

PROTOCOL FOR ANIMAL USE AND CARE*Handwritten forms are not accepted***CNPRC**

EH&S USE ONLY

PROTOCOL # 10348**EXPIRES: _____****Investigator**

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone / Fax:	
After hrs. #:	

Contact

Last Name:	
First:	
Middle:	
email:	
Department:	
Phone:	
After hrs. #:	

Species (common names): _____ **Number:** _____ **Source:** _____

Rhesus macaques	8	CNPRC
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Project Title Effects of Long-acting Interferon- α on Innate and Adaptive Immunity in SIV-Infected Macaques.

Overnight housing location::	CNPRC	Day use only :	
Animals will be maintained by:	<input checked="" type="checkbox"/> Vivarium <input type="checkbox"/> Investigator <i>(If investigator maintained, attach husbandry SOP's.)</i>		

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.

This study will investigate the effects of pegylated (long-acting) interferon- α (peg IFN- α) on the innate and SIV-specific adaptive immune responses in SIV-infected rhesus macaques. Eight animals will be infected with SIV_{mac251} and monitored for 10 weeks. Four animals each will be treated with weekly peg IFN- α or placebo. Animals will undergo phlebotomy for safety measurements and immunologic and virologic parameters. Lymph node biopsies will be performed at weeks 10 and 16. Jejunal biopsies will be performed at week 10. At approximately week 24, all surviving animals will be euthanized and undergo necropsy.

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.

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Other instructions for animal care staff: (check applicable entries)

Sick Animals	Dead Animals	Pest Control
<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator	<input checked="" type="checkbox"/> Call Investigator
<input type="checkbox"/> Clinician to treat	<input checked="" type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input checked="" type="checkbox"/> Necropsy	<input checked="" type="checkbox"/> Necropsy	

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

Infectious Agents?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Agent(s):	Simian Immunodeficiency Virus-mac ₂₅₁
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	NCCFAR	Previously approved?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	

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

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	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 pctlillman@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.

Objectives: The mechanisms of action for the antiviral – both HIV and SIV – activity of interferon- α (IFN- α) have been investigated in both *in vitro* and *in vivo* settings. As a component of the immune response to infection, the effect of HIV-1 and SIV on patterns of interferon production has also been described. However, despite the observation that high doses of IFN- α reduce viral load in the setting of HIV-1 and SIV infection, an intensive investigation of the impact of high dose IFN- α on the innate and adaptive immune response to HIV-1 and SIV has not been reported in the literature. Thus, the experiments proposed in this project will measure multiple parameters of the innate immune system and SIV specific adaptive immune responses to high dose IFN- α therapy in the setting of chronic SIV infection. Collection of lymphocytes from multiple compartments, including lymph node and gastrointestinal sites during the course of therapy and at necropsy will provide new insights into the role of IFN- α in immune regulation as well as support it's possible therapeutic uses in the setting of HIV-1 infection. **Hypothesis:** The hypothesis that this pilot project pursues is that IFN- α initiated immune modulation contributes significantly to the virologic suppressive effects of therapeutic IFN- α . We propose that the proximal arm of those effects relies on augmented innate immune response that leads to broader, more effective adaptive immune responses. In order to address this hypothesis, the two primary objectives of the project are (1) To measure the effect of long-acting IFN- α on SIV_{mac251}-infected macaques on innate immune responses and the development of SIV-specific CD4⁺ and CD8⁺ mediated immune responses, and (2) To measure the virologic effects and the pharmacodynamic and pharmacokinetic parameters of IFN- α on SIV_{mac251}-infected macaques treated with long acting IFN- α for 14 weeks. **Experimental Design:** Eight juvenile macaques will be infected with SIV_{mac251} intravenously following baseline measurements and monitored for SIV disease progression. At 10 weeks, when viral set-point generally occurs, 4 control animals and 4 experimental animals will receive weekly injections of saline and pegylated IFN- α (Pegasys®, Roche Pharmaceuticals), respectively. Peripheral blood (x12), lymph node (x2) and small bowel biopsy samples (x1) will be obtained periodically through week 24, at which time all surviving animals will undergo necropsy. In addition to lymph node, spleen, and liver cells, special attention will focus on obtaining gastrointestinal associated lymphoid tissue for extensive evaluation of the effects of IFN- α on mucosal immunity function at the time of necropsy. Parameters of innate immunity to be measured include NK cell distribution and function, cytokine and chemokine tissue levels, and toll-like receptor measurement. Parameters of adaptive immunity to be measured include SIV specific immunity (as measured by ELISPOT) for CD8⁺ T-cell function and lymphocyte proliferation responses for CD4⁺ T-cell function. Lymphocyte subset analysis and distribution including dendritic cell measurement will be performed in peripheral blood, lymph node, and gastrointestinal tissues by FACS analysis (flow cytometry). Virologic, pharmacokinetic and pharmacodynamic parameters will also be collected to establish dose effect relationships. **Data analysis:** Each animal will serve as their own control as well as animals will be grouped by treatment intervention. The primary endpoint is development of CD8⁺ mediated SIV specific immunity – breadth and magnitude. Correlations will be sought within animals and between treatment groups as a function of innate immune parameters. Tissue and plasma samples will be preserved for additional studies as indicated. As a pilot protocol, this effort will result in preliminary data to be used for obtaining additional grant support for future projects. Effective immunomodulatory therapy could have multiple applications in the treatment of HIV disease, including as strategies to prolong treatment free periods in strategic treatment interruption (STI) regimens or as part of an induction antiretroviral regimen prior to therapeutic vaccine treatment. In addition, the authors are in the process of designing a clinical trial treating early stage HIV-infected subjects with

pegylated IFN- α in a phase I dose escalation design. Comparative analysis between species is an exciting and novel opportunity that will also result from these studies.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

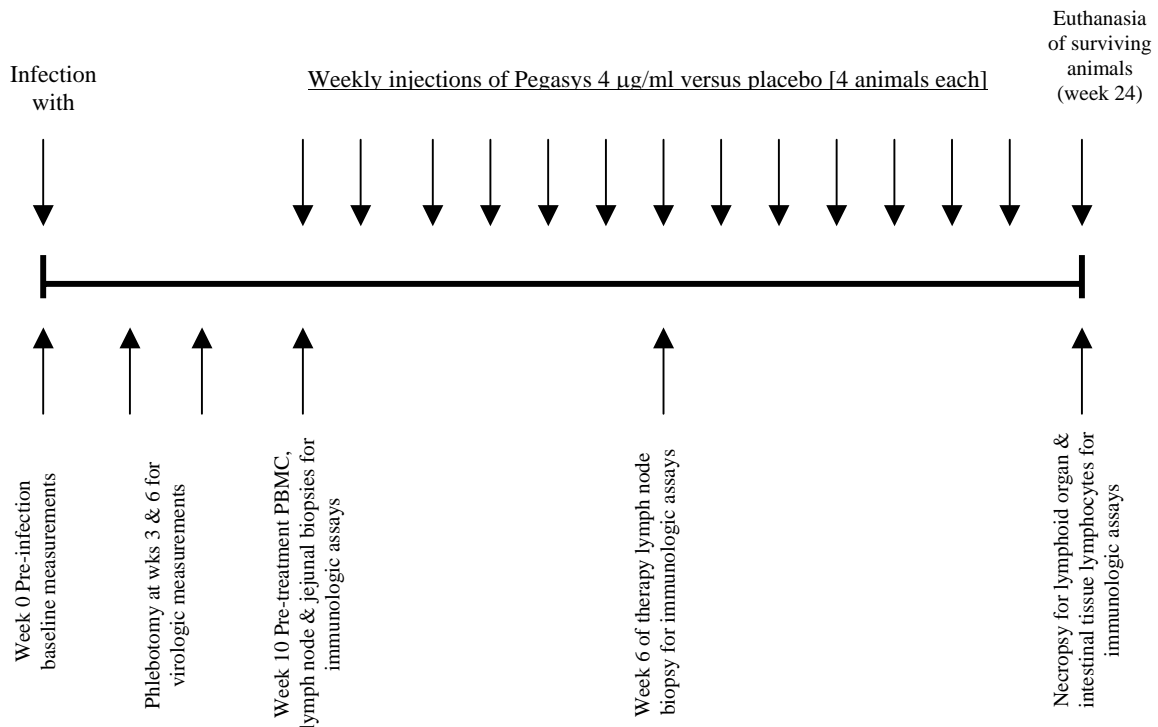
- | | | |
|---|---|---|
| <input type="checkbox"/> Monoclonal Antibody Production ** | <input type="checkbox"/> Food or water restriction | <input type="checkbox"/> Special diets; food or water treatment. |
| <input type="checkbox"/> Polyclonal Antibody Production ** | <input type="checkbox"/> Non-recovery surgical procedures | <input checked="" type="checkbox"/> Induced illness, intoxication, or disease |
| <input type="checkbox"/> LD 50 or ID50 studies. | <input type="checkbox"/> Survival surgical procedures | <input type="checkbox"/> Death as an endpoint (see i below) |
| <input checked="" type="checkbox"/> catheters, blood collection, intubation | <input type="checkbox"/> Multiple survival surgery | <input type="checkbox"/> Trapping, banding or marking wild animals |
| <input type="checkbox"/> Prolonged restraint. (8 hrs+) | <input type="checkbox"/> Behavioral modification. | <input checked="" type="checkbox"/> endoscopy |
| <input checked="" type="checkbox"/> Fasting prior to a procedure. | <input type="checkbox"/> Aversive conditioning. | <input type="checkbox"/> |

**** 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.

A total of 8 animals will be used in this study. 8 juvenile colony bred Rhesus macaques will be infected with 1000 TCID₅₀ (tissue culture infectious dose causing 50% cell death) of SIV_{mac251} administered intravenously (IV) to assure infection. All animals will have bimonthly blood draws of 2 mls of blood for the determination of virus replication levels and peripheral blood lymphocyte (PBL) subset analysis. At the viral set point around 10 weeks following infection (verified by plasma viral RNA determinations), 4 animals will be treated with weekly pegylated IFN- α (Pegasys®, Roche Pharmaceuticals) at 6 μ g/Kg subcutaneously (sq) and 4 will receive weekly placebo (normal saline) sq injections. All animals will undergo lymph node biopsy procedures and small intestinal biopsies prior to initiation of injections. Intestinal biopsy will consist of 10 pinch biopsy pieces for histochemical assays and cellular flow cytometric analysis. Lymph node biopsies will be rendered into single cell suspension for lymphocyte subset analysis by flow cytometry and immune function studies. All animals will have weekly blood draws (consisting of 3 ml of blood) from pre-infection (week 10) to week 24 of infection for safety analysis and PBL isolation for immune function studies. The primary safety laboratory measurements will be complete blood cell counts and the liver transaminase ALT. Dose adjustment of Pegasys® will be made as necessary.

All animals will have weekly weight determination. Daily CNPRC staff observation of the specially housed, infected animals will be utilized to monitor the animals. At week 24 the animals will be necropsied. Animals will be euthanized as specified in the CRPRC guidelines "criteria for euthanasia of retrovirus infected macaques". A complete necropsy will be performed for each animal and peripheral and systemic lymphoid tissues will be prepared for histological, immunohistochemical, flow cytometric, bDNA and PCR 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.

Group	Procedures / Drugs	Number of Animals	Category
1	After 10 weeks of SIV _{mac251} infection, subcutaneous injection of placebo weekly until study termination. Pretreatment phlebotomy	4	3

	to measure status of SIV infection at 2 time points. Weekly post treatment blood collection, lymph node (x2) and jejunal (x1) biopsies, necropsy. Necropsy will be performed week 24.		
2	After 10 weeks of SIV _{mac251} infection, subcutaneous injection of pegylated IFN- α (Pegasys [®] , Roche Pharmaceuticals) at 6 μ g/Kg weekly until study termination. Pretreatment phlebotomy to measure status of SIV infection at 2 time points. Weekly post treatment blood collection, lymph node (x2) and jejunal (x1) biopsies, necropsy. Necropsy will be performed week 24.	4	3

Categories of invasiveness

Category	Description
1	Little or no discomfort or stress Examples: domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral.
2	Minor stress or pain of short duration Examples: cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress
3	Moderate to severe distress Examples: major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation
4	Severe pain near, at or above the pain tolerance threshold Examples: exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress; Surgical experiments which have a high degree of invasiveness.

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

SIV and SHIV infection of nonhuman primates remains the optimal model for studying HIV immunopathogenesis and for testing novel therapeutic strategies. Access to large numbers of lymphoid tissue cells during and after treatment will afford extensive and detailed analysis of the immune response in the setting of peg IFN- α treatment. In consultation with a statistician, four animals in each arm will provide sufficient power to detect differences as a pilot study.

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.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus macaques	telazol	5 mg/kg	IM	before biopsy procedure

Rhesus macaques	oxymorphone	0.15 mg/Kg	IM	as analgesic/ 3 times daily if needed, discretion of CNPRC vets
Rhesus macaques	Buprenorphine	0.1-0.3 mg/kg	IM	BID for 3 days, discretion of CNPRC vets

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)

Discomfort may accompany lymph node and intestinal biopsies, however animals are anesthetized during the entire procedure. Intestinal biopsies may lead to intestinal perforation, bleeding and death.

Blood collection may be associated with minimal discomfort.

Animals will be euthanized according to CNPRC criteria for euthanasia of SIV infected macaques. This would include weight loss of >15% in 2 weeks, persistent leukopenia, total WBC<3,000, opportunistic infections that do not respond to therapy, dehydration >7% and not responsive to oral hydration therapy for 3 days, lymphopenia, abdominal lesions and severe depression (obtusion).

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.

Analgesics or any post-operative procedures, such as bowel resection in the case of intestinal perforation, may be utilized as deemed necessary by the attending 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 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?

October, 2002

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1990 - present	primates, interferon, SIV
Current Contents/	1993 to 2002	primates, interferon, SIV
BIOSIS Previews	1969 to 2002 week 44	primates, interferon, SIV

What were your findings with respect to alternative methodologies?

There are no known alternatives to the procedures used in this study.

Has this study been previously conducted?

Yes 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?

At the end of the treatment period and/or animals with SAIDS 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.

Species	Method	Drug	Dose (mg/kg)	route
rhesus macacques	deep ketamine anesthesia followed by barbiturate overdose	Sodium pentobarbital	60 mg/kg	I.V.

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

N/A

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.

David M Asmuth, MD	Assistant Professor of Medicine	October 1, 2002
<i>Principal Investigator</i>	<i>Rank / Title</i>	<i>Date</i>

Committee Use Only Below

** Conditions necessary for Committee Approval:
Final Disposition of this protocol: <input type="checkbox"/> Approved <input type="checkbox"/> Not Approved <input type="checkbox"/> 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.

_____		_____
<i>Campus Veterinarian</i>		<i>Date</i>

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ANIMAL ROOM SAFETY INFORMATION

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

PROTOCOL # 10348**EXPIRES:** _____

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RUA#: _____

BUA#: pending

CCA#: _____

Identity of Hazard:

SIV

Investigator Last Name:

Department:

First Name:

Phone:

Email:

Fax:

Provide a short description of the agent:

SIV (simian immunodeficiency virus) is a blood born lentivirus that causes fatal immunodeficiency (AIDS) in rhesus macaques. It is genetically similar to HIV. SIV can infect humans but it is unknown whether it can cause disease.

This agent / material is hazardous for: Humans only Animals only Humans and Animals
 For which Animal Species? **Non-human primates**

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

Describe any human health risk associated with this agent:

SIV can infect humans; thus, it could possibly cause fatal AIDS-like disease in humans. SIV-infected humans have generated infectious virus and antibodies to SIV. There have been no reports of disease seen in SIV-infected humans.

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:
- Cages must be autoclaved before cleaning.
 Label cages and remove label after decontamination.
 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 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:
 Lab Coat/Coveralls Shoe Covers/Booties
 Disposable Gloves Head Cover
 NIOSH Certified Dust Mask Disinfectant footbath
 Eye Protection/Face Shield
 Fitted Respirator Type:
 Other: Describe: **disposable gown/coveralls**
- 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:

Biosafety level 2+ (BSL2+) precautions must be followed at all times

