Last update 10/18/10 **ORIGINAL**

NATIONAL INSTITUTES OF HEALTH **ANIMAL STUDY PROPOSAL** Rec'd on new form 11/16/2009 Sent out for pre-review 12/10/2009 Rec'd. back 12/18/2009 Sent to PI 12/18/2009	Leave Blank Proposal # **LMVR 13** Approval Date**:6/1/10** Expiration Date: **5/31/13** **Annual Review Due**: 1st Year:**5/31/11** 2nd Year**: 5/31/12**

For information about NIAID ACUC policies and procedures, see NIAID Animal Care and Use Committee
For general information, see NIH Policy Manual 3040-2.
See the NIAID ACUC ASP Form with instructions for detailed information regarding completion.

Emergency Treatment and Animal Care instructions (IEATC) shall be provided on the attached form at the end of this document.

A. ADMINISTRATIVE DATA:

Institute or Center	**NIAID**
Principal Investigator	**Thomas E. Wellems**
Building/Room	**Twinbrook III, Room 3E-10D**
Telephone	**(301) 496-4021**
Fax/Email	**(301) 402-2201 / twellems@niaid.nih.gov**
Division, Laboratory, or Branch	**Laboratory of Malaria and Vector Research**
Project Title	**Genetic cross of chloroquine-resistant and -sensitive *P. vivax* parasites in a chimpanzee**

Initial Submission [x]

List the names of all individuals authorized to conduct procedures involving animals under this proposal, and complete a T & E Form for each person; identify key personnel (i.e., Co-investigator(s)).
A brief summary of the training and/or experience for procedures each co-investigator will be expected to perform in this ASP must be documented and available to the ACUC. The name(s) of the supervisor, mentor, or trainer who will provide assurance each co-investigator is/has achieved proficiency in those procedures shall be included in that documentation (T & E Form).
For guidance see, NIAID ACUC Training & Safety Requirements OACU Training History Search

PI (if doing hands-on animal work):

Co-investigator(s): **Robert Gwadz, Juliana M. Sa**

Other investigators: ~~**Evan Kessler**~~ **(Removed 08/10)** **Gloria Tavera** **(added 10/10),** **Sarah Kaslow** **(added 12/10)**
Anthony Cook, DVM, MS, DACLAM (BIOQUAL RB), Charlene Shaver, LAT (BIOQUAL RB),

Other personnel: **Animal Facility (BIOQUAL RB) animal care personnel Raymond Tchoua, LAT, Zach Pippens, LAT**

B. ANIMAL REQUIREMENTS:

Species:	***Pan troglodytes***
Stock/Strain:	**N/A**
Age/Weight/Size:	**Various**
Sex:	**Male/female**
Source(s):	**NIH-approved sources**
Animal Holding Location(s):	**BIOQUAL RB, Gaithersburg, MD.**
Animal Procedure Location(s):	**BIOQUAL RB, Gaithersburg, MD.**

Number of Animals:

(amended 6/10, 08/10)

Species	Year 1	Year 2	Year 3	Total
Pan troglodytes	**~~1~~ ~~2~~ 1**	**~~1~~ ~~2~~ 1 (same animal, carried-over)**	**0**	**~~2~~ ~~4~~ 1 animals~~;~~ ~~2~~ ~~4~~ 2 chimpanzee-years**

C. TRANSPORTATION: Transportation of animals must conform to all NIH and Facility guidelines/policies. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within the Clinical Center, also include the route and elevator(s) to be utilized.

No transportation is required under this ASP.

D. STUDY OBJECTIVES:

1. Provide no more than a 300 word summary of the objectives of this work. Why is this work important/interesting? How might this work benefit humans and/or animals? This should be written so that a non-scientist can easily understand it. Please eliminate or minimize abbreviations, technical terms, and jargon. Where necessary, they should be defined.
2. For renewal proposals, provide a brief summary of recent progress made using the last three-years' animals.
3. For new and renewal proposals, a brief synopsis of the scientific rationale or foundation for the study may optionally be provided.

Approximately 2.6 billion people live at risk of infection by *P. vivax*, the most widely distributed human malaria parasite, resulting in an estimated 70-390 million clinical cases every year. Treatments for *P. vivax* infections include chloroquine against the parasites inside red blood cells, and primaquine against the dormant stages of the parasites inside liver cells (i.e., the parasites called hypnozoites).

Chloroquine has been the first line treatment against *P. vivax* for over 50 years, even though chloroquine-resistant parasites have been documented since 1989 and are continually spreading from Papua New Guinea to Indonesia, Myanmar, India and South America. Increased tolerance to primaquine has also been observed in *P. vivax* parasites from Southeast Asia, Oceania, and South America.

The genetic locus of chloroquine resistance is unknown in *P. vivax*, in part because research is severely limited by the lack of a continuous *in vitro* cell culture system. Previous genetic crosses in *Plasmodium falciparum* successfully identified a transporter gene (*pfcrt*) linked to chloroquine (Fidock DA *et al.*, 2000), quinine (Ferdig MT *et al.*, 2004) and amodiaquine resistance (Sa JM *et al.*, 2009) in that species. However, *pfcrt* does not appear to be the determinant of chloroquine resistance in *P. vivax* (Nomura T *et al.*, 2001; Sa JM *et al.*, 2005).

The aim of this proposal is to perform a genetic cross between two *P. vivax* clones, one chloroquine-sensitive and another chloroquine-resistant, entirely within non-human primates. Completion of our proposed *P. vivax* cross should allow us to identify the gene(s) linked to chloroquine resistance among the independent recombinant progeny, and should also provide the foundation for linkage studies of determinants involved in other distinguishable *P. vivax* phenotypes, for example primaquine resistance, and early and late hyponozoite relapse.

An activity of this study is the cryopreservation of viable sporozoites from the *P. vivax* cross. This cryopreservation will be performed at Sanaria Inc., where a successful and proprietary method to cryopreserve sporozoites has been developed. We will transfer the infected mosquitoes containing recombinant parasites to Sanaria Inc. and receive the cryopreserved parasites to re-inoculate the chimpanzee. Sanaria for its part of the study will retain a portion of the sporozoites for drug screening and infectivity studies in human hepatocyte cell monolayers. (amended 08/10)

E. RATIONALE FOR USE OF ANIMALS: 1) Explain your rationale for animal use. 2) Justify the appropriateness of the species selected. 3) Justify the number of animals to be used.

1. **Because of the lack of continuous *in vitro* culture for *P. vivax* erythrocytic stages and gametocytes, we require non-human primates in order to produce gametocytes and perform this genetic cross. The plan is to obtain recombinant progeny from cross-fertilization of the gametocytes of a chloroquine-sensitive and a chloroquine-resistant line co-transmitted through *Anopheles* mosquitoes. In order to genetically map the determinants of drug resistance, we must analyze genetic recombinants, which are only generated in the mosquito during the production of sporozoites. The only way to recover infectious recombinants is by transmission of the sporozoites by mosquito bite to a live, susceptible animal. A number of *P. vivax* lines are adapted to non-human primates, including *Aotus* monkeys (owl monkeys) and *Pan troglodytes* chimpanzees. (Amended 08/10)**

   We recently established the parameters of drug resistance and drug sensitivity in ~~five~~ six of these lines in *Aotus*: Salvador-1 and Vietnam-IV (both chloroquine- and primaquine-sensitive), AMRU-I ~~and~~ Indochina-XIX, and Indonesia-I/CDC (~~both~~ chloroquine-resistant and primaquine-sensitive lines), and Brazil1/CDC (a chloroquine-sensitive and primaquine-tolerant line). These lines have also been genetically characterized using a number of available genetic typing markers for *P. vivax*. All lines were ~~are~~ able to produce gametocytes in *Aotus* ~~and infect~~, but infectivity to mosquitoes ~~fed on infected animals, as judged by oocyst production (~~was ~~is~~ unacceptably low ~~(less than 0.5% in *Anopheles stephensi, An. freeborni* and *An. dirus* mosquitoes)~~. In order to genetically map the determinants of drug resistance, we must analyze genetic recombinants, which

are only generated in the mosquito during the production of sporozoites. Between February 2009 and June 2010 we attempted to transmit malaria from Aotus to mosquitoes by either letting them feed directly on the animal or by performing *in vitro* feeds with infected blood in the LMVR Secure Insectary. A total of 27 Aotus developed blood stages of one of the 6 different *P. vivax* strains described above. Approximately 12,000 mosquitoes were used from all available species reared in the LMVR Insectary, also representative from different geographic regions: *Anopheles dirus* (from South East Asia), *An. albimanus* (from Central America), *An. stephensi* (from India), *Anopheles freeborni* (from North America), and *An. gambiae* (from Africa). We dissected roughly half of the mosquitoes and used the other half to "re-infect" 5 *P. vivax* naïve Aotus. Very rarely we found an infected mosquito (less than 0.05% infectivity), and when it happened it presented a single oocyst, which was validated by PCR. Further, none of the 5 animals exposed to bites from potentially infected mosquitoes developed parasitemia over more than 200 days of follow up. We have recently obtained other *P. vivax* strains (Salvador-II, Achiote, Panama), which have been adapted to Aotus by other groups (Collins *et al.* 1973a, Collins *et al.* 1973b, Collins *et al.* 1976, Collins *et al.* 1979). Despite the previously low success rate, we will test the infectivity from Aotus to mosquitoes of these strains as well. ~~The only way to recover infectious recombinants is by transmission of the sporozoites by mosquito bite to a live, susceptible animal.~~ (amended 6/10, 08/10)

2. **Although we have been able to ~~infect mosquitoes with~~ obtain *P. vivax* gametocytes from *Aotus*, we have not been able to infect mosquitoes at the desired level and moreover, have not been able to infect *Aotus* with *P. vivax* sporozoites ~~from mosquitoes~~ despite multiple tries. This ~~lack of~~ low success ~~may be due to an inability of~~ has been observed in other studies, which has led to a report that gametocytes produced in splenectomized chimpanzees are more infective to mosquitoes than gametocytes produced in Aotus (Sullivan *et al.* 1996; data for spleen-intact chimpanzees are not available). Gametocyte development includes several stages, and only fully mature gametocytes can develop ookinetes. There is the possibility that gametocytes produced in *Aotus*, which are not as closely genetically related to humans as chimpanzees, do not mature adequately. Further, *P. vivax* sporozoites may lack the ability to invade and complete a replication cycle in the *Aotus* owl monkey liver cells, which are necessary for any infection from sporozoites to progress to erythrocytes in the bloodstream. Evidence for a liver-stage restriction in *Aotus* was also seen in our recent *P. falciparum* cross, which yielded abundant recombinant progeny from mosquito infection of a chimpanzee, but not from mosquito feeding on either of two *Aotus* (Hayton K *et al.*, 2008; unpublished results). For these reasons, we will need to complete the *P. vivax* cross through a chimpanzee~~s~~. (amended 6/10, 08/10)**

   ~~Moreover, we will test whether a *P. vivax* cross may be possible in a spleen-intact chimpanzee, because *P. vivax* do not sequester away from the human spleen as *P. falciparum* parasites must during human bloodstream infection. If *P. vivax* parasites can tolerate the chimpanzee spleen just as they can tolerate the human spleen, infection of mosquitoes with the *P. vivax* parent gametocytes from chimpanzees, and of a chimpanzee with mosquitoes carrying progeny sporozoites from the cross, may be possible with just two chimpanzees.~~ (Deleted 08/10)

   ~~Of all non-human primate laboratory models, the chimpanzee is the best available animal to receive recombinant *P. vivax* sporozoites from mosquitoes and carry them through to recombinant erythrocytic-stage parasites that can be used for quantitative trait loci analysis of *P. vivax* chloroquine resistance.~~

   Of all non-human primate laboratory models, the chimpanzee is the best available animal to produce gametocytes for infection through mosquitoes (Sullivan *et al.* 1996), receive *P. vivax* sporozoites from mosquitoes, and carry them through to recombinant erythrocytic-stage parasites that can be used for quantitative trait loci analysis of *P. vivax* chloroquine resistance. As noted above, all chimpanzees used by Sullivan *et al.* (1996) were splenectomized and spleen-intact chimpanzees were not tested. ~~For reasons stated above we will first test whether spleen-intact chimpanzees can be used to infect mosquitoes and then receive progeny of the cross.~~ (amended 6/10, 08/10)

   Considering the high risk of not developing parasitemia in a spleen-intact animal, we propose to use a single splenectomized chimpanzee. We will inoculate the splenectomized chimpanzee with a mixture of blood-stage chloroquine-sensitive and chloroquine-resistant parasites, monitor the presence of gametocytes from both strains by PCR, and feed mosquitoes directly on the animal and by *in vitro* membrane feed. (Amended 08/10)

   After mosquito dissection and verification of recombinant oocyst presence in the mosquito midgut, the chimpanzee will be treated with Malarone for 3 days in order to eliminate the parasite parental clones. This drug is rapidly metabolized and will allow the development of parasites infected as sporozoites within a week of the treatment. This approach will be used in order to increase the chances to obtain a recombinant progeny from fresh sporozoites. We will obtain a first pool of recombinant progeny by re-infecting the chimpanzee using part of the recombinant sporozoites found after salivary gland dissection of the mosquitoes. (added 10/10)

   The other part of the ~~Part of the R~~recombinant sporozoites will be cryopreserved by Sanaria. After development and

cryopreservation of the first pool of the recombinant progeny, the chimpanzee will be cured and ~~while the chimpanzee is cured and~~ housed for a vacation period ~~after the mixed infection~~ (4-9 months). This period is necessary to diminish the immune response of the chimpanzee against another ~~second~~ infection with *P. vivax* parasites.
A second pool of progeny will be developed by ~~The~~ sporozoites that will be thawed and inoculated to the treated and recovered animal in order to develop blood stages of the progeny. (Amended 08/10, 10/10)

Because *Aotus* spp. can sustain *P. vivax* infection when the erythrocytic-stage parasites are inoculated into the bloodstream, once progeny broods are obtained from the *P. vivax* cross in the chimpanzee, we plan to return to the *Aotus* monkeys under the ASP LMIV 8E and use them for isolation of independent recombinant progeny, determination of inherited drug responses and mapping of the genetic loci involved.

Other previously reported models capable to develop *P. vivax* blood stages include Saimiri (Collins WE 2002) & Saguinus monkeys (Rossan RN 1973; Collins & Skinner 1982). However, no evidence supports the use of these models to infect mosquitoes and develop parasitemia from a sporozoite infection. Further, we have recently attempted to use a humanized SCID mouse model to develop *P. vivax* parasites in collaboration with Dr. David Fidock from Columbia University. However, after several attempts using three *P. vivax* strains (AMRU-I, Salvador-I & Vietnam IV), none of the animals developed parasites. (Amended 08/10)

3. ~~**If the first attempt at the *P. vivax* cross in the chimpanzee chimpanzees is successful, only one animal two animals (to be received at BIOQUAL in January on April 2010) will be used. However, we are requesting approval for two four chimpanzees, in case the first attempt initial attempts does not produce an adequate progeny brood for our genetic studies. It is possible that only one progeny clone or just one of the two *P. vivax* parents will predominate in the cross attempt. In such a case we will try once again with different choices of *P. vivax* parents. We are optimistic for an adequate progeny brood, because our previous crosses showed large numbers of recombinants in the progeny that did not require repeated attempts [Walliker D *et al.*, 1987; Wellems TE *et al.*, 1990; Hayton K *et al.*, 2008].**~~
We will use one animal, cure the animal after the ~~collection~~ verification of oocyst presence to re-infect the first time with recombinant sporozoites and develop a first pool of parasite progeny. The animal will be cured a second time after the cryopreservation of recombinant progeny and let it rest for 4-9 months. The animal will then be infected with cryopreserved sporozoites to develop a second pool of progeny. ~~of sporozoites from mosquitoes after feeding on the animals's blood and, a few months after full recovery of the animal (4-9 months), introduce the sporozoites to the animal for development.~~ After collection of the blood-stage parasites from the animal, it will again be cured and retired from the study.
(amended 6/10, 08/10, 10/10)

F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES: Briefly explain the experimental design and specify all animal procedures performed. This description should allow the ACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study. Specifically address the following:

1. **Briefly explain the experimental design.** An outline, table or flowchart presentation might be helpful.

The *P. vivax* lines we plan to use for the genetic cross are from Central America, (Salvador-I, chloroquine and primaquine sensitive) and from Papua New Guinea (AMRU-I, chloroquine-resistant and primaquine-sensitive). Because *P. vivax* cannot be continuously grown *in vitro*, we need to induce carry out gametocyte production, infection of mosquitoes, progeny isolation and drug response characterizations in *Aotus* monkeys and chimpanzees.

For gametocyte production and mosquito infection, we will infect ~~*Aotus*~~ one splenectomized chimpanzee with both drug-sensitive and drug-resistant parental clones in order to produce the gametocytes ~~(under ASP LMIV 8E)~~. Once gametocytes are detected, we will feed *Anopheles* mosquitoes ~~(100 mosquitoes/animal/day for 4 days)~~ by either presenting them to the abdominal skin of an anesthetized ~~and splenectomized *Aotus* to take their~~ chimpanzee or by feeding them blood ~~(under ASP LMIV 8E).~~withdrawn from the chimpanzee. These mosquitoes will be checked for evidence of recombinant *P. vivax* oocysts in their midguts (6-10 days post-infection by PCR assay, see ~~Hayton K~~ Ranford-Cartwright *et al.*, ~~2008~~ 1991) and for eventual development of sporozoite-stage parasites in the salivary glands (~~10-14~~12 - 16 days post-infection). After verification of oocysts in the mosquito midgut, the animal will be cured with Malarone, a drug active against the parental clones of *P. vivax* and which is rapidly metabolized. This will allow the development of a first pool of recombinant progeny via mosquito bites from some of the mosquitoes that present sporozoites.

This approach will increase our chances to obtain recombinant clones from fresh sporozoites, and allow us to map the chloroquine-resistance locus. The rest of the mosquitoes will be sent to Sanaria for cryopreservation of sporozoites. After cryopreservation of the first pool of recombinant progeny, the animal will be cured and let it rest for 4 to 9 months. A second pool of recombinant progeny will be generated by re-infection of the chimpanzee with the cryopreserved sporozoites

from Sanaria.
~~We will cure the animal and transfer the infected mosquitoes to Sanaria Inc. to prepare cryopreserved stocks of sporozoites that will be used a few months after full recovery of the animal (4-9 months).~~ (Amended 06/10, 08/10, 10/10)

Because *Aotus* monkeys are not susceptible to sporozoite infections and development of the liver stage parasites (Section E2), we will then need to use ~~another~~ the same splenectomized chimpanzee ~~(splenectomized and vaccinated with Pneumovax 23 (Pnu-Imune 23))~~ in order to carry the cross and provide recombinant progeny. The splenectomy ~~is essential to might be~~ is critical for the success of this study because the spleen is responsible for the removal of the parasitized red blood cells from the circulation as a response of the organism to repress the infection. (amended 6/10, 08/10)

For this purpose, the sporozoites from approximately 1000-2000 mosquitoes ~~carrying sporozoites~~ will be ~~presented~~ provided to chimpanzee, ~~either by presenting the mosquitoes to the abdominal skin of the anesthetized chimpanzee. The blood~~ or by recovery of the sporozoites from dissected mosquitoes and direct~~ly~~ inoculation into the bloodstream. ~~Blood-fed mosquitoes will be then counted, examined for engorgement and destroyed.~~ Stained blood samples from the chimpanzee will be followed for evidence of infection daily, beginning on day 10 after mosquito feeding (in previous crosses with *P. falciparum*, blood stage parasites were observed ~20 days after mosquito feeding). (Amended 06/10, 08/10)

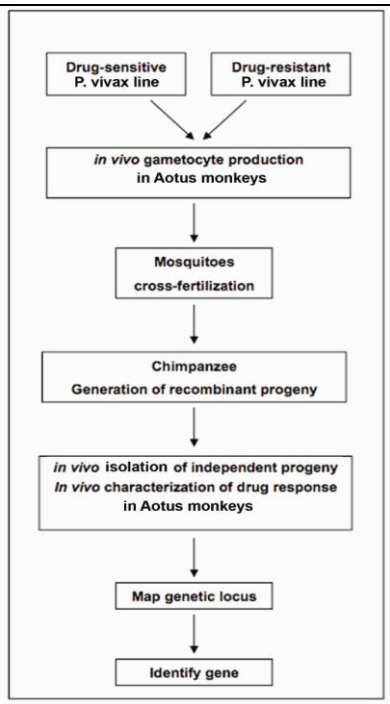
Once parasitemia is detected, chimpanzee blood samples will be drawn by venipuncture, aliquoted and cryopreserved. Because *P. vivax* causes only very mild malaria clinical signs in non-human primates, there is little risk to the life of the chimpanzee from progeny of the cross. Nevertheless, if parasitemia reaches 2.0%, or the chimpanzee develops clinical illness such as diarrhea (more than 500 ml of liquid stool within 24 hours), lethargy (due to multiple factors such as anemia or fever) or anorexia, the chimpanzee will be cured of its infection immediately. Supportive care (anti-inflammatories, fluid supplementation, etc.), at the attending veterinarian's discretion, may be given to animals with clinical illness.

To eliminate the parasite parental clones from the first infection (after detection of recombinant oocysts in the mosquitoes' midguts), the animal will receive 3 doses of Malarone® (GSK; Atovaquone/Proguanil HCl). To eliminate chloroquine-sensitive erythrocytic-stage *P. vivax* parasites from the bloodstream, the chimpanzee will be initially treated via NG tube with three daily doses of chloroquine phosphate (pharmaceutical-grade, Global®). Once chloroquine-resistant progeny are selected, blood samples will be drawn by venipuncture, aliquoted, and cryopreserved for identification of the chloroquine-resistance locus using the method of linkage group selection (Carter R *et al.*, 2007). (amended 10/10)

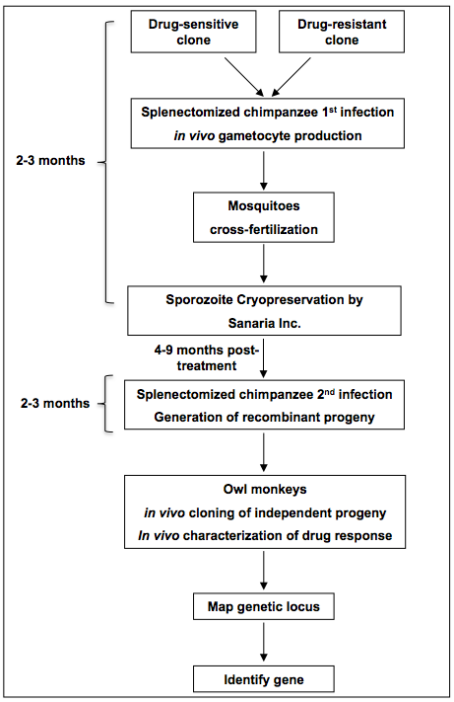
Finally, to eliminate *P. vivax* chloroquine-resistant progeny and initiate the cure, a single dose of mefloquine will be given for complete cure (pharmaceutical-grade, Lariam; 25 mg salt/kg, nasogastric administration; mefloquine is supplied in 250 mg salt = 228 mg base tablets). The erythrocytic-stage parasites may also be eliminated by treatment of the animal for 3 days with a daily oral dose of Malarone® (GSK, Atovaquone/Proguanil HCl). Alternatively, the erythrocytic-stage parasites will be treated twice a day for 3 days with artemether-lumefantrine (pharmaceutical-grade, Coartem, Novartis, supplied as tablets containing 20 mg artemether and 120 mg lumefantrine). Depending on the weight of the animal, 3 or 4 tablets will be given orally at 0, 8, 24, 32, 48, and 60 hr (total 18 or 24 tablets). Mefloquine (Lariam) and Coartem tablets can be crushed, mixed with water and given together or followed by milk (or other liquid such as Ensure or Boost). Malarone® (Atovaquone/Proguanil) will be administered orally for 3 days according to the animal's weight: 11-20 kg: 62.5 mg/25 mg; 21-30 kg: 125 mg/50 mg; 31-40 kg: 187.5 mg/75 mg; >40 kg 250 mg/100 mg. Malarone pills will be macerated and mixed with milk, Ensure or Boost for the oral treatment.
As a ~~third~~ fourth alternative, 3 days of quinine treatment might be applied (25 mg/kg/day IM at twice-daily intervals). (amended 10/10)

At the end of the study, any dormant liver stages will be eliminated with 14 daily doses of primaquine, supplied in 15 mg base = 26.3 mg phosphate salt tablets (pharmaceutical-grade, UPS, doses 0.25 mg base/kg/day or 0. 5 mg base/kg/day; 25 mg/kg/day IM at twice-daily intervals can be substituted). The animal will then be retired from the study.

~~Following is a schematic diagram of the steps in this plan:~~ (Amended 08/10)



(Deleted, August 2010)



New experimental design. (Amended 08/10)

2. **Injections or inoculations** (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)

P. vivax-infected erythrocytes from _Aotus_ monkeys will be washed and resuspended in incomplete RPMI-1640 (Roswell Park Memorial Institute 1640) medium and injected via saphenous vein to produce gametocytes from each _P. vivax_ parent in a chimpanzee. _P. vivax_-infections of _Anopheles_ mosquitoes will be generated by allowing the mosquitoes to feed on the abdominal skin of the ~~splenectomized chimpanzee. The infection will not be performed until at least two weeks post-splenectomy~~ chimpanzee. 1000-2000 mosquitoes (est. 10-15% carrying sporozoites) will be used in the feeding. (amended 6/10)

3. **Blood withdrawals** (volume, frequency, withdrawal sites, and methodology)

Daily thick and thin smears will be performed on microscope slides with blood obtained by pricking the ear or heel of the chimpanzee, 10 days after sporozoite infection or 4 days after blood infection.
In addition, blood samples (approximately 5 ml) will be collected approximately every other day when patent parasitemia is present, depending upon the parasitemia and the recombination events.

When the parasitemia appears to be at maximum (~1%, no more than 2%), a larger sample may be collected. The maximum bleed at one time will be 4 ml per kg, and will not exceed a cumulative volume of 12 ml/kg/month, depending upon the health of the animal. The blood volume of a chimp is around 75 ml per kg.

Blood will be drawn from the animal under Ketamine (10 mg/kg) or Telazol (2.4 - 2.6 mg/kg) sedation.

4. **Minor surgical procedures** (that do not penetrate and expose a body cavity)

None

5. **Non-survival surgical procedures** (Provide details of survival surgical procedures in Section G.)

None

6. **Radiation** (dosage and schedule)

None

7. **Methods of restraint** (e.g., restraint chairs, manual restraint, chemical restraint, collars, vests, harnesses, slings, etc.)

We will determine if the animals will consume mefloquine (Lariam) or Coartem. If operant training is unsuccessful, they will be anesthetized with ketamine (IM, 10 mg/kg) one time for NG administration of mefloquine (Lariam) or twice a day for 3 days (8 or 12 hours apart) for NG administration of Coartem.

Ketamine (IM; 10 mg/kg) will be administered to sedate (chemically restrain) the animal for mosquito-feed experiments.

8. **Animal identification methods** (e.g., ear tags, tattoos, collar, cage card, etc.)

Tattoo.

9. **Other procedures** (e.g., breeding, genotyping, etc.)

~~**Not applicable.**~~
The following procedures will be performed on anesthetized animals. The animal will be fasted overnight (but allowed water) prior to anesthesia. The animal will be anesthetized with ketamine 10-15 mg/kg IM q 30 min. Additionally, the animal will be given analgesia prior to the biopsy procedure as described below.

Bone Marrow Biopsy: The hair will be clipped over the biopsy site with a #40 surgical blade and the skin over the site will be scrubbed with betadine scrub three times alternating with 70% isoprophyl alcohol. The site will be draped with sterile material and sterile gloves and instruments will be used. 0.5 ml will be taken from the trochanteric fossae or the iliac crest using an Illinois sternal/iliac or Jamshidi bone marrow aspiration needle. Following the biopsy, animals will be observed until they are moving about the home cage and exhibiting normal behaviors. Appropriate analgesics will be given after the procedure, usually Ketoprofen 5 mg/kg IM q 6 -8 hours, over the next 12 – 24 hours. After 24 hours, analgesic will be administered as needed for discomfort, as instructed by the attending veterinarian (BIOQUAL SOP 1528).

~~**Percutaneous Liver Biopsy (direct or ultrasound-guided): The liver biopsy is percutaneous, trans-diaphragmatic, using a liver biopsy 16-gauge needle set. The hair will be clipped and the skin surgically prepped using betadine and 70% isopropyl alcohol, alternating, for a total of three scrubs. Then a betadine solution will be applied and left on. We will use sterile gloves, needles, etc. We do not initially use ultrasound because we use the central part of the right lobe, which is large and there are no anatomical structures to avoid. We do use the ultrasound if we have initial trouble getting sample, because sometimes an animal develops fibrous connective tissue at the site, in which case we use the ultrasound to see how to best position the biopsy needle. There will be a maximum of 3 tries per animal to get liver. There is minimal to no pain involved with this procedure. If noted, appropriate analgesics are given as instructed by the attending veterinarian (BIOQUAL SOP**

~~1510).~~

~~**Percutaneous Spleen Biopsy (direct or ultrasound guided): The spleen biopsy is percutaneous, trans-diaphragmatic, using a spleen biopsy 16 gauge needle set. The skin will be clipped and surgically prepped using betadine and 70% isopropyl alcohol alternating for a total of three scrubs. Then a betadine solution will be applied and left on. We will use sterile gloves, needles, etc. There will be minimal to no pain involved with this procedure. If noted, appropriate analgesics will be given as instructed by the attending veterinarian (BIOQUAL SOP 1510).~~ (added 6/10, parts deleted 08/10)**

10. **Potentially Painful or Distressful Effects, if any, the animals are expected to experience** (e.g., pain or discomfort, ascites production, etc.), by experimental procedure if applicable. For Column E studies provide: a. A description of the procedure(s) producing pain and/or distress; b. Scientific justification why pain and/or distress can not be relieved.

The *P. vivax* infection will reach levels that would be acceptable for human volunteers. If the chimpanzee shows signs of illness such as diarrhea (more than 500 ml of liquid stool within 24 hours), lethargy (due to multiple factors such as anemia or fever) or anorexia, or parasitemia > 2%, it will be cured of its infection immediately.

Splenectomy may cause a requirement for life-long (50-60 years) monitoring and medical management. However, our experiences with ~~a~~ previous ~~eross has~~ crosses of *P. falciparum* have been positive. ~~The chimp was~~ Two chimps were splenectomized, vaccinated with pneumovax, and subsequently did well. (amended 6/10)

***Plasmodium vivax* infections can cause anemia even at lower parasitemia levels, therefore the hematocrit of the animals ~~should~~ needs to be monitored regularly (once a week until it develops parasitemia; every other day with positive parasitemia). Hematocrit values in healthy chimpanzees are very similar to humans and vary between 35-51% (Howell S et al, 2003). Therefore the "10/30" rule for anemia should be applied (minimum acceptable values of 10g/dL hemoglobin concentration and 30% hematocrit; Adam RC et al, 1942). If hematocrit levels drop below 30%, a physical assessment will be made to determine if cure or a transfusion is necessary. If considered appropriate, healthy non-infected chimpanzee blood will be given to the anemic animal via IV transfusion as determined by the attending veterinarian. (amended 6/10)**

To avoid itching or swelling from mosquito bites, topical Diphenhydramine (Benadryl®) should be applied to the skin after the feed.

11. **Experimental endpoint criteria** (i.e., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical signalment, or signs of toxicity) must be specified when the administration of tumor cells, biologics, infectious agents, radiation, or toxic chemicals are expected to cause significant clinical signs or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must always be scientifically justified. List by experimental procedure if applicable.

The chimpanzee will be cured at a parasitemia greater than 2.0 % or if the animal shows signs of illness such as diarrhea (more than 500 g of liquid stool within 24 hours), lethargy (due to multiple factors such as anemia or fever) or anorexia.

12. **Disposition of Animals at the End of the Study** (for those animals not meeting Experimental Endpoint Criteria above, e.g. retired breeders, etc.) not expected to experience clinical signs:

After cure from malaria, the chimpanzees will be returned to general housing, and may be recycled into other approved studies

G. MAJOR SURVIVAL SURGERY - If proposed, complete the following.
NONE __ (check if none)

~~An animal~~ Any mature adult chimpanzees that ~~has already~~ have been splenectomized ~~cannot be used because the splenectomized chimps~~ and available in the United States are ~~mature adults~~ not useful for this study. There are no chimpanzee-holding locations in close proximity to the NIAID insectary capable of holding a mature adult chimpanzee. Transportation of the infected mosquitoes to existing holding locations capable of holding a mature chimpanzee is not a viable option, because the presence of infectious sporozoite-stage parasites in the mosquito salivary glands is time-sensitive.

~~Alternatively to performing the splenectomy locally, the animal may~~ A juvenile chimpanzee will be splenectomized at the site of origin (under the applicable ASP and SOPs there) and allowed to recover prior to shipment to BIOQUAL. (Amended 08/10)

1. Describe the surgical procedure(s) to be performed. Include the aseptic methods to be utilized.

~~For reasons stated above, we will make every attempt to complete the entire *P. vivax* cross with spleen-intact chimpanzees. However, if a~~ **A splenectomized chimpanzee (as used by Sullivan *et al.*, 1996) is required, and we will ~~cannot~~ obtain an already splenectomized and fully recovered animal from the source, New Iberia Research Center (NIRC).** ~~we will proceed with the splenectomy as follows:~~

~~Splenectomy under general anesthesia: Animals have a CBC and serum chemistry profile run prior to surgery. Animals are held off feed, but offered water for 12 hours pre-op. General anesthesia is by Telazol (2 mg/kg), followed by isoflurane 2 to 3%. The surgical site will be clipped and surgically prepared using an antiseptic scrub alternating three times with alcohol. The surgeon will don hair cover and facemask, a sterile gown, and sterile surgical gloves. The site will be draped with sterile surgical drapes. Sterile surgical instruments will be utilized.~~

~~All anesthetized animals have an IV catheter placed and IV fluids administered. Animals are given 15 ml/kg/hour of IV fluids while anesthetized and are closely monitored by the veterinarians. All animals have continuous ECG, noninvasive blood pressure, temperature, respiration, and spO2 monitored with a Critikon Patient monitor. We have electrocautery in our OR as well as state-of-the-art surgical equipment. The animal's abdomen is opened with a midline incision (10 cm). The splenic ligament is clamped off between its attachment to the spleen and to the greater curvature of the stomach. The vessels are tied off with 3-0 or 4-0 absorbable suture (PDS II) and the spleen is removed from the abdomen. The clamps are removed from the ligament and checked for hemorrhage prior to releasing the ligament back into the abdomen. The abdomen is closed in a three layer closure using sterile sutures (PDS II) with skin staples applied to the final skin closure.~~ **(Amended 08/10)**

2. Who will perform surgery and what are their qualifications and/or experience?

~~Dr. Anthony Cook (BIOQUAL, RB) will perform the surgery. Dr. Cook has 7 years of veterinary experience with chimpanzees, and 11 years of experience performing splenectomies on several species of animals.~~ **(Amended 08/10)**

3. Where will surgery be performed (Building and Room)?

~~Dedicated surgery suite at BIOQUAL.~~ **(Amended 08/10)**

4. Describe post-operative care required, including consideration of the use of post-operative analgesics, and identify the responsible individual:

~~Once When the animal is chimpanzee begins to wake from anesthesia and begins moving around, the IV catheter is removed, and the animal chimpanzee is returned to its home cage and placed on disposable pads. A heat lamp or a "snuggle safe" heated disc can be placed in the cage at the discretion of the care staff, to aid recovery. The animal chimpanzee is monitored closely until it is moving about the cage. Gatorade or Tang bottles, or other food treats (e.g., fruits, marshmallows, frozen treats) are offered to the animal chimpanzee. The animal chimpanzee is checked at least twice daily during the whole study by trained technicians and monitored for food and water intake and stool/urine output. Staples or sutures are removed 7 – 10 days post-op. Animals Chimpanzees are given buprenorphine 0.15 mg BID for 48 hours post-op, and then as needed for discomfort. Other analgesics, as approved by the veterinarian, may be substituted, such as pentazocine or butorphanol. Banamine or ketoprofen may be used as adjuncts to the opioid analgesic, at the veterinarian's discretion. (amended 6/10)~~

~~Animals Chimpanzees are watched closely to ensure they are eating, drinking, passing stool and behaving normally during the 10-day post-op recovery period. Antibiotics are given for a minimum of 7 days post-operatively as per the veterinarian's instructions. Packed cell volume (PVC) or Hematocrit (HCT) are checked 48 – 72 hours post-op to ensure there is no significant red blood cell loss. (amended 6/10)~~ **(Amended 08/10)**

5. Has major survival surgery been performed on any animal prior to being placed on this study? Y / N [~~**N**~~**Y**]
If yes, please explain:

We will obtain an already splenectomized and fully recovered animal from the source, New Iberia Research Center (NIRC). (Amended 08/10)

6. Will more than one major survival surgery be performed on an animal while on this study? Y / N / Not Applicable [**NA**]
If yes, please justify: **(Amended 08/10)**

H. RECORDING PAIN OR DISTRESS CATEGORY - The ACUC is responsible for applying U.S. Government Principle IV, contained in Appendix 3 of the NIH Manual 3040-2: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
Check the appropriate category(ies) and indicate the approximate number of animals in each. Sum(s) should equal total from Section B.

IF ANIMALS ARE INDICATED IN COLUMN E, A SCIENTIFIC JUSTIFICATION IS REQUIRED TO EXPLAIN WHY THE USE OF ANESTHETICS, ANALGESICS, SEDATIVES OR TRANQUILIZERS DURING AND/OR FOLLOWING PAINFUL OR DISTRESSFUL PROCEDURES IS CONTRAINDICATED. FOR USDA REGULATED SPECIES, PLEASE COMPLETE THE EXPLANATION FOR COLUMN E LISTINGS FORM AT THE END OF THIS DOCUMENT. THIS FORM WILL ACCOMPANY THE NIH ANNUAL REPORT TO THE USDA FOR ALL OTHER SPECIES, THE JUSTIFICATION FOR SUCH STUDIES MUST BE PROVIDED IN SECTION F. NOTE: THIS COLUMN E FORM, AND ANY ATTACHMENTS, e.g., THE ASP, ARE SUBJECT TO THE FREEDOM OF INFORMATION ACT.

	Number of animals to be used each year		
	YEAR 1	YEAR 2	YEAR 3
USDA Column C – Slight (Minimal), Momentary (Transient), or No Pain or Distress	**0**	**0**	**0**
USDA Column D - Pain or Distress Relieved By Appropriate Measures **(added 6/10, amended 8/10)**	~~**1 2**~~ **1**	~~**1 2**~~ **1**	**0**
USDA Column E - Unrelieved Pain or Distress	**0**	**0**	**0**

Describe in a brief narrative your consideration of alternatives to all procedures listed for Column D and E that may cause more than momentary or slight pain or distress to the animals, and your determination that alternatives that allow the attainment of the goals of the research were not available. Consideration must be given to alternative procedures, refinement of current procedures, and reduction in the number of animals used. Delineate below the methods and sources used in the search for and consideration of alternative procedures and method refinements. **Database references must include databases (2 or more) searched, the date of the search, period covered, and keywords used. Expert consultations must include the consultant's name and qualifications and the date and content of consult.** It is the responsibility of the Principal Investigator to justify why alternative procedures and refinements are contraindicated. Guidance and resources will be found in NIAID ACUC web document Consideration of Alternatives to Painful/Distressful Procedures.

Chimpanzees are not natural hosts of *P. vivax* or *P. falciparum*. In previous genetic crossing studies, we learned that ~~the~~ *P. falciparum* sporozoite infections for a cross would not progress satisfactorily in a chimpanzee with an intact spleen; the presence of a spleen causes the *P. falciparum* parasitemia in the chimpanzee to be unacceptably low and self-cure within a week, therefore preventing production and recovery of a sufficient number of recombinant progeny. (Refer also to Bray, R.S. Studies on Malaria in Chimpanzees. VI. Lavarania falciparum. Am. J. Trop. Med. Hyg. 7 20-24, 1958). In the protocol, ~~we will first test whether *P. vivax* infections, in contrast to *P. falciparum* infections, will progress satisfactorily in a chimpanzee with an intact spleen; if not,~~ we will use a splenectomized chimpanzees~~s~~ to carry *P. vivax*. (Amended 06/10, 08/10)

Database searches:

Database: AWIC Advanced Alternatives Search
Keywords: splenectomy, alternative, malaria infection in chimpanzee
Date: 12/30/09
Period covered: no limits
Results: 6

Database: Pubmed
Keywords: splenectomy, malaria infection in chimpanzee
Date: 12/30/09
Period covered: no limits
Results: 8

No alternatives were found.

Note: Principal investigators must certify in Section N, Certification 5, that no valid alternative was identified to any described procedures which may cause more than momentary pain or distress, whether it is relieved or not.

I. ANESTHESIA, ANALGESIA, TRANQUILIZATION – For animals indicated in Section H, Column D, and for animals anesthetized to prevent distress, specify the anesthetics, analgesics, sedatives or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and frequency of administration.
NONE __ (check if none)

For parasite injection, mosquito feeding, nasogastric drug treatments, and blood smears and blood withdrawal (if necessary), the chimpanzee will be sedated with IM injection of Ketamine (10 mg/kg) or Telazol (2.4 - 2.6 mg/kg). (Amended 06/10)

~~For splenectomy, Telazol (2 mg/kg) followed by isoflurane 2 to 3%.~~

~~Dose and route of intra-op/post-op analgesic: Buprenorphine 0.3 mg/ml, 0.03 – 0.10 ml/kg IM q 6-12 h; Pentazocine 30 mg/ml, 0.06 - 0.16 ml/kg IM q 6-12 h; or Butorphanol 0.1 - 0.3 mg/kg IM q 6-12 h. Banamine (1.1 mg/kg IM, SQ every 12 - 24 hours) or Ketoprofen 15 – 30 mg/kg IM q 12 – 24 h, as adjuncts.~~ **(Amended 08/10)**

J. METHOD OF EUTHANASIA AND/OR DISPOSITION OF ANIMALS AT END OF STUDY Indicate the proposed method, and, if a chemical agent is used, specify the dosage and route of administration. If the method(s) of euthanasia include those not recommended by the AVMA Guideline on Euthanasia, provide scientific justification why such methods must be used, e.g., cervical dislocation of non-anesthetized mice. Indicate the method of carcass disposal if not as MPW

NONE ___ (check if none)

After cure from malaria, the chimpanzees will be returned to general housing, and may be recycled into other approved studies. Cure will be ascertained by daily negative blood smears over a two-week period. (Amended 08/10)

K. HAZARDOUS AGENTS: Use of hazardous agents requires the approval of an IC safety specialist. Registration Documents for the use of recombinant DNA or potential human pathogens may be required to be attached at the discretion of the ACUC. However, in most instances, the NIAID ACUC only requires HPRD and Recombinant DNA RD numbers to have been issued prior to ASP approval.

NONE ___ (check if none)

	Yes	No	List agents and registration document number (if applicable)
Radionuclides:		**X**	
Biological Agents with Human Pathogenic Potential:	**X**		***Plasmodium vivax* (HPRD 313)**
Hazardous Chemicals or Drugs:		**X**	
Recombinant DNA:	**X**	~~**X**~~	**Genetically-modified parasites RD 11-X-03 (amended 11/11)**
Study conducted at Animal Biosafety Level (ABSL):	**ABSL-2**		

Describe the practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study. For studies involving the administration of radioisotopic materials to animals, provide the DRS number for the Authorized User. Also describe methods for removal of radioactive waste and, if applicable, the monitoring of radioactivity. Use of volatile anesthetics requires a description of scavenging methods used.

In the laboratory insectary, mosquitoes will be transferred to an escape-proof mesh (Bridal Illusion Veil) -covered container, securely taped closed, and labeled. Each container will be sealed in a Ziploc® transparent bag, placed into a cardboard box with Styro-foam to prevent movement, and transported to BIOQUAL inside a cooler with a latch and a lock for triple containment, using a Government-owned vehicle.

The chimpanzee will be placed under general anesthesia as described in Section I. The anesthetized chimp will be placed on a cart in the service cove (6 feet x 6 feet) adjacent to the caging unit. Two caging units are served by one service cove. (Or we will use an adjacent hallway or food prep area. This area will be outfitted with a Spin-Sect mosquito trap. Air will be supplied to cove into animal holding space and hallway.)

The feeding area (abdominal surface area) will be clipped to remove hair. The mesh surface of the escape-proof container will be then placed against the clipped area for approximately ten minutes to allow the mosquitoes to feed through mesh. Experienced LMVR personnel will perform the feeding procedure. The escape-proof container of blood-fed mosquitoes will then be placed back into the box. The box will then be sealed closed and returned to the LMVR insectary.

Additional safety considerations: **The chimpanzee will be held in a facility where there is no access to wild Anopheles mosquitoes. Mosquito traps will be provided to the BIOQUAL facility by an LMVR entomologist as an additional precautionary measure. (Amended 08/10)**

Occupational Health and Safety considerations (vaccinations, precautions, etc):

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS (e.g., cell lines, antiserum, etc.): All biologicals to be used in animals must be listed here, regardless of species. Biologicals for use in rodents must be reviewed by the CMB QA Office before they can be used. If the biological is intended for use in rodents, has a CMB Biological Assessment Form for the material been submitted to the CMB QA

Office? If yes, please list the CMB QA assessment code. If no, please use the link above to obtain the submission form.
NONE ___ (check if none)

Material Name:	Source:	Sterile/Attenuated? Y/N	For use in rodents? Y/N If yes, list CMB QA assessment code.
***P. vivax* parasites**	**LMVR**	**N**	**N**

I certify that the MAP/RAP/HAP/PCR tested materials to be used in rodents have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP/RAP/HAP/PCR tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.

____ Initials of Principal Investigator

M. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY: List any special housing, equipment, animal care (i.e., special caging, water, feed, or waste disposal, etc.). Include justification for exemption from participation in the environmental enrichment plan for nonhuman primates or exercise for dogs. NONE ___ (check if none)

~~The chimps will~~ ~~If our attempts to induce *P. vivax* infection in a chimp with an intact spleen fail, w~~We will ~~be~~ use a splenectomized~~. Splenectomy will make the animals~~ chimpanzee~~s~~. Like splenectomized humans, splenectomized chimpanzees may be susceptible to pneumococcal pneumonia post-splenectomy. To prevent ~~this complication,~~ pneumococcal pneumonia, any splenectomized chimps ~~must~~ will be vaccinated with pneumococcal vaccine polyvalent, Pnu-Imune 23, (Pneumovax 23) just prior to or at the time of splenectomy (Single dose - 0.5 ml IM or SC). ~~The animal~~ Chimpanzees can be vaccinated at any time before the surgery, if desired. (Amended 06/10, 08/10)

~~The animal~~ Chimpanzees will be socially housed whenever possible~~ for the experiments~~. During mosquito feedings or if a compatible animal is not available, the animal will be singly housed, but with conspecifics in visual, vocal, and olfactory contact. (amended 6/10)

All animals have a variety of both food enrichment and toys, which are rotated. The enrichment program is administered by a full-time behavior technician working under the direction of BIOQUAL's primatologist, Joseph M. Erwin, PhD. Liquid enrichment such as Boost and Ensure are recommended daily during infection to prevent dehydration, and can be given as treats after procedures that don't require anesthesia.

The facility has an efficient insect control program in place. Mosquito traps will be provided to the BIOQUAL facility by an LMVR entomologist as an additional precautionary measure.
(Amended 08/10)

N. PRINCIPAL INVESTIGATOR CERTIFICATIONS (See instructions for further guidance.):

1. I certify that I have attended an approved NIH investigator training course. OACU Training History Search

   Year of Course Attendance: **March 31, 1988**

   Year (s) of Refresher Training: **December 11, 2009**

2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research. Provide a brief narrative describing the means used in your determination. In addition, for renewal proposals provide citations of your published work under this ASP or describe advances made during the previous 3 year ASP cycle.

   ~~To our knowledge no *Plasmodium*~~ No laboratory cross of *P. vivax* ~~genetic cross~~ has ~~ever~~ previously been reported. This can be confirmed by searching the key words "Plasmodium" "vivax" "genetic" "cross" on databases such as PubMed/NLM or the even more general Google. Research on *P. vivax* is severely restricted due to the lack of a long-term *in vitro* culture system for the parasite, and for the limitation of non-human primates as animal models. Results from the proposed genetic cross will ~~certainly~~ be original, and will ~~contribute to~~ advance the scientific understanding of *P. vivax* mechanisms of drug resistance, leading to improvements in the detections and treatment of millions of people with ~~this infection.~~ chloroquine-resistant *P. vivax* malaria. (amended 6/10)

3. I certify that all individuals working on this proposal who have animal contact are participating in the NIH Animal Exposure Program (AEP), (or equivalent, as applicable, for contract personnel)

4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal; have attended the course "Using Animals in Intramural Research: Guidelines for Animal Users," will complete refresher training as required, and have received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); procedures for reporting animal welfare concerns. I further certify that I am responsible for the professional conduct of all personnel listed in Section A.

5. FOR ALL COLUMN D AND COLUMN E PROPOSALS (see Section H): I certify that I have reviewed the pertinent scientific literature and the sources and/or databases (2 or more) as noted in Section H, and have found no valid alternative to any procedures described herein which may cause more than slight or momentary pain or distress, whether it is relieved or not.

6. For all proposals involving the administration of chemicals and/or bioactive compounds to animals: I certify that whenever possible, in accord with USDA Animal Care Policy 3 and ARAC Guidelines for the Use of Non-Pharmaceutical-Grade Chemicals/Compounds in Laboratory Animals, pharmaceutical-grade chemicals/compounds will be used. The use of non-pharmaceutical-grade chemicals/compounds will be based on scientific necessity and non-availability of acceptable veterinary or human pharmaceutical-grade material, not on cost. Sufficient detail will be provided in the proposal for ACUC review, including the chemical or experimental-use grade of the compound, its formulation, and vehicle.

7. I will obtain approval from the ACUC before initiating any significant changes in this study. I will obtain approval from the NIAID ACUC-authorized official before initiating any minor changes in this study. (See NIH PM 3040-2, F.4.f.d)

   Principal Investigator: **Thomas E. Wellems** Signature:_____ Date: _____

O. CONCURRENCES: Proposal Number **LMVR 13**

Laboratory/Branch Chief certification of review and approval on the basis of scientific merit. The Scientific Director's signature is required for proposals submitted by a Laboratory or Branch Chief.

Name: **Kathryn Zoon** Signature: _____ Date: _____

Division of Occupational Health and Safety Representative certification of review and concurrence. (Required of all studies utilizing hazardous agents.)

Name: **Jeffrey Potts** Signature: _____ Date: _____

Facility Manager certification of resource availability in the facility indicated to support the proposed study.

Facility: **BIOQUAL RB** Name: **Jim Edwards** Signature: _____ Date: _____

COMMENTS: **This NIAID ASP is reviewed by the BIOQUAL, Inc., IACUC. The analogous BIOQUAL ASP, along with all individual animal experimental protocols, are reviewed and approved by the BIOQUAL IACUC before implementation. BIOQUAL is PHS Assured (Animal Welfare Assurance # 3086-01) and AAALAC accredited.**

Facility Veterinarian certification of review:

Facility: **BIOQUAL RB** Name: **Anthony Cook** Signature: _____ Date: _____

Attending Veterinarian certification of review:

Name: **Daniel Paré** Signature: _____ Date: _____

P. FINAL APPROVAL:

Certification of review and approval by the **NIAID** Animal Care and Use Committee Chairperson.

CHAIRPERSON: Ted A. Torrey Signature: _____ Date: _____