## PROTOCOL FOR ANIMAL USE AND CARE

**CRPRC**

### Handwritten forms are not accepted

### EH&S USE ONLY

**PROTOCOL # 9567**

**EXPIRES:**

### Investigator

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### Contact

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### Species (common names):

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus macaque</td>
<td>20</td>
<td>CPRC</td>
</tr>
<tr>
<td>Cynomolgus macaque</td>
<td>20</td>
<td>CPRC</td>
</tr>
</tbody>
</table>

### Project Title

Development and Evaluation of Vaccines in Nonhuman Primates

### Overnight housing location:

<table>
<thead>
<tr>
<th>CPRC</th>
<th>Day use only:</th>
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</table>

### 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.

Animals will be immunized with peptides, proteins, inactivated viruses, DNA plasmids, or live attenuated bacterial vectors, all of which are routinely used components of candidate human vaccines. Some monkeys may be infected with live viruses pathogenic for nonhuman primates, specifically simian immunodeficiency virus (SIV) and SHIV (SIV expressing HIV envelope).

### 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 will be maintained in a BLS-2 facility once they have been infected with SIV or SHIV.

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

#### Sick Animals

- [ ] Call Investigator
- [ ] Call Investigator
- [ ] Call Investigator

#### Dead Animals

- [ ] Clinician to treat
- [ ] Save for Investigator
- [ ] Bag for disposal

#### Pest Control

- [ ] Terminate
- [ ] OK to use pesticides
- [ ] No Pesticides in animal area

#### 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>[ ] No</td>
<td>[ ] No</td>
<td>[ ] No</td>
<td>[ ] No</td>
</tr>
</tbody>
</table>

Agent(s): **SHIV, SIV**
Funding source: State of California  
Previously approved? [X] Yes [ ] No

Is the project already funded? [X] Yes [ ] No

Previous protocol number (if any): 8207

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.

The intent of this study is to evaluate the immunogenicity of candidate human vaccines that are composed of a variety of different components that are known to induce potent cellular and/or humoral immunity. We hypothesize that immunization of macaques with plasmid DNA and with a live attenuated recombinant bacterial vector (rBCG) will preferentially induce a cellular immune response. We are unsure, however, and wish to determine which of these forms of vaccination induces the strongest and most durable cellular immune response. Similarly, we expect that immunization with peptides, recombinant proteins, or whole inactivated viruses will preferentially induce humoral immune responses. Again, we wish to determine which vaccine component induces the strongest, most durable, and most broadly reactive humoral immune response. Strong cellular and humoral immune responses will need to be evoked by any efficacious vaccine against HIV. The data obtained from this study should indicate what is the best combination of vaccine components in a candidate HIV vaccine.

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.
- [X] 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)
- [X] 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.)*

Monkeys will be immunized with peptides, proteins, inactivated viruses, plasmid DNA, or attenuated live bacterial vectors in order to evaluate quantitatively and qualitatively cellular and humoral immunity induced by these vaccine components. Blood (10mls collected in a tube containing no anticoagulants ("red tops") and 30mls collected in tubes containing the anticoagulant EDTA ("purple tops")). Will be obtained on two occasions separated by 1 month prior to any immunizations. In addition, blood will similarly be obtained two weeks after each immunization in order to monitor elicited antibody titers and cellular responses. Animals will be immunized two times with plasmid DNA or rBCG, each immunization separated by one month. Animals will be immunized three times with peptides, recombinant proteins, or whole inactivated viruses, the boosts occurring one and six months after the first immunization.

Specifically, the following assays will be used to measure evoked immune responses in immunized animals: ELISA, T cell proliferation, virus neutralization, antibody-dependent cell cytotoxicity (ADCC), and quantitation of the frequency of antigen-specific T helper cells and cytotoxic T lymphocytes using flow cytometry. Once an appropriate immune response has been elicited and measured, animals will be challenged intravenously with 10 MID50 of previously titered SIVmac251 or SHIV 89.6P obtained from the NIH AIDS Reagent Repository. After challenge, the animals will be followed for up to one year. The monkeys will be monitored for infection, viral load, and protection from disease by cocultivation of plasma samples with a permissive cell line, polymerase chain reaction analysis of animal samples for proviral DNA, assessment of virus-specific antibodies by ELISA and neutralization, measurement of absolute CD4+ T cell count and CD4/CD8 T cell ratio, and general animal health.
<table>
<thead>
<tr>
<th>Group</th>
<th>Immunogen</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
<th>Times Admin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>peptide</td>
<td>500µg</td>
<td>i.m.</td>
<td>0, 1, &amp; 6 months</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>protein</td>
<td>1mg</td>
<td>i.m.</td>
<td>0, 1, &amp; 6 months</td>
<td>6</td>
</tr>
<tr>
<td>C</td>
<td>inact. virus</td>
<td>500µg</td>
<td>i.m.</td>
<td>0, 1, &amp; 6 months</td>
<td>6</td>
</tr>
<tr>
<td>D</td>
<td>plasmid DNA</td>
<td>80µg</td>
<td>i.d.</td>
<td>0 and 1 months</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>bacterial vector</td>
<td>1x10^8</td>
<td>i.v.</td>
<td>0 and 1 months</td>
<td>2</td>
</tr>
</tbody>
</table>

**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>A-E</td>
<td>Rhesus macaques: 4 animals/group immunized with peptide, protein, inactivated virus, plasmid DNA, or attenuated bacterial vector.</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>A-E</td>
<td>Cynomolgus macaques: 4 animals/group immunized with peptide, protein, inactivated virus, plasmid DNA, or attenuated bacterial vector.</td>
<td>20</td>
<td>4</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?

The intent of this study is to evaluate the immunogenicity of candidate human vaccines that are composed of a variety of different components that are known to induce potent cellular and/or humoral immunity. We wish to use macaques because immunogenicity and efficacy data obtained using these animals correlate very well with immunogenicity data in human vaccinees immunized with similar vaccine components. Moreover, macaques are an outbred species like the human population for which the candidate vaccines are intended, unlike most other animal models used for vaccine development. Rhesus and cynomolgus macaques have been selected because they are susceptible to infection with SIV and SHIV, model pathogens against which our candidate vaccines are based. Because of their outbred nature, a minimum number of 4 animals/group is needed to have data that is statistically meaningful given the inherent variation of immune responses seen in these animals. This minimum number of animals has been adopted by investigators in the field worldwide.

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

<table>
<thead>
<tr>
<th>Building</th>
<th>Room</th>
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<tbody>
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</table>

Who will be the surgeon?

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>10-40</td>
<td>i.m.</td>
<td>after each immunization (3-6 times)</td>
</tr>
<tr>
<td>macaque</td>
<td>hydrochloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>Ketamine</td>
<td>10-40</td>
<td>i.m.</td>
<td>after each immunization (3-6 times)</td>
</tr>
<tr>
<td>macaque</td>
<td>hydrochloride</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?

- 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)

If animals challenged with virus become chronically infected, symptoms associated with immunodeficiency syndrome are expected. These include wasting, susceptibility to infection, fever, weight loss, opportunistic infections and neoplasia. Immunization of animals with peptides, proteins, inactivated viruses, or plasmid DNA constructs can result in local redness and induration. Immunization with attenuated live bacterial vectors (rBCG) can also result in fever. Complications may arise from either the immunization or challenge studies, in which case appropriate treatment by CRPRC personnel will be provided and additional scheduled inoculations may be delayed.

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.

The analgesics can and will be administered when necessary and appropriate in order to avoid discomfort for the animals, at the discretion of the Primate Center veterinarians. Ketamine is routinely used at 10mg/kg (im) and Ketamine-Xylazine (100mg/ml ketamine, 20mg/ml xylazine) used at 0.1-0.2ml/kg body weight (im).

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.

Animals that become chronically infected as a result of virus challenge will be treated. If animals do not respond to therapy and develop progressive disease, they will be euthanized. See attached document titled "Criteria for Euthanasia of Retrovirus Infected Macaques" (CRPRC Dated 6/30/96).

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? 5/1/01

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>
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</table>

University of California, Davis
Printed 7/21/2004 11:10:26 AM Page 6
What were your findings with respect to alternative methodologies?

Painful procedures that are not absolutely necessary are excluded from the proposed protocol. CPRC personnel will avoid unnecessary pain to the animals when administering vaccine components by using anesthesia. A comprehensive review of the literature has demonstrated that our proposed studies are novel and do not duplicate previous studies/results.

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? |
| Progressive disease that does not respond to therapy will be the indication for euthanizing a particular animal. See attached document titled "Criteria for Euthanasia of Retrovirus Infected Macaques" (CRPRC Dated 6/30/96). |

| 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 an 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 | Deep Ketamine anesthesia | Barbiturate | Overdose | i.v. |
| Cynomolgus macaque | Deep Ketamine anesthesia | Barbiturate | Overdose | i.v. |

| m) Surplus animals: What will you do with any animals not euthanized at the conclusion of the project? |
| They will be used in future approved projects by us and by other scientists working in vaccine development. |
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.

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<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.

---

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.

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Campus Veterinarian

Date
**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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0464</td>
<td></td>
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</table>

Identity of Hazard: **SIV, SHIV, recombinant BCG**

**Investigator Last Name:** [Name]

**First Name:** [Name]

**Department:** [Department]

**Phone:** [Phone]

**Fax:** [Fax]

**Email:** [Email]

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

SIV and SHIV are pathogenic for nonhuman primates. Recombinant BCG vectors are infectious for nonhuman primates.

This agent / material is hazardous for:

- [ ] Humans only
- [X] Animals only
- [X] Humans and Animals

**For which Animal Species?** Rhesus and Cynomolgus macaques

The agent can be spread by:

- [X] Blood
- [ ] Feces/urine
- [ ] Saliva/nasal droplets
- [ ] Does not leave animal

Describe any human health risk associated with this agent:

SHIV, SIV, and recombinant BCG are all infectious 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:
    - [ ] Incineration
    - [ ] Bag and Autoclave
    - [ ] Biohazardous Waste Container
    - [ ] 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
    - [ ] EH&S will pick-up (2-1493).

**Personal Protective Equipment Required:**

- [X] Lab Coat/Coveralls
- [X] Disposable Gloves
- [X] NIOSH Certified Dust Mask
- [X] Eye Protection/Face Shield
- [ ] Fitted Respirator
- [ ] Other:
  - Type: [Type]
  - Describe:
  - [X] Personal protective equipment must be removed before leaving the room.
  - [X] Personal protective equipment must be discarded or decontaminated at the end of the project
  - [X] Hands, arms, and face must be thoroughly washed upon leaving the room
  - [X] Full shower, including washing of hair, must be taken upon leaving the room.
  - [X] 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:**

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University of California, Davis
Printed 7/21/2004 11:10:26 AM  Page 10