**PROTOCOL FOR ANIMAL USE AND CARE**

*Email to: campusvet@ucdavis.edu*

**CNPRC**

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>Last Name:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>First:</th>
<th>First:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Middle:</th>
<th>Middle:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>email:</th>
<th>email:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Department:</th>
<th>Department:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phone / Fax:</th>
<th>Phone:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After hrs. #:</th>
<th>After hrs. #:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PROTOCOL:** 10544  
**EXPIRES:** 4/24/04

<table>
<thead>
<tr>
<th>Species (common names):</th>
<th>Number:</th>
<th>Source:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus macaque</td>
<td>656</td>
<td>CNPRC</td>
</tr>
</tbody>
</table>

**Project Title:** Production of Pedigreed SPF Rhesus Macaque

**Overnight housing location:** CNPRC  
**Day use only:**

Animals will be maintained by: [x] Vivarium  [ ] Investigator  *(If investigator maintained, attach husbandry SOP's.)*

**Procedures:**

Provide a one or two sentence layman's description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

The intent of the project is to derive a colony of rhesus macaque free of specific pathogens. In addition, these macaques will be genetically characterized.

**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.

Animals must be maintained separate from conventional rhesus macaques.

**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>[ ] Call Investigator</td>
<td>[ ] Call Investigator</td>
<td>[ ] Call Investigator</td>
</tr>
<tr>
<td>[x] Clinician to treat</td>
<td>[ ] Save for Investigator</td>
<td>[x] OK to use pesticides</td>
</tr>
<tr>
<td>[ ] Terminate</td>
<td>[x] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[x] Necropsy</td>
<td>[x] Necropsy</td>
<td></td>
</tr>
</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>[ ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
<td>[ ] Yes</td>
</tr>
<tr>
<td>[x] No</td>
<td>[x] No</td>
<td>[x] No</td>
<td>[x] No</td>
</tr>
</tbody>
</table>

**Agent(s):**

Agent(s):
Funding source: NIH
Previously approved? [x] Yes [ ] No
Is the project already funded? [x] Yes [ ] No
Previous protocol number (if any): 9099

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)
[ ] 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.

Infant monkeys will be produced by natural breeding and embryo transfer technology. These infants will have a blood sample collected for genetic testing and be screened for Herpes B and simian retroviruses to develop a specific pathogen-free (SPF) colony. The SPF status of the animals improves animal health, removes potential confounding variables in research, and improves occupational safety.

b) Procedures employed in this project:

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

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

This project will screen a total of 500 Indian origin rhesus macaques at the Primate Center to determine animal pedigree and their Major Histocompatibility Complex (MHC) alleles. The MHC is an essential immune system component. 400 animals will be from the corrals and 100 indoor housed rhesus macaques. This will be done with a peripheral blood sample not more than 5 ml. Each year 52 infants will be harvested on day of birth for SPF testing. 45 indoor animals will be selected from the indoor group. 14 females with desirable MHC alleles will be superovulated with hormone treatment and oocytes collected nonsurgically using ultrasound. Female rhesus receive twice daily IM injections of 30 IU human urinary FSH (Follicle Stimulating Hormone) (Metrodin, Serono Laboratories, Inc.) for 7 or 8 days beginning on Days 1–4 of the menstrual cycle (Day 1 = first day of menstruation). These animals are given a single IM injection of 1,000 IU hCG (human chorionic gonadotropin) to induce oocyte maturation in vivo. At the CNPRC, follicle aspiration is performed on Ketamine (10 mg/kg IM) or Telazol (5 mg/kg IM) anesthetized females by ultrasound guidance. Briefly, ovaries are imaged on ultrasound and a 20 gauge x 3 inch spinal needle (with stylet removed) is attached to a 3 cc syringe that is inserted through the abdominal wall until its tip is imaged near the ovary. Aspiration is initiated as the needle tip is advanced into the ovary and the tip is monitored as it traverses each follicle. The entire process is continuously monitored by ultrasound and follicles are obtained within 10-15 minutes. 2 male rhesus macaques will have semen collected in chair restraint and serve as sperm donors. Electro-ejaculation will be performed by the CNPRC Standard Operating Procedure. Embryos with desirable MHC alleles will be produced by in vitro fertilization. Embryos will be transferred by cannula into the uterine cavity using ultrasound to female rhesus anesthetized with Ketamine (10 mg/kg). The remaining 29 females will serve as embryo recipients or be used in natural mating. Offspring from both indoor and outdoor mating will be collected at the day of birth and placed in the CNPRC SPF nursery. Beginning at approximately three months of age, the infants will be tested over the next year for Herpes B, Type D Retrovirus, Simian Immunodeficiency Virus, Simian T-cell Lymphotropic Virus, and Simian Foamy Virus using the CNPRC SPF Standard Operating Procedures on 3 ml samples of heparinized blood collected from the femoral vein at three month intervals. Infants will be socialized according to CNPRC standard operating procedures, and at 1 year of age placed in social groups.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Breeding - Outdoor</td>
<td>400</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Semen Collection</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Oocyte Harvest</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Embryo Transfer</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Normal Breeding - Indoor (time-mated)</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>SPF Testing (Infants)</td>
<td>156</td>
<td>1</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 skilful 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?

This project is designed to produce breeding groups of rhesus that are genetically characterized and SPF. It is in response to an NIH request for these types of animals for use in biomedical research. The initial figure of 500 adult animals was chosen as a target number for the production of the 156 SPF infants.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>For oocyte harvest and embryo transfer.</td>
</tr>
<tr>
<td>Rhesus</td>
<td>Telazol</td>
<td>5</td>
<td>IM</td>
<td>For oocyte harvest and embryo transfer</td>
</tr>
</tbody>
</table>

**h) Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:

Why do you need to use a neuromuscular blocking agent?
What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

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

i) Adverse effects:
Describe any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

There is little risk of adverse effects associated with oocyte harvest and embryo transfer based on extensive experience and over 10 years of studies. Animals will be anesthetized with ketamine or Telazol for both procedures.

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.

Although none are anticipated, any adverse effects will be referred to a CNPRC 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 [x] No

(Note: “Death as an endpoint” refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

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

go to 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: mwwood@ucdavis.edu)

What was the date on which you conducted this search? April 5, 2000

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>PubMed</td>
<td>1995-2000</td>
<td>SPF, rhesus</td>
</tr>
</tbody>
</table>
What were your findings with respect to alternative methodologies?

There are no alternatives to use of live animals for an animal production program. The techniques used in SPF screening are standardized in the primate literature.

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

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

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

Animals that are determined not to be SPF will be returned to the conventional colony.

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>Drug overdose</td>
<td>Pentobarb</td>
<td>60</td>
<td>IV</td>
</tr>
</tbody>
</table>

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

Return to the colony.
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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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://ehs.ucdavis.edu/animal/health/](http://ehs.ucdavis.edu/animal/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. Information is available on the world wide web at [http://ehs.ucdavis.edu/](http://ehs.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排名/头衔日期

Committee Use Only Below

** Conditions necessary for Committee Approval:


Final Disposition of this protocol:

___________ Approved

___________ Not Approved

___________ Withdrawn by Investigator

Date of Action: _____ / _____ / ______

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

______________________________  __________________________
Campus Veterinarian日期
Dear [Name],

Thank you for your review of the attached protocol. I have attempted to answer the questions you raised and have incorporated the changes into the attached document (10544R.doc). The changes are highlighted in blue type. Please let me know if I need to make further adjustments or if you need additional information. Thanks again.

Sincerely,

At 12:37 PM 4/9/2003, you wrote:

Hi all,

I have pre reviewed the recently submitted protocol which has been assigned accession number 10544 for future reference. I have attached a copy of the protocol with the number embedded for ease of making revisions to the questions below and returning the doc as an attachment.

For this protocol to be considered on the 4/24 committee agenda, please forward your revised protocol to me on or before noon, Tuesday, April 15th.

Thanks in advance,

Protocol 10544 ( )

1. On page 1, you have stated that you will be using 500 animals. Is that 500 per year or a total of 500 over the three year life of the protocol? In sections c and d you have listed different numbers. Please make sure that the numbers throughout the protocol reflect the same numbers per group the total projected number of animals for the study.

2. In section a, you discussed the overall intent of the study, but did not describe the significance. Please expand section a.

3. In section c, you use the acronym MHC. Please explain what you mean by this acronym.

4. In section c, you mention different numbers of animals, but the groups defined in section d do not always correspond to what is written in section c. Please make sure the groups in section c and d correspond and that the totals in both sections equals that which you have cited on page 1.

5. Where do the 52 infants fit into the total numbers?

6. What is the route for the single injection of HCG?

7. When will the blood sampling begin on the infants if it is supposed to be collected at three month intervals for a year? Please clarify.

8. In section d, there is no reference to indoor or outdoor animals. Please make sure that you have the groups correspond to those discussed in section c.
9. In section e, you have only provided a response to the first question. Please provide a response to the second question on justification of animal numbers.

----- Original Message ----- 
From: 
To: 
Sent: April 11, 2003 1:23 PM 
Subject: Protocol 10544 

> Please find attached a copy of protocol 10544. 
> 
> This is either a new or updated protocol. Please perform a veterinary review, and if you have any questions or concerns, please forward them via email to campusvet@ucdavis.edu 
> 
> Regards,
> 
> 
>