

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY
PROTOCOL # <u>9560</u>
EXPIRES: _____

Investigator	
Last Name:	
First:	
Middle:	
email:	
Department:	
Phone / Fax:	
After hrs. #:	

Contact	
Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaque	6	CRPRC

Project Title Envelope antigen linked to broad neutralization of HIV-1.

Overnight housing location::	<input type="checkbox"/> CRPRC	<input type="checkbox"/> Day use only :	
Animals will be maintained by:	<input type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

Procedures: Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Animals on this project will be inoculated intravenously with SHIV-C. Samples, (blood and lymph node biopsies) will be obtained to assess virus infection and to localize SIV in the tissues.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

Hazardous Materials *(only if in the animal room):*

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	SHIV-C
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NIH	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Previous protocol number (if any):	

What Veterinarian or veterinary clinic will provide care for your animals? (check one)

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	Another Veterinarian

If you checked "Another Veterinarian", please provide:

Veterinarian:		Address:	
Day phone:			
Emergency phone:		Email:	

If your veterinarian is not affiliated with one of the three service units listed above, please contact the campus veterinarian, 2-2357 (email pcutlman@ucdavis.edu) for current information about training and record keeping requirements.

Summary of Procedures:

a) Briefly describe the **overall intent** of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Because of the genetic diversity of HIV, it has been classified into subtypes based on the nucleotide sequences in gag and envelope regions. HIV subtype C is the most prevalent virus strain in Africa, and spreading quickly throughout Asia. Therefore, development of a subtype C specific HIV vaccine is necessary. In using SHIV-C (containing HIV-C envelope) in rhesus macaques we hope to develop an animal model to test subtype-C specific vaccines.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- | | | |
|---|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input checked="" type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input type="checkbox"/> |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

**** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.**

c) Describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

1. SHIV-C immunization, monitor diseases occurrence, and biopsy.

The first step in the experiment is to determine if the SHIV-C is attenuated enough to be used as a vaccine. Adult male rhesus macaques will be inoculated with 1 ml SHIV C (SIV containing the HIV C subtype env) intravenously twice a day (AM and PM, approximately 6 hours apart) for two days. Blood samples (not to exceed 12 ml/kg/month) will be obtained one week prior to immunization, the day of immunization, at weekly intervals for the first four weeks and every month thereafter for six months. Lymph node biopsies (peripheral lymph node: axillary or inguinal, approx. 1 gram/biopsy) and a 3 mm. diameter skin biopsy will be obtained at 90 day intervals beginning the day of inoculation (day 0). If the animals do not progress to SAIDS (within 6 months), they will be boosted with another ml of SHIV-C intravenously twice a day (AM and PM, approximately 6 hours apart) for two days. The same sampling will be done as in the first immunization.

2. Test protect effect from SIVmac239 challenge.

If the animals still do not progress to SAIDS, six months after the boost they will be challenged with 1 ml SIVmac239 intravenously. The animals will continue on the same sample schedule as the immunization. We anticipate culling the animals at 18 months after the first inoculation, or at the development of clinical SAIDS. At the time necropsy, tissues will be collected for immunologic and virologic parameters analysis.

d) **Study Groups and Numbers:** Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; **you may add as many rows as you require.** The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

Group	Procedures / Drugs	Number of Animals	Category
1	SHIV C Inoculation	6	4

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

Rhesus macaques are the only animal model in which reliable data regarding vaccine efficacy for protection against pathogenic viral challenge. Therefore, we request to develop an animal model that will allow testing of recombinant HIV subtype C vaccines in rhesus macaques, starting by testing the infectivity of the SHIV-C virus. We have settled on a group of 6 for this study. Based on a student T test, these are the smallest groups that can be used to determine the relative infectivity of the virus stock, and the efficacy of SHIV-C as a vaccine.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

Building:

Room:

Who will be the surgeon?

g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

Provide the following information about any of these drugs that you intend to use in this project.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
rhesus	Telazol	6-8 mg/kg	IM	Before all procedures
rhesus	Oxymorphone	1mg/kg	IM	As needed in the judgement of CRPRC vets

h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:

Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Any injection or venipuncture has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

SIV infection of rhesus macaques results in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized before, or when, they experience 3 of the following: weight loss >15% in two weeks or >30% in 3 months; persistent hypothermia <96F even with heat supplementation; leukopenia (total WBC<3,000); lymphopenia (lymphocytes <800); anemia (hemoglobin <10); dehydration >10%; nonresponsive to therapy for opportunistic infections; persistent anorexia(>3 days); animal significantly obtunded. These criteria are based on CRPRC guidelines. In addition, the lymph node biopsies will result in some post-procedure pain.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

All possible efforts will be made to minimize animal pain and discomfort. Analgesics have no effect on the proposed studies and they will be administered at the discretion of the CRPRC veterinary staff. The SIV infected animals will be euthanized prior to or at the time they develop clinical signs of AIDS. The decision to euthanize will be based on the judgement of the CRPRC veterinarians.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? Yes No

(Note: "Death as an endpoint" refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. "Alternative methodologies" refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search?

5/9/01

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
Pubmed	unlimited	SHIV-C, SIVmac 239; HIV subtype C, vaccine, immunization
Current contents	unlimited	SHIV-C, SIVmac 239; HIV subtype C, vaccine, immunization

What were your findings with respect to alternative methodologies?

Has this study been previously conducted?

Yes No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

k) **Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

Animals will be euthanized for obtaining samples as noted C. In addition, the SHIV infected animals will be euthanized prior to, or at the time, they develop clinical signs of AIDS. (see 19 a above)

l) **Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

Species	Method	Drug	Dose (mg/kg)	route
rhesus	IV	pentobarbital	60 mg/kg	IV

m) **Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

All animals will be euthanized at the end of this project.

Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator's Statement:

I have read and agree to abide by the *UC Davis Policy and Procedure Manual* section 290-30 (Animal Use and Care). This project will be conducted in accordance with the *ILAR Guide for the Care and Use of Laboratory Animals*, and the *UC Davis Animal Welfare Assurance* on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at <http://ehs.ucdavis.edu/>). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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Committee Use Only Below

<p>** Conditions necessary for Committee Approval:</p>
<p>Final Disposition of this protocol:</p> <p>_____ Approved</p> <p>_____ Not Approved</p> <p>_____ Withdrawn by Investigator</p> <p>Date of Action: ____/____/____</p>

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

_____ <i>Campus Veterinarian</i>	_____ <i>Date</i>
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