



- Laboratory for AIDS Vaccine Research and Development, Duke University Medical Center, Durham, NC, U.S.A.
- Collaboration of AIDS Vaccine Discovery
- Comprehensive Antibody - Vaccine Immune Monitoring Consortium (CA-VIMC)
- Sanofi-Pasteur
- VaxGen, Inc. (now Global Solutions for Infectious Diseases)
- Thai NGO Coalition on AIDS (TNCA)
- The Health and Opportunity Network (HON)
- The Foundation for Service Workers in Group (SWING)

SUMMARY OF FUTURE PLANS AND STRATEGIES

The immediate focus of the coming year is to start the three follow on studies to evaluate and perhaps improve the immunogenicity of the RV144 regimen. Evaluation and down-selection of HIV Env proteins for use in a phase 2B trial is being pursued and may include collaborations with the Gates Foundation and the Department of Veterinary Medicine at AFRIMS for NHP work. Due to the expanding work, capacity, and specimen repository requirements of the department in the upcoming years, additional laboratory, specimen processing, and repository space have been sought through the RTA Medical Department. A phase 2B trial is planned for FY16 to evaluate early efficacy of this vaccine regimen in community risk and high-risk (MSM) populations. In preparation of this phase 2B study, activities for development of cohort studies to characterized suitable populations will begin in FY13. These studies will involve new collaborators at Chiang Mai University.

The Department will continue to serve as one of the MHRP's major testing platforms for phase I-II studies of newer vaccine candidates which will involve further testing of the subtype E (CRF01_AE) MVA vaccine candidate currently in phase I testing and newer DNA vaccine candidate. Further development of populations suitable for more advanced testing in phase 2B and 3 vaccine trials will be pursued through cohort studies of high-risk populations in Chiang Mai, Bangkok and Pattaya.

DEPARTMENT OF VETERINARY MEDICINE

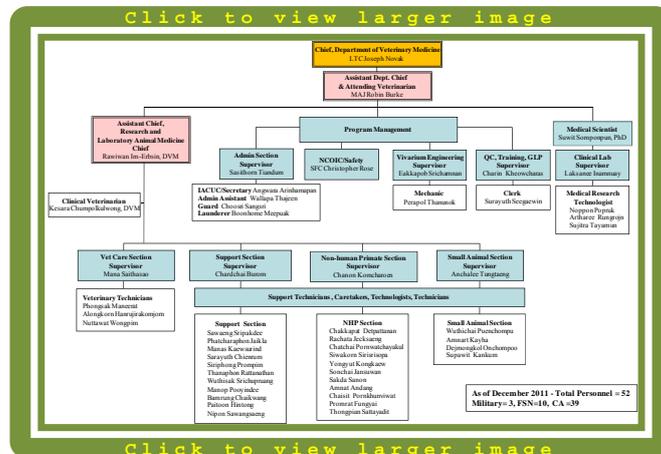
DEPARTMENT MISSION

To protect military personnel and their families against tropical disease threats through pre-clinical product development of new prophylactic and therapeutic drugs and new or improved vaccines.

To fulfill this mission the Department of Veterinary Medicine (DVM) conducts biomedical research in animal models and zoonotic disease surveillance, provides veterinary expertise and research animals that are free of confounding diseases to intra- and extra-mural collaborators, and ensures that all animals receive humane, proper, and safe care and that the USAMC-AFRIMS' Animal Care and Use Program complies with appropriate laws, regulations and guidelines.



PERSONNEL



IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

In-House Training

- American Association for Laboratory Animal Science (AALAS) Certification Training and Official Testing Site (weekly training with annual or biannual certification testing as needed)
- Weekly Staff Training on SOPs, Protocols, Departmental Policies and Other Topics as Appropriate
- The Humane Care and Use of Lab Animals
- Animal Bites and Other Injuries/Reports
- Animal Euthanasia
- B-Virus Post-Exposure Intervention and Prophylaxis
- Animal BSL-3 Hazard & Procedures
- Occupational Health Program & Safety Communication
- Equal Employment Opportunity and Prevention of Sexual Harassment (EEO & POSH)
- Fire Prevention, Protection, Report and Investigation
- Fire Fighting Training
- Accident, Illness and Complaint Reporting, Records and Investigations
- Computer Security Awareness
- Counter Intelligence, Operations Security, and Anti-Terrorism
- Hazard Communication Program
- Chemical Safety
- Safety Equipment Usage
- Biosafety in Laboratory
 - Laboratory Waste Management
 - Respiratory Protection Program
 - Composite Risk Management
- Routine Prophylaxis and Screening and Post-exposure Prophylaxis and Intervention
- Radiation Safety for X-ray Use
- Radiation Safety (General)
- Blood-Borne Pathogens Exposure Control Plan



- GLP Training – Initial and Refresher
- Ethics Training

Outside Training

- 1st Thai National Symposium on Animal Care & Use for Scientific Purposes & Laboratory Animal Trade Exhibition 2011. Bangkok, Thailand. July 2011.
- Nonhuman Primate Ultrasound Imaging Workshop. Bastrop, Texas. July 2011.
- AALAS Laboratory Animal Technologist (LATG) Review Course. Washington, DC. August 2011.
- Association of Primate Veterinarians (APV) Annual Workshop. San Diego, CA. 28 September-1 October 2011.
- American Associate for Laboratory Animal Science (AALAS) National Annual Meeting. San Diego, CA. October 2011.
- Basic Excel Training. Bangkok, Thailand. October 2011 and December 2011.
- Thailand Association for Laboratory Animal Science (TALAS) and USAMC-AFRIMS Institutional Animal Care and Use Committee (IACUC) and Research in Agricultural and Aquatic Animals: AAALAC International Perspectives. Bangkok, Thailand. October 2011.
- American College of Veterinary Pathologists (ACVP) Annual Meeting. Nashville, TN. December 2011.
- American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting. Philadelphia, PA. December 2011.

AWARDS

Non-applicable

CORE RESEARCH ACCOMPLISHMENTS

Malaria Research:

The mouse model for screening new candidate antimalarial compounds for blood stage activity has been used for over 30 years and is very effective for making comparisons between drugs and for choosing promising drugs for advanced development. It is rapid, relatively inexpensive, and makes reliable predictions of how drugs will act in higher mammalian hosts, including humans. In 2011, a total of 38 compounds in 18 experiments were tested for their potential anti-malarial efficacy.

A new and economic mouse model for screening antimalarial compounds for exoerythrocytic (EE) activity (i.e., liver stage) was developed in 2006. Prior to development of this model, an *in-vivo* test for EE screening was not readily available. In 2007 this established model was validated and optimized. In 2011, 32 compounds were screened in 11 experiments.

The ongoing efficacy model for drug development and therapeutics in the non-human primate (*Macaca mulatta*) was very active in 2011. This model provides a mechanism to identify effective new drugs for the enhanced prevention and treatment of malaria infections. Two experiments for one radical curative and one causal curative treatment were conducted in 2011 using 20 monkeys and a variety of antimalarial compounds were administered (chloroquine combination with primaquine and/or 1-aminoben-zotriazole, chloroquine combination with tipranavir/ritonavir, cethromycin or lopinavir/ritonavir, doxycycline, atovaquone-proguanil, mefloquine, chloroquine, WR150008 and chloroquine combination with WR150008 or WR246976).

**Zoonotic Surveillance:**

Due to limited budget and discussion with Thailand National Institute of Animal Health (NIAH), samples collected from the field for surveillance were submitted for leptospirosis, anthrax, melioidosis and brucellosis assays only. A 15% random check (1,630 samples) of samples from Mae Hong Son, Kanchanaburi, and Tak provinces were additionally submitted for a series of tests for *Coxiella burnetti* (Q fever). Results are pending.

Research Support:

The DVM provided research support to five departments at the USAMC-AFRIMS for 19 active animal use protocols studying disease mechanisms of and developing therapeutics and vaccines for tropical disease threats in Southeast Asia.

The DVM maintains breeding colonies of rhesus monkeys and rodents to support the USAMC-AFRIMS research needs. Seventy-eight (78) baby rhesus macaques were born in the colony. 6,689 ICR mice (*Mus Musculus*) were produced and 3,042 mice were used for 6 active protocols. Two hundred fifty (250) ICR mice were used for one protocol to maintain mosquito colonies. The veterinary clinical laboratory is an integral part of the research support for malaria drug development, malaria vaccine testing, drug-drug interaction investigation, dengue anti-viral drug development and diarrhea model development. The laboratory performed nearly 10,751 malaria parasite counts, 1,130 doses of test compounds, 1,988 complete blood counts, 8,660 serum chemistries and 1,166 tissue sections.

ACCOMPLISHMENTS AND CURRENT STATUS

1. Antimalarial Drugs Efficacy Testing in the Rhesus Monkey (*Macaca mulatta*)/ *Plasmodium cynomolgi* Malaria Models: two experiments using 20 monkeys were performed this year. Thirteen compounds were tested.

2. Care and Maintenance of Rhesus (*Macaca mulatta*) and Cynomolgus (*Macaca fascicularis*) Monkeys and Management of Breeding Colonies: Seventy-eight baby rhesus monkeys were born and newly imported male monkeys (received in 2008) were placed as breeders in gang cages.

3. Care and Maintenance of Laboratory Rodents and Rabbits, Maintenance of Rodent Breeding Colonies and Quality Assurance/Quality Surveillance Program: 6,689 ICR mice (*Mus musculus*) were produced, and 3,042 mice were used for six active protocols.

4. A *Plasmodium berghei* - Mouse Model for Screening Blood-Stage Antimalarial Drugs: 38 compounds in 18 experiments were tested for their potential anti-malarial efficacy.

5. *Plasmodium berghei* - *Anopheles dirus* Sporozoite - ICR Mice Malaria Model for Screening Exoerythrocytic Antimalarial Drugs: A total of 32 compounds in 11 experiments were tested for their potential anti-malarial efficacy.

6. A *Plasmodium berghei* – Mouse Malaria Model for Studying Hemoglobin Based Oxygen Carrier (HBOC) Treatment of Severe Malaria Anemia: Pilot experiment was performed and results are still pending. There was no activity or animal use in 2011.

7. Efficacy Testing of Two Novel Anti-viral Compounds against Dengue Fever Virus in the Rhesus Monkey (*Macaca mulatta*): Step 1 was performed and 25 rhesus monkeys were used in the testing of two novel anti-viral compounds. PK blood were collected and shipped to Toyama Company for analysis. The result is high-dose T-705 reduced peak and duration of viremia compared to control. High-dose T-1106 also had a lesser effect on viremia peak and duration but appeared highly toxic to the animals necessitated dosing discontinuation and euthanasia in one animal. There was no activity or animal use in 2011.



8. Mosquito Feeding Using *In Vitro* and *In Vivo* Techniques with Mice (*Mus musculus*) as a Blood Source: 250 mice were used instead of hamster to maintain mosquito colony.

9. Determination and Comparison of Optimum Inoculation Doses of Two *Orientia tsutsugamushi* Strains in ICR Mice: 374 mice were used within this fiscal year.

10. Maintenance of the *Leptotrombidium* Larval Mite Colonies: Chigger Feeding on ICR Mice (*Mus musculus*): 660 mice were used to maintain the chigger mite colony.

11. Continuation of Evaluation of Live, Attenuated Oral *Shigella dysenteriae* 1 Vaccine Candidates WRSd3 and WRSd5 in Rhesus Monkeys (*Macaca mulatta*) in an Intragastric Challenge Model. This protocol was the second Good Laboratory Practices (GLP) study at USAMC-AFRIMS. All animal procedures were completed. The results are still pending.

12. Continuation of "Evaluation of a Tetravalent Dengue Purified Inactivated Virus (TDEN PIV) Vaccine in *Macaca mulatta*, Phase II: Adjuvant Down Selection and Duration of Immunity". This protocol is a follow-up study in rhesus monkeys to test the most effective tetravalent dengue purified inactivated (TDEN PIV) vaccine formulations that confer long term immunity. Sixty monkeys were used to test the dengue vaccine in the different formulation. For positive control group (Live attenuated vaccine-LAV) that plan to use six monkeys was cancelled.

13. Validation of Dengue Virus (DENV) Strains as Challenge Viruses in the *Macaca mulatta* Infection Model. The study was completed by using nine naïve dengue virus rhesus monkeys. The 9 monkeys were inoculated with 3 strains of dengue virus to validate the challenge strain that will be used in the vaccine efficacy study. The result is pending.

14. Comparison of Room Temperature Preservation to Hypothermic Preservation of Renal Autograft in the Rhesus Macaque (*Macaca mulatta*) Renal Transplantation Model. Only four rhesus monkeys were used for this protocol. After surgery, 3 monkeys were found no urination with higher than the normal range of BUN and creatinine level and 1 monkey had thermal burn at caudal thigh. AV and PI recommended to euthanize the monkeys. IACUC decided to convene the investigator for all incidents and request the proper equipment and the appropriate training for the staff before resume the study.

The DVM has directly contributed to 115 published scientific articles dating back to 1966. In 2011, seven journal articles were published with DVM authors.

The DVM organizes and conducts weekly American Association for Laboratory Animal Science (AALAS) certification training and serves as an official testing site. All DVM personnel and personnel from outside collaborating institutions who work with laboratory animals are encouraged to participate in the AALAS certification course. The supervisors and qualified technicians conducted the regular classroom training for staff seeking all levels of AALAS certification. The 3 levels of certification in order from most to least difficult are Laboratory Animal Technologist (LATG), Laboratory Animal Technician (LAT) and Assistant Laboratory Animal Technician (ALAT). Eighteen (18) personnel attended training; 16 from DVM and 2 from Chulabhorn Research Institute (CRI). AALAS certification exam for the year of 2011 has been delayed and will be scheduled for Mar 15, 2012 with 10 personnel testing. There are 7 personnel from AFRIMS, 1 person from CRI, and 2 personnel from Mahidol University, Central Animal Facility to test.



COLLABORATIONS

MOUs

A Memorandum of Understanding (MOU) between WRAIR-ET and the Department of Veterinary Medicine (DVM) was established to allow for primary support to the AFRIMS animal colony in exchange for priority use of our malaria animal models.

HJF, NAVY and AFRIMS-DVM engaged to perform work on study on Hemoglobin-Based Oxygen Carrier (HBOC) treatment of severe malaria anemia.

CTAs

Commercial Test Agreement (CTA) between WRAIR/AFRIMS and the National Center for Natural Products Research (NCNPR), University of Mississippi.

Mahidol University Faculty of Veterinary Science, Sai Yoke Campus, served as site for critical reagent program in sheep to develop antiserum for use in Influenza A assays (CTA) (AFRIMS-Enteric Diseases and AFRIMS-DVM).

CRADAs

Toyama Chemical Company, Toyama, Japan, AFRIMS-Virology and AFRIMS-DVM engaged to perform work on novel anti-viral compounds for dengue fever virus.

GlaxoSmithKline, Belgium, AFRIMS-Virology and AFRIMS-DVM engaged to perform work on novel anti-viral compounds for dengue fever virus using a collaborative research and development agreement.

Novartis, WRAIR-ET, AFRIMS-Entomology and AFRIMS-DVM engaged on work with antimalarial compounds in mice.

Mahidol University, AFRIMS-Immunology and AFRIMS-DVM performed study on safety and immunogenicity of live-attenuated dengue vaccine in monkey.

AFRIMS-EDS and AFRIMS-DVM continued to work on GLP study of evaluation of live, attenuated oral *Shigella dysenteriae* vaccine candidate.

FUTURE PLANS AND STRATEGIES

- Continue to use and improve animal models of malaria in the mouse and monkey for testing new anti-malarial drugs.
- Develop new animal models of human disease including a Rhesus scrub typhus model in collaboration with Mahidol-Oxford Research Unit and a Rhesus dengue vaccine model with mosquito challenge.
- Continue GLP capabilities for preclinical studies.
- Continue to develop partnerships for testing vaccines and therapeutics against Dengue Fever in the Rhesus Macaque model.
- Provide expertise and assistance to support development of a regional primate center in Thailand
- Continue to support the USAMC-AFRIMS research mission by providing veterinary expertise and animal resources for product testing.
- Continue to maintain and exceptional animal care and use program and prepare for the 2014 AAALAC site visit.