

Rabbit polyclonal antibody production sample Animal Use Protocol

End points need to be specified: volumes of sera needed, maximum # immunizations, target titer levels, and maximum duration under experiment.

2) Describe all procedures: *Description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.*

a. Overview of procedures.

Housing and Immunizations. Rabbits are housed in stainless steel cages and allowed free access to complete pelleted rabbit diet and water, and with 12 hour photoperiod. After no less than 7 days acclimation to the environment and observation for signs of disease, rabbits are given the primary immunization with (X ug antigen or agent) in a 1 ml volume emulsion of sterile PBS and Complete Freund's Adjuvant, administered subcutaneously in five locations. Two booster immunizations are given at 21 day intervals with the emulsion prepared using Incomplete Freund's Adjuvant administered subcutaneously in two locations. Rabbits are checked daily for signs of discomfort or local vaccine reactions. Up to two (investigators choice, but specify the maximum that will be given) additional booster immunizations may be given at 3 week intervals if antibodies are detected but the titer found to be low (<200, specify investigators choice). If the rabbit does not respond to immunization after this time it will be humanely euthanized (specify the endpoint).

Blood sampling. Rabbits will have a pre-immune blood sample (1-3 ml) drawn prior to the first immunization from the central auricular artery using a 3 ml syringe and 20 gauge needle. The rabbit will be placed in a rabbit restraining box and the ear swabbed with alcohol. A topical anesthetic ointment (i.e. Lanocaine) is then rubbed on the skin over the artery. After 5 or more minutes the ear is again swabbed with alcohol to disinfect and remove any remaining anesthetic. Using this method the rabbit typically tolerates the blood draw with little reaction. A post-immunization sample (test bleed) is similarly drawn 7 days after the 3rd immunization and checked by western blotting or ELISA to determine antibody titer. If additional immunizations are given, test bleeds are drawn 7 days later to check antibody titer. After a sufficient antibody response has developed a final blood sample is drawn by intra-cardiac (IC) method under anesthesia at a level suitable for surgery. Following the IC blood draw the rabbit is euthanized without recovery from anesthesia.

Alternatively, up to three (investigators choice, specify number) production blood samples may be drawn from the central auricular artery (maximum 25 ml) at two week intervals prior to the terminal bleed. Allowing for several production bleeds prior to euthanasia will assure an adequate supply of immune sera to minimize the number of rabbits required. The amount of immune sera needed will depend on the investigator/laboratories use. For example, the number of diagnostic samples to be tested for rabies may require a large amount of antisera (specify the amount).

- b. Type and duration of restraint. Rabbits will be restrained in a rabbit box.
- c. Name and dose of anesthesia and/or tranquilizer (contact attending veterinarian). Ketamine hydrochloride (30mg/kg) and xylazine hydrochloride (6 mg/kg). Anesthesia will be used at the end of the experiment prior to final blood draw by cardiac puncture. After this procedure rabbits will be euthanized as described in question 5.
- d. Surgical procedures. None.

3) Justification for species chosen (lowest possible species on phylogenetic scale).

Rabbits can supply a suitable amount of immune serum (25-100 ml, specify the amount needed) for the research we are doing as well to develop diagnostic assays and share with collaborators. There are also many readily available anti-rabbit secondary antibodies.

4) **Statistical** justification for the specified number of animals

a. Justification for number of animals per experiment. Three rabbits will be used for each of the (protein or agent) to account for the normal biological variability that could result in a nonresponder. Using three rabbits will also be helpful since individual rabbits may also have differential response to the variety of potential epitopes within the (protein or agent). Since these antibodies will be used for research purposes, this type of variation will be very helpful in determining the region of the protein best suited for vaccine development. Having three rabbits will be sufficient to allow for one to be a nonresponder and still have two likely to produce strong and specific antibodies.

b. Justification for number of experiments per year (as stated on page 2).

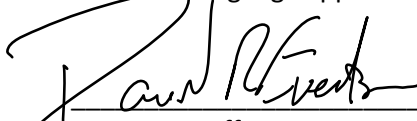
c. Literature cited/reviewed for justification of number of animals proposed

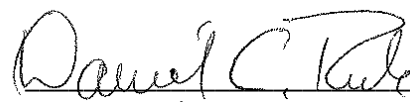
5) Will animals be subjected to euthanasia?

Yes, at the termination of the experiment or if health problems arise.

- a. Method of euthanasia. Rabbits will receive an overdose of sodium pentobarbital IC after the final intra-cardiac bleed. Ref: Current Protocols in Immunology, J.E. Colgin et al., NIH.
- b. Drug and dosage. Beuthanasia, (100mg/kg).
- c. If using drugs for euthanasia, describe disposal of animal remains. Remains will be incinerated at the Wyoming State Veterinary Laboratory.

Standard Language approved by IACUC


 Paul Pfeifers Date 5.26.11
 Veterinary Officer Date


 Daniel C. Tule 5/31/11
 IACUC Chair Date