

## Document Cover Sheet

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**ULAM STANDARD PROCEDURES FOR ANTIBODY PRODUCTION**

The ULAM veterinary technical staff will conduct rabbit polyclonal antibody production efforts for laboratories on a fee-for-service basis. The following sheet describes the standard procedures used by ULAM veterinary technicians, and approved by UCUCA for ULAM use. Variations from these procedures may be acceptable, but they are required to be described in both the Antisera Request Form and the investigator's animal use application (or its modification), and they are subject to review by a ULAM veterinarian.

The UCUCA approval number for the ULAM antibody production protocol is **6972A**. Referral to this approved application can be used as the answer to questions 6 (anesthesia), 7 (euthanasia), 8 (procedures), 9 (adverse consequences), 11 and 12 (animal and species justification), and 23 (housing facilities) in an investigator's individual application. All other questions in the application form must be answered, including the animal numbers request and justification in questions 10 and 13. **Also, any variations from ULAM's standard procedures must be described in an investigator's individual UCUCA application.** Animals will be purchased when the investigator's UCUCA application is approved and will be credited against his/her approval's animal numbers (the ULAM application is for procedures only). Per diem fees will be charged to the investigator's account, as will veterinary technician labor fees and supplies.

**STANDARD PROCEDURES**

Administration of antigen/adjuvant can be by one of three routes - intradermal (ID), subcutaneous (SQ), or intraperitoneal (IP). Specific information on these routes is listed below.

Adjuvant choices include the following: complete Freund's adjuvant (first injection only), incomplete Freund's adjuvant, Hunter's TiterMax, RIBI, or saline.

Antigen/adjuvant administration is given according to the following schedule:

- First injection
- Second injection - two weeks after first
- Third injection - two weeks after second
- Fourth (and subsequent) injections - based on antibody titer (assessed every 2 weeks); booster injections are no more frequent than every eight weeks

Intradermal Route: a maximum of 0.1 ml (100 ul) of antigen/adjuvant mixture is administered per injection site - maximum of 20 sites (2 ml total)

Subcutaneous Route: a maximum of 0.2 ml (200 ul) of antigen /adjuvant mixture is administered per injection site - maximum of 10 sites (2 ml total)

Intraperitoneal Route: a maximum of 0.50 ml (500 ul) of antigen /adjuvant mixture is administered - one injection site only. Can be coupled with intradermal or subcutaneous injections

For titer determinations and/or survival bleeds, rabbits are tranquilized / vasodilated with acepromazine maleate before blood withdrawal. Acepromazine is given at 0.3 mg/kg in the marginal ear vein. For these survival bleeds, blood is withdrawn at a maximum volume of 1% of the body weight every two weeks from the central ear artery.

For terminal exsanguination bleeds, all rabbits are anesthetized with intramuscular ketamine (40 mg/kg) and xylazine (10 mg/kg). Once fully anesthetized, the heart is cannulated, and all possible blood is withdrawn. Once blood withdrawal is complete, sodium pentobarbital is injected intracardiac at 100 mg/kg to ensure death.

**NOTE:** Different antigens may require variations from this standard protocol to achieve acceptable titers. Please share your antibody titer results with the ULAM veterinary technicians so that different approaches may be tried to ensure adequate and useful antibody production.