CHAPTER I

GENERAL INTRODUCTION

Influenza A virus is one of major causes of acute respiratory tracts infections in human populations and animal, generate lots of economic distress in terms of hospitalization, health care systemization and loss of product and work capacities. Seasonal influenza virus epidemic attacks about three to five million cases of world population annually, with 250,000-500,000 deaths [1]. Influenza A virus can circulate worldwide and there is not specific age group in influenza virus infection. Typically, this virus attacks upper respiratory tract, nose, throat and bronchi while it rarely infects lungs, classified as lower respiratory tract organs [2-4]. Influenza virus infection can cause symptoms identified as influenza-like-illness, defined by sudden high fever, nonproductive sore throat, severe malaise, headache, myalgia, runny nose, muscle and joint pain [1,5,6]. However, this typical symptoms expressing influenza A virus infection might dramatically severe, especially in patients with underlying complications, such as metabolic diseases (such as diabetes), elderly (over 65 years old), infants (under 2 years old) and patients with cancer, chronic lung and heart diseases. [7]. Severe complications due to influenza virus infection contain ear infection, sinus infection, dehydration and secondary bacterial infection (pneumonia). Influenza infection can cause the exacerbation of chronic medical complications, such as myocardial infarction,

diabetes and asthma [1,2]. This virus can spread easily via droplet transmission [8-9], which means that when individuals were infected by this virus, the infected droplets were spattered into the air when they sneeze or cough and let other persons can expose. In addition, influenza virus can be transmitted by hand when infected individual touch the surface and object.

Classification, Viral Structure and Replication Cycle of Influenza virus

Influenza virus is member of family Orthomyxoviridae [10]. This virus can display in several shapes,mostly in enveloped, spherical and approximately 80-120 nm in size (Figure 2). Its genetic materialsare covered by lipid bilayer envelope which derived from host's cell membrane with 3 transmembrane proteins, HA, NA and M2 [11-13]. Influenza A virus genome consists of eight segments of negative-sense RNA strands and encoded into 10-11 viral proteins which have different functions important for virus survival, shown in Table 1 [14,15]

Table 1. Characteristics and functions of several genes of influenza

Segment	Length(bp)	Gene Product	Size (a.a.)	Function
1	2350	PB2	759	Cap binding, endonuclease
2	2350	PB1	757	RNA polymerase
3	2250	PA	716	RNA polymerase subunit, proteolysis
4	1780	HA	560	Attachment to sialic acid, membrane

				fusion
5	1575	NP	498	Encapsidates RNAs, nuclear import of RNA
6	1420	NA	450	Sialidase activity, release of virus
7	1050	M1	252	Major structural protein, virus assembly
		M2	96	Proton channel, viral uncoating
8	900	NS1	230	Down-regulate anti-viral response
		NS2	121	Nuclear export of RNPs

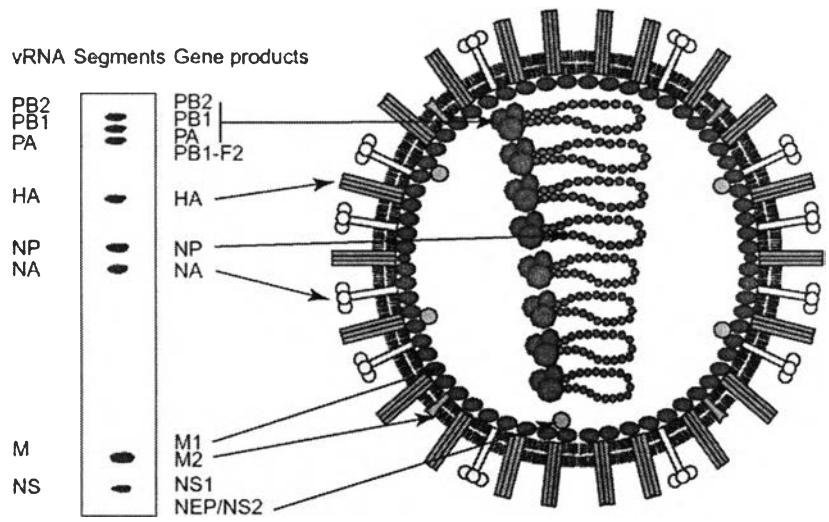


Figure 1. Influenza A virus particle. (a) An electrophoretic separation express different sizes of the influenza virus genome, which consists of eight RNA segments of negative polarity. Each segment is named according to the viral protein(s) it encodes. (b) The location of the different structural proteins within the virus particle. The nucleoprotein (NP) associates with each of the eight viral RNA (vRNA) segments to form ribonucleoprotein (RNP) complexes. The subunits of the viral RNA-dependent RNA-polymerase complex PB1, PB2 and PA are also associated with the RNPs. The viral glycoproteins, hemagglutinin (HA) and neuraminidase (NA), are exposed on the virus surface as trimers and tetramers, respectively. Matrix-protein-1 (M1) is derived from a colinear mRNA of segment 7 and forms the inner layer of the virion. A spliced mRNA of the same segment gives rise to the transmembrane M2 protein, which is a pH-dependent ion channel. Alternative splicing of segment 8 yields the non-structural protein 1 (NS1) and the nuclear export protein (NEP; also known as NS2). (Modified from Ludwig, 2003)

Three largest gene segments of influenza A virus, named as Polymerase basic 2 (PB2), Polymerase basic 1(PB1), and Polymerase acidic (PA), encoded protein subunits of viral polymerase complex, involved in RNA transcription and replication. The polymerase complex is responsible for transcribing messenger RNAs (mRNAs), for synthesizing positive-sense complementary RNA templates (cRNAs), and for transcribing the cRNAs into the virion RNA segments (vRNAs) that are incorporated into progeny viruses. Hemagglutinin (HA) which is major surface glycoprotein of virus plays 2 crucial roles, serving as a receptor-binding site for binding with sialic acid (SA) receptors of the host cell in target recognition mechanism and facilitating of influenza virus entry to cell cytoplasm through a mechanism also known as receptor- mediated endocytosis. The nucleoprotein (NP) of influenza virus encapsulates viral RNA genome to form ribonucleoprotein for stabilizing viral genome and controlling the RNA trafficking from the cytoplasm to nucleus of the infected cell. Nuraminidase (NA) is crucial enzyme for viral survival by cleaving the cellular sialic acid residues linked the influenza bud to host cell membrane to release viral progeny in the end of influenza life cycle. The seventh segment generates two gene products, the matrix protein (M1), and the ion channel (M2) protein. M1 mRNA is an unspliced transcript, and its product is the most abundant structural component in the virion and it is thought to play a fundamental role in virus assembly. The M2 is a small transmembrane protein derived from spliced mRNA. It has proton channel activity that aids in virus disassembly during the initial

stages of infection. The eighth gene segment also encodes two proteins due to alternative splicing. These proteins are referred to nonstructural proteins (NS1) and (NS2). The NS1 has several functions including a regulator of both mRNA splicing and translation, and it also plays a critical role in the modulation of interferon responses to viral infection. The NS2 function is to mediate the export of newly synthesized ribonucleoproteins (RNPs) from the nucleus, so it is also referred to as a nuclear export protein (NEP) [16].

Currently, influenza A viruses can be classified into various subtypes based on their antigenic differences between their two surface antigenic glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Serologically, 17 subtypes of HA (H1-H17) and 9 subtypes of NA (N1-N9) have been identified. [16,17] The receptor-binding efficiency of HA is specific and depend on the type of host SA and its specific linkage of oligosaccharide of the receptor. Therefore, the receptor binding efficiency of HA protein determines the host range restriction of influenza virus [18-20]. Wild aquatic birds are natural reservoirs for influenza A viruses of all known hemagglutinin (HA) and neuraminidase (NA) subtype [21]. Interspecies transmission of influenza viruses are occasionally found in other species, such as domestic poultry, sea mammals, pigs, horse, including human (Figure 2). Nowadays, only six subtypes are reported to infect humans (H1N1, H2N2, H3N2, H5N1, H7N7 and H9N2) while only four subtypes that can

infect swine (H1N1, H1N2, H3N2 and H4N6). By the way, human influenza A virus subtypes H1N1 and H3N2 are greatly different from H1N1 and H3N2 swine influenza virus.

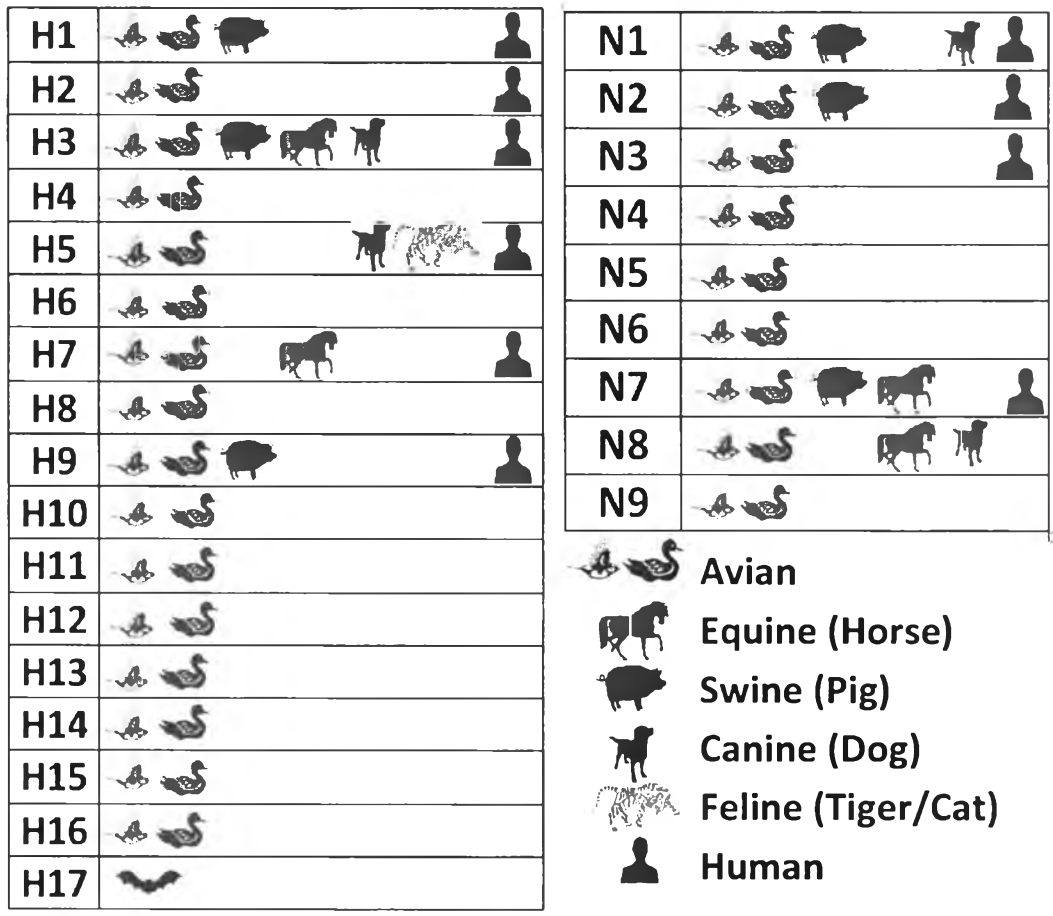


Figure 2. Host Range Restriction of Influenza A virus in 17 HA and 9 NA

The replication process of influenza virus consists ofmany complicates mechanisms which mainly focus on host cell invasion and hijacking the host cell mechanism for its own purposes. There are 5 major steps in influenza life cycle, therefore (Figure3); [22]

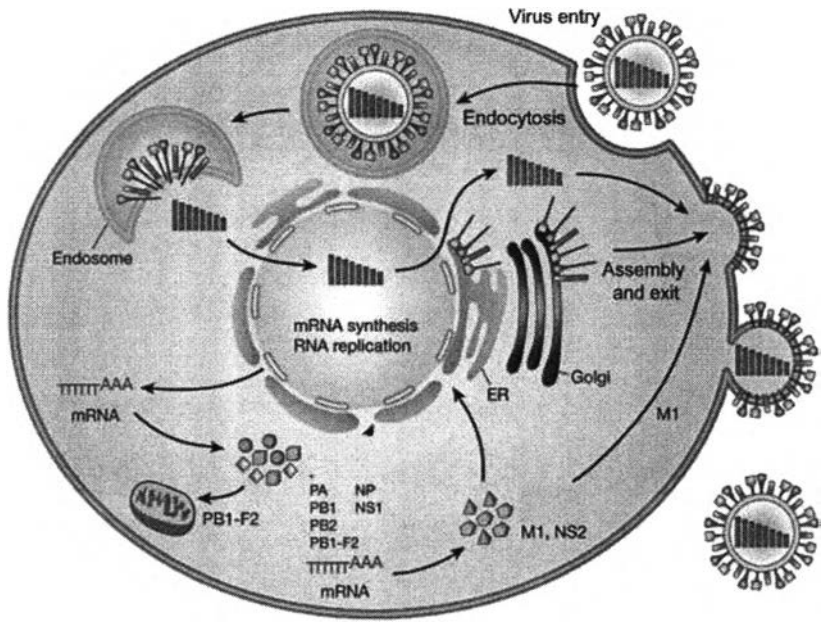


Figure 3. Influenza virus Life Cycle (Arias CF, 2009)

1. Viral entry step

Influenza A virus can attach to the host cell membrane by using the specific binding between viral surface protein called HA and sialic acid receptor of host cell [23]. The host cell restriction of influenza A virus follow upon the specificity of HA molecules in binding to cell surface sialic acid receptor. Typically, avian influenza virus binds specifically to α-2,3sialic acid receptor while human influenza virus can recognize α-2,6 linkages [23,24]. Swine influenza virus can interact with both types of sialic acid receptor, therefore, there is a chance that pig can be infected by at least 2 strains of virus at the same time, leading to the genetic reassortment events occurred in swine. Hence, swine is considered to be the "mixing vessel" of influenza virus [25,26].After specific binding with sialic acid residue, the virus invades into

host cell by receptor-mediated endocytosis, forming acidic endosome. After endocytosis, acidic environment affects the release of viral ribonucleoplrtein (vRNPs) into the host cell's cytoplasm.

2. vRNPs entry step into nucleus

After release from acidic endosomal membrane, the vRNPs must invade into nucleus of host cell, in which viral genome can be replicated and transcribed. This process can be occurred by using cellular nuclear import machinery and the support of nuclear localizing signal (NLS) proteins [27].

3. Transcription and replication process

Following the internalization of vNRPs into the nucleus,many comprehensive mechanisms occurred for replication and transcription process of RNA virus. Known as negative-sense RNA virus, influenza virus has to synthesize a complementary positive RNA strand. Many sophisticated approaches were used to "hijack" or "steal" the host cell mechanism for virus's purposes, such as addition of 5'methylated cap to increase the capability in viral RNA transcription by using the important role of 3 polymerase proteins (PB2, PB1 and PA) called "cap-snatching mechanism" [28-35]. Some genes of influenza virus can decode into more than one protein by alternative splicing. Interestingly, this virus can utilize the host cell splicing mechanism to generate both of its proteins [36]. Many studies discovered that NP and NS1

proteins involved in this mechanism and also blocked out the splicing mechanism of cellular mRNAs [37-39].

4. vRNPs transportation

The vRNPs can be transported through the nuclear pore by using the "daisy chain" structure formation. Many viral proteins such as M1, NP and NEP and a host conserved nuclear transport signal called CRM1 are responsible for vRNPs export [27,40,41]. Recently, there was a study tried to figure the live image of viral proteins transportation and their results showed that vRNPs transportation was the polarized exit because of the preferential localization of NP protein of influenza virus in the apical side called "Genome Gating" [42,43].

5. Assembly and budding process

Assembly and budding mechanism is essential for virus replication before the virus can attack neighboring cells. Due to influenza is enveloped virus, this virus needs to utilize the host plasma membrane to synthesize its lipid bilayer envelope before budding process will be occurred in the apical side of host membrane [13]. From previous study, the apical localization of HA, NA and M2 surface protein was correlated with the polarization of budding mechanism of influenza virus. M1 protein and several host factors play the important role in the last step of closing and budding of virus while M2

protein is also known as crucial factor in virus assembly and particle formation [13,44,45]. The deletion of M2 coding region can cause the elongated influenza virus particles [46]. There were 2 theories suggest the model of packaging process; the random packaging model [47,48], which described that the influenza virus can assemble randomly and the specific packaging model [49], which suggested that the virus needs specific signals in virus segments to control which direction or signal are to be packed in the virions. Finally, NA protein is essential for "pinch-off" process by cleaving sialic acid receptors before the viral particle will be free from the host cell and can infect others [50].

The specificity of infection of this virus has changed over time resulted from genetic mutations which can cause the evolution of virus. Two major processes lead to influenza virus mutations are called antigenic drift and antigenic shift respectively(Figure4) [51]. The first mutation process, antigenic drift, is the mutation caused by the accumulation of point or minor mutations that can change the properties of viral proteins due to the properties of RNA-dependent RNA Polymerase (RDRP) enzyme of influenza virus that lack of proofreading mechanism. Especially, this process usually occur in HA and NA surface proteins which have to generate new strain to avoid the host's immunity system. While the other process, antigenic shift, is a process

happening due to a genetic reassortment when at least two influenza virus infected within the same cell. This mutation process can generate the new reassortant virus that contains combined genetic materials of original strains [52]. The hybrid virus, with the resulting major antigenic alteration, can be especially virulent, because it is not restrained by host immunity to previously prevalent strains of the virus (Figure4). However, when the outbreak was prolonged,pandemic influenza virus has become a seasonal influenza virus. This circumstance suggest that it is resulted from the selection pressure from host immune response affect the mutation accumulation of 2 genes, HA and NA, coding for surface glycoproteins of influenza virus. The virus has gradually adapted in its antigenic sites to avoid the host's immune response [53].

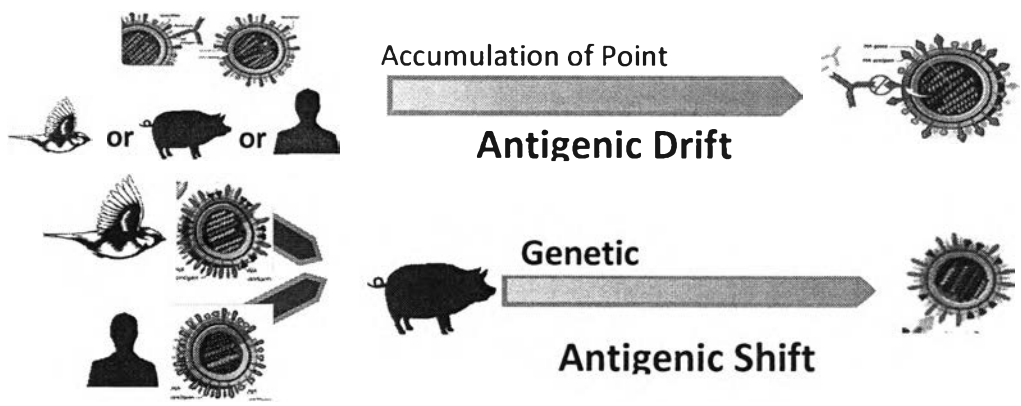


Figure 4. Antigenic drift and antigenic shift in influenza virus mutation processes

Historical Perspective of Influenza A virus Pandemics and Outbreaks

In the last century, there were many evidences supported that influenza virus had been continuously spread. The study of previous influenza pandemics will

lead to the better understanding of the current pandemic or the next pandemic in the future in terms of evolutionary trend and health policies to control the pandemic. The timeline of influenza virus pandemics and outbreaks from 19th Century – Present was shown (Figure 5); therefore, [54,55]

The Spanish Influenza Pandemic (1918-1919)

The first important influenza pandemic started in 1918. This pandemic was the most fatal cases in the history, world population were killed 50 million approximately [56]. The reassorted virus was modified from avian influenza virus. Interestingly, this virus caused disease severity that was not different

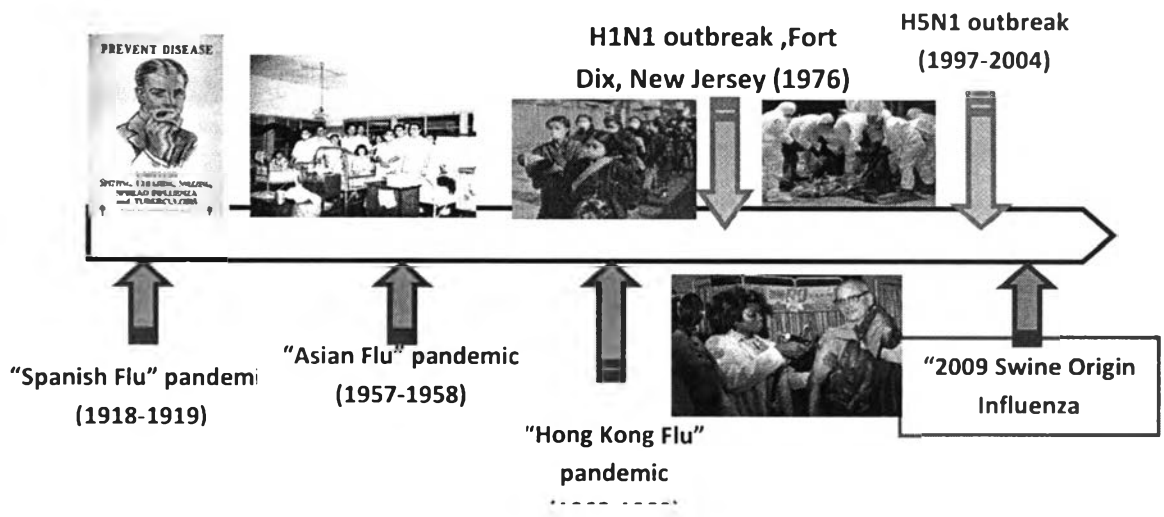


Figure 5. Timeline of Influenza Pandemics and Outbreaks

from others pandemic influenza, but individuals infected individuals were founded the severe pneumonic complications due to secondary bacterial infection [57]. In epidemiological aspect, 1918 Spanish influenza showed the unusual mortality pattern, which demonstrated that the most mortality

rate by 1918 influenza virus infection were between 20 to 40 years old [58]. The difference clinical and epidemiological aspects of this virus were still unclear. By the way, this strain of influenza virus decreased severity and became seasonal influenza after 1920.

- Asian Flu Pandemic (1957 -1958)

The pandemic of this virus had emerged between 1957-1958.The reassortant virus, which was the descendants of Spanish Flu, started from Southeast Asia before the pandemic was throughout the world. Their genome was placed with avian-like segment in HA and NA surface proteins, becoming H2N2 subtypes. Besides, avian-like gene segment was also replaced in novel virus in PB1 gene [59,60]. Although this strain of virus derived from 1918 virus, the outstanding features in age-specific mortality and severe pneumonia infection were not identified. This virus became seasonally spread after 2 years of the pandemic and became distinctive in the next 11 years after the emergence of Hong Kong Flu. Until now, this influenza strain has not returned.

- Hong Kong Flu Pandemic (1968-1969)

Replacing the circulation of H2N2 Asian Flu, the new pandemic influenza virus subtype H3N2 was appeared in 1968. Like the previous pandemic, this strain of influenza virus was modified from the last pandemic, Asian Flu, by

substitution events in HA and PB1by those from avian-like influenza virus. The mortality from the pandemic was increased from Southeast Asia before turned into endemic and sporadic outbreak rapidly. It was assumed that although this pandemic strain contained the novel HA and PB1 genes, the other genes including NA were preserved from 1957 pandemic flu [59,60]. This event affected the limited replication efficiency of this pandemic strain and less severity caused by mild antibody response to H3N2 influenza virus [61]. However, this H3N2 virus remains circulate globally until now.

- **H1N1 outbreak, Fort Dix, New Jersey, 1976**

The primary characterization of human pandemic influenza virus (pH1N1 or pdm flu)in 2009 suggested that this virus probably derived from swine influenza virus. Therefore, the pH1N1 is also called swine-origin H1N1 influenza virus (S-OIV). The classical swine influenza virus has circulates worldwide approximately 80 years. The last interesting outbreak of H1N1 swine influenza virus in human which also shared common ancestor with pH1N1virus was emerged in 1976 in Fort Dix Army, New Jersey. The outbreak of the new swine influenza virus A/New Jersey/76 resulted in 13 hospitalizations of soldiers, including 1 death. Serologic studies indicated that this virus also attacked 230 soldiers approximately. This outbreak concerns the government and health organizations about the presence of

H1N1 influenza virus after it had been disappeared since 1958 Spanish flu outbreak. However, this strain of virus seemed to be failed in human-to-human transmission, indicated by low basic reproductive number (R_0) which was less than 1.5 [62]. Many evidences suggested that this H1N1 swine influenza virus was the zoonotic peculiarity and it might be brought into the army after holidays. The cold weather and crowded community in the army support the outbreak in cramped population in the training. This virus vanished after its circulation nearly a month [63].

- **High Pathogenic Avian Influenza (HPAI) virus H5N1 outbreak (1997-2008)**

The first H5N1 HPAI virus was initially isolated in 1996 from the poultry farm in Guangdong, China [64] before the outbreak expanded throughout the live-bird markets in Hong Kong [65]. This outbreak caused the poultry culling in outbreak area. After H5N1 outbreak in poultry, CDC announced the first case of H5 HPAI virus in human with sever disease, three years-old child in Hong Kong in May, 1997 [66-68], followed by 18 human cases of H5N1 infection including 6 lethal cases [69,70] with the severe respiratory tract syndromes associated pneumonia and many complications with multiorgan failure. After depopulation all of poultry in Hong Kong, the HPAI H5N1 virus inversed the outbreak again in 2003 and attacked the poultry farms throughout Southeast Asia such as China, Viet Nam, Indonesia, including

Thailand. The H5N1 also spread in both of wild birds and poultry farms in some European countries, such as France, Germany, Austria and Hungary [71]. In Thailand, there were three waves of H5N1 outbreak in poultry from January, 2004 – December, 2005 and backyard poultry farming was responsible for the spread of influenza virus. There were 25 confirmed cases with 17 deaths of H5N1 infection in Thailand, most of them was caused by avian-to-human transmission while human-to-human transmission instance of this virus was rare [72-75]. Not only the human and poultry that can be infected by the H5N1, this virus can also transmit to mammals even feline, such as tigers and leopards by feeding the infected poultry carcasses from local abattoir [76]. Results from previous study showed that felids infected with H5N1 HPAI infection had many clinical signs, such as respiratory distress, high fever and hemorrhage in several organs. The H5N1 outbreak brought about mass poultry culling all over Asia to control the virus, which affected the loss of economic productivity and health concerns.

- Human Pandemic Influenza Virus (2009-now)

In mid-March 2009, WHO authority had announced the triple-reassorted virus which is later called human pandemic influenza virus H1N1 (pdmH1N1 or pH1N1). Genome of this pdmH1N1is composed of gene segments from human, avian, including North American and Eurasian swine influenza virus

lineages, therefore, Polymerase basic-2 (PB2) and polymerase A (PA) genes are descended from North American avian origin, hemagglutinin (HA), nuclear protein (NP) and nonstructural protein (NS) genes from classical swine influenza virus, neuraminidase (NA) and matrix genes are from Eurasian swine influenza virus while polymerase basic-1 (PB1) is originated from human H3N2 influenza virus (Figure6) [77].

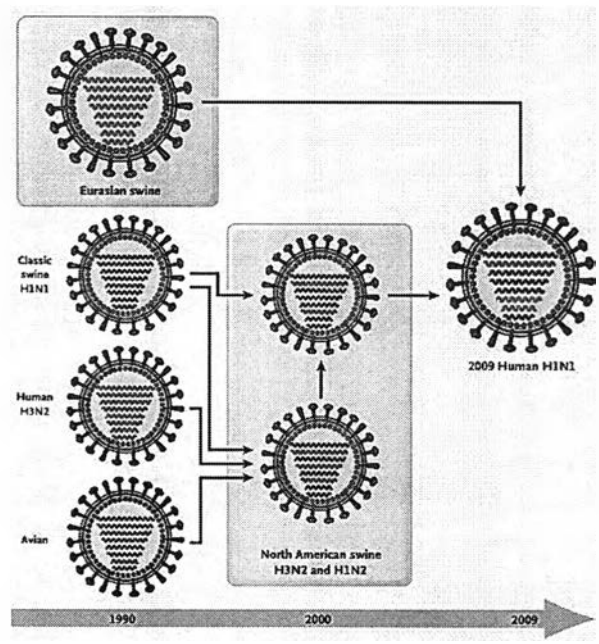


Figure 6. History of Reassortment Events in the Evolution of the 2009 Influenza A (H1N1) Virus (Trifonov V, 2009)

Prevalence and Activities of pandemic H1N1 influenza virus in Thailand

To date, more than 18,398 persons have died from pH1N1 infection with over 220,000 laboratory-confirmed cases [78]. Thus, various strategies have been devised to control outbreaks including vaccine use, which is one of the most effective means to

protect people from pH1N1 infection. However, along with vaccine development, populations should be screened for antibodies against pH1N1 in order to ascertain sufficient protection by the vaccine.

In Thailand, pH1N1 virus outbreak was occurred in May 2009, two cases were reported pH1N1 infection in Bangkok and Pattaya by the Bureau of Emerging Infectious Diseases, Department of Disease Control, Ministry of Public Health. Previous study suggested that overcrowded population can provide the chance of virus to spread and become and outbreak [79]. To date, pandemic H1N1 in Thailand has been predominated, attacked 40% of Influenza-like-illness (ILI) patients. The pH1N1 virushas peaked in 3 waves, therefore in July,2009, early 2010 and again between June and August 2010 (Figure7), which were during or instantly after rainy season. Interestingly, after three waves of pH1N1 virus in 2009 and 2010, the influenza virus subtype H3N2 was ruled over the influenza virus activity in 2011 while the seasonal H1N1 virus has been rarely identified. Unlike other human seasonal influenza viruses, pH1N1 virus mainly attacked people in younger age group (5-24 years old), while seasonal flu usually targeted in elderly (50-64 years old)[80].

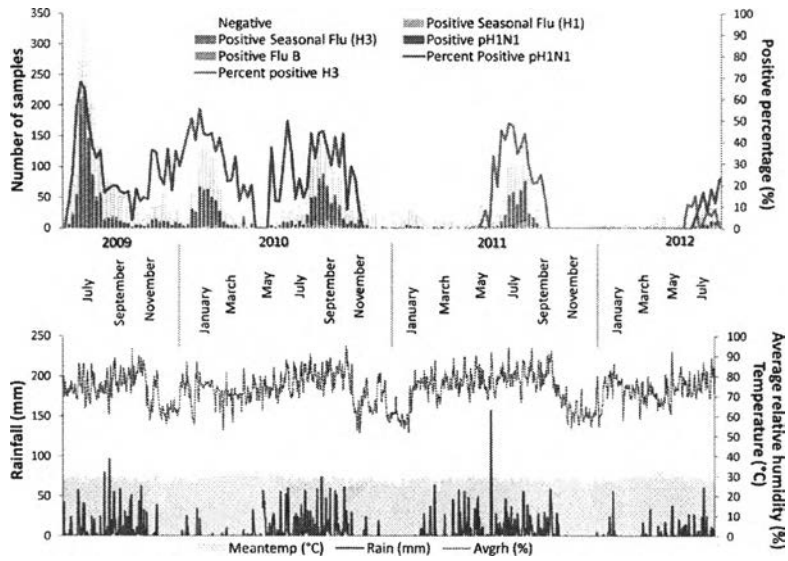


Figure 7. Influenza A viruses activity in Thailand since 2009-2012, correlated with relative humidity and temperature (Prachayangpreecha S, 2012)

Molecular Evolution and Genetic Signature of Influenza Virus

Previous studies have reported the phylogenetic evolution of pH1N1 virus since early period of pandemic [81-83], indicating that pandemic influenza virus isolates were separated into 7 clades in the first place of outbreak (Figure8). Many studies tried to observe the molecular signature of the viral genome to figure out the factors or mutations that can explain the virus emergence in human, cross-species transmission and virus pathogenicity [81-88]. According to this intense pandemic, the attempt to predict the emergence of influenza virus circulating to develop vaccine in both hemispheres has become critical. From September 2010 to January 2011, the major strains of H1N1 influenza virus infecting human are pH1N1. Hence, the vaccine virus A/California/7/2009 was included in recommended composition of influenza virus vaccines for use in 2011-2012 northern hemisphere in WHO declaration.[89] However, monitoring the adaptive mutation of pandemic influenza virus should be performed continuously to elucidate the evolutional trend for vaccine designing.

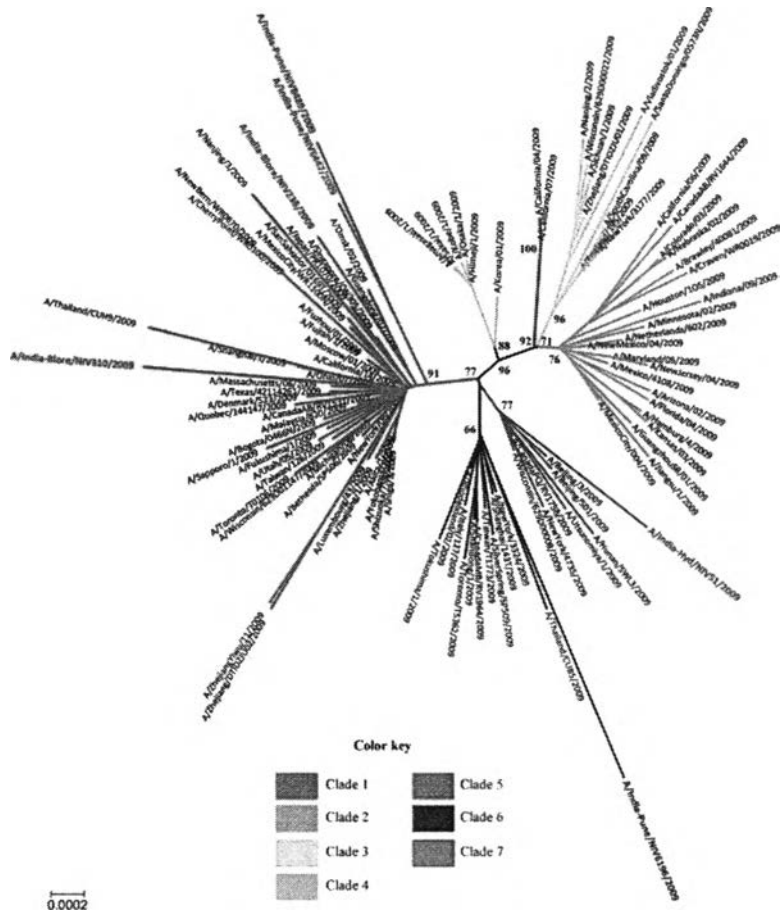


Figure 8. Phylogenetic tree demonstrate the molecular evolution of influenza A virus (Potdar VA, 2010)

Typically, influenza A virus has showed different values of dN/dS, the non-synonymous mutation and synonymous mutation ratio which can be primarily measure a relative importance of selection and genetic drifts in causing amino acid substitution [90]. In the first year of the pandemic, the dN/dS value of pH1N1 was comparatively higher than those in seasonal influenza virus (0.2-1.6 for pH1N1 virus [91]). Also like nucleotide substitution rate values, those in pH1N1 virus were relatively higher than those in seasonal influenza virus (1×10-3 to 8×10-3 substitution per site per year- [92]).

Molecular Techniques to diagnose, investigate the prevalence and neutralization antibody of influenza virus

According to this intense pandemic, the attempt to predict the emergence of influenza virus circulating to develop controlling policies has become critical. The rapid, sensitive and accurate methods needed to be invented and evaluated. The gold standard of influenza virus infection diagnosis is the real-time reverse transcription polymerase chain reaction (real-time RT-PCR) which is used to amplify the amount of specific regions of targeted viral RNA [93]. This method can be used to evaluate the genetic materials of influenza virus in terms of qualification and quantitation, even the detection of drug resistant strain [94]. However, real-time RT-PCR method is quite time consumed (3-4 hours per reaction) and the reagents used in this method are expensive. The real-time RT-PCR may not suitable in some cases needed the rapid diagnosis e.g. in patients with complications. The Rapid Diagnosis Influenza Diagnosis Tests (RDITs) are auxiliary helps but the tests needed to be usually evaluated due to the gradual mutation of influenza virus.

After the first wave of intense pandemic, many health organizations of the entire world provided many strategies to prepare themselves for the second wave of pandemic. Vaccination is one of the effective strategies for preventing influenza virus infection. Initially, molecular characterization of the human pandemic virus has shown that the vaccine for human seasonal influenza virus (H1N1) cannot boost the specific antibody against the new strain of virus due to the distinct antigenic differences between the human pandemic influenza (H1N1) and human seasonal influenza (H1N1) [95,96] while the hemagglutinin antigenic property of the human pandemic influenza is quite similar to the virus isolated from New York in 1976. A study has suggested that people above the age of 60 years could have the antibody titer required to combat the novel

pandemic strain. Hence, evaluating the antibody response to human pandemic influenza (H1N1) among Thai people will be essential for vaccine management which would be more effective. To establish the diagnosis of recent influenza infection even when the result from viral titer measurement is negative due to the clearance of viral particles from host' immunity, the serological methods are used to demonstrated the significant increase of antibody titers in sera (equal or more than 4 fold) between acute phase (day1-7 after infection) and convalescent phase (on day 21-30 after infection) of viral infection, including the evaluation of vaccine immunogenicity.

Various candidate assays can be used for detecting specific antibody titers in response to virus, such as microneutralization (MN) assay, enzyme-linked immunosorbent assay (ELISA) and hemagglutination inhibition test (HI Test). Among those techniques, microneutralization assay is the gold standard for measuring the antibody titer due to influenza virus infection [97]. However this method is the most laborious and requires expertise for infection of virus into cell culture and interpretation of the cytopathic effect (CPE), whereas ELISA is easier to process but more expensive and is occasionally prone to misinterpretation. HI test can be used for detection of specific antibody blocking the unique properties of HA to agglutinate the red blood cells. HI test represents a simple and inexpensive method which would be feasible and attractive for large-scale analysis. The advantages and disadvantages of each technique used in validating HI titer are listed in Table 2.

Table 2. Properties of techniques measuring antibody against influenza virus

Method	Advantages	Disadvantages
Microneutralization assay(MN)	High Specificity. Used as gold standard for measuring antibody level against flu	Time-consuming, require specialists to perform cell culture, analyzing CPE, qualitative, not usually quantitative
Enzyme-linked immunosorbentassay ELISA	High sensitivity, easy, more rapid than MN	Sensitive, no specific kit for pH1N1, expensive
Hemagglutination Inhibition (HI) Test	More rapid, inexpensive, easy, quantitative	May cause cross antibody, less specificity

Due to different types of glycoprotein and glycolipid composition of sialic acid receptors in each species, the influenza A virus preferentially binds to specific host erythrocytes [18-20]. Hence, human influenza A virus preferentially binds to sialyloligosaccharides containing N-acetyl neuraminic acid α2,6-galactose (NeuAcα2,6 Gal) by α2,6-galactose linkage while avian and equine influenza viruses favorably bind to oligosaccharides containing N-acetylneuraminic acid and N-glycolylneuraminic acid α2,3-galactose respectively by α2,3-galactose linkage [23,24]. According to a previous study, there are more NeuAcα2, 6 Gal molecules than NeuAcα2, 3 Gal molecules on the surface of human erythrocytes (O-blood type), turkey, pig and guinea pig erythrocytes, in contrast to chicken and goose erythrocytes [98]. Therefore, the human pandemic influenza A H1N1 may display a preference in its ability to agglutinate erythrocytes depending on the host species of origin.

Principle of virus neutralization

Virus neutralizing assay is an assay detecting the loss of infectivity of virus resulted from the specific interaction between viral antigenic epitope and specific antibody. In addition, novel pandemic virus can be identified using specific antibody. The measured antibody can be quantitated. There are many biological methods for measuring the loss of infectivity of virus, such as tissue culture or embryonated chicken eggs, but the most generally used because of its availability, capability in large-scale used and support the wide types of virus.

Principle of hemagglutination inhibition test (HI test)

The hemagglutination inhibition (HI) test is one of various laboratory techniques performed to validate the antibody titer against the pH1N1 virus. The hemagglutination technique (HA test) is based on the HA protein's ability to agglutinate red blood cells, and thus facilitates quantification of viral antigen prior to performing the hemagglutination inhibition assay [97,99]. The HI test relies on using the HA antigen to suppress hemaggutination in serum in the presence of antibodies specific to pH1N1 (Figure9).

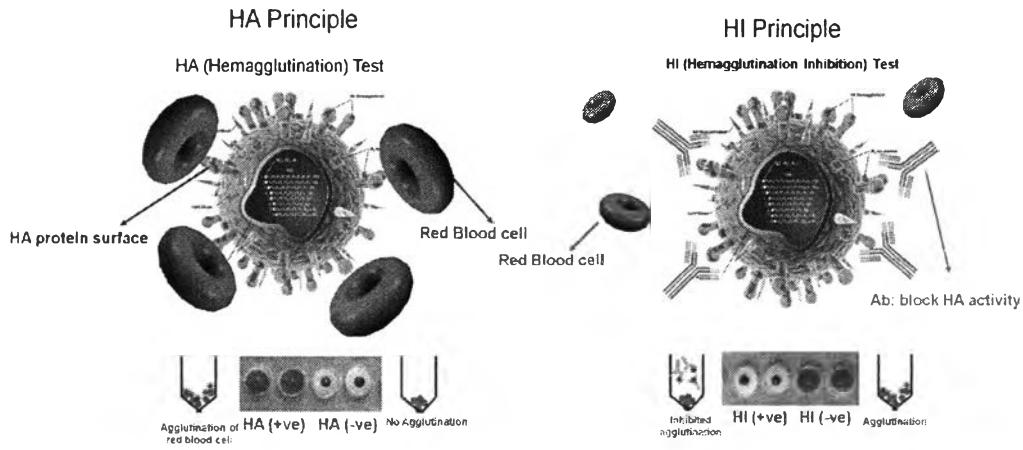


Figure 9. a) Principle of hemagglutination (HA) test b) Principle of hemagglutination inhibition (HI) Test

Part I: Whole genome characterization and phylogenetic analysis of human pandemic influenza virus H1N1 in Thailand

Research Questions

1. Are there some variations in genomic signatures between human pandemic influenza H1N1 virus isolates circulating in Thailand and those circulating in the other countries?

2. How is the genetic relatedness and phylogenetic evolution among human pandemic influenza virus circulating in Thailand and other countries over time?

3. Can vaccine developed against pH1N1 still protect Thai people from pH1N1 infection? Are there any adaptation can cause vaccine ineffectiveness?

Objectives

1. To evaluate the potential positions of mutated residues in viral virulence and pathogenesis of pH1N1 virus

2. To perform whole genome characterization to describe the evolutional trend analysis of human pandemic influenza virus which has been circulated in Thailand over three waves

3. To compare genetic similarity between pH1N1 isolates circulating in Thailand in three waves and pH1N1 used as vaccine strain

Part II: Evaluation of Influenza Virus Rapid Testing in Patients with Influenza-like Illness in Thailand

Research Questions

1. Compare to gold standard method, real-time RT-PCR, the detection of rapid influenza detection test is reliable?
2. Which trademark of RIDTs, which usually be used in Thailand, are more efficient for influenza detection and diagnosis in ILI patients in Thailand in terms of accuracy, specificity and sensitivity?
3. Can efficiency of RIDTs be deviated by any factors, such as influenza season or age groups of patients?

Objectives

1. To evaluate the efficiency of 2 trademarks of RIDTs which are extensively used in Thailand, compared to the gold standard (real-time RT-PCR) in ILI patients in Thailand
2. To monitor any factors that can distort the efficiency of RIDTs, such as age group of patients, influenza activity.

Part III: Antibody response to avian influenza virus H5N1 and human pandemic H1N1 virus

Research Questions

1. Which erythrocyte species have the most binding preference with human pandemic influenza virus H1N1?

2. From the outbreak of H5N1 avian influenza virus, are people in high risk groups, such as workers in swine farm, tiger zoo and chicken executioners infected H5N1 influenza virus?

3. Can this human pandemic virus have prenatal transmission property?

4. Can antibody titers against pH1N1 influenza virus in influenza-like illness (ILI) patients be measured by using hemagglutination inhibition (HI) test? Is ther any cross-reactivity between antibody against pH1N1 and human H1N1 seasonal influenza virus?

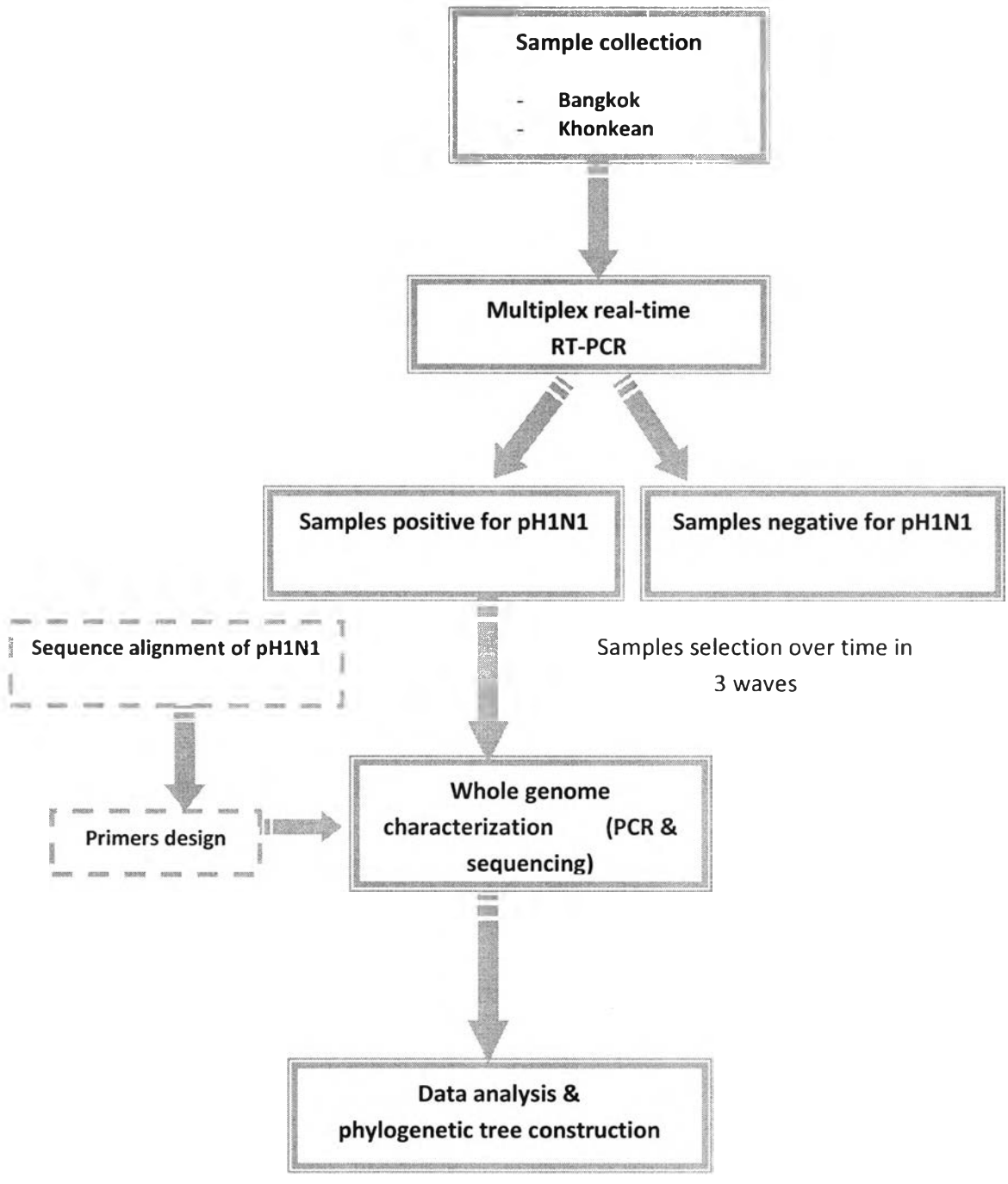
Objectives

1. To investigate the species preference in erythrocyte binding ability of pH1N1 and whether the various erythrocyte binding efficiency can affect HI titers in HI tests.

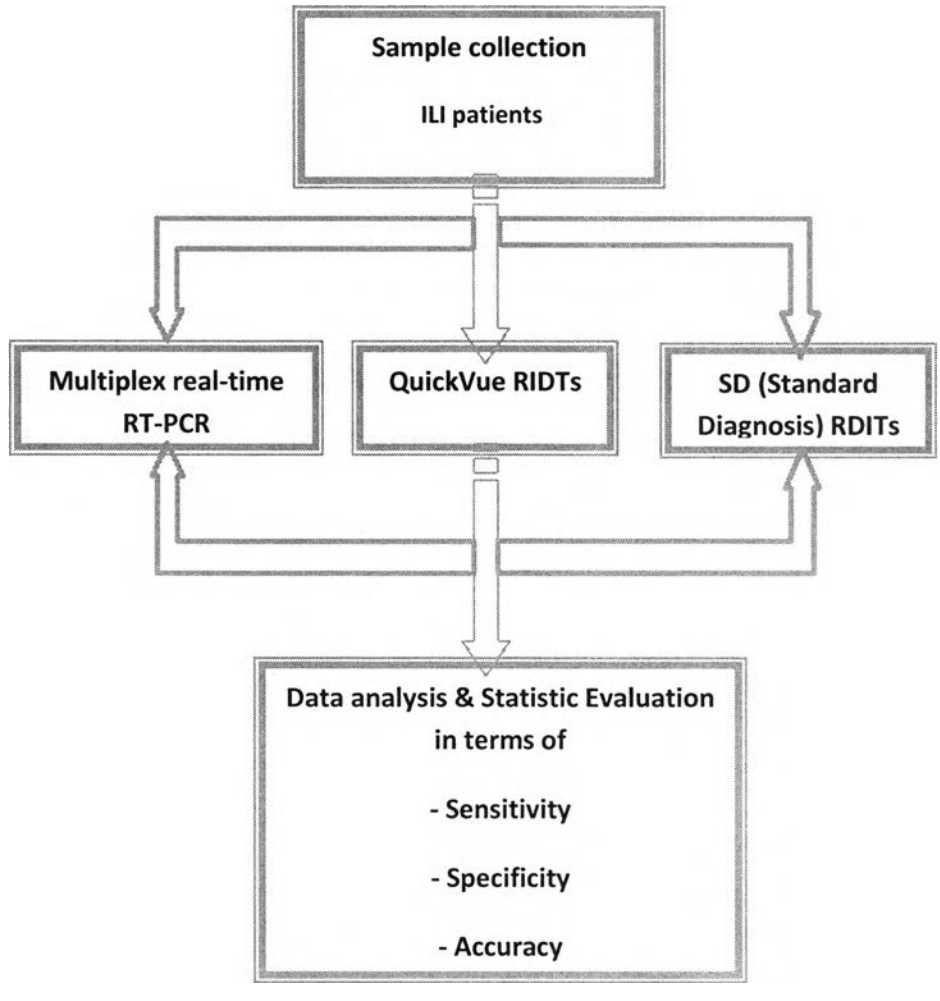
2. To investigate the antibody titers against pH1N1 in people in high risk group in Thailand

3. To evaluate the antibody titers against human pandemic influenza (H1N1) in Thai people with influenza-like illness (ILI), with and without pH1N1 infection

Conceptual framework part I



Conceptual framework part II



Conceptual framework part III