17. Experimental Protocol

a) In this section describe your experimental protocols, outside of normal husbandry, to be performed on the animals. **This response should provide the committee with a clear understanding of what specifically happens sequentially to each animal or group of animals and over what time period.** It is not necessary to repeat the surgical description that is provided in question 28, but the timing of the surgery within the experiment should be indicated. Be sure to include: all drugs given, including dosage range, routes and frequency of administration; nutritional intervention; social or environmental manipulation; method and amount of biological samples taken; methods of antibody production; use of radioactive materials, blood or other fluid sampling including method and amount, etc. Specify the expected sequence, frequency and duration of these procedures. **If this protocol is to cover an animal colony, use this section to detail breeding procedures/methods.** (Append additional page(s) if necessary)

Many times it is necessary to provide preliminary blood or other samples in order to screen animals for specific projects. This protocol will be used to facilitate preliminary screening of numerous animals (naive, previously treated or SIV infected)) to determine which animal is appropriate for which project.

The *following will be the major procedures and methods* commonly used to screen animals for specific projects:

1. Blood drawing: Blood samples will be collected for analysis by venipuncture from either the saphenous or femoral veins. The monkey will be anesthetized with ketamine hydrochloride at an accepted restraint dose (10 mg/kg, intramuscular), with additional small aliquots administered, if required. The animal will be observed throughout this period and until it has completely recovered from the anesthesia.

a. Blood volumes will vary, depending on research needs and limited by animal size/frequency of bleeding. Guide lines for maximum blood volumes drawn are as follows: Based on 60mls of circulating blood volume per kg body weight; allowable volumes would be 20%, if drawn monthly; 10%, if drawn every two weeks; and 5%, if drawn weekly. We do not encourage long term weekly blood drawing. Blood samples will be drawn for screening tests such as: *Complete Blood Count, Blood Chemistry, Virus Titers, Flow Cytometry, Antibody titers or PCR.*

b. Total blood volumes per blood draw and per month will be monitored to insure that an inappropriate amount of blood is not drawn. If clinical observations or blood work indicate problems with the animal's health and well being (as determined by a veterinarian), the total blood amounts drawn will be adjusted downward or temporarily discontinued.

2. Lymph node biopsies: We will occasionally need to perform lymph node biopsies from naïve, vaccinated/drug treated or SIV infected animals. The monkey will be anesthetized with ketamine hydrochloride at twice the normal restraint dose (20 mg/kg, intramuscular), **or an alternative anesthetic as recommended by a veterinarian**. Animals are monitored daily for 10 days or until the wound is healed. Sutures are removed when appropriate. Topical antibiotic cream is used as needed. No more than two biopsies are collected from any one subject. The interval between biopsies is at least one month and the second biopsy is a distinct site. We will collect from a single biopsy site in a single procedure.

3. Biopsies of the vagina/cervix: We will occasionally need to obtain biopsy samples to determine the cellular composition and function of mucosal immuncompartment that resides in the vagina or cervix The monkey will be anesthetized with ketamine hydrochloride at twice the normal restraint dose (20 mg/kg, intramuscular), **or an alternative anesthetic as recommended by a veterinarian**. We will then take biopsies from two different sites using a baby Tischler biopsy device. Size of biopsies will be approximately 1x1x1 mm, and the interval between any biopsies will be least one month with a maximum duration time of six months. No other biopsy samples will be taken simultaneously.

4. Fine needle aspiration biopsies of lymph nodes: To define the cellular composition of lymph nodes before or after infection/treatment we will occasionally need to perform fine needle aspiration biopsies. The monkey will be anesthetized with

ketamine hydrochloride at twice the normal restraint dose (20 mg/kg, intramuscular), **or an alternative anesthetic as recommended by a veterinarian**. One lymph node will be sampled 3-4 times during one occasion. Local bleeding will be stopped by applying pressure to the vessel. Fine needle aspiration biopsies of lymph nodes may need to be performed two separate times from an animal before any infection and/or treatment to assess the variability of the samples for individual animals. The interval between biopsies is at least one month and the biopsies will be taken from different sites.

5. Euthanasia: If required because of poor health, pain or in order to obtain required test tissue and organ specimens, an animal would be euthanized by an IV overdose (1 ml per 5kg body weight) of sodium pentobarbital (Beuthanasia). Death would be defined by stoppage of the heart as determined by a qualified and experienced person using a stethoscope to monitor heart sounds from the chest area. As required at the Center, a necropsy would then be performed by a qualified and experienced person.

b) Do any animals undergo any type of restraint beyond normal housing methods? YES [NO] If YES, indicate method, length of restraint, and justification for such restraint. If the design of the study requires continuous restraint for longer than 12 hours without the opportunity for exercise, be sure the justification addresses need for such an extended period and include the maximum length of time the animals will be restrained. Include any plans for providing additional enrichment and any steps taken to avoid physical discomfort during the restraint. (See Campus Policy on Non-human Primate Chairing if applicable - available on the web at: www.rarc.wisc.edu

c) Are any animals subjected to fluid or food restriction? [YES] NO If YES, discuss level of restriction, expected consequences, and justification for such restrictions.

Food is routinely withheld from animals the morning of bleeding or until other minor manipulations have been completed. When minor surgery is required, animal food intake is restricted the afternoon before.

d) Will any animals require nonstandard husbandry exemption (e.g. exercise exemption, extended cage cleaning periods, etc.)
[YES] NO If YES, indicated nonstandard husbandry required and justification for this practice.

Most animals will be socially housed, except for temporary quarantine and occasional logistical problems.

However, SIV infected animals will require continued separation to prevent any possibility of interanimal virus transmission through bites. Because of the varying natural viral selection detectable through each animal, this holds true even for monkeys initially infected with the same virus stock. Equally important, single housing decreases the chance of spreading secondary infections that are inevitably observed in the latter part of the viral infection (SAIDS).