### Investigator Information

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

### Contact Information

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<tr>
<th>Last Name:</th>
<th>First:</th>
<th>Middle:</th>
<th>Email:</th>
<th>Department:</th>
<th>Phone:</th>
<th>After hrs. #:</th>
</tr>
</thead>
</table>

### Species Information

<table>
<thead>
<tr>
<th>Species (common names):</th>
<th>Number:</th>
<th>Source:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cynomolgus macaque</td>
<td>3</td>
<td>CNPRC</td>
</tr>
<tr>
<td>Cynomolgus macaque</td>
<td>100</td>
<td>CNPRC</td>
</tr>
</tbody>
</table>

### Project Title

Hepatitis B-like Virus Infection in Nonhuman Primates

### Overnight Housing Location:

<table>
<thead>
<tr>
<th>Primate Center</th>
<th>Day use:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primate Center</td>
<td></td>
</tr>
</tbody>
</table>

Animals will be maintained by:

- [ ] Vivarium
- [ ] Investigator

### Procedures

Three cynomolgus macaques will be inoculated with serum from Mauritius cynomolgus macaques naturally infected with an HBV-like virus, and will be followed for twelve weeks. Serum samples will be collected weekly and analyzed for liver transaminases and for presence of HBsAg. Specific sera will be used for viral DNA amplification. Serology will be performed on 100 macaques to survey for natural infection.

### Special Husbandry Requirements

Animals will be housed in infectious housing (BSL-2+).

### Other Instructions for Animal Care Staff

- [ ] Sick Animals
  - [X] Call Investigator
  - [X] Call Investigator
  - [X] Call Investigator
- [X] Dead Animals
  - [X] Call Investigator
  - [X] Bag for disposal
  - [ ] No Pesticides in animal area
- [X] Pest Control
  - [X] OK to use pesticides
  - [X] Necropsy

### Hazardous Materials

- Infectious Agents? [X] Yes [ ] No
  - Agent(s): HBV-like virus
- Radioisotopes? [ ] Yes [X] No
  - Agent(s):
- Chemical Carcinogens? [X] Yes [ ] No
  - Agent(s):
- Toxic Chemicals? [ ] Yes [X] No
  - Agent(s):
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.

**Hypotheses:**
a) *M. fascicularis* can harbor HBV–like simian virus; and b) *M. fascicularis* can be chronically infected with a HBV-like virus.

**The objectives of our study are:**
1) to evaluate the presence of a natural HBV-like virus infection in *M. fascicularis* at the CNPRC. The presence of natural HBV-like virus infection in *M. fascicularis* will be investigated in a random sample of *M. fascicularis* (100 animals) housed at the CNPRC; and 2) an HBV-like virus recently identified in *M. fascicularis* from Mauritius Island will be experimentally inoculated to HBV-negative *M. fascicularis* at the CNPRC to assess its infectivity and pathogenic potential.

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
- [ ] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Behavioral modification.
- [ ] Death as an endpoint (see i below)
- [ ] 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.)

**Evaluation of the presence of a HBV-like virus in *M. fascicularis***

The presence of natural HBV-like infection will be investigated in 100 *M. fascicularis* serum samples from the CNPRC colony. The serum samples (4.0 ml whole blood) will be collected by femoral venipuncture at the time of routine physical examination. These serum samples will be tested at UC Davis for liver transaminases and for presence of HBsAg by ELISA. The search for HBV-like virus DNA will be performed at INSERM unit 271 in Lyon, France using specific and highly sensitive nested PCR using primers from two regions (S and C) of viral genome. The results of PCR will determine whether HBV-like infection is present among *M. fascicularis* housed at the CNPRC.

**Transmission of HBV-like virus isolated from naturally infected *M. fascicularis* Mauritius***

HBV-like virus identified in the serum of *M. fascicularis* from Mauritius will be used as inoculum for experimental infection of 3 HBV-negative juvenile male *M. fascicularis*. Animals will be followed for 12 weeks (84 days). To determine whether HBV-like agent is transmitted to *M. fascicularis* serial serum samples (4.0 ml whole blood/week) will be taken weekly during the 12 weeks of follow up. These serum samples will be analyzed for liver transaminases and for presence of HBsAg at UC Davis. In addition, a specific PCR will be used for viral DNA amplification, as described above.

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>Blood collection for serology/DNA testing</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Inoculation with HBV-like virus and blood collection for serology/DNA testing</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Categories of invasiveness**

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

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?

<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>Cynomolgus macaque</td>
<td>Ketamine HCl</td>
<td>10mg/kg</td>
<td>IM</td>
<td>Immobilization for blood sampling and inoculation</td>
</tr>
</tbody>
</table>

M. fascicularis from Only known species to be naturally infected with this newly discovered HBV-lik virus. Inoculation of three animals will allow us to assess the level of infectivity of this virus in naïve animals. This is a pilot study, and use of 3 animals will minimize the potential influence of interanimal variability in susceptibility to infection. We are seeking a yes/no answer regarding infection, and not necessarily statistical significance.

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.

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)

Potential local irritation at inoculation site. Except for a transient elevation of serum transaminase levels, infection with HBV-like virus in cynomolgus macaques is expected to be asymptomatic.

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.

Infection should be mild enough not to require treatment. Indication for use of analgesics will be determined by the attending veterinarian.

---

Note: If any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure? [ ] Yes [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...
Although severe disease is not expected, any animals developing severe
disease, unresponsive to specific and supportive therapy will be
euthanatized. Decision regarding euthanasia will be made by the attending
veterinarian.

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?  May 21, 2003

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>1989-2003</td>
<td>Hepatitis B virus and Nonhuman Primates</td>
</tr>
<tr>
<td>BioMedNet</td>
<td>1966-2003</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
<tr>
<td>Biosis</td>
<td>1969-2003</td>
<td>&quot; &quot; &quot; &quot;</td>
</tr>
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</table>

What were your findings with respect to alternative methodologies?

HBV-like viruses have not previously been reported in macaques, and past
attempts to infect macaques with human-HBV have not been successful

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.

N/A

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

Those inoculated will be euthanized at the end of the study.

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>Cynomolgus</td>
<td>Barbiturate overdose</td>
<td>Pentobarbital</td>
<td>60mg/kg</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?

The 100 animals sampled will remain in the colony. Inoculated animals will
be maintained in infectious housing to determine the persistence and
chronicity of infection. Inoculated animals will not be returned to the
colony, but will be euthanized at the end of the 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>
</tr>
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<tbody>
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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/](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.

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
</tr>
</thead>
</table>

Committee Use Only Below

** Conditions necessary for Committee Approval:

<table>
<thead>
<tr>
<th>Condition</th>
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</table>

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.

<table>
<thead>
<tr>
<th>Campus Veterinarian</th>
<th>Date</th>
</tr>
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</table>
## ANIMAL ROOM SAFETY INFORMATION

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

**PROTOCOL # 10665**  
**EXPIRES:** ________

**RUA#:**  
**BUA#:**  
**CCA#:**

<table>
<thead>
<tr>
<th>Identity of Hazard:</th>
<th>Hepatitis B-like virus</th>
</tr>
</thead>
</table>

**Investigator Last Name:**  
**First Name:**  
**Department:**  
**Phone:**  
**Email:**  
**Fax:**

### Provide a short description of the agent:

Novel Hepatitis B-like virus of cynomolgus macaques. Agent is a DNA virus, genetically related to human-HBV.

**This agent / material is hazardous for:**  
- [ ] Humans only  
- [ ] Animals only  
- [X] Humans and Animals

**For which Animal Species?**

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

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

This is a newly recognized Hepatitis B-like virus (HBV-like) virus. Infectivity for humans is unknown. HBV infection in humans may cause acute or chronic hepatitis. Effective vaccine is available against human HBV.

### The precautions checked below apply to this experiment:

- [X] 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  
  - [X] Stall  
  - [ ] Water Bottle  
  - [X] Animal Carcasses
  - [ ] Bedding  
  - [ ] Other:

- [ ] Cages must be autoclaved before cleaning.  
- [ ] Label cages and remove label after decontamination.
- [X] Animal carcasses must be labeled and disposed of as follows:
  - [X] Incineration  
  - [ ] Biohazardous Waste Container  
  - [ ] Bag and Autoclave  
  - [ ] EH&S will pick-up (2-1493).
- [X] All contaminated waste (soiled bedding or other animal waste) must be properly labeled and disposed of as follows:
  - [ ] Incineration  
  - [X] 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] Shoe Covers/Booties  
  - [X] Disposable Gloves  
  - [X] Head Cover  
  - [ ] NIOSH Certified Dust Mask  
  - [ ] Disinfectant footbath  
  - [X] Eye Protection/Face Shield  
  - [ ] Fitted Respirator  
  - [X] Other  
  - [ ] Surgical mask

**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.  
- [ ] 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:

HBV and related viruses are transmitted by blood or other body fluids in contact with mucous membranes (splash) or inoculation (e.g. needlestick)
Hi,

I have received and pre reviewed the following protocol which has been assigned accession number 10665 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 10665 ( )

1. You have listed the use of ketamine in section c, but have not listed it in section g. Please expand and include the use of your agents in section g as well.

2. In section i, adverse effects, you state that there is "potential local irritation at inoculation site" but have not mentioned anything about the infection. Is the infection expected to be asymptomatic? Please clarify.

3. In section j, you are asked to provide more than one database searched. Since you have only provided one database, please expand this section to include one or more databases searched or consulted when reviewing for development of this study.

4. On the Animal Room Safety Information sheet, the last page of the protocol, you were asked to provide a short description of the agent. This box was left blank. Please expand to include information about the agent.

Attached is the revised protocol in response to pre-review questions.

Thanks,
I finally spoke with Dr.       .

Answers to the questions are as follows:
1. Inoculated animals will be maintained in infectious housing (BSL-2+).

2. The 100 cynos sampled will remain in the colony. Inoculated animals will be maintained in infectious housing to determine the persistence and chronicity of infection. Inoculated animals will not be returned to the colony, but will be euthanized at the end of the study.