**Protocol for Animal Use and Care**

**Handwritten forms are not accepted**

### Investigator

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<tr>
<th>Last Name:</th>
<th>Contact</th>
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<th>After hrs. #:</th>
<th>Phone:</th>
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### Species (common names):

<table>
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<tr>
<th>Rhesus Monkey</th>
<th>Number: 18/total</th>
<th>Source: CPRC</th>
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### Project Title

Modulation of Simian AIDS by Opioids

### Overnight Housing Location:

CPRC

### 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.

Monkeys will be stabilized on drug injection schedules of morphine or methadone, the opioid. Once the monkeys are stabilized, they will receive an effective dose of SIVmac239 and the course of the viremia will be followed to conclusion.

### 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.

The animals need to be caged in BSL2 isolation after SIV infection. Biscuit intake of each animal should be noted.

### Other Instructions for Animal Care Staff:

(Check applicable entries)

**Sick Animals**

<table>
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<tr>
<th>[x] Call Investigator</th>
<th>[x] Call Investigator</th>
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**Dead Animals**

<table>
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<tr>
<th>[ ] Clinician to treat</th>
<th>[ ] Bag for disposal</th>
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**Pest Control:** STANDARD

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<tr>
<th>[ ] OK to use pesticides</th>
<th>[ ] No Pesticides in animal area</th>
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</table>

### Hazardous Materials (Only if in the animal room):

**Infectious Agents?**

[ ] Yes [x] No

Agent(s): Simian Immunodeficiency Virus

**Radioisotopes?**

[ ] Yes [x] No

Agent(s):

**Chemical Carcinogens?**

[ ] Yes [x] No

Agent(s):
Toxic Chemicals? [ ] Yes [x] No
Agent(s):
The objective of these experiments is to evaluate the interaction between the cyclical disturbances of regulatory function induced by chronic administration of opioids and the development of a simian AIDS-like syndrome. In this proposal, induced opioid dependency and induced simian AIDS in rhesus monkeys will be used as a model system to study opioid dependency in human AIDS patients. The progression of the viremia involving the loss of antiviral and antibacterial endogenous defenses as well as cell-mediated immune mechanisms will be monitored. In addition, the interaction between the viremia and opportunistic bacteremia/infections will be characterized in an effort to further understand the molecular mechanisms involved in AIDS.
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.)

Animals will be trained to accept injections and to submit to the drawing of venous blood. We will treat the animals (monkeys) with opioids (I.M./subcutaneous injections of morphine or methadone, 0.7 ml volume) and then experimentally infect the animals with SIVmac239. Saline-injected and SIVmac239-infected monkeys will serve as a control group. Six animals in each group and a total of 18 animals will be used. All animals will receive three injections (at 7 a.m., 3 p.m. and 11 p.m.) per day and seven days a week. The injection will be given throughout the project. Before the opioid studies, it will take one month of saline injections to establish baseline measurements. Morphone or methadone treatment will begin at 1 mg/kg per injection and increase to a stabilization dose of 5 mg/kg per injection (a total of three months is needed for establishing opioid dependency). The animals will imbibe water from the CRPRC’s Automatic Lixit Device and will be weighed once per week. Blood samples will be drawn at periodic intervals (normally, 6-15ml blood per animal every 3-4 weeks.) Aliquots will be evaluated for polymorphonuclear cells (PMN) and T-lymphocyte activity and the expression of opioid receptors. At selected intervals (one to two times a year), estimates on the levels of circulating opioids in the blood samples drawn will be made (0.5 to 1 ml is enough for this purpose). Behavioral ratings will be made using our previously published rating scales (see Appendix I) which was originally developed at the University of Michigan for the description of the effect of opioids on M. mullata. Briefly speaking, the cage behavior of male rhesus monkeys will be annotated using bar codes to describe as many aspects of the behavior of monkeys as possible. The observer is prompted by a program on a notebook computer. The observer selects the appropriate bar code and strokes the code with the input wand. The data are stored in the notebook computer until the end of the observation session. The notebook computer is then attached to a larger laboratory computer and the data transferred from the notebook computer to the laboratory computer. Behavioral profiles will be gathered two times a week throughout the project, each time for 30 minutes before and after the 3 p.m. injection and during feeding, blood drawing procedures and later on after SIVmac infections. Approximately three months are needed to establish opioid dependency. When signs of stability of the opioid dependency emerge, animals will be inoculated via the intravenous route with SIVmac239 (1.58x10^3 rhesus monkey infectious dosage50/animal). These signs include lack of constant weight gains, change of normal eating behaviors and sleep patterns, and reduction in sexual activities (Masturbating behavior decreases in incidence.) Morphine dependency can easily be detected when morphine injection was inadvertently missed and withdrawal syndrome – nausea, vomiting, sweating, gooseflesh (goose pimples), diarrhea, tremor, chills, and fever – occurs. SIV viremia will be followed by assaying SIV P27 core antigen of infected monkey plasma, co-cultivation of monkey PBMC (peripheral blood mononuclear cells) with CEM x174 cells to isolate SIV virus, and by determining the viral sequence in monkey PBMC using the polymerase chain reaction (PCR). Evaluation of PMN chemotaxis/phagocytosis activities, T-lymphocyte functions (CD4+ cell proliferation, CD8+ cell-mediated antiviral activities) and humoral immune response (anti-SIV antibody formation) of the infected animals will be studied. All these assays/studies have previously been performed in our laboratory. Clinical signs of smian AIDS may include generalized lymphadenopathy, splenomegaly, neutropenia, lymphopenia or histologic lymphoid depletion, weight loss greater than 10%, anemia, abnormal peripheral blood monocytes, bone marrow hyperplasia, persistent diarrhea, chronic skin infections, opportunistic infections and tumor formation. The average life expectancy for SIV-infected monkeys is 18-20 months. However, other factors such as drug treatment, weekend schedules of feeding and care, emergence of intercurrent diseases transmitted by the colony and procedures (human handling), etc., will also affect the life
expectancy of the experimental animals. Nevertheless, by serial monitoring of each treatment group changes may be recognized and used to verify the validity of our experimental data. With respect to those animals survived from the SIVmac239 infection, they will be maintained in the colony for long-term survivor studies.

The proposed increment of drug dosages (0 to 5 mg/kg) is according to blood levels and comportment (behavior) changes established by previous studies over years here and elsewhere by others. By the proposed procedure of a gradual increase of opioid dose to establish opioid dependency, there will not be any incident of opioid overdose. All monkeys will initially be injected I.M./subcutaneously with saline (0.9% NaCl, 0.7 ml), 3 times a day. Sites of I.M./S.C. injections will be rotated often to alleviate muscle sores. The monkeys to be used as control monkeys will continue to receive saline at the times when the treatment groups receive opioids. The opioid treatment groups will receive morphine or methadone 3 times daily, starting at 1 mg/kg per administration and increasing to 3 mg/kg and a maximum of 5 mg/kg per administration. All 18 animals to be used in our study will be subjected to SIVmac239 infection. Pre-selection of these 18 animals is based upon serological studies which show that the animals have not previously been exposed to SRV, STLV-1 or SIV. Animals will be selected and placed into three different experimental groups, based upon their body weights and other criteria such as our pre-infection T-cell and PMN functional studies. Each group will consist of heterogeneous populations of animals with regard to these criteria. With respect to the numbers (6/group for a total of 18) of animals used, statistical considerations have been applied. The proposed study uses primates as the experimental subjects. When small laboratory species are used, even quite subtle differences in activity and behavior may often be detected by increasing the numbers of animals and thus decreasing within-group variability by a factor roughly proportional to the square root of the increase in group size. However, a considerable increase in group-size is impractical with larger species particularly when those species are primates, since both the cost of purchase, care, and experimental manpower set limits on animal numbers. We use six (6) animals per treatment group and 18 animals per experiment (three treatment groups: saline, morphine and methadone). Given that two independent groups of subjects are to be compared on the frequency of occurrence of a particular all-or-nothing factor (e.g. death or survival, presence or absence of a symptom), and with each group (sample) being given a different treatment (e.g. infected or non-infected, control or drug-treated), it can be shown by the application of Fisher’s exact probability test to hypothetical models that significant data can be obtained if the sample size used is 6, no matter how extreme the differences are between expected (control) and observed (treated) frequencies. In any event that the opioid-dependent animals need to be released from the project, a detoxification procedure will be used. Detoxification by a gradual, progressive and slow reduction of opioid dose (5 mg/kg→3 mg/kg→1 mg/kg→0 mg/kg) will not induce withdrawal syndrome in the animals. This procedure is clinically used in reducing the opioid dependency of human addicts.

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>saline injections</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>morphine injections</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>methadone injections</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Allow Stabilization of Drug Treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1. Inoculate saline group with SIV  
2. Inoculate morphine group with SIV  
3. Inoculate methadone group with SIV

### 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 the species choice was appropriate and the number of animals in the groups above was the minimum number necessary to achieve sound scientific results?

*M. mulatta* is the most appropriate species for this project because of the pathological similarities between human AIDS and simian AIDS induced in the rhesus monkey. Eighteen (18) monkeys will be used in this study in order to obtain data which are statistically significant (see page 3, “c”).

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

Building: N/A  
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 monkeys</td>
<td>Opioid: morphine</td>
<td>0 to 5 mg/kg</td>
<td>I.M./subQ</td>
<td>3 times/day, at 8 hr intervals</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 to 0 mg/kg</td>
<td>I.M./subQ</td>
<td>3 times/day, at 8 hr intervals, only for detoxification purposes</td>
</tr>
<tr>
<td></td>
<td>Opioid: methadone</td>
<td>0 to 5 mg/kg</td>
<td>I.M./</td>
<td>3 times/day, at 8 hr intervals</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th></th>
<th>subQ</th>
<th>I.M./subQ</th>
<th>3 times/day, at 8 hr intervals, only for detoxification purposes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 to 0 mg/kg</td>
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**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?

N/A

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)

No adverse or side effect, except common discomfort during the venipuncture and injection, is expected to occur; no unusual pain or discomfort is expected for these procedures. Alterations in growth and weight gain, however, will occur. Based upon the data of our previous experiments, as the dependency state is being established, the animals recede food intake and weights plateau. Once the dependency state is fully established, food in-take increases but not back to the pre-dependency state. Other symptoms (diarrhea, etc.) following SIV infection of the animals will be monitored by veterinarian staff.

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.

There is nothing in the research protocol that should produce pain or discomfort other than the viremia and sequellae. As with our previous studies, veterinary interaction occurs at anytime with discussion and with notification of the investigators for the use of anesthetics and/or analgesics in a timely fashion.

**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:

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? September 15, 2001

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>Melvyl Medline</td>
<td>1990-present</td>
<td>Opioids, AIDS, Rhesus monkeys, simian AIDS</td>
</tr>
<tr>
<td>Keith Killam</td>
<td>1988-1997</td>
<td>Same</td>
</tr>
<tr>
<td>Wallace Winters</td>
<td>1990-present</td>
<td></td>
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</table>

What were your findings with respect to alternative methodologies?

SIV-infected monkeys are still presently the closest animal model available for the study of human AIDS.

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?

We believe that if excessive suffering, extensive disease, and severe weight loss are observed in the animal, veterinary staffs should be consulted for either treatment or euthanasia to be done on the animal. For euthanasia, standard clinical criteria will be utilized by CPRC’s experienced veterinarians in consultation with the PIs to determine when the animals 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>Barbiturate overdose</td>
<td>Pentobarbital</td>
<td>60 mg/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?

Animals not euthanized at the conclusion of the project will be kept for PIs’ next phase of studies with consultation with the veterinary staff.
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.

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<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/ 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/.
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.
ANIMAL ROOM SAFETY INFORMATION
Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

PROTOCOL #_______
EXPIRES: ________

RUA#: 0781
BUA#: 0404
CCA#: NA

Identity of Hazard: SIV-infected monkey blood samples

Investigator Last Name: [ ]
First Name: [ ]
Email: [ ]
Fax: [ ]

Provide a short description of the agent:

SIVmac239 will be used throughout the project. SIVmac239 is a molecular clone of simian immunodeficiency virus (SIV). SIV is a monkey counterpart of human immunodeficiency virus (HIV). SIV causes in monkeys immunodeficiency syndromes in a similar way HIV causes AIDS in human. So far no scientific evidence of AIDS-like diseases may be induced in humans by SIV. In this study, the CPRC standard procedures for SIV handling will be followed.

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

For which Animal Species?

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

Describe any human health risk associated with this agent:
No human disease related to SIVmac239 has ever been described. However, SIV can infect human cells in tissue culture cell lines.

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.

[x] Cage
[x] Bedding
[ ] Stall
[ ] Water Bottle
[ ] Animal Carcasses
[ ] Other:

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

[x] Incineration
[ ] Bag and Autoclave
[ ] 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:

[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:

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

Describe:

[x] Personal protective equipment must be removed before leaving the room.
[x] 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.
[ ] 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:

P2 (BSL2) facilities for SIV-infected animals.