staff
- i. Use an adjuvant to increase the immunological response to poor antigens.
NOTE: When used with a strong antigen, the adjuvant may induce an overt local inflammatory response.
 - ii. Use Freund's Incomplete Adjuvant (FIA) and Freund's Complete Adjuvant (FCA). In case of an overt reaction, other adjuvants are available for use.
 - iii. Administer FCA **ONLY ONCE** for the primary injection. Do not repeat. Use FCA with a concentration of 0.5 mg/ml of mycobacteria or less.
 - iv. Use FIA for all secondary immunizations.
 - v. Combine the antigen and the adjuvant in a syringe and emulsify by vortex until it no longer separates.
 - vi. Record immunogen, adjuvant, route, sites, volume and date of injection in the medical record of the animal.
 - vii. Record blood collection volume and site as well as body weight, general condition and appearance of the injection sites.
 - viii. Use young adult female Specific Pathogen Free rabbits (about 3.5–4 kg) whenever possible.
 - ix. Allow a minimum of 7 days of acclimation after the arrival of the animals.
 - x. Tranquilize rabbits with acepromazine (0.5 mg/kg IM) + buprenorphine (0.01 mg/kg IM) for initial immunization and blood collections.
 - xi. Apply topical anesthetic over the ear on the blood collection site 3–5 minutes before puncture. If there is a lot of hair, shave the ear with small hair clippers.
 - xii. Collect 2–10 mL pre-immune blood sample from the ear artery.
 - xiii. Primary immunization:
 - (a) Combine the 0.5 mL antigen sample with 0.5 mL of FCA adjuvant.
 - (b) Rinse the injection site with chlorhexidine. Note that we do not shave the rabbit because we have observed increased incidence of fighting wounds in group-housed rabbits that were shaved.
 - (c) Inject the 1 mL sample subcutaneously into 10 sites of 0.1 mL per site bilaterally along the thoracic-lumbar region of the spine. Injection sites must be sufficiently distant to prevent coalescence of the local inflammatory response.
 - (d) Do not contaminate the needle track with resulting intradermic deposition of the mixture.
 - xiv. Wait the 3–4 week period necessary to build up a primary immunological response.
 - xv. Secondary immunization: **DO NOT REPEAT FREUND'S COMPLETE ADJUVANT (FCA)**. If FCA was used in primary immunization, use Freund's *incomplete* adjuvant (FIA), or another adjuvant.
 - (a) Give booster injections in the vicinity of the initial sites as long as there is no indication of inflammatory reaction from the initial injection.

- (b) Proceed as indicated for the primary immunization except the volume can be increased to 0.25 mL/site in four sites.
- xvi. Collect a 10-mL blood sample after 3–4 weeks to determine the titer.
 - xvii. Repeat secondary immunization and titer determination every 3–4 weeks. In most cases, the antibody titer reaches an acceptable level after two boosters.
 - xviii. Observe animals for a minimum of 15 minutes post-injection and contact CARE veterinary staff in case of an abnormal reaction.
 - xix. Observe the animals daily for responses at the injection sites in particular and for overall health or distress in general.
 - xx. If the titer is sufficient, euthanize the animal by exsanguination under general anesthesia, or collect blood on a regular basis according to [SOP CARE 403 Recommended Blood Collection Volume and Frequency](#).
 - xxi. Administer a dose of 0.2–0.4 mL/kg IM of a cocktail combining 10 mL of ketamine, 100 mg /mL mixed with 1 mL of acepromazine, 10 mg/mL for general anesthesia.

4. Safety

- **FCA** is a human biohazard: accidental self-innoculation or splashing in the eye can cause painful inflammatory lesions, abscesses, hypersensitivity reactions, and sensitization to tuberculin.
- When working with **FCA**
 - **Always** use gloves, gowns and protective eyewear
 - **Always** use Luer-lock syringes
 - **Avoid** needle sticks
 - **Do Not** Re-Cap Needles
- Refer to [CARE SOP 711 Sharps Precautions](#).
- Working with research animals can trigger allergies; refer to CARE Allergens web page.
- Refer to the CARE Zoonosis web page for zoonotic information specific to the species with which you are working.
- Observe CARE SOP 707 for animal-related injury.

5. Contingencies

- Emergency: for emergency veterinary assistance (available 24/7), call 1-800-349-2456 and request to have the CARE group paged.
- Nonemergency: report any adverse reactions or general health issues to CARE veterinarians at 607-253-4378 or care@cornell.edu.

6. References

- CARE SOP 403 Recommended Blood Volume Collection and Frequency
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE403.pdf>
- CARE SOP 707 Animal Related Injury
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE707.pdf>
- CARE SOP 711 Sharps Precautions
<http://www.research.cornell.edu/CARE/documents/SOPs/CARE711.pdf>
- CARE Allergens Prevention web page

- <http://www.research.cornell.edu/Care/documents/OSH/AllergyPreventionFactSheet.pdf>
- *Guidelines on Antibody Production*; Canadian Council on Animal Care: Ottawa, Canada, 2002:
http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Antibody/antibody.pdf

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