**PROTOCOL FOR ANIMAL USE AND CARE**

*Handwritten forms are not accepted*

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**Investigators**

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<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone:</th>
<th>Fax:</th>
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**Contact**

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<th>Department:</th>
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**Species** (common names):

<table>
<thead>
<tr>
<th>Number</th>
<th>Source</th>
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<tbody>
<tr>
<td>20</td>
<td>Primate Center</td>
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</tbody>
</table>

**Project Title**

Role of Delayed Hypersensitivity to SIV

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

Animals will be maintained by:

1. [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.

16 juvenile or adult rhesus monkeys will be vaccinated by one of two strategies to stimulate a delayed hypersensitivity immune response to SIV. The monkeys will then be challenged with pathogenic SIVmac. The animals will be bled periodically and lymph node biopsies and skin tests will be performed 3 times/year.

**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.

For BCG or SIV inoculation, animals will be housed in infectious housing with standard biosafety level 2 precautions.

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

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Agent(s):</th>
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<tbody>
<tr>
<td>[X] Yes</td>
<td>SIV, Bacille Calmette Guerin (BCG)</td>
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<tr>
<td>No</td>
<td></td>
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</table>

<table>
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<tr>
<th>Radioisotopes?</th>
<th>Agent(s):</th>
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<tr>
<td>[ ] Yes</td>
<td></td>
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<tr>
<td>[X] No</td>
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<table>
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<tr>
<th>Chemical Carcinogens?</th>
<th>Agent(s):</th>
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<tr>
<td>[ ] Yes</td>
<td></td>
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<tr>
<td>[X] No</td>
<td></td>
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</table>
Toxic Chemicals? [ ] Yes [X] No  
Agent(s):
Is the project already funded? [ ] Yes [ X ] No

Previously approved? [ X ] Yes [ ] No

Proposed Funding Source: NIH
Previous protocol number (if any): 8139

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

[ ] Lab Animal Health Clinic (2-0514)
[ X ] 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.

The hypothesis is that delayed hypersensitivity (DH) elicited by vaccination with SIV antigens delivered by two novel strategies will protect rhesus monkeys against simian AIDS upon challenge with pathogenic SIVmac. DH is a cellular immune response elicited by some intracellular pathogens. The classic examples are infection of humans with tuberculosis and infection of mice with listeria in which DH is thought to be the controlling immune response. To my knowledge, DH is not elicited to HIV-1 in humans or to SIV in rhesus monkeys, so these viruses will not have evolved strategies to evade this immune response. If the host is primed by vaccination to respond with an SIV-specific DH response, then the initial encounter with SIV should result in only transient or low-level persistent infection without progression to simian AIDS.

The two proposed vaccination strategies are the use of a BCG recombinant or of autologous, antigen-pulsed blood dendritic cells to deliver the SIV gag protein in a context that should induce primarily a DH response to SIV. Animals will receive multiple inoculations by various routes and host immunity will be assessed by gag-specific T cell proliferation and secretion of the pro-inflammatory cytokines γ interferon and TNF α. Antibody and cytotoxic T cell responses will also be measured. If these responses are demonstrated, the animals will be challenged by mucosal inoculation of pathogenic SIVmac. The course of SIV infection will be monitored by viral load over 6 to 12 months. Animals that develop the signs of simian AIDS will be culled before they become moribund. The endpoint of protection will be prevention of simian AIDS, but plasma viral RNA levels will be used as a predictor of outcomes.

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 ** [ ] Food or water restriction [ ] Special diets; food or water treatment.
[ ] Polyclonal Antibody Production ** [ ] Non-recovery surgical procedures [ X ] Induced illness, intoxication, or disease
[ ] LD 50 or ID50 studies. [ X ] Survival surgical procedures [ ] Death as an endpoint (see h below)
[ X ] catheters, blood collection, intubation [ ] Multiple survival surgery [ ] Trapping, banding or marking wild animals
[ ] Prolonged restraint. (8 hrs+)
[ ] Behavioral modification.

** 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.)

All the animals will be phlebotomized under Ketamine anesthesia after overnight fasting for blood not to exceed 12 ml/kg body weight per month. The phlebotomy cite is usually the femoral vein, but this is the discretion of the animal health technicians. After vaccination, animals will be bled biweekly times two, then once monthly. After SIV challenge, will be bled weekly times 4, then biweekly times 2, then once monthly. CBCs will be monitored for anemia. Axillary or inguinal lymph node biopsies will be performed under Ketamine anesthesia three times over animals one year. One large or two normal-sized nodes (5 mm long dimension) will be removed. The biopsy cite is usually axillary, but may be inguinal at the discretion of the animal health technician. Mantoux-type skin tests (like the PPD skin test for TB in humans) will be performed on three occasions (every 4 months per year) with tetanus toxoid, SIV gag protein and TB antigens. All 3 antigens will be given at 100µg in 0.1 ml diluted in saline. At 72 hrs after the skin test application, a 6 mm punch skin biopsy will be performed to assess the histology of DH. For immunogenicity controls, all animals will also be immunized 3 times over 3 months with tetanus toxoid using the standard human vaccine and protocol at the CRPRC (0.5ml vaccine IM), or with diphtheria/tetanus/pertussis (DTP) standard human vaccine (0.5ml vaccine IM). The choice of vaccine will be determined by efficacy: tetanus toxoid alone is preferred, but other investigators have used DTP for rhesus macaques. Finally all the animals will be inoculated with pathogenic SIVmac251, 10^5-10^6 tissue culture infectious doses, by the IV, oral or vaginal route, in two doses over 24 hrs. Four animals per year will be culled experimentally at 3 mos. after SIV challenge to assess pathology and viral load in tissues. All SIV-infected monkeys that survive will be culled at 1 or 2 years after challenge because these animals cannot be returned to the colony.

1. BCG group: 8 monkeys will be inoculated with either the parental, attenuated organism or the recombinant expressing SIV gag. In year 1, the route of inoculation will be ID in the interscapular region. We have observed no toxicity with this protocol to date.

2. 8 monkeys will be inoculated with antigen-pulsed dendritic cells. Each animal will be bled for 30 ml to obtain mononuclear cells that are cultured in vitro to differentiate into “blood dendritic cells”. At termination of culture, the cells are incubated overnight with SIV gag produced in baculovirus or a control baculovirus protein and with autologous serum. The cells are harvested and washed, resuspended in normal saline in a volume of 0.1 ml. The cells are then inoculated intradermally (ID) in 0.1 ml on the upper arm. For two animals, the cells will be labeled with the vital dye PKH-26. An axillary lymph node is biopsied 12 hrs later to track the dendritic cells in the lymph node paracortex. The immunization protocol with antigen-pulsed dendritic cells will include a booster at 1 month with transfused cell numbers ranging from 10^5 to 10^8. If the ID route fails to induce a DH response or the cells don’t migrate to the regional node, then other routes will be tested: SQ., IM or IV in years 2 and 3.

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>control BCG</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>BCG expressing SIV gag</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Category</td>
<td>Description</td>
<td></td>
<td></td>
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</tbody>
</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?

Colony-bred rhesus monkeys are an available resource at the CRPRC. The macaque infected with SIV is the only animal model of human AIDS available for vaccine experiments. Our experience and that of other AIDS investigators is that the smallest practical number of animals in an experimental group is 4, with the use of statistics for small samples.

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

<table>
<thead>
<tr>
<th>Building:</th>
<th>Room:</th>
</tr>
</thead>
<tbody>
<tr>
<td>animal housing</td>
<td>treatment room in animal housing</td>
</tr>
</tbody>
</table>

Who will be the surgeon?  
Only minor surgery is proposed, conducted by veterinarian-trained animal health technicians

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 or skin biopsy</td>
</tr>
<tr>
<td>rhesus</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)

No adverse effects of vaccination are anticipated. Mild discomfort may occur with lymph node and skin biopsy, and this would be observed daily by the animal health technicians and veterinarians. The effects of inoculation of pathogenic SIVmac are well known and the veterinarians have developed a diagnostic list of the signs of simian AIDS that permit medical cull before an animal experiences extreme distress. The animals will be euthanized before, or when, they experience 3 of the following:

- weight loss >15% in two weeks or >30% in 3 months;
- persistent hypothermia <96F even with heat supplementation;
- leukopenia (total WBC<3,000);
- lymphopenia (lymphocytes <800);
- anemia (hemoglobin <10);
- dehydration >10%;
- nonresponsive to therapy for opportunistic infections;
- persistent anorexia (>3 days);
- animal significantly obtunded.

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.

Moder analgesic, oxymorphone for post-surgical pain, and antibiotics for some opportunistic infections are administered as needed by the veterinarian.

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? 5/11/01

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>1996 to 2001</td>
<td>HIV and DH, SIV and DH</td>
</tr>
<tr>
<td>Reference Update</td>
<td>1991 to 2001</td>
<td>HIV and DH, SIV and DH</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

The only alternate methodology in the literature is to treat HIV-infected individuals with whole-inactivated virus stripped of the envelope protein and adjuvanted with alum. This methodology was for the immunotherapy of HIV infection and not as an immunoprophylactic vaccine. The results were modest.

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

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

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

Animals will be euthanized at 1 or 2 years after inoculation of SIV.

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?

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

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

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:
Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at http://clueless.ucdavis.edu/health/ 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

CRPRC Director  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

University of California, Davis
ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 9557
EXPIRES: ________

RUA#: BUA#: 0477 CCA#

Identity of Hazard: SIV, BCG

Investigator Last Name: [ ] Humans only [ ] Animals only [ X ] Humans and Animals
For which Animal Species?

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

Describe any human health risk associated with this agent:
SIV is a monkey AIDS virus and it is transmitted by secretions or animal bites. BCG is the standard TB vaccine and it can cause persistent infection and lymph node swelling in humans.

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

Describe any human health risk associated with this agent:
SIV is not a known human pathogen but it is genetically related to HIV. There are two documented cases of humans infected with SIV, neither of whom have developed AIDS. BCG is a human TB vaccine which could cause disease in an immunosuppressed human.

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

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

[ ] Incineration [ ] Biohazardous Waste Container
[ ] Bag and Autoclave [ ] EH&S will pick-up (2-1493).
[ ] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:

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

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

[ X ] Lab Coat/Coveralls [ X ] Shoe Covers/Booties
[ X ] Disposable Gloves [ ] Head Cover
[ ] NIOSH Certified Dust Mask [ ] Disinfectant footbath
[ X ] Eye Protection/Face Shield [ ]
[ ] Fitted Respirator Type: ________________________________
[ ] 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
[ ] 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:
Ketamine anesthesia prior to animal handling