

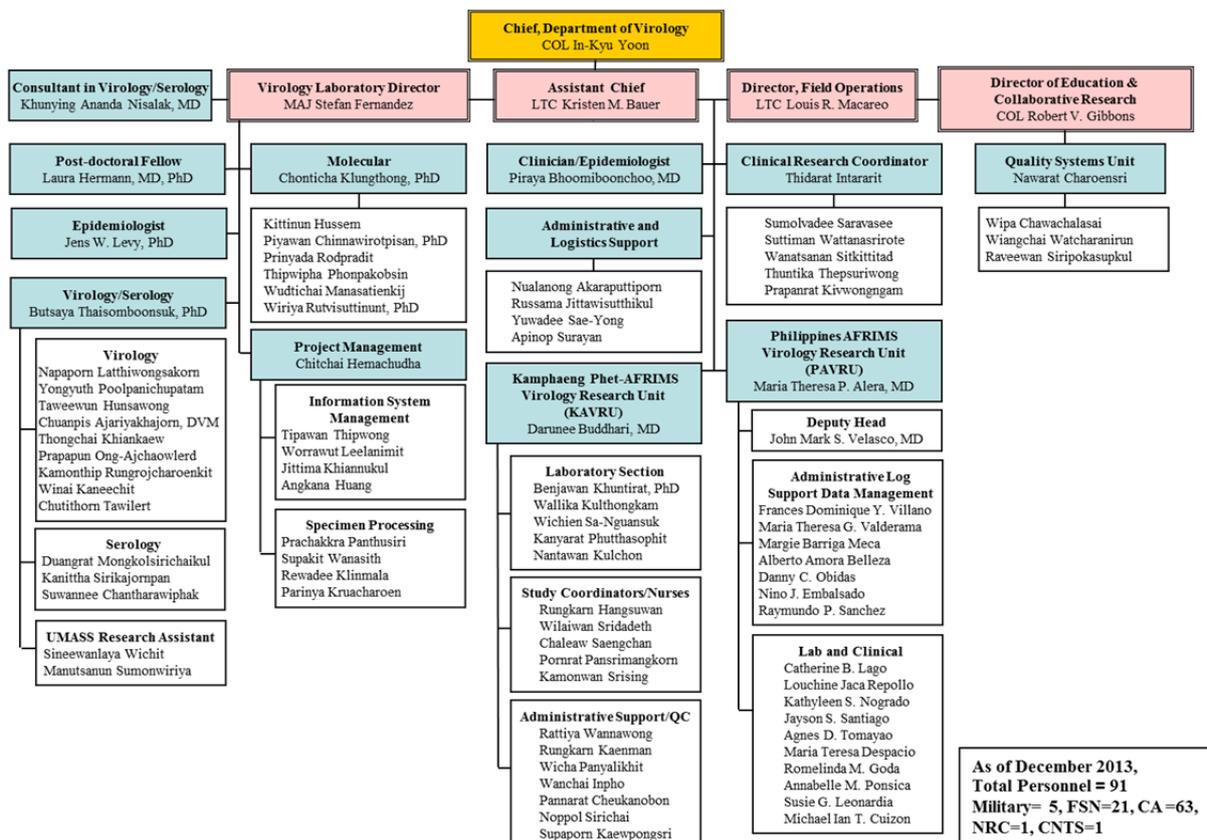


## DEPARTMENT OF VIROLOGY

### DEPARTMENT MISSION

To develop and evaluate products, and collect epidemiologic data to protect the soldiers and citizen from infectious diseases.

### PERSONNEL



### IN-HOUSE TRAINING PROGRAMS AND OUTSIDE TRAINING OF PERSONNEL

#### In-House Training of Personnel

- AFRIMS Annual Occupational Health, Safety and Biosurety Programs
- Animal Biosafety Level-3 (ABSL-3) Hazard Training
- AT Level 1 Awareness Training (AT/FP)
- Basic Life Support and Automated External Defibrillator Training
- BSL-3 Laboratory Requirement
- CITI Training
- CLG004 DoD Government Purchase Card Refresher Training Section 891
- CLG005 Purchase Card Online System (PCOLS) Section 889
- Combating Trafficking in Persons (CTIP)
- Competency Assessment of Lab Personnel on Qualitative RT-PCR Assay for Influenza

#### Viruses

- Competency Assessment of Lab Personnel on RT-PCR for Dengue Virus Genome
- Comptrollers Accreditation & Fiscal Law Course
- Contract Execution CON124 Section 312



- Development of the Live Attenuated Tetravalent Dengue Vaccine TV003: from Baltimore to Bangkok
- DoD Information Assurance Awareness
- EEO & POSH (Equal Employment Opportunity and Prevention of Sexual Harassment)
- Essential OHS Topic for Supervisors Training
- Essentials of GCP for the New Coming Investigator
- Ethics Training
- Fire Fighting Training
- Fundamentals of International Clinical Research Training
- Global Assessment Tool (GAT)
- Human Genomic Research in the Era of Next-Generation Sequencing
- Human Subjects Protection (HSP) and Good Clinical Practice (GCP) Training:  
Refresher
  - Human Subjects Protection (HSP) and Good Clinical Practice (GCP) Training:
- Introduction
  - IMM-QC-004-01 Specimen Destruction
  - Intermediate Medical Acquisition Course (IMAC)
  - Primary Hazard Training
  - Principal Investigator (PI) Training
  - Privacy Act and HIPAA Clinical Refresher
  - Progressing the Global Antimalarial Portfolio: Finding Drugs Which Target Multiple *Plasmodium* Life Stages
- issues
  - Propensity Score: What is it?
  - Receipt of Test Articles: major and minor protocol amendments and noncompliance
- management
  - Requirement for Document Amendment
  - Respirator Fit Test
  - Series of lecturers in hospital speaking with patients and reviewing their clinical
- Class
  - Sexual Harassment/Assault Response and Prevention (SHARP)
  - Sexual Harassment/Assault Response Prevention Training
  - Survival, Evasion, Resistance, and Escape (SERE) 100.1
  - Temperature Sensitive Medical Products (TSMP) Training
  - The AHLTA-T, Medical Communications for Combat Casualty Care (MC4)
  - The Humane Care and Use of Lab Animals
  - Upgrade Intelligence Temperature Monitoring and Alarm System Software Training
- Version 8 April 2013
  - Virology-AFRIMS Protocol for Detection of MERS-CoV by Real Time RT-PCR
  - WHO Real-time RT-PCR Protocol for the Detection of A (H7N9) Influenza Virus
- Spread of Zoonosis
  - WNSF-Personally Identifiable Information (PII) Course
  - WNSF-Phishing Awareness Course
  - WNSF-Portable Electronic Devices and Removable Storage Media
  - WNSF-Safe Home Computing Course
  - WNSF-Thumb Drive Awareness Virtual Training
  - English Language Program
  - Mandatory Command/QAU SOPs
  - Mandatory Departmental SOPs
  - PMK-AFRIMS Research Collaborative Discussion: Emergence, Reemergence and



- PMK-AFRIMS Research Collaborative Discussion: H7N9 Avian Influenza - Are We Prepared?
- PMK-AFRIMS Research Collaborative Discussion: Microbial Pathogenesis
- PMK-AFRIMS Research Collaborative Discussion: The Canine Heartworm Disease, a Whisper from Your Beloved Pup
- PMK-AFRIMS Research Collaborative Discussion: Update on Malaria Prophylaxis
- PMK-AFRIMS Research Collaborative Discussion: Evidence Based Medicine

#### **Outside Training of Personnel**

- 19th Annual Convention Sexier and Healthier
- 2nd PSMID-Cebu Chapter Biennial Postgraduate Course
- American Society of Tropical Medicine and Hygiene Meeting
- Bioinformatic Sequence Analysis Training (Hands On)
- Bioinformatics and Population Genetics
- Cold Chain Management Awareness course (CCM Training)
- International Conference on Dengue and Dengue Haemorrhagic Fever
- National Workshop on Estimating the Burden and Economic Cost of Dengue in the Philippines
- NIAID Post Award Grants Policy and Management Training
- Seminar Workshop on NEQAS
- Surveillance and Rapid Response Team (SRRT)
- The Dimagi CommCare Workshop
- The Next Generation Sequencing Workshop
- Performance Management & Award Brief for LE Staff
- Recruitment Workshop for Locally-Employed Staff
- Virology Association Conferences
- 3rd International Conference on Dengue and Dengue Haemorrhagic Fever "Global Dengue: Challenges and Promises"

#### **AWARDS**

Non-Applicable

#### **ACCOMPLISHMENTS**

##### **1. Long-Term Follow-Up Study of Enrollees in the Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue**

Ninety seven (97) subjects who were previously enrolled in the study entitled, “The Dengue Hemorrhagic Fever Project III: Continued Prospective Observational Studies of Children with Suspected Dengue”, were originally eligible for enrollment. Following the IRB approval process, 53 subjects remained eligible (42 subjects fell out of the 3 year follow up period window due to the length of the approval process and 2 subjects were lost to follow up due to relocation). The project was implemented 7 June 2010. From 7 June to 15 November 2010, 53 subjects were enrolled. Fifty three (53) subjects have completed all clinical activities. Clinical activities were completed on 13 October 2011. Long term follow-up activities have been completed. All specimens have been collected and distributed to the appropriate laboratories to complete protocol defined testing. Data analysis and laboratory testing are ongoing. At the time of this report there are no results to summarize.

**Future Plans:** We have completed all follow up. The protocol remains open in order for the samples and data to continue to be tested and analyzed.



## 2. The Dengue Hemorrhagic Fever Project IV: Continued Prospective Observational Studies of Children with Suspected Dengue

During 2013, an additional 28 cases were enrolled. The purpose of the limited enrollment in 2013 was to evaluate the use of handheld ultrasound machine to detect plasma leakage and to validate the findings against a larger portable ultrasound machine. Presently, there are 256 enrollees in the study: 55, 51, 122, and 28 cases were enrolled in 2010, 2011, 2012, and 2013 respectively. Clinical, serologic and molecular diagnostic studies have been completed. There were 216 dengue cases and 40 non-dengue cases in the first 3 years of the study. The dengue cases were classified into 139 dengue fever (DF) cases and 14, 36, and 28, and 1 DHF grade I, II, III, and IV respectively. Infecting viruses were identified by PCR in 84% of dengue cases. DENV1, 2, 3 and 4 made up 26.8%, 25.4%, 30.5%, and 1.3% of dengue cases. All DHF cases had serologic evidence of a secondary infection while 15% of DF cases had a primary infection.

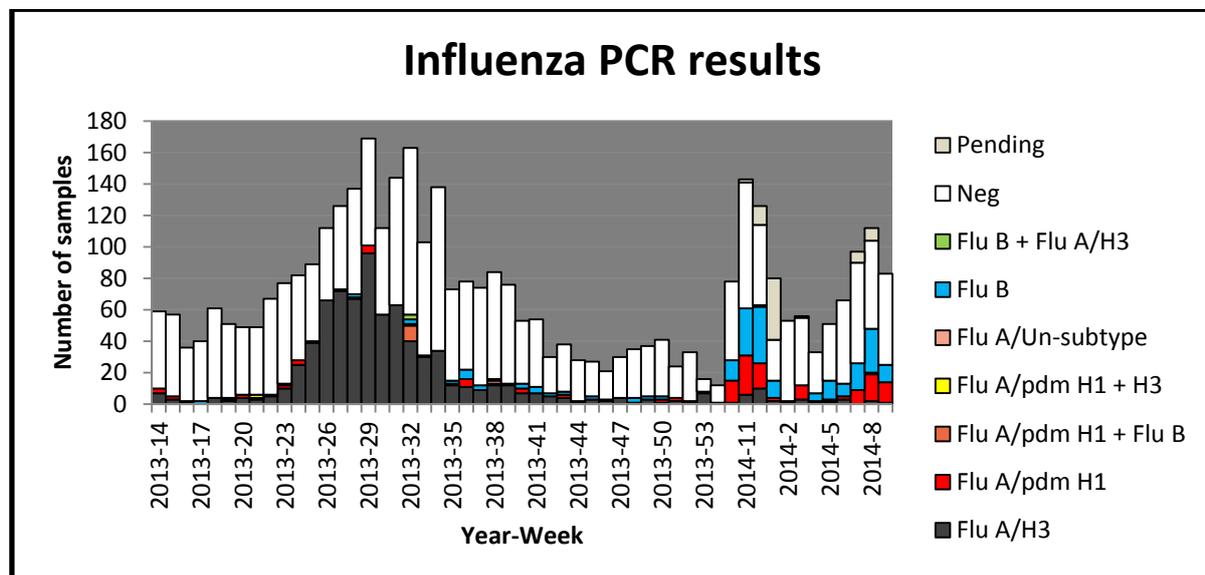
Plasma viral RNA levels were measured from samples from 2010-2013. Quantitative RT-PCR revealed similar viral RNA levels cases classified as DF or DHF. The measurement of NS-1 levels in samples from 2012-2013 enrollees is on-going. The NS-1 levels were not different between cases with either a primary or secondary DENV infection. We have performed antigen-antibody complex uncoupling of plasma samples to determine if this would improve NS-1 antigen detection. This complex dissociation step did not significantly increase the detectable levels of NS-1.

Determination of cardiac enzyme levels including CPK-MB and troponin-T has not shown elevated enzyme levels. Hemodynamic and cardiac function indices were different in DF compared to DHF cases and were for the most part related to plasma leakage and clinical severity.

**Future Plans:** We plan to further analyze the relations between indicators of viral load (viral RNA levels, NS-1 levels) and dengue disease severity, and further characterize the changes in cardiac functions in dengue cases. Studies continue on the analysis of adaptive immunity in dengue. Conduct data analysis and interpretation.

## 3. Sentinel Influenza Surveillance in Asia

From April 2013 to March 2014, 3,763 samples were collected from Bhutan, Nepal, Philippines and Thailand. Of these, 30.7% were influenza positive. Selected samples collected in the duration were sent for isolation. To date, we have completed isolation for 1,246 of the selected samples.





**Future Plans:** Continue to conduct sentinel surveillance and characterization of influenza virus infections for early warning of events of public health importance.

#### **4. Prospective Study of Influenza A Transmission from and to Household Animals in Thailand**

During the year 2013, 251 ILI subjects were enrolled. Of these subjects, 104 were positive for influenza A (all were H3 subtype), and 2 for influenza B (Figure 1). Of the 104 influenza A subjects, 13 live outside the study site (Muang District, Kamphaeng Phet Province), 40 did not have any pet or livestock, 16 could not be reached, and 4 did not permit the study staff to conduct household animal investigation. Of the remaining 31 subjects, there were 30 households that allowed study staff to collect animal samples from their pets/livestock (some subjects reside in the same houses). A total of 131 animals (6 chickens, 28 fighting cocks, 13 ducks, 6 cats, 46 dogs and 14 rodents) were sampled. Respiratory or cloacal samples were collected for virus detection and isolation, and serum specimens for detection of antibody to influenza viruses. Preliminary result of virus detection from respiratory/cloacal samples sent to the NIAH laboratory using real-time RT-PCR technique was negative. Virus antigen to be used in the serology test of the blood samples is in preparation.

**Future Plans:** Sentinel human influenza surveillance of patients presenting to Kamphaeng Phet Provincial Hospital will continue to monitor for evidence of influenza A infection with positive influenza A cases triggering household animal contact investigations. Testing of respiratory/cloacal and serum samples at the NIAH laboratory will also continue.

#### **5. Collaborative Electronic Disease Surveillance Project in the Philippines**

The surveillance system has been operational in CHD. It was implemented in 84 BHCs. An internal evaluation of the system was conducted to enhance or make modifications to improve functionality. An iterative development approach was used based on feedback of the Area Medical Officers (AMOs) in order to improve usability and utility to support public health practice.

A username and password-protected webpage presented case-level and aggregate data at the citywide and health area levels (Figure 1 & 2). AMOs were given a username and password. The dashboard was simplified to present number of cases per health area upon login (Figure 3). CHD preferred weekly analysis. The website shows output of the weekly counts of SMS fever cases with standard deviation at the citywide and per health area level (Figure 4 & 5).

The program enhancement shows that distribution of syndromic fever information to frontline AMOs increase the usefulness and relevance of SMS-FS. Strong political support by decision-makers and acceptability of the SMS-based reporting resulted in rapid expansion of the program and later institutionalization. Results of internal evaluation were discussed with CHD senior managers. Other recommendations were to assign an IT in CHD to manage the system, to have a pool of trainers, recognize good performance of BHCs, and sustain credit loads which is very crucial at this stage. CHD expressed willingness in continuing the program.

A formal evaluation is ongoing. During the planning stage, stakeholders were consulted and involved in drafting the evaluation framework. External consultants were recruited to conduct specific components of the system. The evaluation charter explicitly assigned task to each consultant. Preliminary results of systems evaluation by external IT experts are:

- Users can add other disease/s syndromes.
- Users can view the trend of different disease syndromes on the web.
- Monitor trends daily, weekly and monthly aside from yearly.
- View reports /graphs of specific disease syndromes daily, weekly and monthly.
- Set threshold for alert.
- Send alert notification to end-users.
- Reporting of cases through the web is feasible in urban BHCs with internet access.



Figure 1 City-Wide Time Series

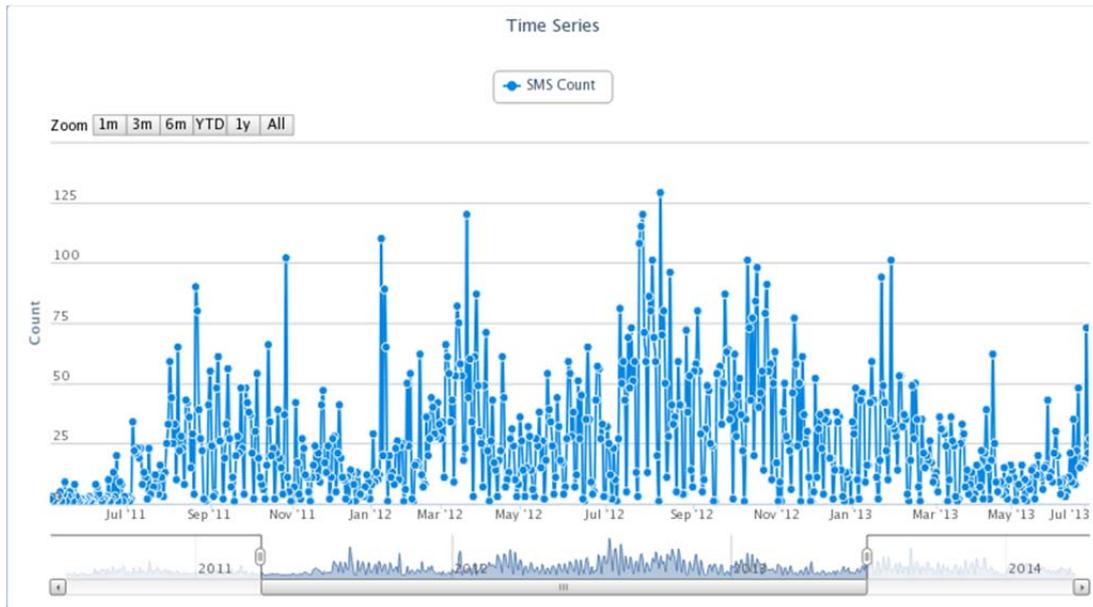


Figure 2 Health Area Time Series

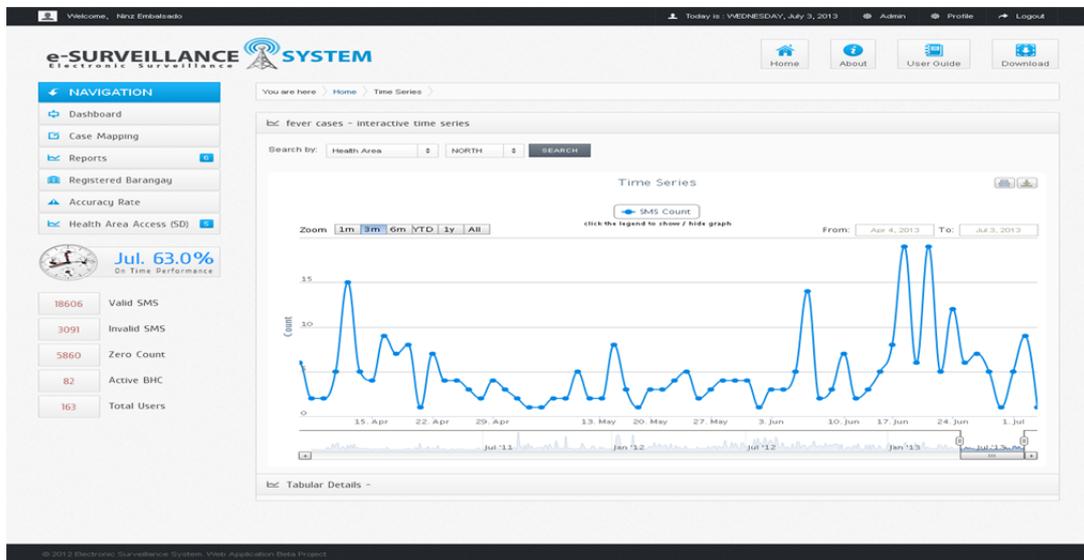




Figure 3 Dashboard

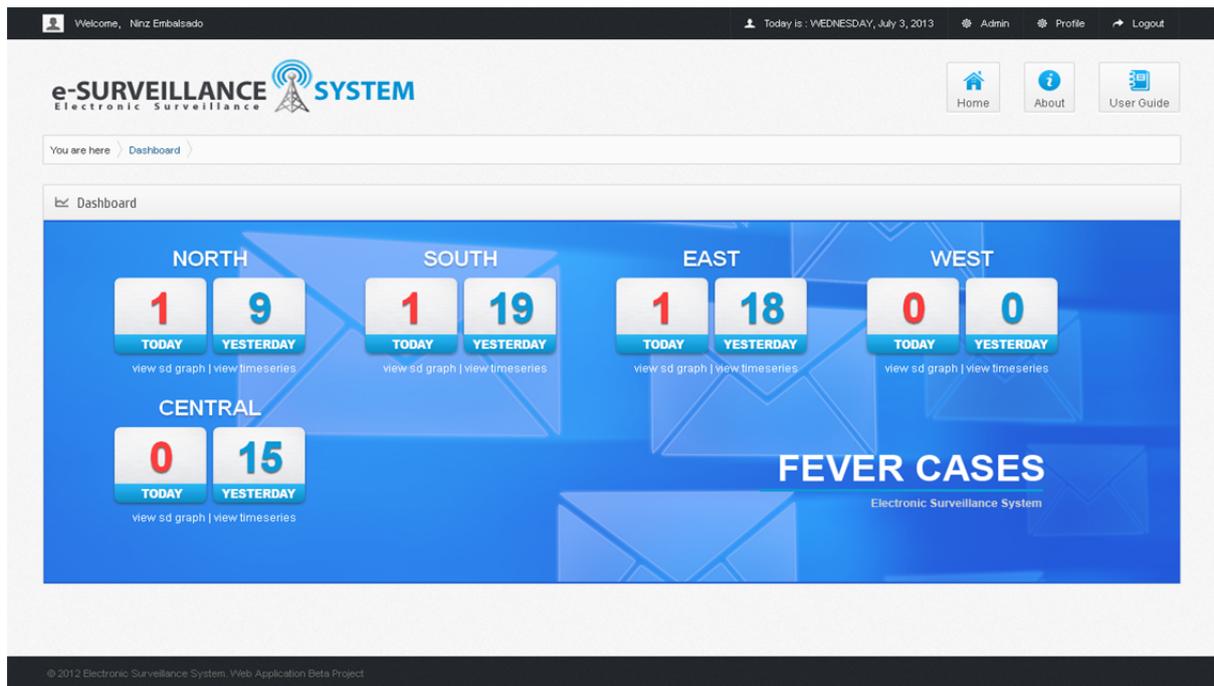
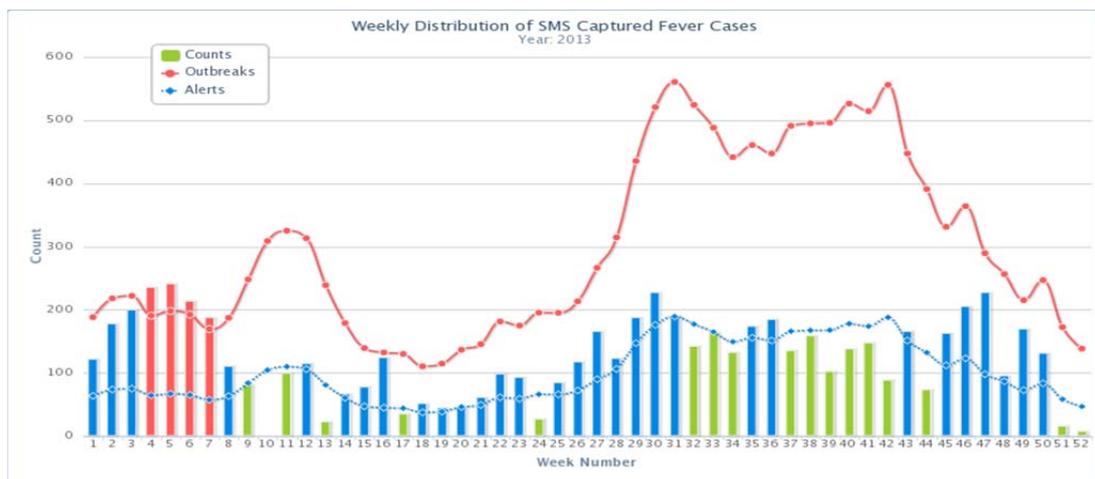
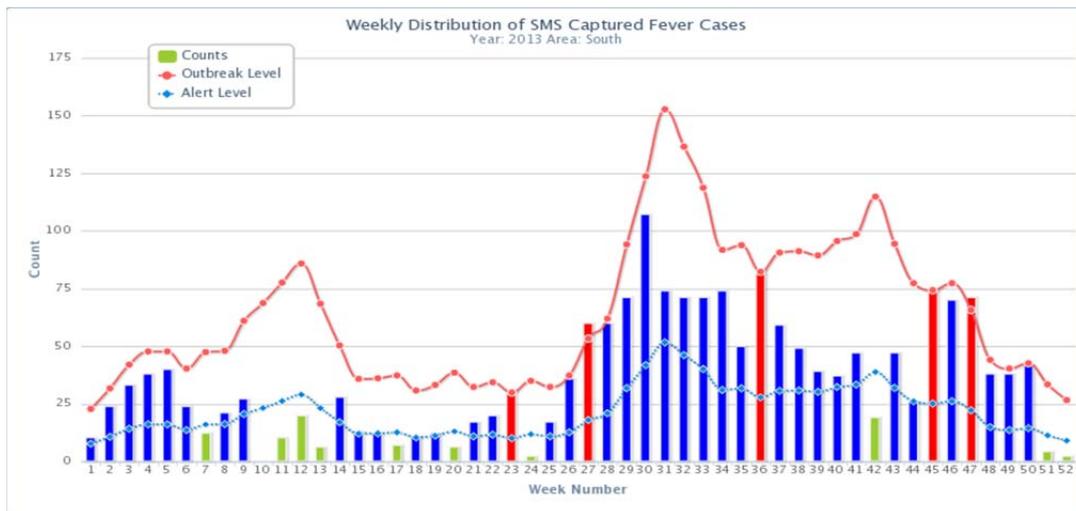


Figure 4 Citywide SMS Weekly Counts with SD





**Figure 5** Health Area SMS Weekly Counts with SD.



EDE Integration into Philippines National Surveillance Programs

Updates on the e-surveillance projects (i.e., EDE, Cebu SMS reporting system, twitter analysis, dengue prediction system) and results of publications from these collaborations were presented to the RESU officers from different regions of the Philippines who attended the National Program Implementation Review (PIR) meeting held on Oct 14-18, 2013.

**6. Efficacy and Safety of a Novel Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 14 years in Asia (CYD14)**

Kamphaeng Phet Site

Subject enrollment was completed in October 2011. Five hundred eighty-five subjects were enrolled. The third (and final) dose vaccination for all subjects was completed in October 2012. Active surveillance for febrile episodes was completed in October 2013. Hospital-based surveillance continues.

Cebu Site

Subject enrollment was completed in December 2011. Nine hundred thirty-four subjects were enrolled. The third (and final) dose vaccination for all subjects was completed in December 2012. Active surveillance for febrile episodes was completed in November 2013. Hospital-based surveillance continues.

**Future plans:** Hospital-based surveillance will continue as outlined in the approved protocol. Detection of suspected dengue cases is ongoing. Preliminary efficacy and safety results are expected at the end of 2014.

**7. Dengue Virus Circulation, Evolution, Virus-Vector, and Virus-Host Interaction in Kamphaeng Phet Province, Thailand**

**7.1 Hospital-Based Study:** The cumulative number of subjects enrolled in the study was 326. Three subjects were found to be PCR negative from repeat PCR, quantitative PCR and viral isolation. So the total data from 323 subjects was used for data analysis. Of these, 75 were DENV-1, 195 were DENV-2, 37 were DENV-3 and 16 were DENV-4. Serologically, there were 6 acute primary DENV infections, 4 recent secondary DENV infections, 296 acute secondary DENV infections, 6 acute secondary flavivirus infections, and 11 without serologic diagnosis due to availability of only a single specimen (unpaired).

**7.2 Cluster Investigations:** There were 326 cluster investigations conducted corresponding to the number of hospital-based subjects; 323 cluster investigations were used for data analysis. A total of 1,246 contacts were enrolled; of these, 33 were positive for DENV-1, 67 for DENV-2, 28 for DENV-3, 6 for DEN-4, and 1,112 were PCR negative PCR on enrollment day. Thirty-nine contact enrollees reported fever before the convalescent blood collection and, therefore, underwent a second acute blood sample collection. PCR results on the fever day (from Day1-Day19) revealed 4 DENV-1, 15 DENV-2, 3 DENV-3, and 17 were PCR negative. Serologically, there was 1 acute DENV infection, 31 acute primary DENV infections, 235 acute secondary DENV infections, 21 recent secondary DENV infections, 1 acute secondary flavivirus infection, 5 JEV infections, 1 recent JEV infection, and 909 without evidence of a recent flavivirus infection. No serologic diagnosis was available for 42 subjects due to single specimen.

**7.3 Vector Studies:** A total 17,283 houses were mapped and 9,322 *Aedes* mosquitoes were collected from 323 cluster investigations. PCR of the female mosquitoes (which transmits DENV) was completed for 9,322 mosquitoes; of these, 13 mosquitoes were positive for DENV-1, 87 for DENV-2, 28 for DENV-3 and 3 for DENV-4. The mosquito infection rate was, therefore, 1.41%. Almost all PCR-positive mosquitoes were found to have the same DENV serotype as the subject who lived in the house where the mosquito was collected.

**Future plans:** Complete database check and analysis.

## 8. Pathological and Immunological Events in Fatal Dengue Based on Autopsy Evaluations

Three autopsy cases have been obtained from Vicente Sotto Memorial Medical Center: 2 dengue cases and 1 control non-dengue case. Laboratory evaluations are currently being performed on these tissue samples at AFRIMS, Bangkok.

**Future Plans:** Enrollment of fatal dengue cases will continue.

## 9. Prospective Cohort Study of Influenza and Dengue Infection in Children and Adults, Cebu City, Philippines

**Surveillance Phase:** There were 162 subjects who had acute fever illness events. The table below shows the total acute fever illness events by age group:

Age Groups/Acute Fevere Illness Events	6 mos-5 yrs.	>5-15 yrs.	>15-30 yrs.	>30-50 yrs.	50 yrs. Above	Male	Female	Total
Age groups of subjects with 1 AFI event	38	20	14	11	13	51	45	96
Age groups of subjects with 2 AFI events	23	13	1	3	4	25	19	44
Age groups of subjects with 3 AFI events	9	4	0	2	0	8	7	15
Age groups of subjects with 4 AFI events	1	0	1	1	0	2	1	3
Age groups of subjects with 5 AFI events	1	0	0	1	0	1	1	2
Age groups of subjects with 6 AFI events	0	0	0	1	0	1	0	1
Age groups of subjects with 7 AFI events	0	0	0	1	0	1	0	1

Thirty Six out of 162 were FLU RT-PCR (+). Of the positives, Flu A/pH1: 14/36 (38%); A/H3: 11/36 (31%); and Flu B: 11/36 (31%).

**Future Plans:** The second year annual follow-up visits were initiated in March 2014. 343 subjects already had their annual follow-up visits in the month of March. Cohort subjects will complete their second year annual follow up visits in 2014.



## 10. Immunological Correlates of Clinical Outcomes in a Tetravalent Dengue Vaccine Cohort - an Ancillary Study (Cebu City, Philippines)

### Study Activities for Pre-Vaccination Volunteers

Date enrollment started	14 Oct 2011
Date enrollment completed (V01)	17 Nov 2011
Number of subjects screened	114
Number of subjects enrolled	114
Number of subjects who completed V01	112
Date V02 (28 days after V01) started	11 Nov 2011
Date V02 completed	26 Dec 2011
Number of subjects who completed V02	112
Date V03 started	27 Mar 2012
Date V03 completed	19 May 2012
Number of subjects who completed V03	112
Date V04 (28 days after V03) started	24 Apr 2012
Date V04 completed	20 Jun 2012
Number of subjects who completed V04	112
Date V05 started	28 Sep 2012
Date V05 completed	19 Nov 2012
Number of subjects who completed V05	112
Date V06 started	26 Oct 2012
Date V06 completed	21 Dec 2012
Number of subjects who completed V06	112
Date V07 started	07 Oct 2013
Date V07 completed	30 Nov 2013
Number of subjects who completed V07	112
Withdrawal from the study	2
a. Number of subjects withdrawn by investigator	0
b. Number of subjects who voluntarily withdrew from the study	2
Number of active volunteers	112
Total Number of Serious Adverse Event	0
Total Number of Unanticipated Problems	0
AE related to the study procedures	0

The post-vaccination enrollment was done at Visit 06 (28 days after the last dose or dose 3) which started from 14 September 2012 to 27 December 2012 as shown in the table below.



### Study Activities for Post-Vaccination Volunteers

Date enrollment started (V06)	14 Sep 2012
Date enrollment completed (V06)	27 Dec 2012
Number of subjects briefed	812
Number of subjects enrolled	502
Number of subjects who completed enrollment procedures	501
Date V07 started	02 Sep 2013
Date V07 completed	30 Nov 2013
Number of subjects who completed V07	499
Withdrawal from the study	4
a. Number of subjects withdrawn by Investigator	0
b. Number of subjects who voluntarily withdrew from the study	4
Number of active subjects	498
Total Number of Serious Adverse Event	0
Total Number of Unanticipated Problems	0
AE related to the study procedures	1

There were no unanticipated problems reported since the start of the study for both pre- and post-vaccination volunteers. There was one (1) adverse event related to the study procedures (vasovagal reaction related to blood draw) involving a post-vaccination volunteer only. There were six (6) subjects who voluntarily withdrew from the study since the enrollment started. Two (2) were pre-vaccination volunteers and four (4) were post-vaccination volunteers.

#### Reconsenting for CYD14

Update for pre- and post-vaccination volunteers who re-consented/did not re-consent for CYD14 protocol amendment 3 (subject will not continue V11 and V12)

	Pre-vaccination	Post-vaccination
• No. of subjects who <u>re-consented</u> for CYD14 protocol Amendment 3	102	485
• No. of subjects who <u>did not re-consent</u> for CYD14 protocol Amendment 3	9	10
• No. of subjects who <u>withdrew re-consenting</u>	0	1
• <u>For re-consenting</u>	1	2

#### Acute Febrile Episodes

There were six hundred thirty (630) cases of acute febrile episodes since the start of the study, with two hundred twenty one (221) cases occurred for 73 pre-vaccination volunteers and four hundred nine (409) cases occurred for 239 post-vaccination volunteers.



## Summary of AFE per number of episodes

Acute Febrile Illness Episode/s (Total No. of Episodes)	Pre-vaccination		Post-vaccination	
	No. of Subjects with AFE	Total AFE	No. of Subjects with AFE	Total AFE
	N=73	N=221	N=239	N=409
1 Acute febrile episode	21	73	144	239
2 Acute febrile episodes	16	52	48	95
3 Acute febrile episodes	15	36	29	47
4 Acute febrile episodes	9	21	9	18
5 Acute febrile episodes	4	12	8	9
6 Acute febrile episodes	2	8	1	1
7 Acute febrile episodes	0	6		
8 Acute febrile episodes	2	6		
9 Acute febrile episodes	2	4		
10 Acute febrile episodes	1	2		
11 Acute febrile episodes	1	1		

**Future plans:** Visit 09 (last vaccination + 24 months) and Visit 10 (last vaccination + 36 months) will be scheduled Aug 2014 and Aug 2015 respectively. At each visit, a clinical examination will be conducted at the investigator's discretion based on the health status of the subject and a blood sample will be taken for dengue neutralizing antibody evaluation.

The Active surveillance to monitor acute febrile episodes will continue throughout the duration of the study.

#### 11. Sentinel Human Influenza Surveillance in Royal Thai Army Recruits

Two hundred and seventy one acute respiratory samples were collected from 484 active subjects enrolled in the study this past year; 278 blood samples were collected for the routine baseline and six-month follow-up visits. Despite the low acute influenza positivity rate, HAI 4-fold seroconversions of the influenza HAI titers provided evidence of the circulation of influenza virus within the study population.

Radio-frequency identification (RFID) technology was initiated to compare with recall diaries. Bed mapping methods were used to track transmission patterns of respiratory disease within the study setting.

**Future Plans:** Complete laboratory testing as outlined in the protocol, and analyze and report the results. Continue the implementation of RFID technology within the study to gain longitudinal data of respiratory disease transmission patterns within the military barracks.