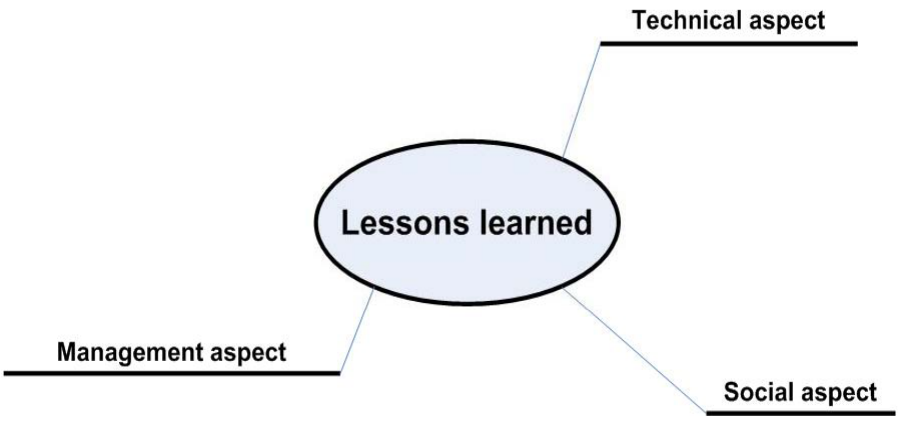
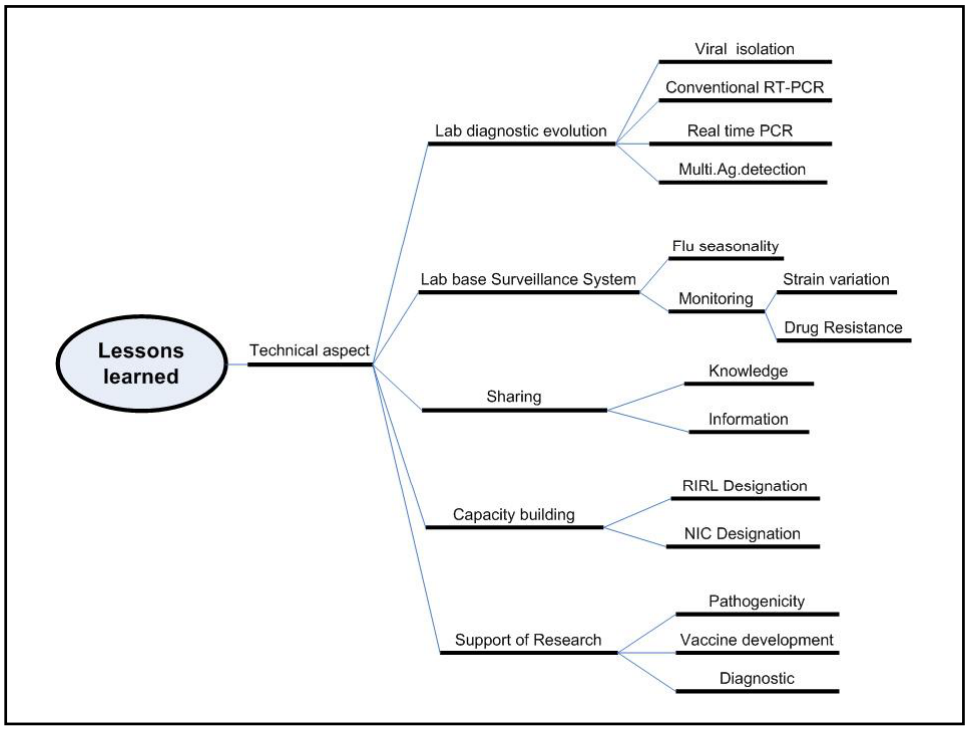
Lessons Learned Phase I year 2004-2009 DMSc

Sirima Pattamadilok
National Institute of Health
Department of Medical Sciences

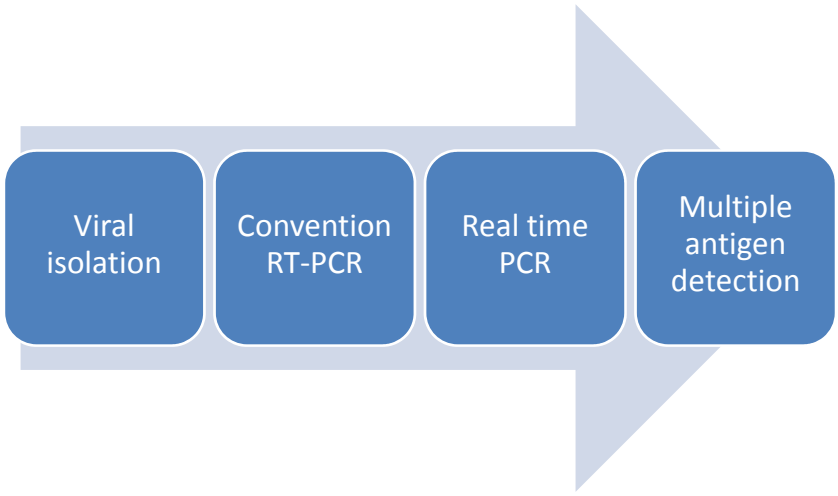
Present at
Sentinel Hospital Influenza Surveillance Project Annual Meeting
Holiday INN, Chang Mai
October 26, 2010

Lessons learned

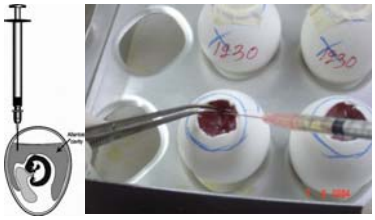




Lab diagnostic evolution

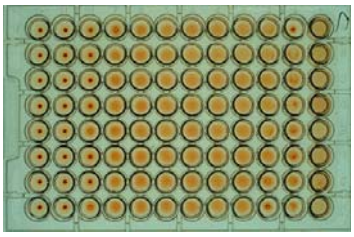


A/H5 isolation by egg inoculation



By amniotic & allantoic route

Egg incubation at 37°C



Strain analysis by HAI test

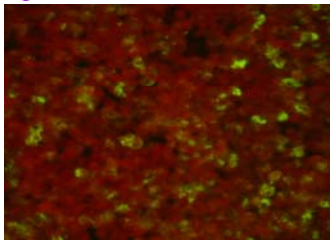
5

A/H5 isolation by cell culture

Inoculation using MDCK

Observed CPE

under microscope



Identify type & subtype by IFA

6

Influenza gene detection by RT-PCR

Extraction RNA from respiratory secretion

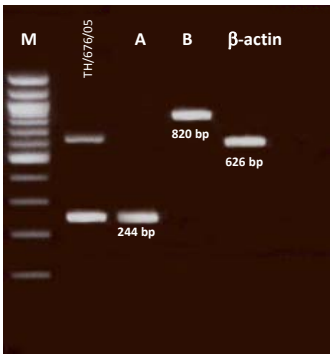
Polymerase chain reaction using PCR thermocycler

PCR product visualized by Gel-electrophoresis

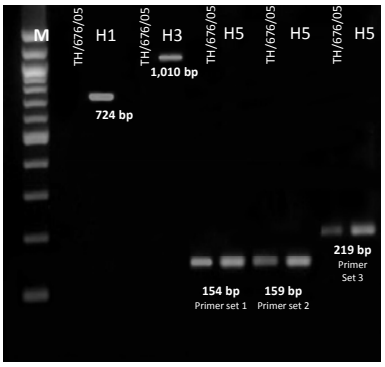
7

PCR product visualized in agarose gel by UV transluminator

Screening test

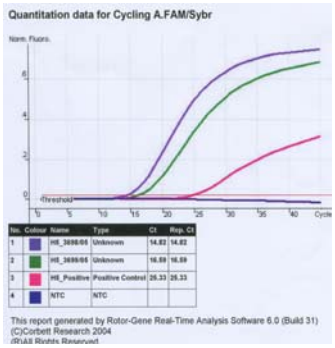


Subtype Identification



8

Typing and subtyping Influenza A by real time RT-PCR



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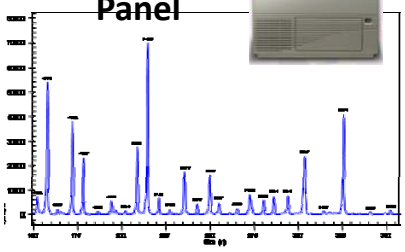
Beckman Coulter Respiratory Virus Identification Solution

swab

respiratory virus RNA

XP-PCR Panel



Nucleic Acid Isolation

Amplification & Detection

Thai NIH,2010

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Facility in Mobile Lab, DMSc, Thailand

Real time RT-PCR

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Strengthen DMSc Laboratory network

★ Regional medical science center (14 RMSc)

Mobile Laboratory (7 mobile labs)

DMSc

RMSc Chaing Mai

RMSc Chaing Rai

RMSc Pitsanulok

RMSc Udornthani

RMSc Ubonrachathani

RMSc Khonkhen

RMSc Nakornrachasima

RMSc Nakornsawan

RMSc Chonburi

RMSc Samutsakorn

RMSc Trang

RMSc Suratthani

RMSc Songkhla

RMSc Phuket

K. supawat,Thai NIH

12

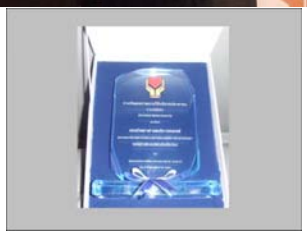
1. Thailand award for public service quality, 2007

"Virological laboratory Testing Process for Suspected Avian influenza cases"

รางวัลคุณภาพการให้บริการประชาชน
รางวัลดีเด่น ประเภทรายกระบวนงาน

กระบวนการงานการตรวจวิเคราะห์ทางห้องปฏิบัติการด้านไวรัสวิทยา กรณีผู้ป่วย ต้องสงสัยด้วยไข้หวัดนก

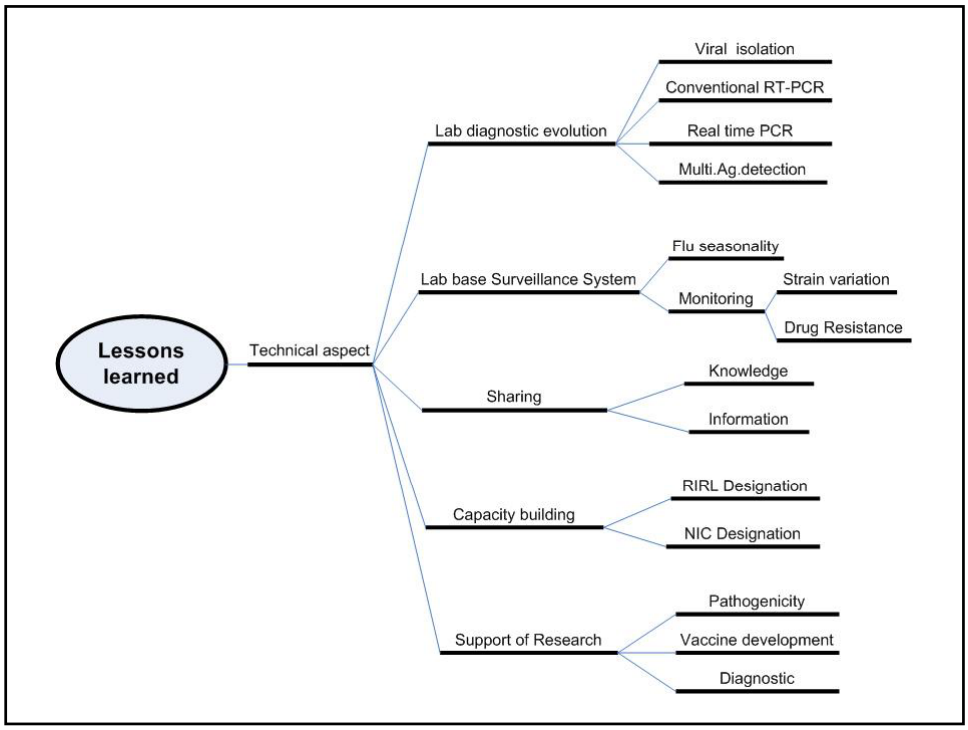
คณะกรรมการพัฒนาระบบราชการ (ก.พ.ร)
พ.ศ. 2550



Thai National Influenza Center

2. Submit for UN award,2008

Lab Based Surveillance System



Laboratory-based Influenza Surveillance network
Five years project partly supported by US-CDC
(Phase 1 : Sep 15, 2004-Sep 14, 2009)

•Virological surveillance

Influenza like illness (ILI) outpatient

– Geographically representative with 11 sites across country
– Five ILI patients / week / site
– Collect nasal and throat swab
–Screening influenza cases using rapid test kit at sentinel site
–Viral isolation at Thai NIH
– Strain analysis and drug resistant testing at Thai NIH

Sentinel sites

Phase I, First year

- ❖ Mae Sot hospital,Tak
- ❖ Nong Khai hospital
- ❖ Phra Pok Klao hospital, Chantaburi
- ❖ Hat Yai hospital, Songkhla
- ❖ Health Center 17, Bangkok

Second year

- ❖ Mae Chan hospital, Chiang Rai
- ❖ Chiang Saen hospital, Chiang Rai

Third year

- ❖ Koh Chang hospital Trad
- ❖ Koh Samui hospital, Bangkok
- Hospital Samui, Surat Thani

Phase II, First year (Sep 2009 – Sep 2010)

- ❖ Bamrasnaradura, Nonthaburi

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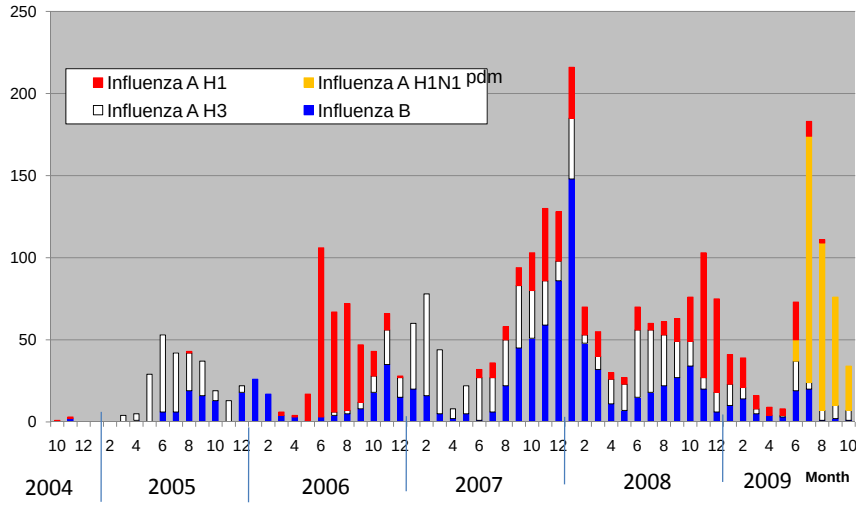
Influenza Positive cases by subtype from 10 sentinel sites, by month, phase I: 2004 – 2009 (5 years)



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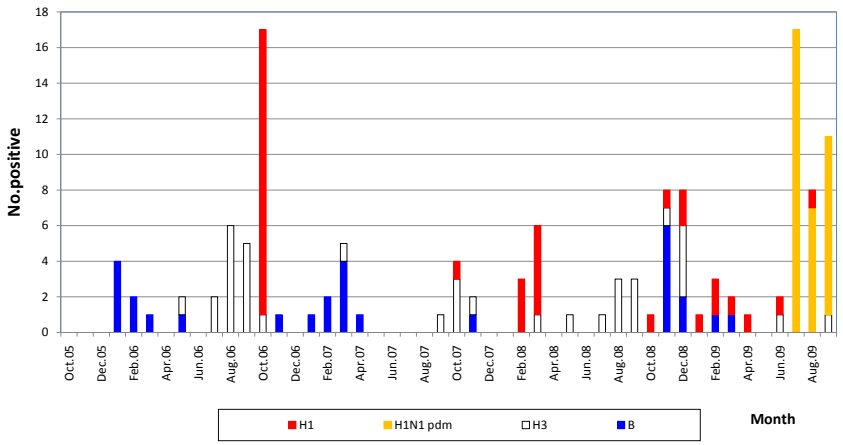
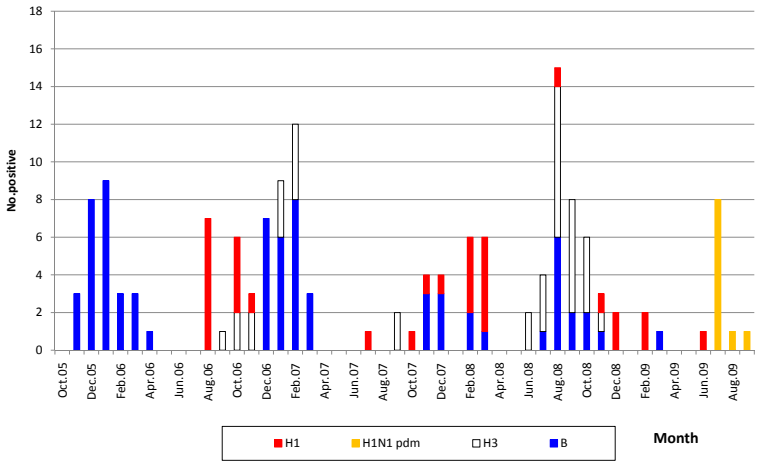
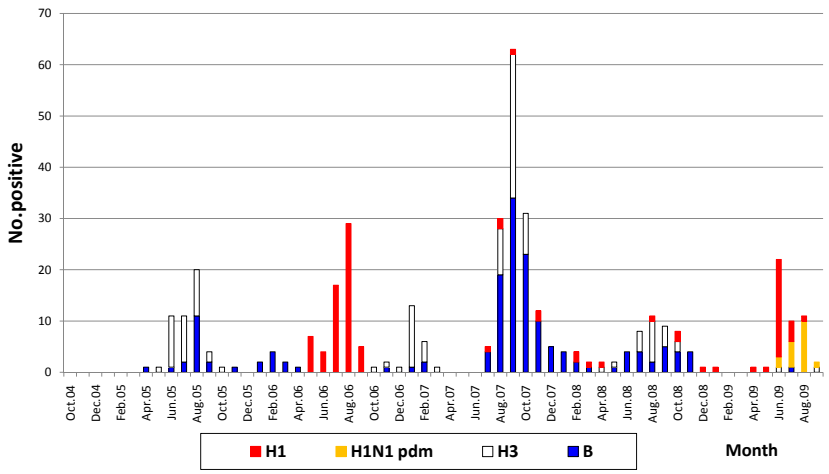
Monthly influenza isolates, Chiang Saen, Chiang Rai Province



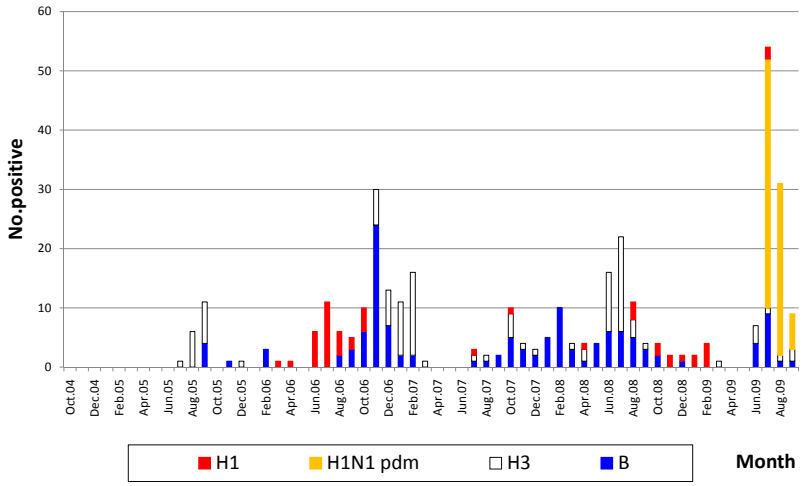
Monthly influenza isolates, Mae Chan, Chiang Rai Province



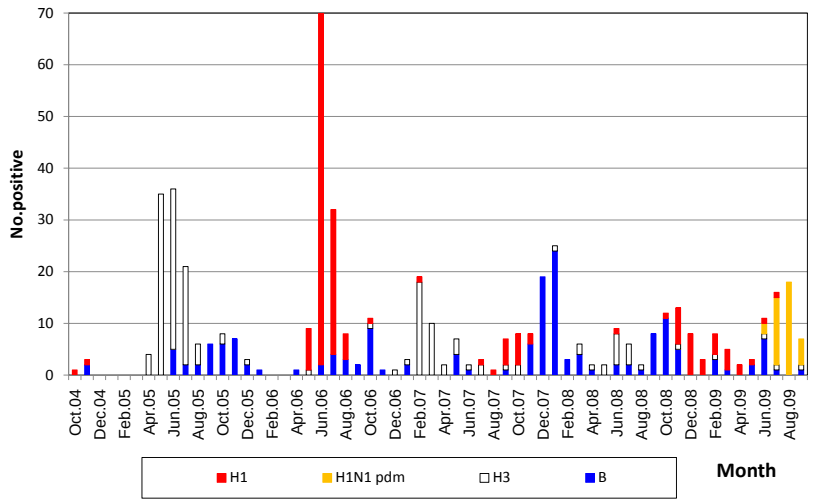
Monthly influenza isolates, Mae Sot, Tak Province



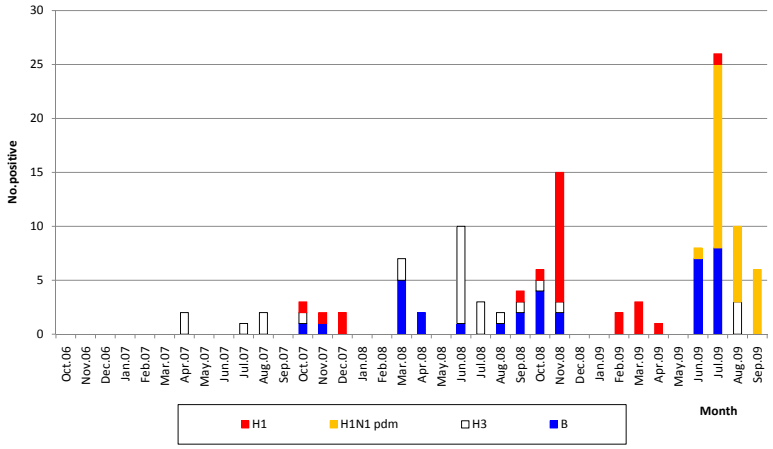
Monthly influenza isolates, Nong Khai, Nong Khai province



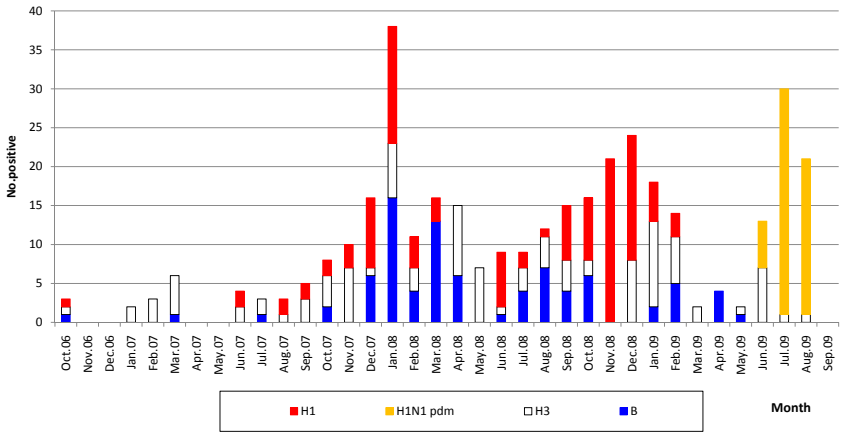
Monthly influenza isolates, Phra Pok Klao Hospital, Chantaburi province



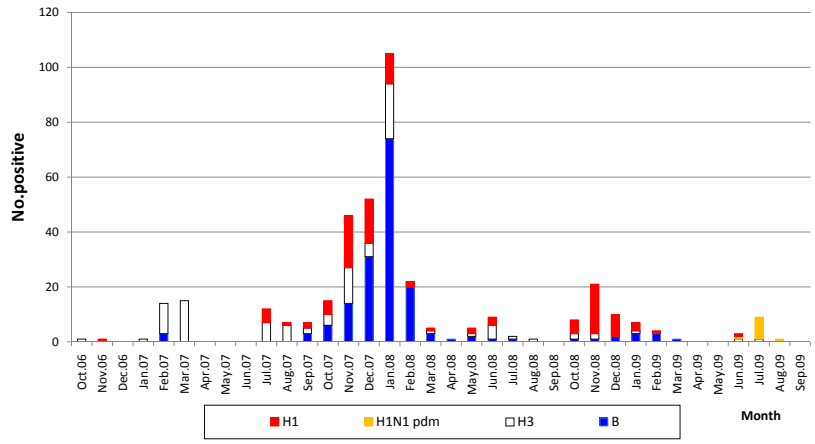
Monthly influenza isolates, Koh Chang, Trat province



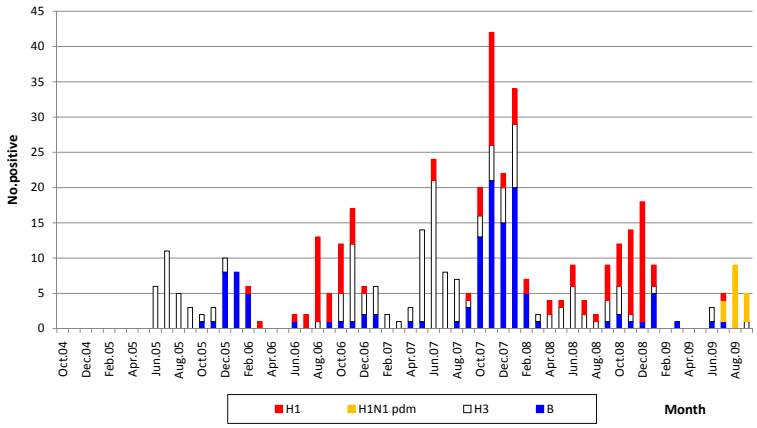
Monthly influenza isolates, Bangkok Hospital Samui, Surat Thani province



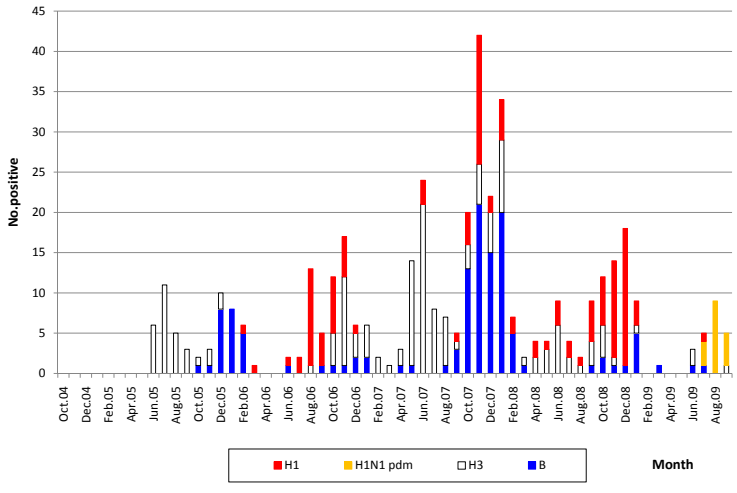
Monthly influenza isolates, Koh Samui , Surat Thani province



Monthly influenza isolates, Hat Yai , Songkhla province



Monthly influenza isolates, Health Center 17th , Bangkok province



Predominant subtypes of Influenza in Thailand, 2005-2009

2005: A / (H3)

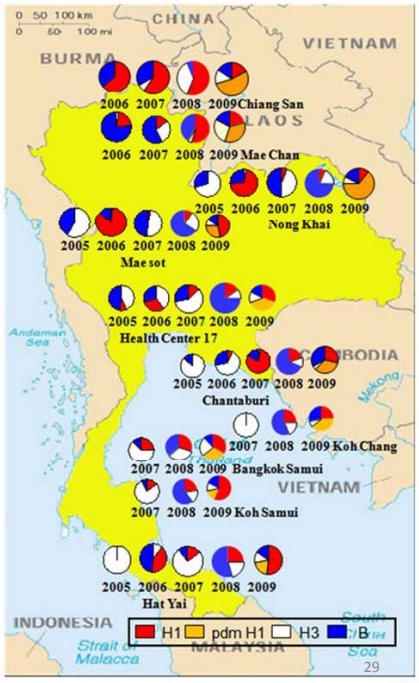
2006: A / (H1)

2007: A / (H3)

2008: B

2009: A/(pandemic H1)

Thai NIH, DMSc, Thailand



K. supawat,Thai NIH

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Positive Specimens by subtype for Influenza virus Phase I : fiscal year 2004-2009 (5 year)

Year	No.test	No.pos.	Flu A (H1)	Flu A (H3)	Flu B
2005	1,478	282(19.1%)	9(0.6%)	197(13.3%)	76(5.1%)
2006	2,763	385(13.9%)	262(9.5%)	16(0.6%)	107(3.9%)
2007	2,800	369(13.2%)	42(1.5%)	210(7.5%)	117(4.2%)
2008	4,077	1,028(25.2)	208 (5.1)	287(7.0)	533(13.1)
2009	3,428	811(23.7)	553 (16.1)*	105(3.1)	153(4.5)
Total	**14,546**	**2875(19.8%)**	**1074(7.4%)**	**815(5.6%)**	**986(6.8%)**

2009 H1 = Seasonal

K. supawat,Thai NIH

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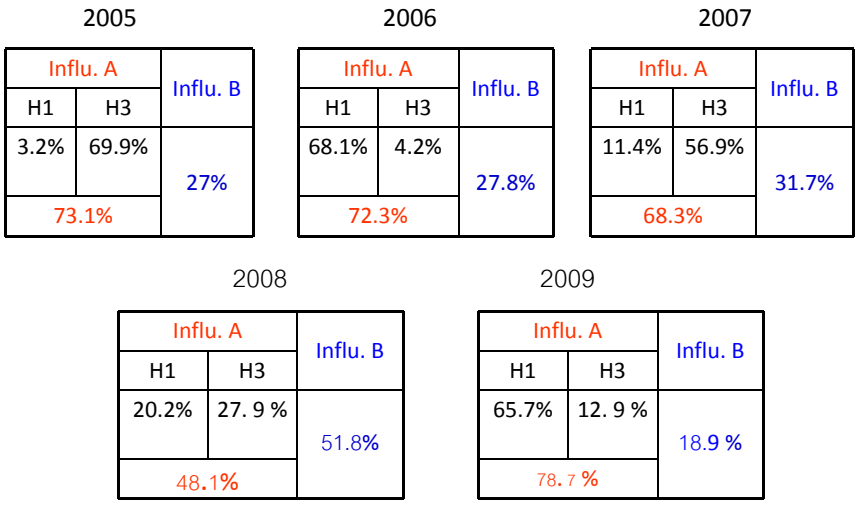
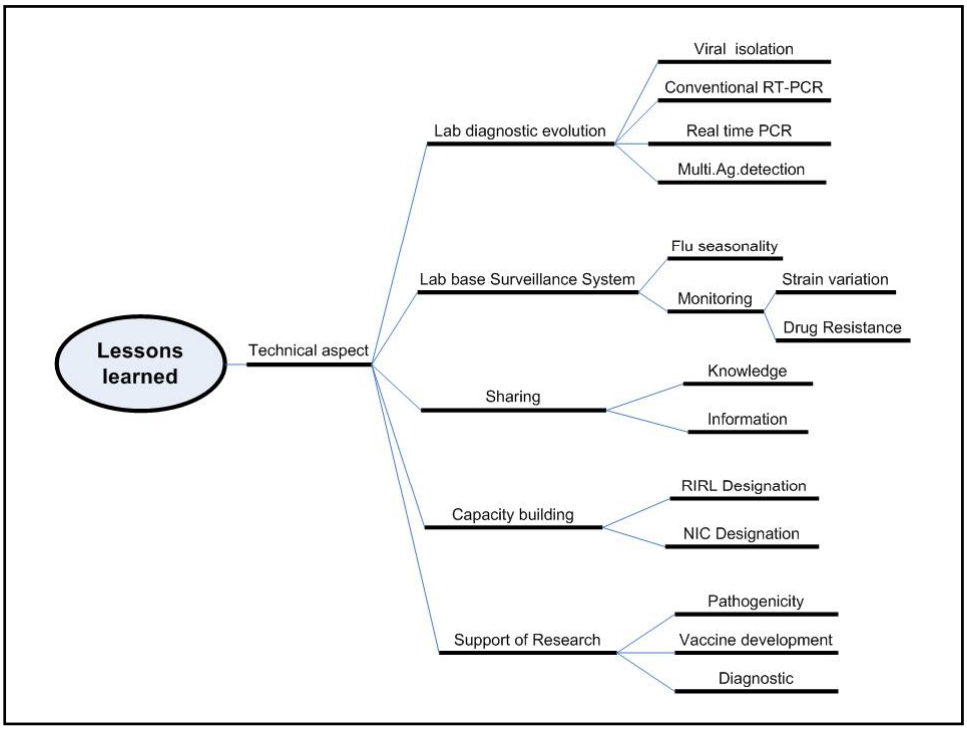
Propotion of positive Influenza cases by subtype phase I: fiscal year 2004-2009 (5 Years)



Monitoring



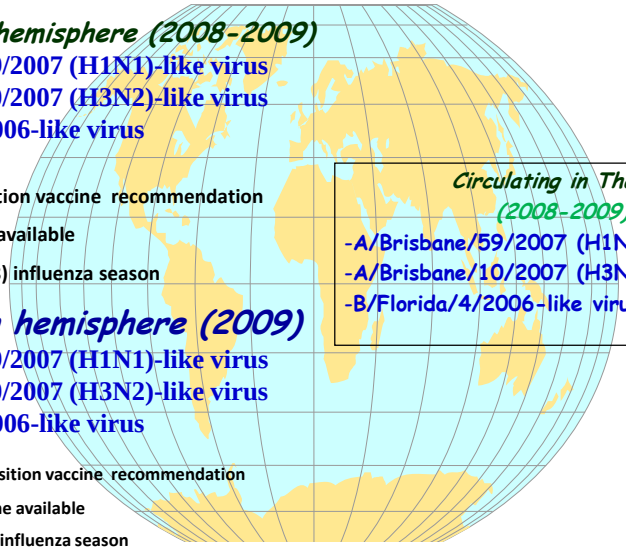
Result of strain analysis: 2005-2007

	2004-2005		2005-2006		2006-2007	
H1N1	0.6%	A/New Caledonia/20/99	9.5%	A/New/Caledonia/20/99	1.5%	A/New Caledonia/20/99 A/Hawaii/15/2001 A/Solomon Island/03/2006
H3N2	13.3%	A/California/7/2004 A/Wellington/1/2004	0.6%	A/Califonia/7/2004 A/Wisconsin/67/2005	7.5%	A/Wisconsin/67/2005 A/Brisbane/10/2007
B	5.1%	B/Hong/Kong/330/2001 B/Hawaii/33/2004 B/Shanghai/361/2002	3.9%	B/Hong/Kong/330/2001 B/Malaysia/2506/04 B/Shanghai/361/2002	4.2%	B/Malaysia/2506/2004 B/Ohio/1/2005 B/Shanghai/361/2002 B/Florida/4/2006
Neg.	80.9%		86.1%		86.8%	

Result of strain analysis: 2007-2009

	2007-2008		2008-2009	
H1N1	5.1	A/Brisbane/59/2007 A/Solomon Island/3/2006	7.4	A/Brisbane/59/2007
H3N2	7.0	A/Brisbane/10/2007	3.1	A/Brisbane/10/2007
B	13.1	B/Florida/4/2006 B/Malaysia/2506/2004 B/Ohio/01/2005	4.5	B/Florida/4/2006
Neg.	74.8		76.3	

WHO Influenza vaccine recommendation



Northern hemisphere (2008-2009)

A/Brisbane/59/2007 (H1N1)-like virus
A/Brisbane/10/2007 (H3N2)-like virus
B/Florida/4/2006-like virus

- Feb 07 :Composition vaccine recommendation
- Sep 07 :Vaccine available
- (Nov. 07 – Apr 08) influenza season

Southern hemisphere (2009)

A/Brisbane/59/2007 (H1N1)-like virus
A/Brisbane/10/2007 (H3N2)-like virus
B/Florida/4/2006-like virus

- Sep 07 :Composition vaccine recommendation
- May 08 :Vaccine available
- (May – Oct 08) influenza season

Circulating in Thailand (2008-2009)

-A/Brisbane/59/2007 (H1N1)-like virus
-A/Brisbane/10/2007 (H3N2)-like virus
-B/Florida/4/2006-like virus

Monitoring of drug resistant strain

Assay Workflow

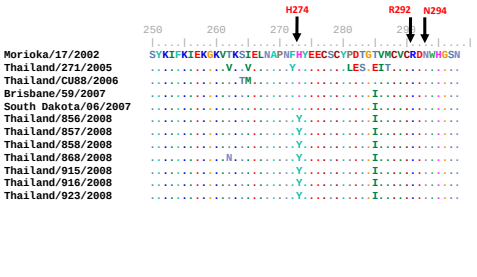
Throat swabs from sentinel sites → **Rapid Screening by Real-time PCR**

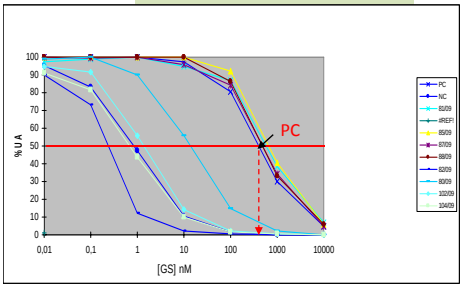
Respiratory secretion from routine service → **Rapid Screening by Real-time PCR**

Rapid Screening by Real-time PCR → **Isolation of viruses**

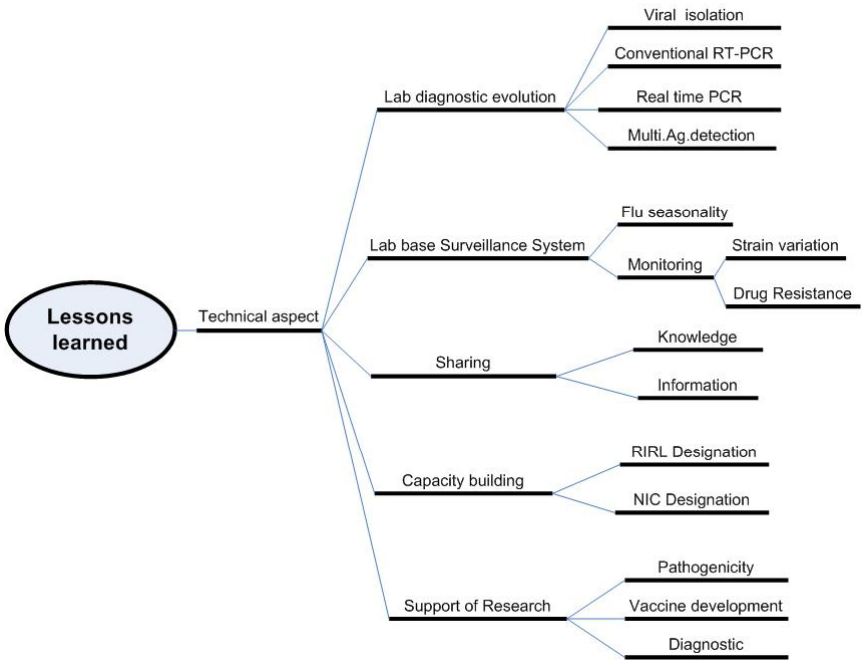
Isolation of viruses → **Identification of NAI resistant by a partial NA sequence**

Isolation of viruses → **Fluorometric Neuraminidase Inhibition Assay**

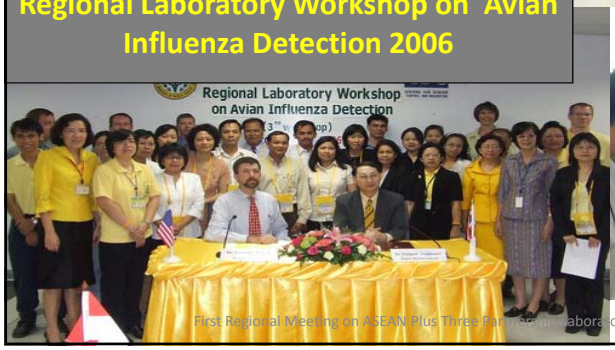




Sharing


Lessons learned
Technical aspect
Lab diagnostic evolution
Viral isolation
Conventional RT-PCR
Real time PCR
Multi.Ag.detection
Lab base Surveillance System
Flu seasonality
Monitoring
Strain variation
Drug Resistance
Sharing
Knowledge
Information
Capacity building
RIRL Designation
NIC Designation
Support of Research
Pathogenicity
Vaccine development
Diagnostic

Regional Laboratory Workshop on Avian Influenza Detection 2006



Regional workshop on PCR base diagnosis of influenza A (H1N1) June 2009

Training Workshop on Influenza Drug Resistance by Multiplex Realtime PCR For 14 RMSc
7-8 April 2010

World Health Organization

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Visit
Center for Laboratory and Epidemiology
Ministry of Health,Vientiane , Lao PDR

First time

27 Jun 06

Second time

24 Aug 07

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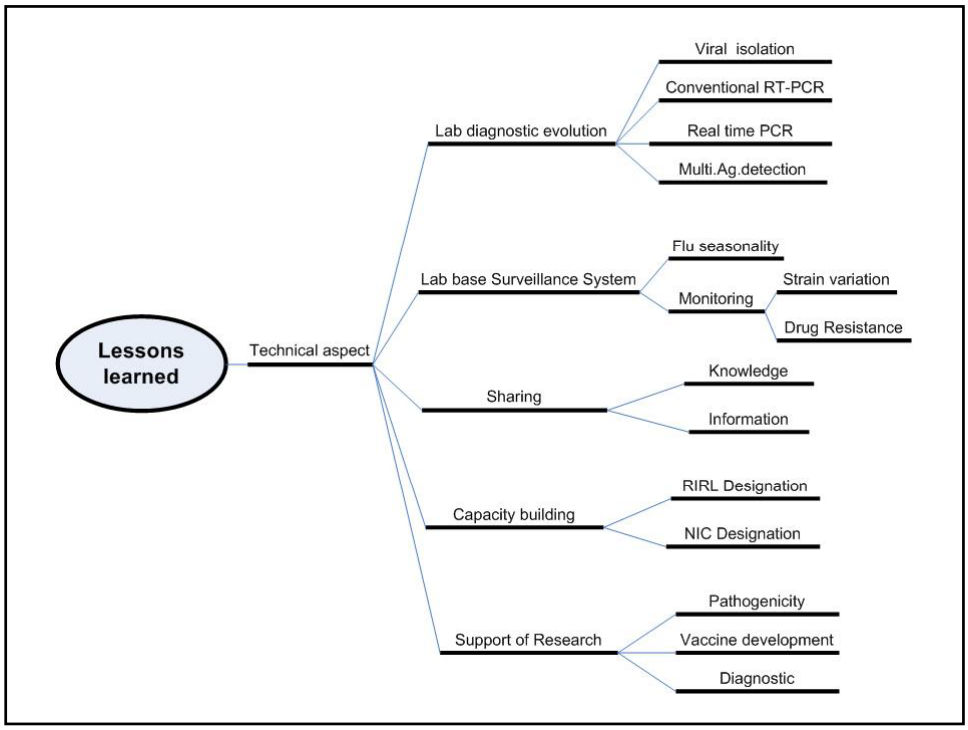
Collaborative meeting Thailand-Cambodia

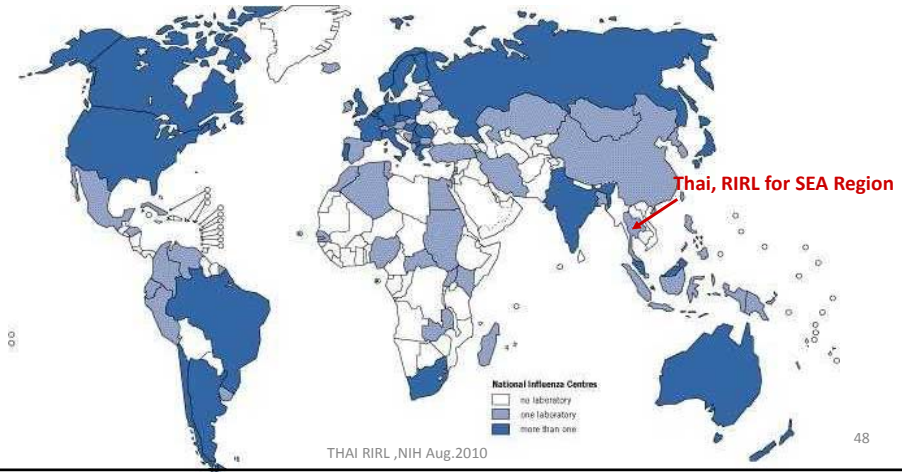
Capacity Building



WHOGlobal Influenza surveillance network

WHO National Influenza Center, Thai NIC
has been established since 1972 at NIH, DMSc, Thailand

Regional Influenza Reference Laboratory (RIRL) for SEA Region
has been designated by WHO since 22 July 2010



THAI RIRL ,NIH Aug.2010

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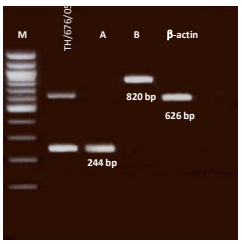
Strengthen lab. Capacity

Thai NIC, DMSc Laboratory network

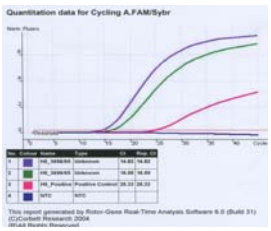
Location of
13 regional Medical Science Center

H5N1 detec

RT-PCR



real time RT-PCR



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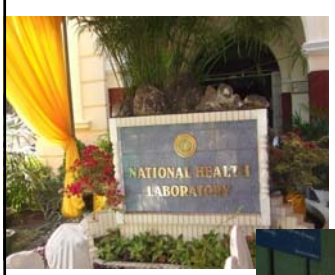
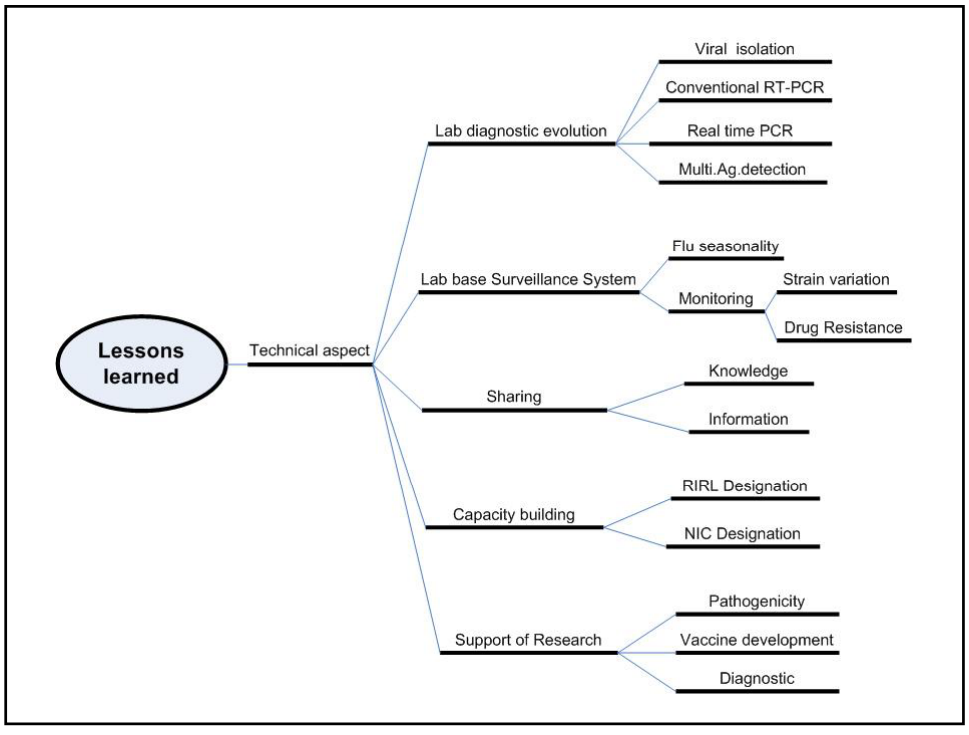
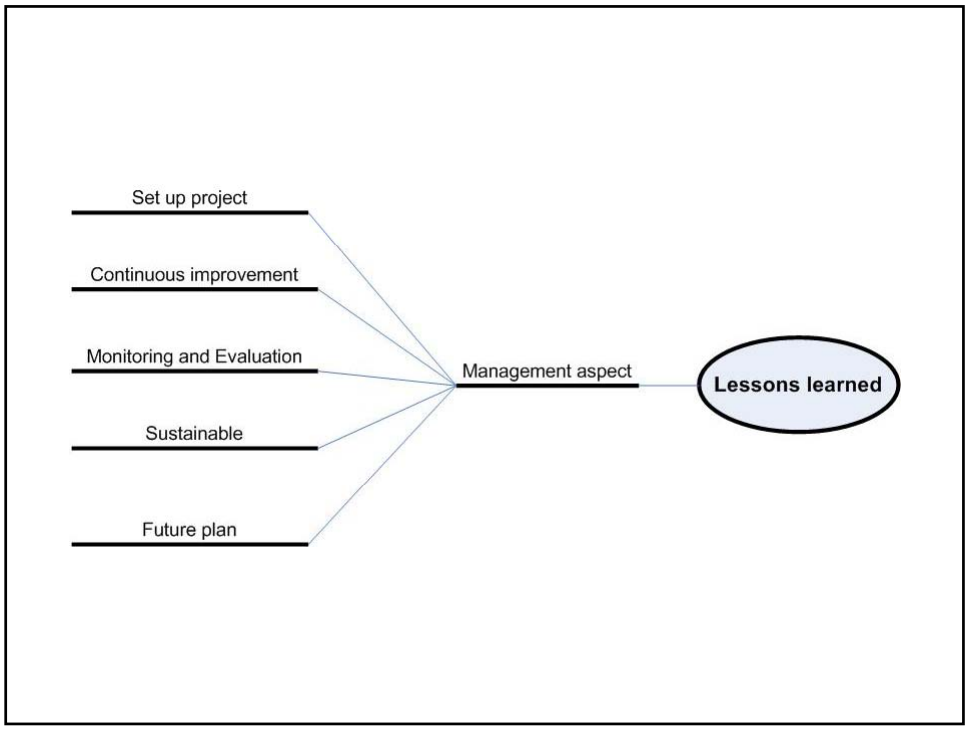
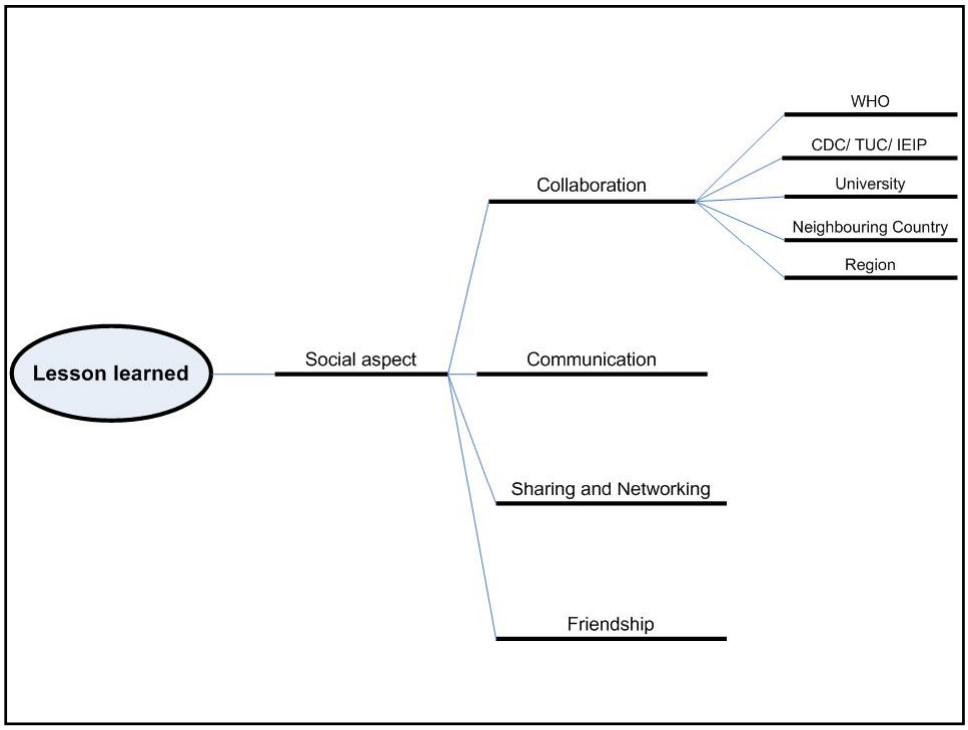
The Accreditation of National Health Laboratory as National Influenza Centre, Myanmar
26 February 2008



Support of Research




Set up project
Continuous improvement
Monitoring and Evaluation
Sustainable
Future plan
Management aspect
Lessons learned


Lesson learned
Social aspect
Collaboration
WHO
CDC/ TUC/ IEIP
University
Neighbouring Country
Region
Communication
Sharing and Networking
Friendship

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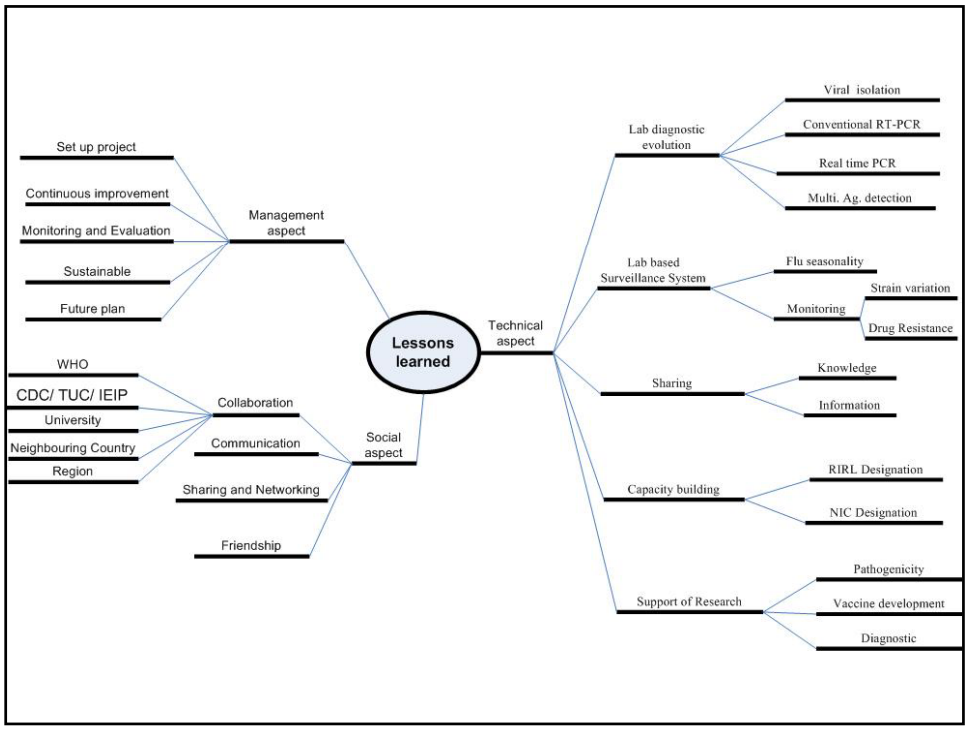
Lessons learned
Management aspect
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Social aspect
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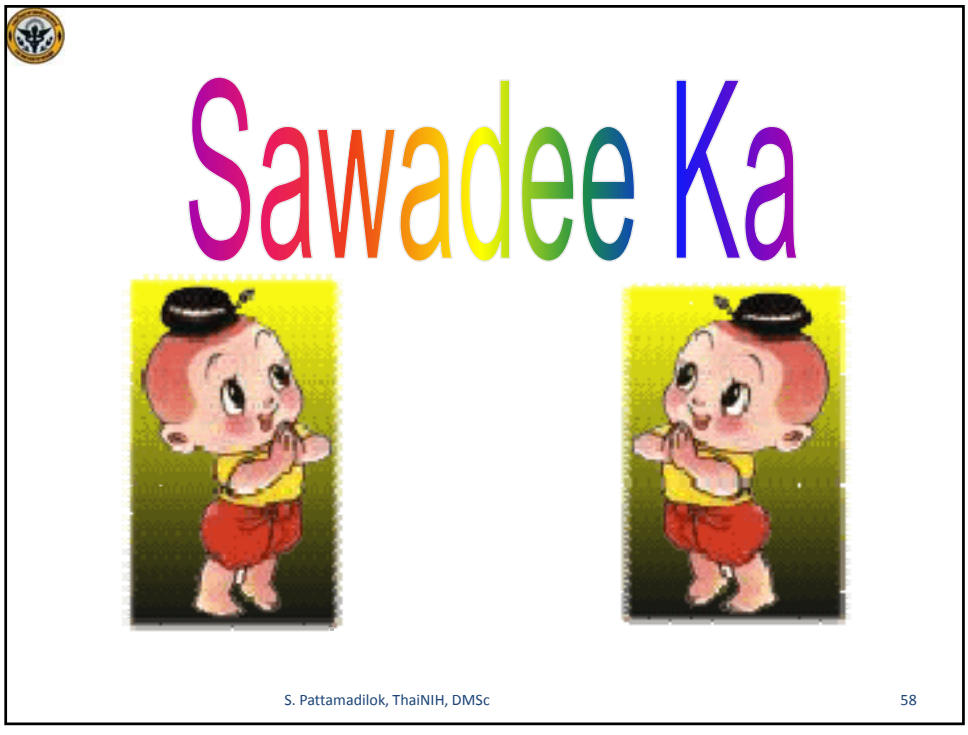
Neighbouring Country
Region
Communication
Sharing and Networking
Friendship
Technical aspect
Lab diagnostic evolution
Viral isolation
Conventional RT-PCR
Real time PCR
Multi. Ag. detection
Lab based Surveillance System
Flu seasonality
Monitoring
Strain variation
Drug Resistance
Sharing
Knowledge
Information
Capacity building
RIRL Designation
NIC Designation
Support of Research
Pathogenicity
Vaccine development
Diagnostic


Sawadee Ka
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