**PROTOCOL FOR ANIMAL USE AND CARE**

*Handwritten forms are not accepted*

**CNPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
</tr>
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<tbody>
<tr>
<td>Last Name:</td>
<td>Last Name: same</td>
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<td>Phone / Fax:</td>
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<td>After hrs. #:</td>
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**Species (common names):** Rhesus macaque  **Number:** 61  **Source:** Primate Center

**Project Title:** Preclinical testing in rhesus macaques of an HIV DNA vaccine expressing the envelope of an Indian clade C virus.

**Overnight housing location:** Primate Center  **Day use only:**

**Animals will be maintained by:** [ ] 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.

The goal is to develop a DNA vaccine specific for HIV viruses that are circulating in India. Juvenile male rhesus will be inoculated with an HIV/SIV chimeric virus (SHIV) in Years 1 and 2. It is likely that the virus will be attenuated initially, but it will be passaged by blood through at least 3 pairs of monkeys to make it cause simian AIDS. This virus will then be used as a challenge for additional juvenile male monkeys that have been given a DNA vaccine and then protection from AIDS will be monitored by clinical signs, viral loads and CD4+ cell counts.

**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.

No specific requirements.

**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>[ ] Call Investigator</td>
<td>[ x] Call Investigator</td>
<td>[ x] Call Investigator</td>
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<tr>
<td>[ ] 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>
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<tr>
<td>[ ] Necropsy</td>
<td>[ ] Necropsy</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>[ X] Yes  [ ] No</th>
<th>Agent(s): SHIV (BAUA #0477)</th>
</tr>
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<tbody>
<tr>
<td>Radioisotopes?</td>
<td>[ ] Yes  [ x] No</td>
<td>Agent(s):</td>
</tr>
<tr>
<td>Chemical Carcinogens?</td>
<td>[ ] Yes  [ x] No</td>
<td>Agent(s):</td>
</tr>
<tr>
<td>Toxic Chemicals?</td>
<td>[ ] Yes  [ x] No</td>
<td>Agent(s):</td>
</tr>
</tbody>
</table>
Funding source: NIH
Previously approved? [X] Yes [ ] No
Previous protocol number (if any): 9104

Is the project already funded? [X] Yes [ ] No

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)
[ ] California Primate Research Center (2-0447)
[ ] 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.

Epidemic HIV infection is emerging in the Indian subcontinent and the prevalent viral strains are different from those in the U.S., by envelope gene sequence they are clade C types. The goal is to develop a DNA vaccine specific for HIV in India. Preliminary results of clinical trials with DNA vaccines have indicated that humoral and cellular immune responses can be induced in humans. However, experimental results produced in mice do not predict antigenicity in humans, as mice are often more responsive to immune stimulants than primates. We hypothesize that rhesus monkeys will respond to a DNA vaccine expressing the clade C strain of HIV-1, and that they will be protected from challenge with a pathogenic SHIV expressing the clade C envelope gene.

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.

[ ] 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.
1. All animals will be bled for 10ml prior to assignment for viral screen and for pre-treatment samples.
2. Serial animal passage to obtain a pathogenic SHIV-C. Two juvenile male macaques will be inoculated by the iv route (femoral vein) with $1 \times 10^5$ tissue culture infectious dose (TCID) of SHIV-C virus stock (first pass) and the animals will be bled for 10ml on days 7, 14, 28 and 90 for viral load measurement and for CD4+ T cell counts. Axillary or inguinal lymph node biopsies will be taken at 3 months to measure viral load and CD4+ T cell counts. Lymph node biopsy is a standard minor surgical procedure at the CRPRC performed under Ketamine anesthesia by trained animal technicians. Animals will be culled at 6 months after inoculation or sooner if they develop simian AIDS (opportunistic infection, anemia, neurologic signs) or weight loss > 10% of baseline. For second to third passage of SHIV, 2 macaques per passage (ie. 4 monkeys) will be inoculated iv with 3ml plasma taken and stored from the first and previous pair of monkeys. The animals will be bled (blood samples will be taken on days 3, 7, 14, 21 and then monthly post inoculation) and the signs of AIDS monitored as above. A final 2 monkeys (total n=8) will be inoculated orally with a SHIV stock ($10^5$ TCID in 1ml normal saline) that has been amplified in rhesus peripheral blood mononuclear cells and monitored for AIDS as described above. Note that a second or third animal passage may not be necessary if the animals in passage 1 develop AIDS or CD4+ T cell decline within the 6 month time frame. But experience with clade B SHIVs suggests that 3 passages will be required. Two different SHIVs will be passaged based on 2 clade C viruses from India designated C2 and C3.

3. HIV DNA vaccination followed by challenge with pathogenic SHIV-C. The HIV envelope genes including tat and rev accessory genes have been cloned from clade C2 and C3 isolates by Indian collaborators. These genes will be cloned into the pND mammalian expression vector as a DNA vaccine. Control animals (groups 3 and 5, n=9) will be vaccinated with the same vector DNA expressing the β-galactosidase gene. Animals will be vaccinated with 100µg DNA in 100µl normal saline by the intradermal (id) route. The monkeys will be boosted at 2, 4 and 6 months by the same dose and route. At 8 months, the monkeys will be challenged with SHIV-C2, SHIV-C3 or with a SHIV-B isolate (groups 4, 6 and 7, n=9) by the oral route with $10^5$ TCID in 1ml normal saline. Ten to 15 ml blood will be taken at days 7, 14 and 28 and then bi-monthly and the signs of simian AIDS will be monitored as described above. Animals will be culled at 6 months if high viral loads and low CD4+ T cell counts are sustained in the absence of other signs of AIDS.

**NOTE:** The following procedure is performed AFTER chemical restraint of the animal. See CNPRC SOP# FF-1: Restraint Procedures: Chemical.

5.1 Wear protective clothing and equipment per current CRPRC Infection Control Policy. Check the animal’s tattoo to verify that it is the correct animal. 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.

5.2 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. **Care must be taken not to inject any of the anaesthetic intravascularly.**

5.3 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.

5.4 The skin is then closed using suture and/or sterile surgical adhesive.
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>Inoculation of SHIV-C and serial animal passage</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Inoculation of SHIV-C and serial animal passage</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Immunization with control DNA followed by challenge with SHIV C2</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Immunization with clade C2 DNA followed by challenge with SHIV C2</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Immunization with control DNA followed by challenge with SHIV C3</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>Immunization with clade C3 DNA followed by challenge with SHIV C3</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Immunization with alternate clade C2-3 DNA followed by challenge with SHIV B</td>
<td>9</td>
<td>4</td>
</tr>
</tbody>
</table>

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Little or no discomfort or stress</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
<tr>
<td>2</td>
<td>Minor stress or pain of short duration</td>
</tr>
<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to severe distress</td>
</tr>
<tr>
<td></td>
<td>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</td>
</tr>
<tr>
<td>4</td>
<td>Severe pain near, at or above the pain tolerance threshold</td>
</tr>
<tr>
<td></td>
<td>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.</td>
</tr>
</tbody>
</table>

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?

The rhesus monkey develops simian AIDS after inoculation with pathogenic isolates of SIV or SHIV, this is the most practical animal model of HIV infection and AIDS pathogenesis. In addition, DNA vaccination, while very effective in rodent models, has been suboptimal in humans and other primate species. Thus, if a DNA vaccine is to be tested in human trials, preclinical testing is valid in the rhesus monkey.

Nine animals per group are required to ensure sufficient statistical power to determine vaccine failure (no difference in number of animals infected, virus load, or disease in vaccinated and unvaccinated controls) as well as vaccine efficacy (partial or complete protection). Nine animals per group are also needed to ensure that we can distinguish statistically significant as well as biologically meaningful differences among the vaccine regimens.

The numbers of animals per group was calculated based on the need to detect with biologically acceptable statistical power (~80%) a minimum of 30-fold difference in measures of vaccine success between controls and vaccinated animals. In consultation with Dr. who has expertise in biostatistics, we used estimated
variances of parameters from our recent vaccine experiments in rhesus neonates conducted at the CRPRC and followed the methods outlined in Motulsky, 1995, *Intuitive Biostatistics*.

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

<table>
<thead>
<tr>
<th>Building:</th>
<th>infectious housing</th>
<th>Room:</th>
<th>work room</th>
</tr>
</thead>
</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</td>
<td>10</td>
<td>IM</td>
<td>phlebotomy, lymph node biopsy</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?

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)

The signs of simian AIDS are expected. These include weight loss, anemia, neurologic deficits and opportunistic infections.

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.

The veterinarian will use appropriate and specific antibiotic therapy, medication and fluid support.

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.

A SHIV-infected animal will be euthanized when the criteria of simian AIDS are established by the veterinarian and before the animal suffers severe distress.

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?  4/29/03

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>
</tr>
</thead>
<tbody>
<tr>
<td>Medline</td>
<td>1993-2003</td>
<td>SHIV-C and pathogenesis, vaccination</td>
</tr>
<tr>
<td>Reference Update</td>
<td>1998-2003</td>
<td>SHIV-C</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are no reliable alternative methodologies to in vivo vaccination to test immunogenicity of novel vaccine preparations for HIV and no studies have been conducted with a clade C HIV isolate.

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.

Vaccine and pathogenesis studies have been conducted with several SHIV-B isolates expressing the clade B envelope common in the U.S. and Europe.  But the behavior of a SHIV-C virus cannot be predicted from these studies.

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

All the animals will be euthanized or else assigned to other SIV pathogenesis projects that can utilize these animals.

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>pentobarbital</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?
Not applicable.
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

Committee Use Only Below

** 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 # _10605__
EXPIRES: ________

Identity of Hazard:  SHIV

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

Provide a short description of the agent:

SHIV is a chimeric virus based on SIV expressing the envelope gene of HIV-1. It causes progressive immunodeficiency in infected rhesus macaques.

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

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

For which Animal Species?  

Describe any human health risk associated with this agent:

This is a potential human pathogen although no known human infection has occurred.

The precautions checked below apply to this experiment:
- [ ] 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  
  - [ ] Water Bottle  
  - [X] Animal Carcasses  
  - [ ] Bedding  
  - [ ] Other:  

SOPs as per CRPRC.

[ ] Cages must be autoclaved before cleaning.
[ ] Label cages and remove label after decontamination.
[ ] 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  
  - [X] 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  
  - [X] Eye Protection/Face Shield  
  - [X] Fitted Respirator  
  - [X] Shoe Covers/Booties  
  - [X] Head Cover  
  - [ ] Disinfectant footbath  
  - [ ] Fitted Respirator  
  - [ ] Other: 

Describe:

[ ] 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:


1. In section c, you mention that lymph node biopsy is a standard minor surgical procedure, but we are now asking for a description of the procedures as per direction from a recent USDA inspection. Please include a description or if it is already written up, the SOP for lymph node biopsy can be attached.

   Lymph Node Biopsy Procedure:

   NOTE: The following procedure is performed AFTER chemical restraint of the animal. See CNPRC SOP# FF-1: Restraint Procedures: Chemical.

   5.1 Wear protective clothing and equipment per current CRPRC Infection Control Policy. Check the animalís tattoo to verify that it is the correct animal. 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.

   5.2 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. Care must be taken not to inject any of the anaesthetic intravascularly.

   5.3 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.

   5.4 The skin is then closed using suture and/or sterile surgical adhesive.

2. In section c, 2., you mention that neurologic signs is one thing you will look for when an animal develops simian AIDS. What type of neurologic signs do you anticipate seeing? Please expand to include what you mean by neurologic signs. Neurologic signs would include head tilt, flaccid paralysis, obfuscation, or nystagmus.

3. At what time point(s) will you be collecting blood? Please expand to include blood collection time points. Blood samples will be taken on days 3, 7, 14, 21 and then monthly post inoculation.