

PROTOCOL FOR ANIMAL USE AND CARE

Handwritten forms are not accepted

CRPRC

EH&S USE ONLY

PROTOCOL # 10043

EXPIRES: _____

Investigator		Contact	
Last Name:		Last Name:	
First:		First:	
Middle:		Middle:	
email:		email:	
Department:		Department:	
Phone / Fax:		Phone:	
After hrs. #:		After hrs. #:	

Species (common names):	Number:	Source:
Rhesus macaques	35	CRPRC Colony
Cynomolgus macaques	15	CRPRC Colony

Project Title

Overnight housing location: CRPRC Day use only :

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.

A group of rhesus and cynomolgus macaques will be maintained for the purpose of providing donor blood for transfusions and blood or bone marrow for use as normal reference samples for investigators.

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)

Sick Animals	Dead Animals	Pest Control
<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator	<input type="checkbox"/> Call Investigator
<input checked="" type="checkbox"/> Clinician to treat	<input type="checkbox"/> Save for Investigator	<input checked="" type="checkbox"/> OK to use pesticides
<input type="checkbox"/> Terminate	<input type="checkbox"/> Bag for disposal	<input type="checkbox"/> No Pesticides in animal area
<input type="checkbox"/> Necropsy	<input type="checkbox"/> Necropsy	

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

Infectious Agents?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Radioisotopes?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Chemical Carcinogens?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	
Toxic Chemicals?	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Agent(s):	

Funding source:	CRPRC	Previously approved?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Is the project already funded?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Previous protocol number (if any):	8597

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

<input type="checkbox"/>	Lab Animal Health Clinic (2-0514)	<input checked="" type="checkbox"/>	California Primate Research Center (2-0447)
<input type="checkbox"/>	VMTH Large Animal Field Service (2-0292)	<input type="checkbox"/>	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.

A group of nonhuman primates will be characterized, monitored, and maintained for the purpose of providing blood as needed for clinical transfusions and for providing blood and bone marrow samples for use as normal references for investigators. The availability of such samples from a pool of prescreened, characterized animals greatly reduces the overall number of animals needed as controls for individual investigators. In addition, samples from this group of animals are made available to selected investigators off site, in support of NIH-funded research projects as part of the Primate Center's mission to provide the global research community, access to our primate resources.

b) Procedures employed in this project:

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

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

**** 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.)

Prior to inclusion in the donor pool, blood is drawn (6 cc from a cephalic vein, using arm-pull technique) for CBC, clinical biochemistry, and antibody screening for SIV, SRV. And STLV.

Selected animals are maintained according to CRPRC animal husbandry SOPs, with the addition of daily multiple vitamins with iron ("Kiddie Chews"). Each month, animals are weighed and 1 cc of blood is drawn for CBC. Animal health records, weights, and CBC results are monitored carefully.

As requested, samples are drawn from donors as follows:

Blood samples, up to 15 mls: taken from chephalic vein, using arm-pull technique

Blood samples, over 15 mls: animals are fasted for 12 hours, immobilized with Ketamine (10mg/kg IM), and blood drawn from a femoral vein.

Bone marrow: animals are fasted for 12 hours, immobilized with Ketamine (10 ml/kg IM) or Telazol (5-8 mg/kg IM), the area over the iliac crest or humerus is shaved and surgically prepped. Local anesthetic (lidocaine or bupivacaine, 0.1-0.2 ml perfused SQ) is applied. An 18 or 20ga spinal needle is inserted into the marrow cavity, and the appropriate volume of marrow is withdrawn. Several sites may be sampled if more than 3-5 mls of marrow is requested. Oxymorphone, (0.15mg/kg, IM, TID x 2 days) or Buprenorphine (0.01-0.03 mg/kg BID x 2 days) may be given post procedure at the discretion of the CRPRC vets.

A logbook is maintained in the Research Services office detailing each animal's donation history, CBC's and weights. Monthly blood draw limits are calculated for each animal based on current weights at the beginning of each month to insure that donation will not exceed limits (see attached samples of blood donor log sheet). These records are monitored carefully and animals removed from the donor pool and referred to the vet staff for clinical evaluation as indicated (i.e. reduction in red cells indices, weight loss, chronic health problem). All blood and marrow draws will comply with the CRPRC blood draw guidelines.

Each blood or marrow request will be reviewed by an AUCAAC member prior to animal sampling. Records of all approved blood request will be maintained in the Research Services office with the blood donor records (see attached samples of blood, marrow request form).

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.

Group	Procedures / Drugs	Number of Animals	Category
1	Blood and bone marrow sampling	50	2

Categories of invasiveness

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

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 species chosen (rhesus and long tailed macaques) are the two species maintained at the Primate Center and therefore used in ongoing research. The majority of requests are for control blood samples from these two species. The number of animal maintained in the donor pool will depend on demand for samples. We currently have 35 animals in the donor pool, but request the flexibility to add animals if demand increases (up to a maximum of 50) in order to keep the blood draw levels well within our guidelines for any one individual. If demand is reduced, animals will be released from the donor pool to the CRPRC colony.

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.

Species	Drug	Dose (mg/kg)	Route	When and how often will it be given?
Rhesus, cynomolgus	Ketamine	10	IM	Once per donation (max of weekly)
Rhesus, cynomolgus	Telazol	5-8	IM	Once per marrow donation (max of monthly)
Rhesus, cynomolgus	Bupivacaine, 0.25% solution	0.1-0.2 ml total	SQ	Once per donation (max of monthly)
Rhesus, cynomolgus	Lidocaine, 5% solution	0.1-0.2 ml total	SQ	Once per donation (max of monthly)
Rhesus, Cynomolgus	Buprenorphine	0.01-0.03 mg/kg	IM	BID x 2 days, max of monthly

Rhesus, cynomolgus	oxymorphone	.015	IM	TID x 2 days, max of monthly
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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 adverse effects include anemia and discomfort from bone marrow sampling.

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.

Animals are given iron supplements daily, CBCs are taken and reviewed monthly. Animals showing signs of anemia are removed from the pool and treated as deemed appropriate by the CRPRC vet staff. In our experience of over 14 year with donor animals, this is an extremely rare occurrence.

Animals are given a local analgesic prior to bone marrow sampling and may be treated with analgesics post sampling at the discretion of the attending vet.

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 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:

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?

3/22/02

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

Database Name	Years Covered	Keywords / Search Strategy
PubMed	1980-2002	Blood alternatives

Medline	1980-2002	Blood alternatives
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What were your findings with respect to alternative methodologies?

Since the majority of requests are for blood samples to be used as controls in biological assays, synthetic blood does not represent a suitable alternative.

Has this study been previously conducted?

Yes No

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

The availability of such samples from a pool of prescreened, characterized animals greatly reduces the overall number of animals needed as controls for individual investigators. In addition, samples from this group of animals are made available to selected investigators off site, in support of NIH-funded research projects as part of the Primate Center's mission to provide the global research community, access to our primate resources.

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

Animals will be euthanized at the discretion of the CRPRC vet staff.

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.

Species	Method	Drug	Dose (mg/kg)	route
Rhesus and cynomolgus	Overdose	Pentobarbital	60 mg/kg	IV

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

Animals are returned to the CRPRC colony after removal from the donor pool.

TITLE: Bone Marrow Aspiration

Purpose: To remove bone marrow for transplant.

Description:

1.0 **Persons Responsible:**

Clinical Therapeutic Technician
Veterinarian
Staff Research Associate

2.0 **Frequency:** As requested or per protocol.

3.0 **Documentation:** This procedure will be noted in the animal's health jacket.

4.0 **Materials Required:**

bone marrow aspiration needles or spinal needles 18-20 gauge 1 to 1 1/2 inch
clippers
sterile gloves
sterile prep materials
syringe
heparin or other appropriate media
sterile drape (optional)

5.0 **Procedure:**

- 5.1 Wear protective clothing and equipment per current CRPRC Infection Control Policy. Check the animal's tattoo to verify that it is the correct animal, then immobilize the animal with ketamine HCl according to SOP FF-1 (Restraint Procedures: Chemical).
- 5.2 The area over the iliac crest or head of the humerus is shaved and surgically prepped according to SOP II-1 (Surgery Preparation). A sterile drape can be placed.
- 5.3 Topical analgesia is recommended via a subcutaneous infiltration of Bupivacaine (0.1-0.2 ml of 0.25% solution) or Lidocaine (0.1-0.2 ml of 5% solution). **Care must be taken not to inject any of the anesthetic intravascularly.**
- 5.4 The anterior dorsal rim of the iliac crest or head of the humerus is identified by palpation. An 18 or 20 gauge 1-1 1/2 inch bone marrow aspiration needle is advanced in a rotating manner 5-15 mm depending on the size of the animal.
- 5.5 After the stylet is removed a syringe (usually heparinized) is attached to the aspiration needle.
- 5.6 Suction is applied by a steady full extension of the plunger to create back pressure. After each pull the needle should be rotated 90 degrees to redirect the direction of aspiration. If marrow does not appear, the stylet is replaced and placement of the needle is changed.
- 5.7 The amount of marrow taken from all aspiration sites should not exceed the guidelines for a blood draw.
- 5.8 Post-procedure analgesics may be administered, per SOP II-5 (POST PROCEDURE ANALGESIA), at the discretion of the veterinarian.

Purpose:

To establish guidelines for blood sampling and ensure that each animal's health is maintained.

Description:

1.0 **Persons Responsible:**

- Animal Care Staff
- Veterinary Staff
- Therapeutics
- Staff Research Associates

2.0 **Frequency:**

- Any time blood is taken from a colony animal.

3.0 **Documentation:**

All blood taken from an animal will be recorded in the animal's health jacket. Blood withdrawn may also be recorded in the study file as applicable.

4.0 **Materials Required:**

- Blood volume guideline chart
- Animals' weight and age

5.0 **Procedure:**

5.1 The amount of blood that can be taken depends on the weight of the animal and the frequency of collection. A chart has been provided to aid in these calculations. The basic conservative guideline for healthy animals is: 5% of blood volume can be removed each week or 10% every 2 weeks, not to exceed 20% in one month. A veterinarian should be consulted for approval of any blood draw over 10% at any one time. Blood volume is estimated at 60 ml/kg of body weight.

Example: 2 kg. (body weight)

2 kg. X 60ml/kg (animal blood volume) = 120 mls total blood volume

5% x 120 mls = 6 mls

10% x 120 mls = 12 mls

20% x 120 mls = 24 mls

5.2 Animals being bled at the maximum levels will be given oral iron supplementation daily for at least one month following cessation of bleeding. Therapeutics will be notified of the animals requiring therapy.

5.3 If more than 10% of the blood volume is taken at one time, fluid replacement may need to be instituted. Consult with a veterinarian prior to blood draw.

5.4 An electronic CBC will be performed at least one time per month during iron therapy.

5.5 See attached guidelines for infant and adult volumes.

