**PROTOCOL FOR ANIMAL USE AND CARE**

*Handwritten forms are not accepted*

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**CRPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
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<tr>
<td>Last Name:</td>
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<tr>
<td>Phone / Fax:</td>
<td>Phone:</td>
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<tr>
<td>After hrs. #:</td>
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</table>

**Species (common names):** *Macacca mulatta*

**Number:** 40

**Source:** CRPRC

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**Project Title**

*Non-Human Primate Model of Neuroborreliosis*

**Overnight housing location:** CRPRC

**Day use only:**

Animals will be maintained by:

- [x] Vivarium
- [ ] Investigator

(If investigator maintained, attach husbandry SOP’s.)

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**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.

Rhesus monkeys will be infected with the agent of Lyme disease and assessed for neurologic disease for up to 6 months using behavior, clinical chemistry, nerve and muscle function and other tests. In order to efficiently infect monkeys, most will be transiently immunosuppressed prior to inoculation.

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**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.

None.

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**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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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Infectious Agents?</th>
<th>Radioisotopes?</th>
<th>Chemical Carcinogens?</th>
<th>Toxic Chemicals?</th>
</tr>
</thead>
<tbody>
<tr>
<td>[x] Yes</td>
<td>[ ] Yes</td>
<td>[x] Yes</td>
<td>[ ] Yes</td>
</tr>
</tbody>
</table>

Agent(s): *Borrelia burgdorferi*
Funding source: NIH/NIAID

Previous protocol number (if any): 8327

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

[ ] Lab Animal Health Clinic (2-0514)
[ ] VMTH Large Animal Field Service (2-0292)
[x] California Primate Research Center (2-0447)
[ ] 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 overall intent of this study is to determine if rhesus monkeys develop neuroborreliosis, and if so, to optimize the model so that future studies can investigate the complexities of disease pathogenesis, and to test the efficacy of selected antibiotic regimens for treatment of this disease.

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 **
[ ] Polyclonal Antibody Production **
[ ] LD 50 or ID50 studies.
[x] catheters, blood collection, intubation
[ ] Prolonged restraint. (8 hrs+)
[ ] Fasting prior to a procedure.
[ ] Food or water restriction
[ ] Non-recovery surgical procedures
[x] Survival surgical procedures
[ ] Multiple survival surgery
[ ] Induced illness, intoxication, or disease
[ ] Lethal illness or disease
[x] Death as an endpoint (see i below)
[ ] Trapping, banding or marking wild animals
[ ] Behavioral modification.
[ ] Prolonged restraint. (8 hrs+)
[ ] Aversive conditioning.

** 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 may 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.)

This project will define and optimize the rhesus monkey model for neuroborreliosis, then use the model to test efficacy of a selected antibiotic regimen that will be dictated by NIH Program staff. The project has been ongoing, and is divided into 4 major experiments:

I. Determine if the brain of rhesus monkeys can be reproducibly infected by tick borne exposure or by syringe. To date, experiments have shown that although tick-borne exposure is effective, monkeys are more consistently infected, and their brains are more consistently infected, when inoculated by syringe. In addition, our studies have shown that immunosuppression with dexamethasone prior to inoculation results in more consistent brain infection. Dexamethasone treatment consists of 2 mg/kg sid (orally) for 1 week, then 1 mg/kg sid for 3 weeks. At this point, drug dosages will be tapered off and ceased. During this treatment, monkeys are inoculated with 1,000,000 cultured Borrelia burgdorferi spirochetes intradermally in a single site in a volume of 0.1 ml (which makes a small intradermal bleb). Prior to completion of dexamethasone treatment, monkeys will be anesthetized. Skin biopsies near the inoculation site and skeletal muscle biopsies will be obtained by an aseptic open muscle biopsy technique (10 x 10mm) on the anterior thigh. These will be obtained by CRPRC veterinary staff. Skin and skeletal muscle have been found to be accurate sites for verification of infection by Borrelia. At 0,4,6,8, and 12 weeks, cerebrospinal fluid (CSF), urine and serum will be collected by CRPRC staff under ketamine (7 mg/kg/medatomadine 3 mg/kg) anesthesia. Blood (5 ml) will be collected from the femoral vein, CSF (1 ml) from the cisterna magna (surgically prepped site) and urine from the bladder by catheter. Blood, CSF, and urine will be collected from all monkeys. Skin and muscle biopsies will be taken at an early interval (4 weeks) to determine infection status; an interval while monkeys are anesthetized for other collections. Monkeys will be euthanized and necropsied at 12 weeks, and tissues (brain, spinal cord, heart, skin, muscle) will be collected for culture, PCR, serology, and histology. 8 additional monkeys will be needed to complete this experiment. This number of animals is needed to determine trends in infection kinetics and disease because of the variable nature of infection.

II. Determine the optimal spirochete isolate for infection studies, using the intradermal inoculation. Four groups of 3 monkeys (12 total) will be immunosuppressed and infected with 4 different Lyme disease spirochetes that have been pre-selected for potential neurovirulence by in vitro testing and testing in mice (other studies, another protocol). Monkeys will be necropsied at 12 weeks after inoculation, and samples collected and processed as above. Since this experiment simply seeks to determine an optimal isolate, we will use only 3 animals per group. Based upon past experience, this number is sufficient to test for infectivity.

III. Examine the long-term neurologic sequelae during chronic infection. Twelve monkeys will be infected and 8 monkeys will be sham-inoculated (using optimal strain selected above), then the course of neurologic disease will be followed for up to 6 months. If chronic infection can be verified, then the model will have been validated as a model for chronic neuroborreliosis. At that time, further support will be sought. Monkeys in these studies will be treated as in the above experiments, with additional collections of CSF, serum and urine at 4 and 5 months, with necropsy at 6 months. Samples will be collected and processed as above. Treatment group sizes are larger than Phase I because of the even more variable nature of chronic infection, thus it is assumed that 12 infected monkeys will suffice. Eight controls are needed to determine background pathology that may be misinterpreted as infection-related in the principal group.

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>I</td>
<td>See I, above</td>
<td>8</td>
<td>2-3</td>
</tr>
<tr>
<td>II</td>
<td>See II, above</td>
<td>12</td>
<td>2-3</td>
</tr>
<tr>
<td>III</td>
<td>See III, above</td>
<td>20</td>
<td>2-3</td>
</tr>
</tbody>
</table>
### Categories of invasiveness

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

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

#### e) Rationale for species and numbers

How did you determine that 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?

Although rodents, and mice in particular, are outstanding models for Lyme disease investigation, they do not develop neurologic infection or disease. Rhesus monkeys are the only known animal model for investigation of neuroborreliosis, but the model needs to be optimized. The numbers for each study are based upon the minimal numbers needed to identify monkeys with neuroborreliosis, taking into consideration the variable rates of CNS infection, rates of disease, rates of infection and >10 years of experience with the monkey model by the PI (Dr. , an MD neurologist).

#### f) Surgery

If the project involves survival surgery, where will the surgery be conducted?

- **Building:** CRPRC  
  **Room:**  
  **Who will be the surgeon?** CRPRC staff

#### 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/meda, meda</td>
<td>7mg/kg and 3mg/kg</td>
<td>i.m.</td>
<td>See section C, above</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Oxymorphone or buprenorphine</td>
<td>To effect</td>
<td>i.m.</td>
<td>As indicated by CRPRC veterinary staff</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Tylenol or ibuprofen</td>
<td>To effect</td>
<td>oral</td>
<td>As indicated by CRPRC veterinary staff</td>
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</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?

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What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

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Under what circumstances will incremental doses of anesthetics-analgesics be administered?

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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)

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Lyme disease can cause a variety of relatively mild clinical signs and symptoms, the most severe being oligoarticular arthritis (which has been documented in monkeys). Monkeys rarely experience transient stiffness or lameness that is generally mild. More significant adverse effects might occur in response to dexamethasone treatment, which may result in anorexia, Cushingoid changes, etc. Post surgical discomfort may occur following skin and muscle biopsies. Biopsy sites should be monitored closely for postsurgical infection.

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.

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Monkeys should be monitored daily. If discomfort is noted, monkeys should be treated with analgesics (above) as indicated by veterinary staff. If infection of biopsy sites occurs, veterinary staff should contact the PI, as antibiotic treatment will negate the experiment. Nevertheless, if antibiotic treatment is warranted, the welfare of the animal holds higher priority.

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j) Literature search

This section is specifically required by Federal law. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this study, or 2) there are alternative methodologies, but these are not appropriate for your particular study. *Alternative methodologies* refers to reduction, replacement, and refinement (the three R's) of animal use, not just animal replacement. You must also show that the study is not unnecessarily duplicative of other studies.

What was the date on which you conducted this search? September 9-12, 2001

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</table>
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>NIH-Sponsored Workshop on Neuroborreliosis</td>
<td>Past to present</td>
<td>3 day, intensive meeting of European and US scientists discussing neuroborreliosis in humans and animal models.</td>
</tr>
<tr>
<td>MedLine</td>
<td>&quot;</td>
<td>neuroborreliosis</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There is a pressing need for an animal model of neuroborreliosis, as there is a considerable void of knowledge in this important human disease.

Has this study been previously conducted? [X] 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?

Monkeys will be euthanized at 3 to 6 months after infection, as outlined above.

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>pentobarbitol</td>
<td>60mg/kg</td>
<td>i.v.</td>
</tr>
</tbody>
</table>

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

None
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/](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/](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

** Conditions necessary for Committee Approval:


Final Disposition of this protocol:

___________ Approved

___________ Not Approved

___________ Withdrawn by Investigator

Date of Action: _____ / _____ / _____

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

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

<table>
<thead>
<tr>
<th>RUA#</th>
<th>BUA#</th>
<th>CCA#</th>
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</table>

Identity of Hazard: **Borrelia burgdorferi**

**Provide a short description of the agent:**

Agent of Lyme disease

**This agent / material is hazardous for:**

- [ ] Humans only
- [x] Animals only
- [x] Humans and Animals

**For which Animal Species?**

- [ ] Blood
- [x] Saliva/nasal droplets
- [x] Does not leave animal

- [ ] Tick vector

**Describe any human health risk associated with this agent:**

Laboratory exposure to cultured agent, sharps accidents involving infected material, and infected ticks. Humans can develop Lyme disease.

**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
  - [x] 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:**

- [x] The following personal protective equipment must be worn/used in the room:
  - [x] Lab Coat/Coveralls
  - [x] Disposable Gloves
  - [x] NIOSH Certified Dust Mask
  - [x] Eye Protection/Face Shield
  - [x] Fitted Respirator
  - [x] Shoe Covers/Booties
  - [x] Head Cover
  - [ ] Disinfectant footbath
  - [ ] Other: Describe:

- [x] 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:**

Personal protective equipment commensurate with risk of handling rhesus monkeys far exceeds need for protection against Lyme disease agent.