**PROTOCOL FOR ANIMAL USE AND CARE**

**CNPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<tbody>
<tr>
<td>Last Name:</td>
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<td>Phone / Fax:</td>
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<tr>
<td>After hrs. #:</td>
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**Species (common names):** Rhesus macaque  
**Number:** 16/yr  
**Source:** CNPRC

**Project Title:** SIV and SHIV Clones in Macaques

**Overnight housing location:** CNPRC  
**Day use only:** CNPRC

**Animals will be maintained by:** [X] Vivarium  [ ] Investigator  *(If investigator maintained, attach husbandry SOP's.)*

**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.

Juvenile macaques will be inoculated with SIV or SHIV by either the intravenous or mucosal membrane routes (vaginal mucosal membranes). At 2 to 4 week intervals, 10-20cc of peripheral blood is collected for virological and serological studies. At 8 week intervals, axillary or inguinal lymph nodes are obtained by truncutaneous biopsy under anesthesia.

**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.

**CNPRC protocols of SIV-infected macaques in BSL-2 housing.**

**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>[ ] 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>
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</table>

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Agent(s): Simian Immunodeficiency Virus (SIV) and Simian-Human Immunodeficiency Virus (SHIV)</th>
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<tbody>
<tr>
<td>[X] Yes  [ ] No</td>
<td>Agent(s):</td>
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<tr>
<th>Radioisotopes?</th>
<th>Agent(s):</th>
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<tbody>
<tr>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
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<tr>
<th>Chemical Carcinogens?</th>
<th>Agent(s):</th>
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<tbody>
<tr>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
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</table>

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<tr>
<th>Toxic Chemicals?</th>
<th>Agent(s):</th>
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<tbody>
<tr>
<td>[ ] Yes  [X] No</td>
<td>Agent(s):</td>
</tr>
</tbody>
</table>
Funding source: NIH-R01-AI38530  
Previously approved?  [X] Yes  [ ] No  
Previous protocol number (if any):  #8966

What Veterinarian or veterinary clinic will provide care for your animals? (check one)
[X] Lab Animal Health Clinic (2-0514)  
[ ] California Primate Research Center (2-0447)  
[ ] VMTH Large Animal Field Service (2-0292)  
[ ] 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 pctillman@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.

Background: AIDS is a fatal disease caused by infection with the human Immunodeficiency virus type 1 (HIV-1). No vaccines for preventing HIV-1 infection and no effective therapies or cures for AIDS are available. An appropriate animal model for investigating mechanisms of HIV-1 infection and pathogenesis is not available. Selected strains of simian immunodeficiency virus (SIV), a primate lentivirus genetically related to HIV, causes a fatal AIDS-like disease in macaques.

Hypothesis: The hypothesis is that functions of HIV-1 genes can be analyzed in vivo by constructing SIV and SHIV clones and testing such clones in susceptible macaques.

Objectives and significance: The objective of this project is to test the pathogenic potential of SIV and SHIV clones in juvenile rhesus macaques. This project will produce molecular clones of these 2 viruses with mutations in specific viral genes. Macaques inoculated with viral mutants will be monitored for virus load, antiviral immune responses, and clinical signs of immunodeficiency. Thus, the significance of this project is that we will be able to identify which viral genes contribute to disease. Ultimately, drugs and/or vaccines targeted against a viral gene may be developed; such drugs and vaccines may inhibit viral replication and prevent or delay disease progression.

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+)  
[X] Fasting prior to a procedure.  
[ ] Food or water restriction  
[ ] Non-recovery surgical procedures  
[ ] Survival surgical procedures  
[ ] Multiple survival surgery  
[ ] Behavioral modification.  
[ ] Special diets; food or water treatment.  
[ ] Induced illness, intoxication, or disease  
[ ] Death as an endpoint (see i below)  
[ ] Trapping, banding or marking wild animals  
[ ] Aversive conditioning.  

** 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.)

Macaques will be inoculated once with 1 cc of virus (from a molecular clone of SIV* or SHIV*) in tissue culture fluid by intravenous (IV) inoculation with a hypodermic syringe. To study mucosal membrane transmission, 1 cc of virus in tissue culture medium will be topically applied to vaginal mucosa of females (Ivag). Macaques will be anesthetized for inoculations by intramuscular injection with ketamine (10 mg/Kg body weight). Two groups of animals will be inoculated with SIV by the IV or Ivag routes. Two groups of animals will be inoculated with SHIV by the IV or Ivag routes.

On the day of virus inoculation, a prebleed sample (10 ml) of peripheral blood will be collected. Only viruses which replicate in cultures of rhesus PBMC will be used to inoculate macaques. After inoculation, 10 to 20 ml of peripheral blood will be collected from macaques by venipuncture at 1, 2, 4, 8, 12, 16, 20, 24, 32, 42 and 52 weeks post-exposure. Axillary or inguinal lymph nodes will be obtained from each juvenile macaque by transcutaneous biopsy at 2, 8, 24, and 52 weeks post-inoculation; biopsies will be performed by CNPRC staff according to CNPRC SOPs. Macaques will be anesthetized for blood and lymph node collections by intramuscular injection with ketamine (10 mg/Kg body weight). Peripheral blood lymphocytes and lymph node cells will be tested for virus load (co-culture assay, plasma viremia, plasma antigenemia) and anti-viral antibody responses. Complete blood counts (CBC), including CD4 and CD8 lymphocytes, will be determined at the timepoints listed above on peripheral blood samples to monitor potential hematologic abnormalities that may accompany SIV infection. When animals are bled, weights will be determined. CNPRC guidelines outlining criteria for euthanasia for retrovirus infected macaques will be followed.

* Our intention is to use a single animal protocol for the in vivo macaque studies of either SIV or SHIV clones because the procedures for inoculation and sample collection are identical for animals inoculated with SIV or SHIV. The choice of SIV versus SHIV clone depends on the results of in vitro cell culture analysis of these clones prior to inoculation.

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>IV inoculation with SIV clone</td>
<td>4 juveniles</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Ivag inoculation with SIV clone</td>
<td>4 juveniles</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>IV inoculation with SHIV clone</td>
<td>4 juveniles</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>Ivag inoculation with SHIV clone</td>
<td>4 juveniles</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?

1. Rhesus macaques have proven to be a valuable non-human primate model for investigating many human infectious agents and closely related simian pathogens. Macaques exhibit a fatal AIDS-like disease after exposure to SIV or SHIV. Accordingly, macaques are an important model for analyzing mechanisms of pathogenesis that occur in HIV infection and AIDS. Additionally, the immune system of macaques shares many similarities to that of humans. A non-human primate model will be essential for developing and testing antiviral drugs and vaccines aimed at controlling or preventing viral infection and associated disease.

2. Our previous studies at CNPRC on SIV and SHIV clones and variants indicates that statistically valid information can be obtained from a group of 4 animals inoculated with one virus.

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.

<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</td>
<td>IM</td>
<td>Per CNPRC SOPs</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Midazolam</td>
<td>10</td>
<td>IM</td>
<td>Per CNPRC SOPs</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Medetomidine</td>
<td>30-50 mcg/kg</td>
<td>IM</td>
<td>Per CNPRC SOPs</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Atipamezole</td>
<td>0.15</td>
<td>IM or IV</td>
<td>Per CNPRC SOPs</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Ketoprofen</td>
<td>20</td>
<td>IM</td>
<td>Per CNPRC SOPs</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Lidocaine</td>
<td>0.01</td>
<td>SubQ</td>
<td>Per CNPRC SOPs</td>
</tr>
</tbody>
</table>
Rhesus Buprenorphine 0.01-0.03 IM Per CNPRC SOPs

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)

   SIV or SHIV infection of susceptible macaques produces a progressive fatal immunodeficiency disease characterized by hematologic abnormalities, lymphocyte depletion, weight loss and cachexia, an infection with opportunistic pathogens. CNPRC guidelines outlining criteria for euthanasia for virus infection will be followed.

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 CNPRC 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.

   This project will assess the virulence of novel SIV and SHIV clones; accordingly, death is not an endpoint.

   Infected animals will be euthanized prior to or at the time they develop clinical signs of SAIDs. The decision to euthanize will be based on the judgment of the CNPRC veterinary staff.

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?  Feb. 10, 2003
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>
</tr>
</thead>
<tbody>
<tr>
<td>PubMed</td>
<td>1990-2003</td>
<td>SIV, SHIV, simian AIDS, viral pathogenesis in macaques</td>
</tr>
<tr>
<td>Symposium on Non-Human Primates in AIDS (Monterey, CA)</td>
<td>2002</td>
<td>SIV, SHIV, simian AIDS, viral pathogenesis in macaques</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

As yet, there is no tissue culture system for analyzing SIV, SHIV, or HIV-1 pathogenesis, comparing routes of transmission or evaluating anti-viral drugs and vaccines. Such studies can be done only in vivo in experimentally infected macaques.

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?

Macaques will be euthanized at 24 months after inoculation. If clinical judgement decides that sick animals cannot be stabilized, then they will be euthanized.

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>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Overdose</td>
<td>Sodium Pentobarbitol</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

If the animals do not show any signs of disease at 24 months post-inoculation, then these animals may be included in other ongoing virology projects.
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://ehs.ucdavis.edu/animal/health/](http://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at [http://ehs.ucdavis.edu/](http://ehs.ucdavis.edu/).
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/](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.

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**Conditions necessary for Committee Approval:**

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Final Disposition of this protocol:

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

Date of Action: _____ / _____ / _____

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

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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.

Identity of Hazard:
- SIV (Simian Immunodeficiency Virus)
- SHIV (Simian-Human Immunodeficiency Virus)

Provide a short description of the agent:
Retrovirus, obtained by recombining simian and human immunodeficiency virus clones.

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

For which Animal Species?
[ ] Rodents
[ ] Canines
[ ] Felines
[ ] Equines
[X] Primates
[ ] Non-human Primates
[ ] Other:

The agent can be spread by:
- [X] Blood
- [ ] Saliva/nasal droplets
- [ ] Feces/urine
- [ ] Saliva/nasal droplets
- [ ] Does not leave animal
- [X] Other: mucosal (eye/mouth/nose/genital)

Describe any human health risk associated with this agent:
Potential for causing immunodeficiency disease. Related viruses are fatal in monkeys and humans.

The precautions checked below apply to this experiment:
- [X] The researcher or his/her technicians are responsible for the feeding and care of these animals.
- [X] The following items must be assumed to be contaminated with hazardous material and must be handled only by the researcher or his/her technicians.
  - Cage
  - Stall
  - Bedding
  - Water Bottle
  - Animal Carcasses
  - [ ] Other:
    - [X] Incineration
    - [ ] Bag and Autoclave
    - Biohazardous Waste Container
    - [ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required:
- [X] The following personal protective equipment must be worn/used in the room:
  - Lab Coat/Coveralls
  - Disposable Gloves
  - NIOSH Certified Dust Mask
  - Eye Protection/Face Shield
  - Fitted Respirator
  - Type:
    - [X] Other: Describe: plastic disposable gown/coverall
  - Shoe Covers/Booties
  - Head Cover
  - Disinfectant footbath

Provide any other information needed to safely work in this room:
CNPRC staff will provide all animal care and will follow standard CNPRC procedures for infectious agents (SIV and SHIV).