PROTOCOL FOR ANIMAL USE AND CARE
Handwritten forms are not accepted

CNPRC

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<td>Phone / Fax:</td>
<td>Phone:</td>
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<td>After hrs. #:</td>
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Species (common names): Rhesus macaque
Number: 6
Source: CRPRC

Project Title: Immunology Core Positive Controls

Overnight housing location: Primate Center
Day use: Primate Center
Animals will be maintained by: [ X] Vivarium [ ] Investigator (If investigator maintained, attach husbandry SOP's.)

Procedures:
These animals will be maintained as a sample source for "positive controls" of immunologic assays performed by the Immunology Core at the Primate Center. They must remain in good health and it will be important to notify the contact person of any health problems.

Special Husbandry Requirements:
Infectious housing for SIV-infected monkeys.

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

<table>
<thead>
<tr>
<th>Sick Animals</th>
<th>Dead Animals</th>
<th>Pest Control</th>
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<tbody>
<tr>
<td>[ x ] Call Investigator</td>
<td>[ x ] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[ x ] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[ x ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[ ] Necropsy</td>
<td>[ x ] Necropsy</td>
<td></td>
</tr>
</tbody>
</table>

Hazardous Materials (only if in the animal room):

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Radioisotopes?</th>
<th>Chemical Carcinogens?</th>
<th>Toxic Chemicals?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ x ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
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<tr>
<td>[ ] No</td>
<td>[ x ] No</td>
<td>[ x ] No</td>
<td>[ x ] No</td>
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</tbody>
</table>

Agent(s): SIV
Funding source: Primate Center Base Grant

Previously approved? [x] Yes [ ] No

Previous protocol number (if any): 9231

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

[ ] Lab Animal Health Clinic (2-0514)
[ ] VMTH Large Animal Field Service (2-0292)
[x] California Primate Research Center (2-0447)
[ ] Another Veterinarian

If you checked “Another Veterinarian”, please provide:

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

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.

In order to maintain quality control of immunologic assays provided as a service to other investigators by the Immunology Core at the Primate Center, it is necessary to maintain SIV-infected positive control animals for specific SIV test antigens. The animals will be bled periodically, not to exceed 12mL/kg/month, and peripheral lymph node biopsies will be obtained under local anesthesia.

b) Procedures employed in this project:

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

[ ] Monoclonal Antibody Production **
[ ] Polyclonal Antibody Production **
[ ] LD 50 or ID50 studies.
[ ] catheters, blood collection, intubation
[ ] Prolonged restraint (8 hrs+)
[ ] Fasting prior to a procedure.

[ ] Food or water restriction
[ ] Non-recovery surgical procedures
[ ] Survival surgical procedures
[ ] Multiple survival surgery
[ ] Behavioral modification.
[ ] Aversive conditioning.

[ ] Special diets; food or water treatment.
[ ] Induced illness, intoxication, or disease
[ ] Death as an endpoint (see i below)
[ ] Trapping, banding or marking wild animals

** 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.)
A total of 6 rhesus monkeys will be maintained throughout the time course of this protocol. The animals will be infected with SIV. Any animal that becomes ill will be promptly treated by the veterinarian and the animal will be euthanized for medical reasons if it develops serious, recurrent or untreatable illness, e.g. simian AIDS.

The monkeys will be inoculated IV with $10^3$ TCID50% of SIVmacΔnef with 1 pre-bleed on the day of inoculation. This virus is an attenuated molecular clone that establishes low-level persistent infection in rhesus monkeys but does not cause simian AIDS. In fact, about 10% of monkeys will develop AIDS with this virus over a 2-year period. At the earliest time of AIDS diagnosis (criteria are listed in section “i”, below), the animal would be euthanized and replaced with another animal. Animals will undergo phlebotomy on a weekly or bi-weekly basis with the cumulative volume of blood not to exceed 12ml per kg body weight per month. Axillary or inguinal lymph nodes will be biopsied (1 node, approximately 10mm in length) every 2 months by a skilled animal technician. The biopsy site is determined according to the location of the enlarged lymph node for clinical procedures and according to the project protocol for experimental procedures. Topical analgesia is attained via a subcutaneous infiltration of Bupivicaine (0.1 - 0.2 ml of 0.25% solution) proximal and medial to the lymph node to be biopsied. The site is surgically prepared. The skin over the node is incised with a scalpel blade. The node is exposed by blunt dissection. The node can either be removed in its entirety by a combination of blunt and sharp dissection, or the node can be clamped with hemostats and a portion removed by sharp dissection. The skin is then closed using suture and/or sterile surgical adhesive. Bone marrow aspirates will be taken every 6 months by needle aspiration according to CNPRC SOP and conducted by CNPRC staff. Immobilize the animal with ketamine HCl. The area over the iliac crest or head of the humerus is shaved and surgically prepped. Topical analgesia is recommended via a subcutaneous infiltration of Lidocaine (0.1-0.2 ml of 2% solution). The anterior dorsal rim of the iliac crest or head of the humerus is identified by palpation. An 18 or 20 gauge 1-1 1/2 inch bone marrow aspiration needle is advanced in a rotating manner 5-15 mm depending on the size of the animal. After the stylet is removed a syringe (usually heparinized) is attached to the aspiration needle. Suction is applied by a steady full extension of the plunger to create back pressure. After each pull the needle should be rotated 90 degrees to redirect the direction of aspiration. If marrow does not appear, the stylet is replaced and placement of the needle is changed. The amount of marrow taken from all aspiration sites should not exceed the guidelines for a blood draw. Post-procedure analgesics may be administered at the discretion of the veterinarian. Lymph node and bone marrow samples are taken from overnight-fasted animals under ketamine anesthesia.

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SIV inoculation</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>
## Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 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?

Only rhesus monkeys can be utilized because they are the subject species for all projects that will use the Immunology Core services at the Primate Center. Six is a practical animal number for the determination of statistical significance in immunologic assays.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
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</thead>
<tbody>
<tr>
<td>anteroom of animal housing</td>
<td></td>
</tr>
</tbody>
</table>

Who will be the surgeon?  
trained animal technician

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhesus</td>
<td>ketamine HCl</td>
<td>10 mg/kg</td>
<td>IM</td>
<td>before all procedures</td>
</tr>
<tr>
<td></td>
<td>oxymorphone</td>
<td>0.15</td>
<td>IM</td>
<td>as needed for pain</td>
</tr>
</tbody>
</table>

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?

no
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 and wound contamination. The animals are immobilized with ketamine for the procedure and should not experience pain. Wound infections are treated with antibiotics and local care by the veterinarian.

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 2 weeks or >30% in 3 months; persistent hypothermia <96°F 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 and bone marrow 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 postoperative 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.

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 [x] 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:

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

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit
http://trc.ucdavis.edu/jawelsh/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu)

or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search? 7/25/03

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

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
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<tbody>
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What were your findings with respect to alternative methodologies?

There are no new technologies in vitro that can replace the requirement for SIV-infected rhesus monkeys for positive controls in SIV immunologic assays.

Has this study been previously conducted?  [ ] Yes  [x] 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 after diagnosis of simian AIDS.**

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
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</thead>
<tbody>
<tr>
<td>rhesus</td>
<td>IV</td>
<td>pentobarbital</td>
<td>60mg/kg</td>
<td>IV</td>
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</table>

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

**There will be no surplus animals.**
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don’t need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
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.

Principal Investigator | Rank / Title | Date

**Conditions necessary for Committee Approval:**

Final Disposition of this protocol:

- [ ] Approved
- [ ] Not Approved
- [ ] Withdrawn by Investigator

Date of Action: _____ / _____ / _____

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

Campus Veterinarian | Date
ANIMAL ROOM SAFETY INFORMATION

Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL # 10796

EXPIRES: ________

<table>
<thead>
<tr>
<th>RUA#</th>
<th>BUA#</th>
<th>CCA#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0477</td>
<td></td>
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</table>

Identity of Hazard: SIV

Investigator Last Name: ____________________________  Department: ____________________________
First Name: ____________________________  Phone: ____________________________
Email: ____________________________  Fax: ____________________________

Provide a short description of the agent:
SIV is a primate lentivirus that is genetically similar to HIV and causes fatal immunodeficiency (AIDS) in infected rhesus macaques. SIV can infect humans, but it is unknown whether SIV causes human disease.

This agent / material is hazardous for: [ ] Humans only  [ ] Animals only  [X] Humans and Animals

For which Animal Species?

The agent can be spread by: [X] Blood  [ ] Feces/urine  [ ] Saliva/nasal droplets  [ ] Does not leave animal

All mucosal secretions can be contaminated.

Describe any human health risk associated with this agent:
SIV can infect humans; thus, it is possible that SIV could cause a fatal, AIDS-like disease in humans. Infectious virus and SIV antibodies have been detected in SIV-infected humans but there have been no reports of disease in SIV-infected people.

The precautions checked below apply to this experiment:
[ ] The researcher or his/her technicians are responsible for the feeding and care of these animals.
[ ] The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.

- [X] Cages must be autoclaved before cleaning.
- [ ] Label cages and remove label after decontamination.
- [X] Animal carcasses must be labeled and disposed of as follows:
  - [ ] Incineration
  - [ ] Bag and Autoclave
  - [X] Biohazardous Waste Container
  - [ ] EH&S will pick-up (2-1493).
- [X] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows
  - [ ] Incineration
  - [ ] Bag and Autoclave
  - [X] Biohazardous Waste Container
  - [ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required:
[ ] The following personal protective equipment must be worn/used in the room:

- [X] Lab Coat/Coveralls
- [X] Disposable Gloves
- [X] NIOSH Certified Dust Mask
- [X] Eye Protection/Face Shield
- [X] Fitted Respirator
- [ ] Other: ____________________________  Type: ____________________________  Describe: Plastic disposable gown/coveralls

[ ] Personal protective equipment must be removed before leaving the room.
[ ] Personal protective equipment must be discarded or decontaminated at the end of the project.
[ ] Hands, arms, and face must be thoroughly washed upon leaving the room.
[ ] Full shower, including washing of hair, must be taken upon leaving the room.
[ ] Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room:

Biosafety Level 2 precautions must be used at all times.
08/01/03
Pre review Questions Protocol 10796

Hi ,

I have received and pre reviewed the recently submitted protocol, which has been assigned accession number 10796 for future reference. I have attached a copy of the protocol for ease of making revisions. For this protocol to be considered on the Aug 14th committee agenda, please forward your revised protocol to me on or before noon, Tuesday, Aug 5th.

If you have any questions feel free to contact me.

Thank you in advance,

Protocol 10796 ( )

1. On page 1 the following boxes were left blank. Please complete the overnight housing and day use boxes.

2. On page 1, the special husbandry requirements section has nothing listed. Will these animals need to be housed in infectious housing?

3. In section c, you mention that the animals will be inoculated IV with SIVmac... At what time point will this be performed and will there need to be a pre bleed collection?

4. In section c, you go on to state that at the earliest time of AIDS diagnosis, the animal will be euthanized. What signs and what is the approximate time frame?

5. You also discuss lymph node biopsies. Are these performed under anesthesia? If so, please describe what will be done. Will the animals be fasted? Will the bone marrow aspirates be performed under anesthesia and fasting be required? Will post procedure analgesics be needed? Please expand to list everything that will be happening to the animals.

6. In section i, you have a detailed description of the SIV infection, but what about post procedure infection? Is is possible to have post biopsy or bone marrow sampling infection or complications? If so, please provide additional detail.

7. In section j, you only provided one database. Please be advised that you are required by law, per instructions for this section, to list more than one database. Please provide the additional information.

********************************************************************************

08/14/03
Vet Staff questions Protocol 10796

Hi ,

I have received the following comment from the CNPRC vet staff regarding protocol 10796 ( ). The comment is a very good one that will require committee approval. At this time we are not requesting any changes, but may do so if the committee so chooses.

Stay tuned - I am going to request that PI's, in consultation with the CNPRC staff, can reference SOPs for specific procedures.

Protocol 10796 ( )

Dr. is using an old description for bone marrow and lymph node biopsies and its actually primate center central staff that does his procedures. We have very complete SOP's for these- can he just reference the SOPs or does everything need to be clearly described in the protocol?