



- Collaboration of AIDS Vaccine Discovery
- Comprehensive Antibody - Vaccine Immune Monitoring Consortium (CA-VIMC)
- Sanofi-Pasteur
- VaxGen, Inc. (now Global Solutions for Infectious Diseases)

SUMMARY OF FUTURE PLANS AND STRATEGIES

The immediate focus of the coming year is additional laboratory studies of archive RV144 specimens to identify correlates of vaccine efficacy and better understand the immune response. Two advisory groups (Scientific and Product Development) made up of international experts and trial collaborators have been established to advise the sponsor on ways to improve the ALVAC prime gp120 boost vaccine regimen and future clinical development of the vaccines. Plans are underway to begin three follow-up studies to investigate immunologic response following late boosting and intense evaluation of immunologic profile following each vaccination. A phase IIB trial is planned for FY2012 to evaluate early efficacy of this vaccine regimen in community risk and high risk (MSM) population. In preparation of this phase IIB study, activities for development of cohort studies to characterized suitable population will begin in Q1 2011. These studies will involve new collaborators at Chiang Mai University; Phramongkutklao Hospital and Medical College of Medicine of the Royal Thai Army.

The Department of Retrovirology will continue to serve as one of the USMHRP'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 IIB and III vaccine trials will be pursued through cohort studies of high-risk populations in Bangkok and Pattaya.

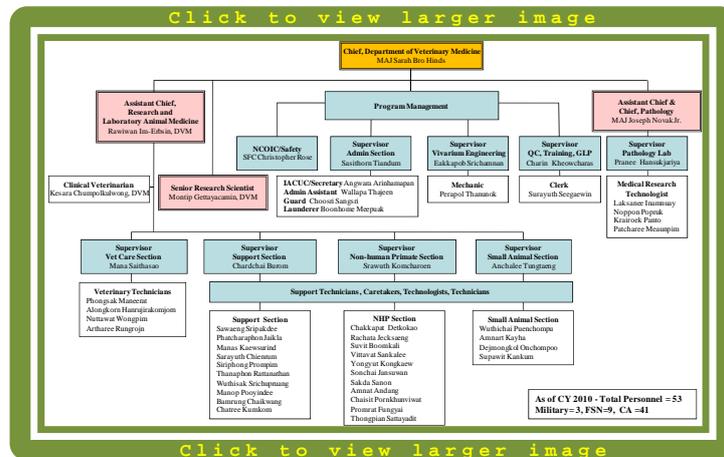
DEPARTMENT OF VETERINARY MEDICINE

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 extramural 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



Dr. Montip Gettaycamin, DVM, DAFLAM, departed the DVM 31 December 2010 after nearly 26 years of dedicated service to AFRIMS. In her tenure here as Assistant Chief, Research and Laboratory Animal Medicine (RLAM) she brought the facility to current standards to attain the required accreditation with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) in 1999, and elevated the program to exemplary status for the 2005 and 2008 site visits. As a scientist, she developed the only validated mouse and monkey exoerythrocytic malaria models used routinely here for pre-clinical testing of malarial therapeutics. She spearheaded zoonotic surveillance efforts in close collaboration with Thai officials. She assumes a full time position as Director of Southeast Asia Programs for AAALAC, located in Bangkok.

A new FSN position for Medical Research Scientist, to serve as the DVM's fulltime malaria scientist, was created in 2010 and is expected to be filled early 2011. This position will reside in the Pathology Section, under the supervision of the U.S. Army Veterinary Pathologist.

Ms. Pranee Hansukjariya, MS, Supervisor Pathology Laboratory, retired from AFRIMS 31 December 2010 after over 38 years of dedicated service. She began work in the Bacteriology (now Enterics) Department, then moved to the DVM as Supervisor of the Pathology Laboratory. She is credited as an author on many DVM publications and poster presentations. Her support in developing the mouse and non-human primate malaria models was especially invaluable.

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 & 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

- Multilateral Emerging Infectious Disease Workshop. Bangkok, Thailand. 3-5 August 2010
- Jackson Laboratory's Mouse Colony Management Principles and Practices. Taipei, Taiwan. 7-8 August 2010
- Association of Primate Veterinarians (APV) Annual Workshop. Atlanta, GA. 6-9 October 2010
- American Associate for Laboratory Animal Science (AALAS) National Annual Meeting. Atlanta, GA. 10-14 October 2010
- Thailand Association for Laboratory Animal Science (TALAS) and USAMC-AFRIMS Institutional Animal Care and Use Committee (IACUC) and Scientists Training Program. Bangkok, Thailand. 27-29 October 2010
- American College of Veterinary Pathologists (ACVP) Annual Meeting. Baltimore, MD. 30 October-3 November 2010
- American Society of Tropical Medicine and Hygiene (ASTMH) Annual Meeting. Atlanta, GA. 3-7 November 2010
- Asia Federation of Laboratory Animal Science Association (AFLAS) Congress Meeting. Taipei, Taiwan. 9-11 November 2010
- Singapore Association for Laboratory Animal Science (SALAS) Regional Annual Conference. Singapore, Singapore. 2-3 December 2010

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 2010 a total of 60 compounds in 28 experiments were tested for their potential antimalarial 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 2010, we screened 34 compounds in 17 experiments.

The ongoing efficacy model for drug development and therapeutics in the nonhuman primate (*Macaca mulatta*) was very active in 2010. This model provides a mechanism to identify effective new drugs for the enhanced prevention and treatment of malaria infections. Two experiments were conducted in 2010 using 32 monkeys and a variety of antimalarial compounds were administered (chloroquine combination with primaquine or tinidazole, or pyrazinamide, tafenoquine, malarone[®] (atovaquone and proguanil), and Ketotifen combination with chloroquine and primaquine, among several other combination therapeutics).

Zoonotic Surveillance:

In 2010 we received money from GEIS to continue sample analysis for samples collected in 2009. The Thailand National Institute of Animal Health (NIAH) analyzed for leptosporosis and brucellosis, with results showing here. BEP monies were awarded in late 2010, which will be applied to final testing of samples for Anthrax (7,835 samples) and Melioidosis (7,835 samples) from both Tak and Mae Hong Son provinces. Analysis of those samples commences early 2011.

Reports on the analyses from 2010 show the following:

Table 1. Data summary on samples collected 2009 in Mae Hong Son province[†]

Disease/ Year	Anthrax	Brucellosis	Leptospirosis	Melioidosis	Tuberculosis
2009	pending	3.1% (3305)	41% (3305)	pending	pending

Table 2. Data summary on samples collected 2009 in Tak province[†]

Disease/ Year	Anthrax	Brucellosis	Leptospirosis	Melioidosis	Tuberculosis
2009	pending	2.1% (4520)	15% (4520)	pending	pending

[†] A positive result is a titer of $\geq 1:160$ by HAI. Total number of samples in parentheses.

A BEP proposal to fund zoonotic surveillance as a collaborative effort between the Departments of Veterinary Medicine (DVM) and Epidemiology and Disease Surveillance (EDS) (formerly GEIS) will be submitted in early 2011.



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-five (75) baby rhesus macaques were born in the colony. 8,634 ICR mice (*Mus Musculus*) were produced and 2,756 mice were used for 6 active protocols. 55 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 15,000 malaria parasite counts, 420 doses of test compounds, 2,700 complete blood counts, 5,150 serum chemistries and 1,000 tissue sections.

ACCOMPLISHMENTS AND CURRENT STATUS

1. **Antimalarial Drugs Efficacy Testing in the Rhesus Monkey (*Macaca mulatta*)/ *Plasmodium cynomolgi* Malaria Models:** Two experiment using 38 monkeys were performed within this year. More than 30 compounds were tested.
2. **Care and Maintenance of Rhesus (*Macaca mulatta*) and *Cynomolgus* (*Macaca fascicularis*) Monkeys and Management of Breeding Colonies:** Forty-four baby rhesus monkeys were produced and newly imported male monkeys (received 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:** 8,634 ICR mice (*Mus musculus*) were produced and 2,756 mice were used for six active protocols.
4. **A *Plasmodium berghei*-Mouse Model for Screening Blood-stage Antimalarial Drugs:** 60 compounds in 28 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 34 compounds in 17 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.
7. **Pharmacokinetic (PK) Testing of Two Novel Anti-viral Compounds in the Rhesus Monkey (*Macaca mulatta*):** Six rhesus monkeys were tested with two novel anti-viral compound. PK blood was collected and shipped to the Toyama company for PK analysis. Protocol closure has been submitted.
8. **Efficacy Testing of Two Novel Anti-viral Compounds against Dengue Fever Virus in the Rhesus Monkey (*Macaca mulatta*):** Step 1 was performed and twenty-five rhesus monkeys were tested the two novel anti-viral compounds. PK blood were collected and shipped to Toyama Company for analysis since 2009. 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 necessitating dosing discontinuation and euthanasia in a single animal.
9. **Evaluation of a Tetravalent Purified Inactivated Virus (TPIV) Vaccine for Dengue in *Macaca mulatta*:** Forty-eight (48) rhesus monkeys were test the TPIV vaccine. All work has been completed. Rhesus serum were collected and shipped to WRAIR and GSK for analysis. TPIV in AS03 exhibited the highest geometric mean neutralizing antibody titers after two doses of vaccine and the fewest days of viremia after challenge with wild-type DENV-3.



10. **Safety and Immunogenicity of Two *Plasmodium vivax* Circumsporozoite Protein Vaccine Candidates in Rhesus Monkey (*Macaca mulatta*):** Twenty monkeys were tested the malaria vaccine. There was no activity or animal use in 2010.

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

12. **Study on Safety and Immunogenicity of Live-Attenuated Dengue Vaccine Candidates in Rhesus Macaques (*Macaca Mulatta*):** Sixteen monkeys were tested with live-attenuated dengue vaccine. All work was completed since 2009. All vaccines were safe and rhesus monkey model is suitable for dengue challenge study. Protocol closure has been submitted

13. **Determination and Comparison of Optimum Inoculation Doses of Two *Orientia tsutsugamushi* Strains in ICR Mice:** One hundred and thirty four (134) mice were used within this calendar year.

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

15. **Evaluation of Live, Attenuated Oral *Shigella dysenteriae* 1 Vaccine Candidates in Rhesus Monkeys (*Macaca mulatta*) in an Intra-gastric Challenge Model:** Forty eight rhesus monkeys were use in this *Shigella* vaccine study. This protocol was the first very Good Laboratory Practices (GLP) study at USAMC-AFRIMS. All animal procedures were completed. There was no further activity on this protocol in 2010.

16. ***Ex Vivo* Antimalarial Activity and Pharmacokinetic/Pharmacodynamic (PK/PD) Screening of Antimalarials and Their Metabolites in Rhesus Monkey Models:** Eight monkeys were tested the antimalarial drug and PK blood collection were completely performed.

17. **Safety and Immunogenicity of Protein Recombinant Baculovirus Expressing *Plasmodium falciparum* Circumsporozoite (CSP) Vaccines in Rhesus macaques:** Forty two rhesus monkeys were tested with malarial vaccine and all animal procedures were completed. There was no activity on this protocol in 2010.

18. **Evaluation of Scrub Typhus Vaccine Candidates by Natural and Artificial Challenge Models in ICR Mice (*Mus mucus*):** There was no activity or animal use in 2010.

19. **Continuation of Evaluation of Live, Attenuated Oral *Shigella dysenteriae* 1 Vaccine Candidates WRSd3 and WRSd5 in Rhesus Monkeys (*Macaca mulatta*) in an Intra-gastric 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.

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). In 2010, 19 personnel attended training; 15 from DVM and 4 from host nation organizations (Thai National Institute of Health [NIH], National Laboratory Animal Center [NLAC] and Chulabhorn Research Institute (CRI)). Thirty two (32) personnel attempted the certification, yielding an overall pass rate of 38%, with 5 AFRIMS' personnel and 7 host nation personnel achieving certification. The breakdown in certification was 1 LATG, 8 LAT and 3 ALAT. The DVM had 35 AALAS certified technicians/technologists (12 LATG, 6 LAT, 17 ALAT) at the close of 2010.



COLLABORATIONS

MOU

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 (PN09-05)

CTA

Commercial Test Agreement (CTA) between WRAIR/AFRIMS and the National Center for Natural Products Research (NCNPR), University of Mississippi. (PN07-06 Amd 3,4 Dr. Paktiya & Dr. Walker).

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-Enterics and AFRIMS-DVM)

CRADA

Toyama Chemical Company, Toyama Japan, AFRIMS-Virology and AFRIMS-DVM engaged to perform work on novel anti-viral compounds for dengue fever virus (PN09-07)

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 (CRADA) (PN09-09)

Novartis, WRAIR-ET, AFRIMS-Entomology and AFRIMS-DVM engaged on work with antimalarial compounds in mice (CRADA) (PN07-06 Amd 42)

Mahidol University, AFRIMS-Immunology and AFRIMS-DVM performed study on safety and immunogenicity of live-attenuated dengue vaccine in monkey. (PN09-06 Dr. Sathit – Do not know what type of agreement was – can be CRADA)

AFRIMS-EDS and AFRIMS-DVM continued to work on GLP study of evaluation of live, attenuated oral *Shigella dysenteriae* vaccine candidate (PN10-03 – Dr. Islam).

FUTURE PLANS AND STRATEGIES

- Continue to use and improve animal models of malaria in the mouse and monkey for testing new anti-malarial drugs.
- Streamline department business model to determine and predicts costs associated with providing quality nonhuman primates and mice to support research funded by intra- and extra-mural partners
- Begin new hire: fulltime medical research scientist to support research on the malaria models
- Continue GLP capabilities for preclinical studies.
- Continue to develop partnerships for testing vaccines and therapeutics against dengue fever in the rhesus macaque model.
- Develop partnerships and collaborations for zoonotic surveillance in hoofstock at border provinces under the Biotechnology Engagement Program.
- Provide expertise and assistance to support development of a regional primate center in Thailand

