

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY
PROTOCOL # <u>9773</u>
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 macaque	20	CRPRC

Project Title Bartonella: A Model for an AIDS Opportunistic Infection

Overnight housing location: _____ 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.

This project will utilize both SIV-infected and uninfected macaques to develop a nonhuman primate model of Bartonellosis/Bacillary Angiomatosis (BA) in AIDS. SIV-infected macaques (released from other SIV projects) will be coinfecting with *Bartonella quintana*, *B. heselae*, or *B. bacilliformis*. SIV-negative macaques will be inoculated with *Bartonella spp.* as controls.

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.

All animals assigned to this project will be housed under BSL-2 conditions.

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 type="checkbox"/> Save for Investigator	<input 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 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):	<i>Bartonella spp.; SIV</i>
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, NIAID	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8239

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 pcillman@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 objective of this study is to continue development and utilization of a nonhuman primate model of AIDS-associated bartonellosis, including persistent or relapsing bacteremia. The SIV-rhesus macaque experimental system will be utilized for model development, and for investigation of mechanisms of pathogenesis.

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.)

SIV-infected and uninfected rhesus macaques will be inoculated intradermally with approximately 1×10^5 *Bartonella quintana*, *B. henselae*, or *B. bacilliformis* (total volume <0.5 ml). Blood samples (<5.0 ml, depending on body weight) will be collected in pediatric Isostat tubes under Ketamine anesthesia on days 3,5,7,10,14,21 and 28 post-inoculation, then bi-weekly for 6 months to monitor onset and duration of bacteremia, antibody responses, and development of angiomatous lesions. Complete blood counts and liver function tests will be performed at 2-week intervals (no additional blood). Animals will receive physical examinations at every blood collection. Skin lesions developing at the site of inoculation or at other sites, suggestive of cutaneous BA, will be biopsied using a 6 mm biopsy punch and sterile technique. A single regional lymph node draining the skin lesion site will be biopsied per animal. All biopsies (lymph node and skin) will be performed with the animal under ketamine anesthesia (10 mg/kg IM). Animals will also receive an ultrasound examination every 2 weeks to monitor for development of bacterial valvular endocarditis and/or hepatic peliosis.

For several experiments, a modified *Bartonella* organism expressing green fluorescent protein (GFP) will be used as inoculum. These modified organisms also possess a plasmid for kanamycin resistance. In order to select in favor of the GFP-expressing organisms in experimentally infected animals, this subset of animals will be treated with Kanamycin sulfate (Kantrim) at a dose of 10 mg/kg IM BID (vol= 0.5 ml in 5 kg animal). Alternating IM sites of inoculation will be used to minimize repeated injections at the same site. The period of treatment may extend for 6-8 weeks. Because Kanamycin is potentially nephrotoxic, bi-weekly serum chemistries (creatinine, BUN) will be used to monitor renal function. Kanamycin treatment will be discontinued if signs of compromised renal function are observed.

Miniature transmitters (Minimitter) will be surgically implanted subcutaneously on the back between the scapulae in selected animals to allow remote sensing of body temperature. Surgical implantation of transmitters will be performed by CRPRC staff veterinarians with animals under Ketamine anesthesia. The transmitters, designed for use in nonhuman primates, measure ~5.0 cm in diameter, and 0.5 cm in thickness. Low temperature gas or cold liquid sterilization will be used to sterilize the devices prior to implantation.

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	SIV-negative; <i>B. quintana</i> / <i>bacilliformis</i>	4	2
2	SIV-positive; <i>B. quintana</i> / <i>bacilliformis</i>	10	2
3	SIV-positive; Bq/Green fluorescent protein	3	2
4	SIV-positive; Bq/Promotor trap GFP vector	3	2

Categories of invasiveness

Category	Description
1	<p>Little or no discomfort or stress</p> <p>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.</p>
2	<p>Minor stress or pain of short duration</p> <p>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</p>
3	<p>Moderate to severe distress</p> <p>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</p>
4	<p>Severe pain near, at or above the pain tolerance threshold</p> <p>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.</p>

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?

The SIV/rhesus macaque model is the premier animal model for AIDS research. Our previous studies have demonstrated the development of relapsing bacteremias in SIV and *Bartonella* coinfecting animals. Additional inoculates (total n=10) will provide an adequate sample size for determination of the probability of prolonged bacteremia. The smaller sample sizes for the other groups will provide answers to qualitative rather than quantitative question.

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 macaque	Ketamine HCl	10 mg/kg	IM	Prior to blood sampling and biopsies

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)

Adverse effects may result from SIV infection, including weight loss, diarrhea, fever, lymph node enlargement, neurologic disease, and/or opportunistic infection secondary to immunosuppression. Co-infection with *Bartonella spp.* May result in fever, inappetence, skin lesions (abscesses, BA) lymph node enlargement, valvular endocarditis, and clinical signs associated with systemic BA. Severe disease may result from infection with *B. bacilliformis*, characterized by fever and anemia (Oroyo Fever).

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, anesthetics, antibiotics and other supportive or specific treatments will be administered whenever deemed appropriate 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?

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
Medline	1980-2000	Bartonellosis, HIV, AIDS, Bacillary Angiomatosis, SIV, Animal models, <i>B. bacilliformis</i> , Oroyo fever, nonhuman primates, Macaca
Index Medicus	1920-1980	<i>B. bacilliformis</i> , Oroyo fever, Carrion's disease, nonhuman primate, animal model

What were your findings with respect to alternative methodologies?

BA and relapsing bacteremia are recently recognized manifestations of bartonellosis seemingly unique to patients with AIDS or other severe immunosuppressive disease. Spontaneously occurring BA has not been recognized in animals, nor has any animal model system been developed to study the pathogenesis of this unusual lesion. (1926) successfully induced infection and cutaneous angiomatous lesions in apparently immune competent macaques by intradermal inoculation with *B. bacilliformis*.

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.

Studies of co-infection of macaques with SIV and *Bartonella spp.* Have not been previously conducted. To our knowledge, our studies are unique.

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

All SIV-infected animals will be euthanized at the conclusion of the study. SIV-negative animals recovering from bartonellosis, with not evidence of persistent bacteremia, will be returned to the research pool.

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 macaque	Barbiturate overdose	Pentobarbital	60 mg/kg	IV

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

Animals not infected with SIV, and that recover from bartonellosis, will be returned to the research pool.

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	Adjunct Professor	9-24--01
<i>Principal Investigator</i>	<i>Rank / Title</i>	<i>Date</i>

Committee Use Only Below

<p>** Conditions necessary for Committee Approval:</p>
<p>Final Disposition of this protocol:</p> <p>_____ Approved</p> <p>_____ Not Approved</p> <p>_____ Withdrawn by Investigator</p> <p>Date of Action: ____/____/____</p>

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.
