

COMMENTARY



A review of epidemic preparedness for influenza through local vaccine production: national security for Thailand

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ABSTRACT

Being vigilant of the potential for an influenza pandemic, the Global Action Plan for Influenza Vaccines (GAP) promoted the establishment and maintenance of local vaccine manufacturing capacity. In accordance with this, the Thai government has developed its national strategic plan for influenza pandemic preparedness including the plan for manufacturing influenza vaccine in Thailand. With the support of WHO, the Thai Government Pharmaceutical Organization (GPO) as the developed local production capacity of influenza vaccines. The H1N1 live attenuated influenza vaccine (LAIV) and H5N2 LAIV produced by GPO have been proven to be safe and immunogenic through clinical trials, followed by Thai FDA licensure for pandemic use. The GPO-produced H5N2 LAIV has been proven to have priming effects on an inactivated subunit H5N1 booster vaccine. This Thai local manufacturer is now expanding its capacity to Inactivated Influenza Vaccine (IIV), aiming for sustainable influenza vaccine production for national coverage.

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Current situation of influenza pandemic risk and the 2009 H1N1 pandemic

Since the first human infection of highly pathogenic H5N1 avian influenza in Hong Kong in 1997, sporadic cases have been reported off and on in several countries including in Thailand in 2004. In fact, as of May 2019, there have been 861 confirmed human infections including 455 deaths from avian influenza A (H5N1) reported from 17 countries.¹ Moreover, since March 2013 when the avian influenza A(H7N9) infection was first detected in humans, a total of 1,567 laboratory-confirmed human infections including at least 615 deaths (mainly from China) have been reported to the World Health Organization (WHO).

In 2006, with growing concern that avian influenza viruses could mutate to become transmissible between humans and cause a pandemic, the Global Action Plan for Influenza Vaccines (GAP) was launched by WHO. At the time of launching GAP, egg-based vaccine production had the capacity to produce about 350 million doses of inactivated trivalent vaccine containing 15 µg of hemagglutinin (HA) per dose per year, or with optimization (working three shifts/24 h) up to 500 million doses. Even if manufacturers were to optimize output and expand vaccine production-capacity in the following 2–3 years, it was predicated that by 2009 the estimated maximum production capacity would be 780 million doses of inactivated trivalent vaccine and 2,340 million doses of monovalent pandemic influenza vaccine.² Therefore, one of the major approaches to increasing supplies of pandemic influenza vaccine was capacity building and infrastructure strengthening or new production facilities in developing and/or industrialized countries; several developing country vaccine manufacturers participated in the

GAP technology transfer programme, including the Government Pharmaceutical Organization (GPO), Thailand which signed an agreement with WHO in 2007.

At the time of the influenza A(H1N1) pandemic in 2009, the first imported case of pandemic H1N1 in Thailand was in early May 2009. By the second week of June 2009, there were two influenza outbreak notifications in the central region of Thailand, and then only a few weeks later the influenza virus spread throughout the country.³ At that time, there was no commitment to supplying inactivated influenza vaccines to Thailand during potential pandemics and there was no domestic production of bulk influenza vaccines. In fact, prior to 2007, there was no experience in egg-based vaccine production. By 2010, Thailand was importing 2.1 million doses of seasonal influenza vaccine: GPO-MBP, a joint venture between the Government Pharmaceutical Organization (GPO) and Sanofi Pasteur, supplied approximately 800,000 doses of seasonal influenza vaccine by formulating and filling imported bulk, and the remaining 1.3 million doses were imported as finished products.

In 2005, the Thai government issued the first national pandemic influenza preparedness plans in response to the highly pathogenic avian influenza outbreak in 2004. The second national strategic plan was issued for the year 2008–2010. The first national strategic plan for influenza pandemic preparedness (2005–2007) focused on supporting research and development of vaccines and antivirals during outbreaks.⁴ In the second national strategic plan (2008–2010), it was emphasized that Thailand would set up a local industrial-scale manufacture plant for egg-based pandemic influenza vaccines and train personnel for industrial-scale vaccine research and development processes.⁵

With the support from WHO and the Biomedical Advanced Research and Development Authority (BARDA), the GPO has been able to develop live attenuated influenza vaccine (LAIV) for potential pandemic vaccines (H1N1 and H5N2 LAIV) and inactivated influenza vaccine for seasonal use.

In 2009, the Thai Government Pharmaceutical Organization (GPO) decided to focus on development of LAIV because of the potential advantages of LAIVs compared to IIVs: LAIVs manufacturing does not require down-stream processing and the harvested vaccines can be simply packaged; confer high yields (20–50 doses of monovalent vaccine per an egg) compared to the IIVs; delivered needle-free (intra-nasal spray application) which can be facilitated in resource-poor settings; and induce a broad-immune responses including mucosal, systemic and cross-reactive responses.^{6,7} In May 2009, there was a sub-license agreement with WHO to obtain Russian LAIV technology from the Institute of Experimental Medicine (IEM), Saint Petersburg, Russia. After receiving the influenza A H1N1 (2009) pre-master seed from Russia through the support of WHO, the first pLAIV vaccine concentrate was harvested, and the first pLAIV clinical lot was filled in late August 2009. Based on the WHO recommendations for production and quality control of LAIV, the GMP-certified pilot plant was built at Silapakorn University in Bangkok, Thailand.⁸ Some initial obstacles were encountered such as a lower yield of doses than expected, the need to import a huge number of specific pathogen-free (SPF) eggs and longer time for the optimization processes.⁹ The H1N1 monovalent vaccine was shown to be safe and immunogenic, fulfilling the criteria for licensure for emergency use.⁸

GPO-manufactured live attenuated influenza H5 candidate vaccine

GPO had received master donor seed H5N2 candidate vaccine strain (A/17/turkey/Turkey/05/133) from WHO. The product was assessed in preclinical and nonclinical studies and then moved to a phase I clinical trial. After a safety interim analysis, the Data Safety Monitoring Board agreed to move into a phase II study in healthy participants.

1. Phase I Safety and Immunogenicity of Live Attenuated Influenza H5 Candidate Vaccine Strain A/17/turkey/Turkey/05/133 (H5N2) in Healthy Thai Volunteers

This was a double-blind randomized study in 24 participants aged 18–45 years in 2012, 16 and 8 participants received vaccines and placebo, respectively. The Primary objective was to evaluate safety and reactogenicity of candidate H5 LAIV (strain H5N2) manufactured by GPO in healthy Thais with the humoral immune response by using hemagglutination inhibition (HAI) test and microneutralization assay as a secondary objective. Viral shedding and the stability of the viral strain were measured by polymerase chain reaction (PCR). The vaccine used the cold-adapted temperature-sensitive (*ca-ts*) live attenuated H5 candidate strain. The A/17/turkey/Turkey/05/133 (H5N2) vaccine was administered with 0.25 ml to each nostril by nasal spray on day 1 and day 21. The safety and immunogenicity monitoring were done up to 60 days. Participants were kept isolation and were discharged when nasal swabs

were negative to ensure that there was no risk to the environment.

The A/17/turkey/Turkey/05/133 H5N2 candidate vaccine was safety and immunogenic. There were a total of 51 adverse events by systemic organ classes, 35 adverse events were reported from the vaccine group (16 participants), and 16 events were from the placebo group (8 participants). The most frequent adverse events were respiratory/thoracic/mediastinal disorders both in the vaccine group (16 events) and the placebo group (6 events), followed by gastrointestinal disorders in the vaccine group (6 events) and the placebo group (4 events). Among the total of 47 local reactions (24 after 1st immunization and 23 after 2nd immunization), 31 were from the vaccine group and 16 were from the placebo group. After the 1st immunization, the most frequent local reaction in vaccine group was stiffness of the nose (6 events), while placebo group reported only 1 event of the stiffness of the nose after the 1st immunization. After the 2nd immunization on day 21, the most frequent local reactions in the vaccine group were stiffness of the nose (6 events) and scratchy throat (4 events).

Viral shedding and viral strain stability results

At the 1st immunization, 14 vaccinees out of 16 showed positive PCR results, and 10 vaccinees persisted to day 3. After the 2nd immunization, 13 out of 15 vaccinees had positive PCR results on day 1, and 5 vaccinees persisted up to day 3 following the 2nd immunization. One vaccinee's nasal swab had positive PCR results up to day 5 after the 2nd immunization. The nasal swab culture in eggs showed fewer positive results: 5 out of 16 vaccinees' nasal swab were culture-positive after the 1st immunization and no one had positive culture after day 1. Only one vaccinee's nasal swab was culture-positive on day 1 after the 2nd immunization. No influenza virus was isolated from Madin-Darby Canine Kidney epithelial (MDCK) cell cultures. There were nucleotide/amino acid changes in *NP*, *NA*, and *PB1* genes but not in *PA*, *PB2*, *M*, *NS*, and *HA* genes.

2. Phase II Safety and Immunogenicity of Live Attenuated Influenza H5 candidate vaccine strain A/17/turkey/Turkey/05/133 (H5N2) in healthy Thai volunteers

This was a double-blind randomized placebo-controlled study using the same dose as in phase I study. The study was conducted from January to June 2013. The purpose of this study was to evaluate the immune response and safety of live attenuated influenza H5 vaccine candidate strain A/17/turkey/Turkey/05/133 (H5N2) manufactured by GPO in healthy Thais. One hundred and fifty participants (100 vaccinees and 50 placebos) aged 18–49 years were enrolled. Due to limited space in the isolation ward of the clinical trial unit, Participants were separated in 4 batches; Batch 1: 36 participants (24 vaccinees and 12 placebos) Batch 2: 38 participants (25 vaccinees and 13 placebos) Batch 3: 38 participants (25 vaccinees and 13 placebos) Batch 4: 38 participants (26 vaccinees and 12 placebos). Each batch was admitted to the isolation ward for 5 days after each immunization mainly for safety assessment and to ensure that there was no risk to contaminate the external environment with the vaccine strain. Two doses of live attenuated influenza H5 vaccine candidate strain A/17/turkey/Turkey/05/133 (H5N2) were given by

intranasal route on days 1 and 28. Follow up for nasal swab culture was done on days 2, 3, 5 and participants were discharged if culture-negative. Anyone who was still positive for nasal shedding on day 3 was given oseltamivir. Blood withdrawal was done on days 1 and 28 before immunization and on days 49 and 60, to measure immune responses by HAI, microneutralization, and serum IgG and IgA ELISA. 45 participants were randomized for nasal wash specimen on days 1, 14, 28 and 49. The trial could be terminated by any of those followings: If subject experienced disability or severe adverse event or death which was definitely related to the study vaccine in the investigator's opinion; Data and Safety Monitoring Board (DSMB) judges to terminate the trial. The detailed information about the vaccine and the study procedure was previously described.¹⁰

Characteristics of screened volunteers

Between Feb 4, 2013, and Feb 28, 2013, 256 individuals were screened, of whom 152 participants were enrolled and 104 were excluded because of abnormal laboratory tests, abnormal chest x-rays, and medical history.¹⁰ The characteristics of the screen-failed and enrolled participants were not statistically different in terms of age, height, weight and gender composition (Table 1).

Table 1. Baseline characteristics of screen-failed and enrolled participants in the phase II trial conducted in Thailand.

Personal data	Enrolled fail	Enrolled participants	P-value
	(N = 104)	(N = 152)	
Sex			
– Male	42(40.38%)	60(39.47%)	0.884
– Female	62(59.62%)	92(60.53%)	
Age (years)			
– Mean ± SD	31.20 ± 9.00	31.27 ± 8.54	0.948
Height (cm.)			
– Mean ± SD	162.3 ± 8.46	162.1 ± 7.93	0.893
Weight (kg.)			
– Mean ± SD	61.53 ± 13.69	58.98 ± 9.36	0.102

Safety results

There was a total of 138 adverse events: 95 events from 101 vaccinees and 43 events from 51 placebos. Eighty (84%) of 95 events in the vaccine group and 32 (78%) of 43 events in the placebo group were reported as probably related to vaccination.¹⁰ There was no adverse event reported as definitely related to vaccination.

The most frequent adverse event reported according to the organ classes was of respiratory, thoracic and mediastinal disorders both in vaccine group (36.84%) and placebo group (23.26%), and the second most frequent adverse event was of infections and infestations both in vaccine group (15.79%) and placebo group (18.6%) (Figure 1). The most frequent systemic reaction was post-nasal drippings in both groups after the first immunization (Figure 2).

Immune responses

Humoral immune response was measured using hemagglutination inhibition test (HAI), microneutralization assay (MN), serum IgA and serum IgG. On day 21, 14 of 100 (14%) of participants in the vaccine group had a fourfold or greater rise of antibodies by at least one of HAI, MN, IgA or IaG assays. On days 49 and 60, 36 (36%) and 53 (53%) out of 100 vaccinees, respectively, showed a 4-fold or greater increase of antibodies with at least one of these four assays, while no 4-fold or greater rise was observed in the placebo group on any days.

H5N2 LAIV also induced local antibody responses and cellular immune responses in cytotoxic T cells, different T-helper cell populations, and virus-specific immune memory cells. A recent study conducted in the US by K. Subbarao showed that influenza A(H5N1) pLAIV recipients with poor primary immune responses were actually having a long-lasting immunity and could be boosted with inactivated influenza H5N1 vaccine at 52, 54 or 56 months after the immunization by pLAIV.¹¹

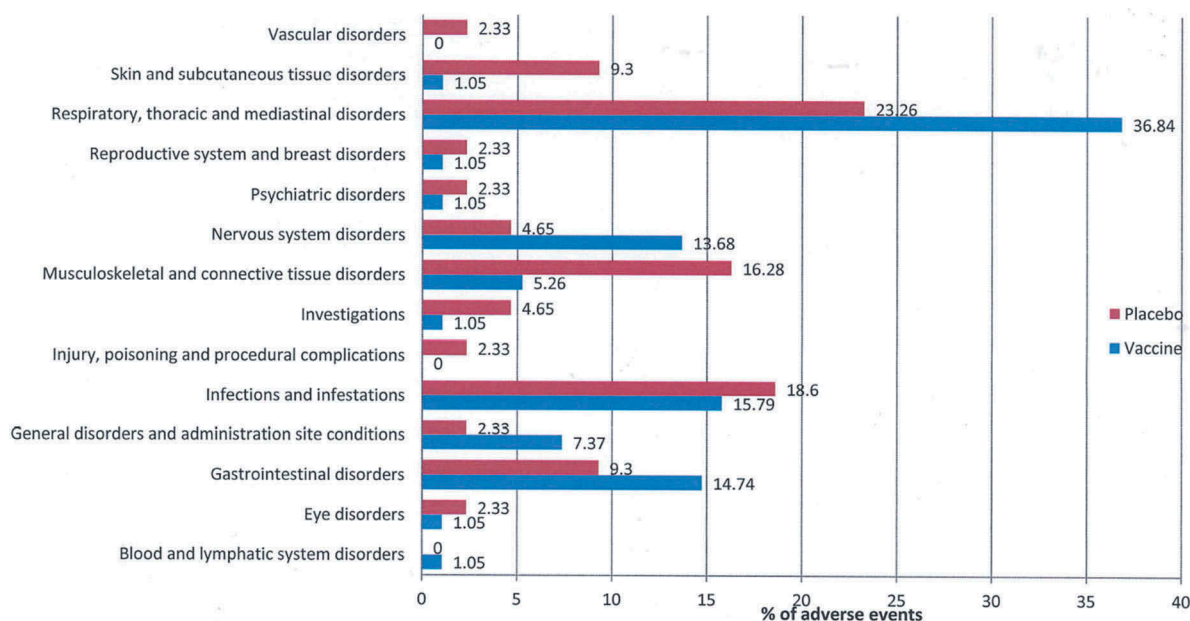


Figure 1. Adverse events by systemic organ classes (%by each group) either receiving H5N2 LAIV vaccine or placebo.

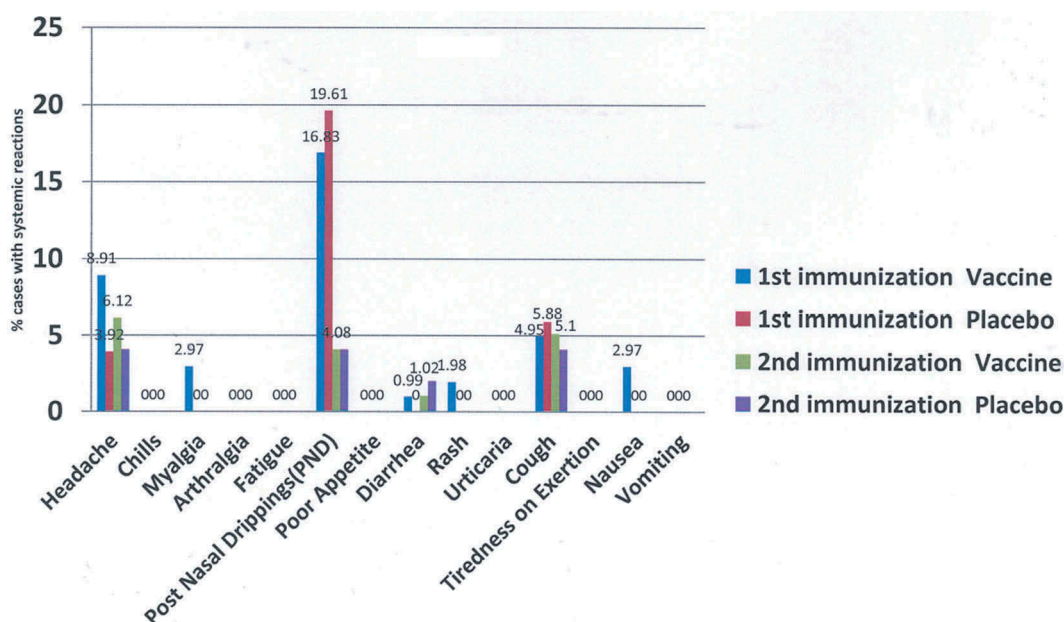


Figure 2. Systemic reactions (%) after 1st and 2nd immunization with H5N2 LAIV vaccine or placebo.

3. Evaluation of Priming Effects of H5N2 pLAI Vaccine on the Subsequent Response to Inactivated H5N1 Vaccine (“OrniFlu”, Federal State Scientific-Industrial Company Microgen for Immunobiological Medicines, Ministry of Health, Russia)¹⁰

This study aimed to evaluate the effect of H5N2 LAIV priming on an inactivated subunit H5N1 booster vaccine with an approximate 1 year of the prime-boost interval.

Materials and methods of the study

The booster vaccine used was subunit aluminum hydroxide adsorbed influenza vaccine containing HA and NA proteins from the influenza virus A/turkey/Turkey/1/05 (H5N1). Sixty participants (40 vaccinees, 20 placebos from the previous phase II study) have been enrolled and received one dose of the H5 inactivated influenza vaccine (IIV) 1 year

after the H5N2 vaccination. The total follow-up time was 90 days.

Immune responses

In the vaccinated group who had been previously immunized with two doses of H5N2 LAIV 1 year before the booster vaccination, 39 (98%) of 40 participants developed a four-fold or greater rise of antibody titres against the strain A/turkey/Turkey/05/133 in HAI assay and 38 (95%) of 40 in MN assay on day 7. All participants had a four-fold or greater increase in antibody titers (peak GMT) against A/turkey/Turkey/05/133 and A/Thailand/1(KAN-1)/04 (similar to wild type H5 virus which was circulated in Thailand) in both HAI and MN on day 28 (Tables 2–4). While in the control group who had received placebo, the four-fold or greater increase

Table 2. Comparing seroconversion rates of 4 fold-rising between previously vaccinated with LAIV H5N2 and naïve subjects by hemagglutinin-inhibition assay (HAI) at day 1, 7, 28 and 90 (Test Virus: A/17turkey/05/133 (H5N2)).

Day	Group	4-fold rising N (%)
Day 1	Vaccinated (N = 40)	0(0.00)
	Naïve (N = 20)	0(0.00)
Day 7	Vaccinated (N = 40)	39(97.50)
	Naïve (N = 20)	3(15.00)
Day 28	Vaccinated (N = 40)	40(100.00)
	Naïve (N = 20)	14(70.00)
Day 90	Vaccinated (N = 40), (n = 39)	39(97.50)
	Naïve (N = 20)	15(75.00)

p- vale = -[3]
p- vale = <0.0001 [2]α
p- vale = <0.0008 [1]α
p- vale = <0.0031 [1]α

SID 93014 in previously vaccinated with LAIV H5N2 group got missing data at day 90 both in HIA and micro NT.

[1] Overall p-value (2-sided) based on Fisher’s exact test.
[2] Overall p-value (2-sided) based on Chi-square test.
[3] No statistic was considered.
- Intention to Treat (ITT)
α – significant difference

Table 3. Comparing seroconversion rates of 4 fold rising between previously vaccinated with LAIV H5N2 and naïve subjects by hemagglutinin-inhibition assay (HAI) at day 1, 7, 28 and 90 (Test virus: rg-H5N1-KAN-1).

Day	Group	4-fold rising N (%)
Day 1	Vaccinated (N = 40)	0(0.00)
	Naïve (N = 20)	0(0.00)
Day 7	Vaccinated (N = 40)	35(87.50)
	Naïve (N = 20)	0(0.00)
Day 28	Vaccinated (N = 40)	40(100.00)
	Naïve (N = 20)	3(15.00)
Day 90	Vaccinated (N = 40), (n = 39)	38(95.00)
	Naïve (N = 20)	4(20.00)

p- vale = -[3]
p- vale = <0.0001 [2]
p- vale = <0.0001 [2]
p- vale = <0.0001 [1]

SID 93014 in previously vaccinated with LAIV H5N2 group got missing data at day 90 both in HIA and micro NT.

[1] Overall p-value (2-sided) based on Fisher’s exact test.
[2] Overall p-value (2-sided) based on Chi-square test.
[3] No statistic was considered.
- Intention to Treat (ITT)

Table 4. Geometric mean of immune response by previously vaccinated with LAIV H5N2 and naïve subjects.

Antibody assay	Test virus	Study group	Day 1 GMT (95% CI)	Day 7 GMT (95% CI)	Day 28 GMT (95% CI)
HAI Assay	A17/turkey/Turkey/05/133	Previously vaccinated	3.92 (3.24, 4.75)	211.12 (134.45, 331.52)	566.89 (436.97, 735.44)
		Naïve	2.59 (2.41, 2.78)	3.66 (2.60, 5.15)	25.49 (11.82, 54.96)
		P – value = 0.0011 ^[5] α	< 0.0001 ^[5] α	< 0.0001 ^[5] α	
	A/Thailand/1(KAN-1)/04	Previously vaccinated	2.59 (2.46, 2.72)	32.49 (22.01, 47.96)	98.49 (75.44, 128.58)
Naïve		2.5 (-)	2.77 (2.46, 3.12)	5.18 (2.90, 9.25)	
		P – value = 0.3254 ^[5]	< 0.0001 ^[5] α	< 0.0001 ^[5] α	
Micro NT	A17/turkey/Turkey/05/133	Previously vaccinated	8.56 (6.48, 11.29)	528.93 (333.88, 837.91)	1,395.8 (1,040.8, 1,872.0)
		Naïve	2.68 (2.42, 2.96)	3.42 (2.47, 4.72)	17.41 (9.05, 33.48)
		P – value = < 0.0001 ^[5] α	< 0.0001 ^[5] α	< 0.0001 ^[5] α	
	A/Thailand/1 (KAN-1)/04	Previously vaccinated	2.5 (-)	25.49 (17.90, 36.30)	52.78 (38.92, 71.57)
Naïve		2.5 (-)	2.5 (-)	3.19 (2.50, 4.06)	
		P – value = 1.0000 ^[5]	<0.0001 ^[5] α	<0.0001 ^[5] α	

SID 93014 in previously vaccinated with LAIV H5N2 group got missing data at day 90 both in HIA and micro NT.

[4] Overall *p*-value (2-sided) based on Independence T-test.

[5] Overall *p*-value (2-sided) based on Wilcoxon rank sum test.

- Antibody titers were compared between study groups.

- Intention to Treat (ITT)

α – significant difference

was observed in 3 (15%) of 20 participants in HAI assay and MN assay on day 7 (both $p < .0001$ vs. vaccine group) on day 28, 14 (70%) or 20 participants and 15 (75%) of 20 participants in the control group developed four-fold or greater increase in antibody titer by HAI assay and MN assay, respectively.¹⁰ These data show the boosting effect from the priming dose of vaccine.

All participants in the previously vaccinated group developed a four-fold or greater increase in HAI and neutralizing cross-reactive antibody titers against clade one H5N1 virus (A/Thailand/1 [KAN-1]/04) on day 28. Almost all (95–100%) of the participants in the previously vaccinated group developed a four-fold or greater increase in the antibody against clade 2.1.3.2 H5N1 (A/Indonesia/05/05) and clade 2.3.4 H5N1 (A/Lao/Nong Khai/1/07) viruses on day 28. The detailed results are described in our previous report.¹⁰

Efforts in manufacturing inactivated influenza vaccines (IIVs)

Following experience with the successful clinical trials of the H5N2 live-attenuated influenza vaccine and obtaining the licensure for its pandemic use from the Thai FDA, the GPO is conducting clinical trials and scaling up the production of IIVs.

The results of the phase I trial using IIVs manufactured by GPO showed the vaccine was safe and immunogenic (personal communication). The seroconversion rate of participants who had received the GPO trivalent-inactivated influenza vaccine 21 days later were 85%, 45%, and 95% against H1, H3, and flu B using HAI antibody assay, respectively. The results of phase II/III trial involving approximately 900 participants are underway.

Summary

The Thai government has developed its local vaccine manufacturer (the GPO) to be able to manufacture its own influenza vaccines as a part of the strategy of self-reliance and sustainability for national security. GPO's vaccine manufacturing plant is in the process of validation and is being built with a budget of 1,116.19 million Baht.

The goal of the vaccine manufacturing plant is in the first instance to have the capacity to produce 2 million doses of seasonal influenza vaccines, then to increase to 10 million doses in the near future and eventually to be able to increase up to 60 million doses during epidemics/outbreaks. Furthermore, it is looking forward to becoming one of the key players in vaccine manufacturing in ASEAN and then to expanding its products into animal vaccines and biological products to be able to be a world-class key player of vaccines & biological products.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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