**PROTOCOL FOR ANIMAL USE AND CARE**

**CRPRC**

**Investigator**

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone / Fax:</th>
<th>After hrs. #:</th>
</tr>
</thead>
</table>

**Contact**

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone:</th>
<th>After hrs. #:</th>
</tr>
</thead>
</table>

**Species (common names):** Cynomolgus macaques

**Number:** 28

**Source:** CRPRC

**Project Title:** Infection of cynomolgus monkeys with feline immunodeficiency virus

**Overnight housing location:** CRPRC

**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.

One group of animals will be infected with FIV-infected autolgous cynomolgus monkey PBMCs and blood taken at two week intervals for testing. A second group will be inoculated with non-infected autolgous PBMCs.

**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.

Infected animals will need to be kept in infectious 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>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Call Investigator</td>
<td>[ ] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[ ] Call Investigator</td>
<td>[ ] Call Investigator</td>
<td>[ ] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[ ] Bag for disposal</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<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>Radioisotopes?</th>
<th>Chemical Carcinogens?</th>
<th>Toxic Chemicals?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
</tr>
<tr>
<td>[ ] No</td>
<td>[ ] No</td>
<td>[ ] No</td>
<td>[ ] No</td>
</tr>
</tbody>
</table>

**Agent(s):**

- **Feline immunodeficiency virus (FIV)**
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.

A recent study suggests that FIV will cause a severe AIDS-like disease in cynomolgus macaques when inoculated in the form of FIV-infected autologous PBMCs. This has created controversy about the use of FIV as a gene delivery vector and about the potential health hazard to humans of FIV. The objective of this study is to repeat this work with larger numbers of animals and with more thorough monitoring of disease signs and virus status in the body.

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
- 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.)*

### Specific Aims

1. Confirm that FIV-Petaluma, grown in autologous cynomolgus macaque PBMCs, will cause AIDS-like signs when infected into adult cynomolgus monkeys. (10 animals)

2. Determine whether FIV related disease in cynomolgus monkeys is similar to acute HIV-1 infection of humans.

3. Determine whether the phenomenon is specific for FIV-Petaluma (a clade A strain), i.e., will an isolate from clade C such as FIV-Cgamma also cause disease in cynomolgus macaques. (10 animals)

4. Determine whether a more severe, and possibly progressive, disease can be induced in newborn Cynomolgus macaques. (8 animals)

Peripheral blood mononuclear cell (PBMC) cultures will be established from each animal prior to the actual experiment. Blood will be taken, PBMC purified by isohypaque gradient centrifugation, and cells stimulated with ConA and human rIL-2. Once cultures are growing well, they will be infected with one of two different strains of FIV (Petaluma of Gammar). Virus replication in the cultures will be monitored by an antigen capture ELISA and by TaqMan for viral RNA and DNA. In Specific Aim #1, monkeys will be inoculated either with normal autologous PBMC or with FIV-Petaluma infected PBMC. In Specific Aim #3, monkeys will be treated identically, but inoculated with either normal PBMC or FIV-Gammar infected PBMC. FIV-Gammar is reportedly a much more virulent virus than FIV-Petaluma. In Specific Aim #4, newborn cynomolgus monkeys will be similarly inoculated with normal or FIV infected autologous PBMC. The strain of virus that will be used will depend on the results of experiments conducted in Specific Aims #1 and 3.

Once inoculated, all of the monkeys (adult and newborn) will be monitored in the same manner. Animals will be weighed daily for the first 4-8 weeks for signs of weight loss (adults) or lack of weight gain (newborns) and observed for other outward signs of illness (e.g., diarrhea, rough coat, vomiting, lymphadenopathy, secondary infections, etc). Examinations will be every 2-3 days thereafter, depending on disease course. Rectal temperatures will also be taken daily at first, and every second or third day after the 4-8 week. Blood will be harvested at two-week intervals starting 2-4 weeks prior to infection and extending through the study, which could be over 20 weeks. Blood will be used for routine CBC and tests for CD4+ and CD8+ T cells (by FACS), viral levels (by culture and TaqMan) in PBMC and plasma, and FIV antibodies.

The animals will be observed for as long as they continue to show signs of infection and/or disease. The original studies indicated that the FIV infection of adult cynomolgus macaques was transient. However, these animals were killed at 10 weeks post-infection, when their conditions were improving but not yet normal. Therefore, we anticipate observing these animals for a longer period of time – 10-20 weeks or more. Animals that are showing severe signs of disease will be euthanatized, a necropsy performed, and tissues collected for virologic and pathologic 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-A</td>
<td>Infected with FIV</td>
<td>6</td>
<td>2-4</td>
</tr>
<tr>
<td>I-B</td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>III-A</td>
<td>Infected with FIV</td>
<td>6</td>
<td>2-4</td>
</tr>
<tr>
<td>III-B</td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>IV-A</td>
<td>Infected with FIV</td>
<td>5</td>
<td>2-4</td>
</tr>
<tr>
<td>IV-B</td>
<td></td>
<td>3</td>
<td>1</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><strong>Examples:</strong> domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skilful 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><strong>Examples:</strong> 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><strong>Examples:</strong> 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><strong>Examples:</strong> 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?

We are trying to use the minimum number to confirm the findings of the original report. We have learned from experience, that you must have at least 6-7 animals in each infection group to demonstrate significant differences. We are limiting the control groups to 4 animals each, which should be adequate given the uniformities in blood counts, etc., in adult cynomolgus monkeys. We are using 5 infected and 3 noninfected controls for the newborn study because there has been no previous experience with this age group, but assume that the disease will be more severe rather than milder.

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>Cynomolgus macaque</td>
<td>Ketamine</td>
<td>10mg/kg</td>
<td>IM</td>
<td>Only if needed to collect blood samples</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)

An acute, and significant (20-30%), loss in weight was described in the two animals reported in the literature. No descriptions were provided about other signs of illness, such as fever, inappetance, diarrhea, vomiting, etc.

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.

Staff veterinarians for the CRPRC will have ultimate responsibility for health issues on these animals. They will follow predefined CNPRC euthanasia guidelines for SIV infected macaques. Those guidelines are provided as an attachment. It is important to remember, however, that these animals will not be infected with SIV, but rather with FIV. Initial reports on xenoinfection indicate that the animals were not outwardly ill, except for signs of weight loss. Signs were transient and disappeared when the virus could no longer be recovered. However, we chose to use SIV guidelines, because they are more vigorous and are already in effect.

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? 4/23/02

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>1988 to present</td>
<td>FIV, SIV, HIV, FIV and cynomolgus monkeys, SIV and cynomolgus monkeys</td>
</tr>
<tr>
<td>Entrez-Pubmed</td>
<td>1990 to present</td>
<td>FIV, SIV, HIV, FIV and cynomolgus monkeys, SIV and cynomolgus monkeys</td>
</tr>
<tr>
<td>Hand search of journal article reference list</td>
<td>1990 –4/01/02</td>
<td>References lists of all relevant articles from above searches were cross-referenced</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

Has this study been previously conducted? [x] Yes [ ] No

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

The original report involved 2 animals and documentation of disease and infection was poor. The results, that FIV can cause disease in nonhuman primates, was also totally unexpected, and there are some questions as to the validity of the results. NIH feels that the findings are of sufficient importance and controversy to bear repeating with more animals and in a more thorough fashion.

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

Animals will be euthanized when it is apparent that their disease state is terminal. We have considerable experience in making this judgment in rhesus monkeys infected with SIV of SHIV. Ultimately, this is a decision reached by the investigator and the CRPRC veterinarian.

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>Cynomolgus macaque</td>
<td>Drug overdose</td>
<td>Barbiturate solution</td>
<td>5-10 ml</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?

All control animals, which will not be exposed to any infectious agent, will be returned to the CRPRC colony.
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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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/).
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.

Professor 4/9/2002

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.

Identity of Hazard: Feline immunodeficiency virus

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

Provide a short description of the agent:

FIV is a naturally occurring pathogen of domestic cats and belongs to the immunodeficiency group of lentiviruses, which includes HIV and SIV

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

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

Describe any human health risk associated with this agent:

There are no known human health risks.

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.
[ ] 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  [ ] Animal Carcasses
[ ] Bedding
[ ] Other:
[ ] Cages must be autoclaved before cleaning.
[ x ] Label cages and remove label after decontamination.
[ ] Animal carcasses must be labeled and disposed of as follows:

[ x ] Incineration  [ ] Biohazardous Waste Container
[ ] Bag and Autoclave  [ ] 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  [ ] Biohazardous Waste Container
[ x ] Bag and Autoclave  [ ] EH&S will pick-up (2-1493).

Personal Protective Equipment Required:

[ x ] The following personal protective equipment must be worn/used in the room:

[ x ] Lab Coat/Coveralls  [ x ] Shoe Covers/Booties
[ x ] Disposable Gloves  [ ] Head Cover
[ ] NIOSH Certified Dust Mask  [ ] Disinfectant footbath
[ ] Eye Protection/Face Shield  [ ]
[ ] Fitted Respirator  [ ]

Type: ____________________________
Other: ____________________________
Describe: ____________________________

[ x ] Personal protective equipment must be removed before leaving the room.
[ ] Personal protective equipment must be discarded or decontaminated at the end of the project
[ x ] 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.
[ x ] 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: