

PROTOCOL FOR ANIMAL USE AND CARE

Email to: campusvet@ucdavis.edu

CNPRC

10817

EH&S USE ONLY

PROTOCOL: 10817
EXPIRES:

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

Species (common names):	Number:	Source:
Rhesus macaques	12	

Project Title Effects of Interferon alpha/beta Pre-Treatment on SIV pathogenesis in Rhesus Macaques.

Overnight housing location:: CNPRC Day use: CNPRC

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.

This study will investigate the effects of pegylated interferon alpha (peg. IFN- α) and Betaseron (interferon-beta) pre-treatment of rhesus macaques on SIV infection. Twelve animals will be infected with SIV_{mac251} and monitored for 6 months. Six of the 12 animals will be treated for 4 weeks before SIV infection with interferon alpha and beta (once a week with peg. IFN- α and every other day with β -interferon-Betaseron). Animals will have blood samples taken for hematological measurements, as well as immunologic and virologic parameters. At approximately week 24 post SIV inoculation, 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.

Infectious housing after SIV inoculation

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 checked="" 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 (SIV _{mac})
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:	NIH	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 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.

Objectives: The mechanisms of action for the antiviral – both HIV and SIV – activity of interferon- α and interferon- β (IFN- α/β) have been investigated in both *in vitro* and *in vivo* settings. As a component of the immune response to infection, the effect of interferon production patterns in HIV-1 and SIV have also been described. Further, IFN- α/β treatment is commonly used in viral infections to reduce viral loads. This has also been shown in the setting of HIV-1 and SIV infection. Despite the knowledge of the antiviral activity of IFN- α/β , IFN- α/β have not been explored as a therapeutic agents to prevent infection or to reduce transmission. Thus, we propose to treat monkeys with a long-lasting peg. IFN- α and Betaseron to alter the pattern of infection after a subsequent high dose challenge with SIVmac. Both drugs are FDA approved for use in humans. **Hypothesis:** Pretreatment of rhesus macaques with long-lasting peg. IFN- α and with Betaseron will either completely prevent infection of these animals with pathogenic SIVmac, or severely reduce replication of SIVmac. In addition, the adaptive immune responses induced by IFN- α/β will contribute to control of virus replication.

Experimental Design: Twelve juvenile macaques will be infected with SIVmac intravenously following baseline measurements and monitored for SIV disease progression and levels of viral replication for 6 months. Six of 12 animals will receive weekly injections of pegylated IFN- α (Pegasys®, Roche Pharmaceuticals) and subcutaneous injections of IFN- β (Betaseron®, Berlex) for 4 weeks before SIVmac inoculation. SIVmac inoculation will be performed 24 hrs. after the last treatment with IFN- α/β . Peripheral blood (12x) and lymph nodes (2x) will be obtained periodically through week 24, at which time all surviving animals will undergo necropsy. Blood and lymphoid tissues will be collected to assess the level of virus replication and host immune responses. **Data analysis:** Animals will be grouped into IFN- α treated and non-treated animals. The main read-out will be the level of virus replication detectable in plasma and lymphoid tissues of the two groups. Further, as a marker of outcome, CD4 T cell counts and anti-SIV immune responses will be compared in both groups. A reduction of viremia in IFN- α/β treated animals will be an indication that IFN- α/β can be used in patients with known repeated exposure to the HIV-1 virus to prevent infection and/ or to reduce the rate of transmission. Further, these results will have important implications for the development of drugs that can stimulate innate anti-viral immune response important in the early reduction of virus replication and spread within the host.

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 type="checkbox"/> |
| <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. *(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.)*

A total of 12 animals will be used in this study. 12 juvenile colony bred rhesus macaques will be infected with 1000 TCID₅₀ (tissue culture infectious dose causing 50% cell death) of SIVmac administered intravenously (IV) to assure infection. Blood samples (10 ml) from all animals will be collected on days 1,3, 7, 14, and 28 p.i., and monthly thereafter (10 ml) for the determination of virus replication levels, peripheral blood lymphocyte (PBL) subset and Chem20 panel analysis. Two blood samples will be collected from each animal to be used as baseline reference values approximately 1-2 months prior to inoculation with SIVmac251. Two inguinal lymph node biopsy will be performed on each animal. One lymph node biopsy will be performed 1 month prior to SIVmac251 inoculation (baseline), and the second lymph node biopsy will be performed two weeks post SIVmac251 inoculation.

Group A: Before infection with SIVmac, 6 of the 12 animals will be treated weekly subcutaneously (sq) with peg.IFN- α at 25 mcg/ animal once a week. In addition, and at the same time, the same 6 monkeys will be treated with Betaseron by subcutaneous injection every other day (25mcg/ animal). Pegylated IFN- α and Betaseron treatment will be administered for a total of 4 weeks. Twenty-four hours after the last treatment, the animals will be inoculated intravenously with SIVmac. A blood sample (10 ml) will be collected on days 1, 3, 7, 14, 21 and 28 of the treatment, prior to SIVmac inoculation. The primary safety laboratory measurements will be complete blood cell counts and the liver transaminase ALT. Dose adjustment of IFN- α / β will be made as necessary. The proposed doses are based on doses used in a 70 kg human.

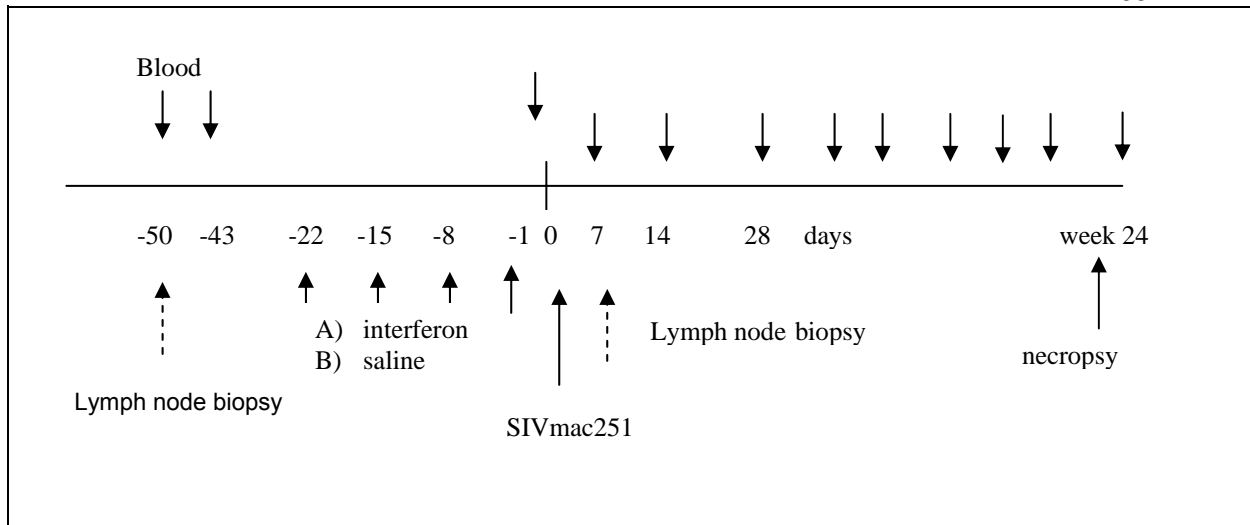
Group B: Six of the 12 animals will be treated for 4 weeks with saline every other day by s.q. injection before infection with SIVmac. A blood samples will be collected prior to SIVmac inoculation.

All animals will have weekly weight determination according to CNPRC guidelines. Daily CNPRC staff observation of the specially housed, infected animals will be utilized to monitor the animals. All animals will be euthanized 6 months after SIVmac inoculation, and blood and lymphoid tissues will be collected. 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.

Note: Animals will be fasted prior to blood collection and lymph node biopsies, and animals will be anaesthetized for the procedure. Ketamine will be used to sedate animals for blood collections, and Telazol for biopsy procedures.

The blood volume will be adjusted according to each animal's weight as not to exceed the allowed blood volume of 12.5 ml/kg/month.

Lymph node biopsy: After anesthesia (medetomidine), the surgical site will be prepared and the skin over the node will be incised with a sterile scalpel blade. Once the node is removed by a combination of blunt and sharp dissection, the skin will be closed using suture and/or sterile surgical adhesive. Post-procedure analgesics will be applied at the veterinarian's discretion; in general ketoprofen will be administered once a day for a total of 3 days.



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 (A)	Before infection with SIVmac, the animals will be treated with weekly injections of peg. IFN- α (25mcg/ animal in 1 ml saline given subcutaneously). In addition, the same 6 monkeys will be treated with Betaseron by s.c. injection every other day (25mcg/animal). All animals will be treated for 4 weeks. 24 hours after the last treatment all 6 animals will be inoculated intravenously with 10^3 TCID ₅₀ of SIVmac. Pretreatment and post-treatment phlebotomy to measure status of SIV infection. Lymph node biopsies will be performed at 1 month prior and 2 weeks after SIVmac inoculation. Necropsy at 6 months after SIVmac infection.	6	3
2 (B)	All 6 animals will be treated with saline for 4 weeks (every other day, sq injections) before intravenous inoculation with 10^3 TCID ₅₀ of SIVmac. Pretreatment and post-treatment phlebotomy to measure status of SIV infection. Lymph node biopsies will be performed at 1 month prior and 2 weeks after SIVmac inoculation. Necropsy at 6 months after SIVmac infection.	6	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 infection of nonhuman primates remains the optimal model for studying HIV immunopathogenesis and for testing novel therapeutic strategies. As a pilot project, the results of this study could suggest novel approaches for future non-human primate and human clinical trials.

We have decided on six monkeys per group, which will allow us to determine statistically significant differences between groups using plasma viral RNA levels post-challenge (et al., 2001).

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 macques	telazol	5 mg/kg	IM	before biopsy procedure
Rhesus macques	ketamine	10 mg/Kg	IM	Prior to all procedures
Rhesus macques	Buprenorphine	0.1-0.3 mg/kg	IM	BID for 3 days, discretion of CNPRC vets
Rhesus macaque	Medetomidine/ Atipamezole	30-35 µg/kg/ 0.15 mg/kg	IM/ IM	Before biopsy procedure/ Immediately after biopsy procedure
Rhesus macaque	Ketoprofen	2 mg/kg	IM	Once a day for 3 days after biopsy procedure

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)

Blood collection may be associated with minimal discomfort.

Peg. IFN- α treatment may result in diarrhea and some weight loss in some monkeys. In that case, the dose of IFN- α will be reduced.

Animals will be euthanized according to CRPRC 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.

Every effort will be made to minimize discomfort and pain.

Analgesics or any post-operative procedures 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:

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: mwood@ucdavis.edu)

What was the date on which you conducted this search?

July 2003

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
Pub Med	1993-2003	SIV, IFN- α/β , non-human primates
Current Contents	1993-2003	SIV, IFN- α/β , non-human primates

What were your findings with respect to alternative methodologies?

There are no alternative methodologies.

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.

_____ <i>Principal Investigator</i>	_____ <i>Rank / Title</i>	_____ <i>Date</i>
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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**PROTOCOL # 10817**

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

EXPIRES: _____

RUA#: _____

BUA#: 0447

CCA#: _____

Identity of Hazard: SIV

Investigator Last Name: _____ Department: _____

First Name: _____ Phone: _____

Email: _____ Fax: _____

Provide a short description of the agent:

SIV is a 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?

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

Describe any human health risk associated with this agent:

SIV can infect humans, and thus, there is the potential it may cause fatal immunodeficiency syndrome. SIV-infected humans develop anti-SIV antibodies. So far, there have been no reports of AIDS-like illnesses caused by SIV infection in 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 gowns/ overall

- 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+ (BSL 2+) precautions must be followed at all times.

Date: Tue, 16 Sep 2003 09:46:35 -0700

To:

From:

Subject: Fwd: AUC 10817

,

enclosed is the revised protocol AUC 10817. The following revisions have been made:

- 1) The box for special husbandry is included.
- 2) In section 2c the following additions have been made:
 - blood volumes are included
 - the lymph node biopsy procedure is described
 - requirements for fasting and anaesthesia prior to procedures have been added
 - agents listed in 2c are now also listed in section 2 g and vice versa
 - treatment with betaseron is explained in section 2c-group A
- 3) In section 2e a reference has been added to justify the requested animal number.
- 4) Ketamine listed in section 2 l is now also listed in section 2g.

Date: Tue, 23 Sep 2003 14:59:50 -0700
To:
From:
Subject: Re: Fwd: additional committee questions protocols
10815/16/17
Cc: sejhahn@ucdavis.edu

Hello,

The treatments proposed in the AUC's 10815/16/17 will result in viral load changes in the SIVmac infected monkeys. It has been shown previously that to detect a difference in plasma viral load of at least 0.5-1 log₁₀ at the peak of viremia and at viral setpoint of SIV Infection, 6-8 monkeys are needed.

Please let me know if this answer is acceptable for you.

Thanks.-

, you can answer these via e-mail. No need to revise the protocol again.

Hi ,

I have received the following additional comment regarding protocols 10815, 10816 and 10817.
Please send the response to: campusvet@ucdavis.edu.

Thanks in advance,

#10815,16,17():

1. Section E (numbers justification) includes a reference as justification for the numbers, but otherwise fails to adequately justify the number of animals. Since we are now asking PIs to remove all reference to other names, I will remove the references and ask that Dr. group provide the statistical justification.