**PROTOCOL FOR ANIMAL USE AND CARE**

**CNPRC**

**PROTOCOL:** 10663  
**EXPIRES:** 7/2/04

**Investigator**

<table>
<thead>
<tr>
<th>Last Name:</th>
<th>Last Name:</th>
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<tbody>
<tr>
<td>First:</td>
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<td>Middle:</td>
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<tr>
<td>email:</td>
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<td>Department:</td>
<td>Department:</td>
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<tr>
<td>Phone / Fax:</td>
<td>Phone:</td>
</tr>
<tr>
<td>After hrs. #:</td>
<td>After hrs. #:</td>
</tr>
</tbody>
</table>

**Species (common names):**  
| rhesus | Number: 78 | Source: CNPRC |

**Project Title:** Heterosexual Transmission of AIDS: A simian model

**Overnight housing location:** CNPRC  
**Day use:** CNPRC (workrooms or animal quarters)

**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 mucosally inoculated with SIV (simian immunodeficiency virus) or SHIV (simian immunodeficiency virus with HIV envelope). Blood, genital secretions, and lymph node biopsy samples will be obtained to assess viral infection and localize the virus in the animals.

**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

**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>[ ] 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>[ ] Bag for disposal</td>
<td>[ ] No Pesticides in animal area</td>
</tr>
<tr>
<td>[ ] 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>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent(s): SIVmac239, SIVmac251, SHIV SF162P, SIVmacDnef, SIVmacDvpx/vpr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radioisotopes?</th>
<th>Yes</th>
<th>X</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent(s):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical Carcinogens?</th>
<th>Yes</th>
<th>X</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent(s):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxic Chemicals?</th>
<th>Yes</th>
<th>X</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent(s):</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Funding source: NIH/NIAID/Rockafellar
Previously approved? [X] Yes [ ] No
Is the project already funded? [X] Yes [ ] No
Previous protocol number (if any): 9206

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.

We hypothesize that unique host and viral factors determine the ultimate outcome of SIV inoculation on a genital mucosal surface. Currently the mechanisms of heterosexual transmission are poorly understood, therefore making progress in protection from the virus difficult. This study is designed to elucidate which host and viral factors play a role in transmission.

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+)
- [X] Fasting prior to a procedure.
- [ ] Food or water restriction
- [ ] Non-recovery surgical procedures
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Special diets; food or water treatment.
- [X] Induced illness, intoxication, or disease
- [ ] Death as an endpoint (see i below)
- [ ] Trapping, banding or marking wild animals
- [ ] Behavioral modification.
- [ ] 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.)

For all groups: We request mature, cycling female rhesus macaques. For all procedures, animals will be fasted 12 hours prior to immobilization using either ketamine (6-10 mg/kg which will be administered intra-muscularly) for bleeds, lavages, and LN biopsies and if available telazol (6-8 mg/kg to be administered intra-muscularly) will be used for SIV/SHIV inoculations and cervical biopsies. If telazol is not available then ketamine will be used in the same method as stated above. Buprenorphine (0.01-0.03 mg/kg) will be administered for post-procedure pain according to the CNPRC veterinary staff.

Group A- Certain strains of SIV and SHIV have unique properties that allow them to infect animals by vaginal inoculation.

One week prior to inoculation (Day -7), blood will be collected (10-20 mls, not to exceed 12 ml/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (byatraumatically inserting a pipette into the vagina and washing with PBS) will be collected for baseline samples. On Day 0, animals will be inoculated (am/pm) intravaginally with 1 ml (by atraumatically inserting a 1 ml syringe with 10^5 TCID_{50} virus in 1 ml saline) SHIV SF162P. Blood, (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein, a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected weekly to monitor the course of infection. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected once prior to inoculation, and after inoculation no more than once a month to monitor for infection. A cervical biopsy (by inserting a lubricated speculum into the vagina and using a biopsy tool which is used in human gynecology to obtain 1 gram of tissue maximum) will be performed no more than once a month to detect initial infection of target cells in the genital tract. We have performed over 25 cervical biopsies in rhesus macaques without any untoward result. We anticipate culling these animals within six months of virus inoculation.

Group B- Certain strains of SIV and SHIV have unique properties that allow them to infect animals by vaginal inoculation.

One week prior to inoculation (Day -7), blood will be collected (10-20 mls, not to exceed 12 ml/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected for baseline samples. On Day 0, animals will be inoculated (am/pm) intravaginally with 1 ml (by atraumatically inserting a 1 ml syringe with 10^5 TCID_{50} virus in 1 ml saline) SIVmac239D_{nef}. Blood, (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein, and a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected weekly to monitor the course of infection. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected once prior to inoculation, and after inoculation no more than once a month to monitor for infection. A cervical biopsy (by inserting a lubricated speculum into the vagina and using a biopsy tool which is used in human gynecology to obtain 1 gram of tissue maximum) will be performed no more than once a month to detect initial infection of target cells in the genital tract. We have performed over 25 cervical biopsies in rhesus macaques without any untoward result. We
anticipate culling these animals within six months of virus inoculation.

**Group C- Certain strains of SIV and SHIV have unique properties that allow them to infect animals by vaginal inoculation.**

One week prior to inoculation (Day -7), blood will be collected (10-20 mls, not to exceed 12 ml/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5), a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected for baseline samples. On Day 0, animals will be inoculated (am/pm) intravaginally with 1 ml (by atraumatically inserting a 1 ml syringe with $10^5$ TCID$_{50}$ virus in 1 ml saline) SIVmac239Dvpx/vpr. Blood, (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein, and a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected weekly to monitor the course of infection. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected once prior to inoculation, and after inoculation no more than once a month to monitor for infection. A cervical biopsy (by inserting a lubricated speculum into the vagina and using a biopsy tool which is used in human gynecology to obtain 1 gram of tissue maximum) will be performed no more than once a month to detect initial infection of target cells in the genital tract. We have performed over 25 cervical biopsies in rhesus macaques without any untoward result. We anticipate culling these animals within six months of virus inoculation.

**Group D- Serial intravenous passage of SIV/SHIV and titration of the resultant stock.**

This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml ($10^5$ TCID$_{50}$ virus in 1 ml RPMI) SHIV 89.6. Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.

**Group E- Serial intravenous passage of SIV/SHIV and titration of the resultant stock.**

This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml ($10^5$ TCID$_{50}$ virus in 1 ml RPMI) SHIV SF162 P. Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals.
within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.

**Group F - Serial intravenous passage of SIV/SHIV and titration of the resultant stock.** This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml (10⁵ TCID₅₀ virus in 1 ml RPMI) **SIVmac239.** Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.

**Group G - Serial intravenous passage of SIV/SHIV and titration of the resultant stock.** This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml (10⁵ TCID₅₀ virus in 1 ml RPMI) **SIVmac251.** Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.

**Group H - Serial intravenous passage of SIV/SHIV and titration of the resultant stock.** This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml (10⁵ TCID₅₀ virus in 1 ml RPMI) **SIVmac239 Dnef.** Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.
**Group I- Serial intravenous passage of SIV/SHIV and titration of the resultant stock.** This group is designed to produce a new stock of infectious virus that is efficiently transmitted. Two animals will be inoculated intravenously with 1 ml (10^5 TCID₅₀ virus in 1 ml RPMI) SIVmac239 Dvpx/vpr. Blood (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein two weeks after inoculation. Plasma will be separated from the blood of the animal with the highest viral load and 1 ml will be used to inoculate a second animal intravenously. This process will be repeated three times using a total of 8 animals. Blood from all inoculated animals (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected every two weeks. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected prior to inoculation and will be obtained no more than once per month after inoculation to monitor for infection. Before the development of clinical SAIDS, the animals will be euthanized. We anticipate culling these animals within 6 months of inoculation. The last animal will be euthanized and serum will be harvested to produce a new infectious virus stock.

**Group J- Normal control group**

Blood, (10-20 ml, not to exceed 12 mg/kg/month in compliance with Primate Center blood collection guidelines SOP GG-5) will be collected from a femoral vein, and a cytobrush will be used to atraumatically sample cells in the cervix of the animals, and vaginal lavages (by atraumatically inserting a pipette into the vagina and washing with PBS) will be collected weekly to monitor normal changes in lymphocyte counts and cytokine secretion. Lymph node biopsies (peripheral lymph nodes: axillary or inguinal approximately 1 gram of tissue according to CNPRC Standard Operating Procedure) will be collected no more than once a month to establish normal values for lymphocyte activation markers and cytokine secretion. A cervical biopsy (by inserting a lubricated speculum into the vagina and using a biopsy tool which is inserted in human gynecology to obtain 1 gram of tissue maximum) will be performed no more than once a month to obtain baseline data in the genital tract. We have performed over 25 cervical biopsies in rhesus macaques without any untoward result. Animals will then be necropsied for normal tissues to compare to SIV infected tissues.

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</td>
<td>SHIV SF162PB inoculation</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>SIVmac239 Dnef inoculation</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>SIVmac239 Dvpx/vpr inoculation</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>SHIV 89.6 inoculation-serial passage</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>SHIV SF 162 P inoculation-serial passage</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>F</td>
<td>SIVmac239 inoculation-serial passage</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>G</td>
<td>SIVmac251 inoculation-serial passage</td>
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<td>3</td>
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<tr>
<td>H</td>
<td>SIVmac239 Dnef inoculation-serial passage</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>SIVmac239 Dvpx/vpr inoculation-serial passage</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>J</td>
<td>Normal controls</td>
<td>6</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 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 SIV-rhesus macaque model of HIV heterosexual transmission has become the recognized standard for studies on pathogenesis and prevention of HIV vaginal transmission.

Groups A, B, and C- These groups are designed to test the transmission characteristics of different strains of SIV/SHIV. 6 animals will be inoculated to account for potential differences in susceptibility among rhesus macaques to the virus. Previous experience in this area tells us 6 animals will be needed to detect differential transmission of virus strains.

Groups D-I- Because of individual animal variation, two monkeys will be used in parallel for each passage. Thus two animals will be inoculated, the plasma from these animals will be mixed and used to inoculate two more animals, then the plasma from those two animals will be used to inoculate the last two animals. This approach minimized the number of animals used, but avoids relying on susceptibility of a single animal to keep the serial passage moving forward. Six monkeys will then be needed to titrate each stock produced and those monkeys are accounted for in Groups A-C of this protocol.

Group J- In order to evaluate what factors in the host play a role in transmission, it is necessary to define what “normal” parameters are in samples taken from rhesus macaques. This group is the minimum number that can be used to account for animal to animal variation in order to determine “normal” values.

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.

<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 HCL</td>
<td>6-10 mg/kg</td>
<td>IM</td>
<td>Prior to all sampling procedures</td>
</tr>
<tr>
<td>rhesus</td>
<td>Telazol</td>
<td>6-8 mg/kg</td>
<td>IM</td>
<td>Prior to all inoculations</td>
</tr>
<tr>
<td>rhesus</td>
<td>buprenorphine</td>
<td>0.01-0.03 mg/kg</td>
<td>IM</td>
<td>As needed in judgement of CRPRC vets</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)

Any injection, venipuncture, or biopsy has the potential to cause minor pain or discomfort, but the animals are immobilized for the procedure and should not experience pain.

SIV infection of rhesus macaques can result in a fatal immunodeficiency and wasting syndrome. The animals will be euthanized when they experience 3 of the following: weight loss >15% in 2 weeks or >30% in 3 months; persistent hypothermia <96F even with heat supplementation; leukopenia (total WBC <3,000); lymphopenia (lymphocytes <800); anemia (hemoglobin <10); dehydration >10%; nonresponsive to therapy for opportunistic infections; persistent anorexia (> 3 days); animal significantly obtunded. These criteria are based on CRPRC guidelines.

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.

All possible efforts will be made to minimize animal pain and discomfort. Analgesics have no effect on the proposed studies and they will be administered at the discretion of the CRPRC veterinary staff.

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 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? 5/29/03

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>1990-present</td>
<td>Primate, SIV, genital transmission</td>
</tr>
<tr>
<td>Reference Update</td>
<td>1999-present</td>
<td>Primate, SIV, genital transmission</td>
</tr>
<tr>
<td>Current Contents</td>
<td>1990-present</td>
<td>Primate, SIV, genital transmission</td>
</tr>
</tbody>
</table>

What were your findings with respect to alternative methodologies?

There are no alternative methodologies for assessing the host and viral factors playing a role in genital lentiviral transmission.

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.

Serial passages and subsequent titrations are needed to replenish virus stocks. The proposed vaginal inoculation studies use the same model as our previous experiments but use different virus strains allowing further analysis of the viral factors affecting genital transmission.

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

Animals will be euthanized after systemic infection is documented or when the animal develops clinical SAIDS.
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>IV</td>
<td>pentobarbital</td>
<td>60 mg/kg</td>
<td>IV</td>
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</tbody>
</table>

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

Animals will be euthanized or recycled to a SIV therapeutic study.
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://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/.
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

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

__________________________
Date

University of California, Davis
Printed 7/21/2004 8:34:46 AM Page 12
ANIMAL ROOM SAFETY INFORMATION
Complete this form if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal room.

Identity of Hazard: SIV, SHIV

Provide a short description of the agent:
SIV is a primate lentivirus that is genetically similar to HIV and causes fatal immunodeficiency (AIDS) in infected rhesus macaques. SIV can infect humans but it is unknown whether SUV causes human disease.

This agent / material is hazardous for:

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

For which Animal Species?

[ X ] Blood
[ ] Feces/urine
[ X ] Saliva/nasal droplets
[ ] Does not leave animal
[ ] All mucosal secretions can be contaminated

Describe any human health risk associated with this agent:
No human disease related to SIV has ever been described. However, there is a potential for SIV to infect 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:

[ X ] Cages must be autoclaved before cleaning.
[ ] Label cages and remove label after decontamination.
[ X ] Animal carcasses must be labeled and disposed of as follows:
[ ] Incineration
[ ] Bag and Autoclave
[ ] 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).
[ ] 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
[ ] Fitted Respirator
[ ] Other:

[ 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
[ ] Hands, arms, and face must be thoroughly washed upon leaving the room
[ ] Decontaminate Room (Inform ARS area supervisor when cage and/or room can be returned to general use).

Provide any other information needed to safely work in this room:
Biosafety level 2+ (BSL2+) precautions must be used at all times.
Date: Tue, 17 Jun 2003 11:51:55 -0700
To: 
From: 
Subject: Fwd: Re: Fwd: pre review questions protocol 10663

Date: Tue, 17 Jun 2003 11:50:44 -0700
To: 
From: 
Subject: Re: Fwd: pre review questions protocol 10663

here is the modified version

Questions from .

Date: Mon, 16 Jun 2003 14:21:42 -0700
To: 
From: >
Subject: pre review questions protocol 10663

Hi ,
I have received and pre reviewed the following protocol which has been assigned accession number 10663 for future reference. I have attached a copy of the protocol for ease of making revisions. For this protocol to be considered on the July 3rd committee agenda, please return the revised document to me on or before noon, Tuesday, June 24th. If you have any questions, feel free to contact me via phone or email.
Thanks in advance,

Protocol 10663 ( )
1. In Groups D, E,F,G,H and I, it appears you will only be using 4 animals according to your explanation, but you have listed 12 animals. Please clarify why you will be using 12 animals per group instead of 4 or clarify the use of the animals within the groups.
2. In Group J, you mention performing cervical biopsies monthly, but do not state the length of time the monthly biopsies will cover. Please clarify.
3. In section e, you mention that "six monkeys will be needed to titrate the stock". Please explain how the stock will be titrated. It is not discussed in section c. Please expand section c to include this description.