Paediatrica Indonesiana

VOLUME 51 January • 2011 NUMBER 1

Original Article

Immunogenicity and safety of a trivalent inactivated influenza vaccine

Eddy Fadlyana [1], Kusnandi Rusmil[1], Novilia Sjafri Bachtiar[2], Rachmat Gunadi[3], Hadyana Sukandar[4]

Abstract

Background Trivalent inactivated influenza vaccines (TIV) containing antigens of two influenza A strains, A(H1N1) and A(H3N2), and one influenza B strain, are the standard formulation for influenza prevention. The vaccines must be updated annually to provide optimal protection against the predicted prevalent strains for the next influenza season.

Objective To assess the immunogenicity and safety of the inactivated influenza vaccine (Flubio®) in adolescents and adults, 28 days after a single dose.

Methods In this experimental, randomized, single-blind, bridging study, we included 60 healthy adolescents and adults. A single, 0.5 mL dose was administered intramuscularly in the deltoid muscle of the left arm. Blood samples were obtained before and 28 days after immunization. Standardized hemagglutination inhibition (HI) test was used to assess antibody response to influenza antigens.

Results From January to February 2010, a total of 60 adolescents and adults enrolled in the study, but two participants did not provide the required blood samples. One hundred percent of the subjects had an anti-influenza titer ≥ 1:40 HI units to all three strains, A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008 (P=1.000) after immunization. The Geometric Mean Titers (GMT) after immunization increased for all strains: A/Brisbane, 76.4 to 992.7, A/Uruguay, 27.6 to 432.1, and B/Brisbane, 19.9 to 312.7. Twenty-eight days after immunization, we found a ≥ 4 times increase in antibody titers in 75.8% of the subjects for A/Brisbane, 84.5% for A/Uruguay, and 77.6% for B/Brisbane. We also observed that 100% of seronegative subjects converted to seropositive for all 3 strains. All vaccines were well-tolerated. There were no serious adverse events reported during the study.

Conclusion In adolescents and adults, the Flubio® vaccine was immunogenic and safe. **[Paediatr Indones. 2011;51:22-8]**.

Keywords: *adolescents, adult, inactivated influenza vaccine, immunogenicity, safety*

Annual influenza epidemics due to influenza A and B viruses remain a substantial cause of morbidity and mortality worldwide. Epidemics particularly affect vulnerable people such as those aged ≥65 years, children <2 years, and people with chronic medical conditions.[1-3] Each year, 3–5 million cases of severe illness and 250,000–500,000 deaths are thought to result from these epidemics worldwide. Among the many subtypes of influenza A viruses, influenza A(H1N1) and A(H3N2) subtypes currently commonly circulate among humans.[4] In Indonesia, few influenza surveillance studies have been published. A study on the epidemiology of influenza in Jakarta and surrounding areas (JABOTABEK: Jakarta municipality, district of Bogor, Bekasi municipality and district of Tangerang) was conducted on 2712 outpatient children and adults from August 2004 to July 2006. Throat and nasal swab analysis showed

From the Department of Child Health, Medical School, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.[1] From Bio Farma Pharmaceuthical Company, Bandung, Indonesia.[2] From the Department of Internal Medicine, Medical School, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.[3] From the Department of Epidemiology & Biostatistics, Medical School, Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.[4]

Reprint requests to: Eddy Fadlyana, MD, Department of Child Health, Medical School, Padjadjaran University, Hasan Sadikin General Hospital, Jl. Pasteur No 38 Bandung 40161, Indonesia. Ph/Fax: +62-22-2035957. E-mail: edfadlyana@yahoo.com

that 8.5% of children below five years of age were infected by influenza viruses.[5] In addition, a study by the Department of Infection Prevention & Control in Riyadh, Saudi Arabia on Indonesian Muslims on pilgrimage reported that 50% of the subjects with a positive viral culture were positive for influenza B, and 5.6% were positive for influenza A.[6]

Trivalent, inactivated influenza vaccines (TIV) containing antigens of two influenza A strains, A(H1N1) and A(H3N2), and one influenza B strain, provide the standard formulation for influenza prevention. Because one or more new, antigenically-drifted variants circulate annually, vaccines must be updated to provide optimal protection against the predicted prevalent strains for the next influenza season.[7,8] The World Health Organization (WHO) and US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research, provide annual guidance for strain selection based on new drift variants detected through a global influenza surveillance network.[7] The current global, seasonal influenza vaccine production capacity is limited (300 million doses annually) and concentrated mainly in Europe, North America, Australia and Japan. In the event of a pandemic, even if all producers switch to the production of a pandemic influenza vaccine, production would be barely sufficient to cover 10% of the world's population. Influenza vaccine production expansion is an urgent measure in preventing widespread disease. However, there is currently no influenza vaccine producer in Southeast Asia, including Indonesia. Ultimately, countries in this region may not have the vaccine if a pandemic occurs.[8] Establishment of an influenza vaccine production facility is vital to cover the vast area of Indonesia. As such, Bio Farma has started facilitating the technology transfer needed to formulate and manufacture the influenza vaccine locally. The aim of this trial was to assess the antigenicity and safety of the newly formulated Bio Farma influenza hemagglutinin (HA) vaccine, Flubio®, in adolescents and adults 28 days after a single dose.

Methods

Study Design and Population

We conducted an experimental, randomized, single-blind, bridging study from January to February 2010 at Hasan Sadikin Hospital, Bandung. Our subjects were healthy individuals aged 12 - 64 years. All subjects gave written, informed consent. We excluded subjects who: 1) were concomitantly or scheduled to be enrolled in another trial; 2)had history of allergy to egg/chicken protein or any other component of the vaccine; 3) had history of uncontrolled coagulopathy or blood disorders contraindicating intramuscular injection; 4) had received treatment which altered their immune response (e.g., intravenous immunoglobulin or other blood-derived products) in the previous 4 weeks, or long-term (> 2 weeks) corticosteroids; 5) were pregnant or lactating; 6) had chronic disease which might interfere with the assessment of the trial objectives; 7) had already been immunized with an influenza vaccine within the past year; or 8) were ill, especially with infectious diseases or fever (axillary temperature ≥ 37.5°C).

Prior to this study, approval was obtained from the Ethics Committee of the Medical School, Universitas Padjadjaran/Hasan Sadikin General Hospital, Bandung and The National Agency of Drug and Food Control. This trial was conducted in accordance with the latest Edinburgh (Scotland) revision of the Declaration of Helsinki, ICH, Good Clinical Practice guidelines[9-11] and local regulatory requirements.[12]

Antigenic characterization and serology

A single 0.5 mL dose was administered intramuscularly in the deltoid muscle of the left arm in each subject. Blood specimens were obtained before and 28 days after immunization. Following serum separation, specimens were blinded and frozen, until tested. Immunogenicity was assessed using validated hemagglutination inhibition (HI) assay methods performed at the Bio Farma Immunology Laboratory, Bandung. A standardized test was used to assess antibody response to influenza antigens. Briefly, 25μl of antigens from the vaccine strains of an hemagglutination assay (HA) titer of 8 HA units were mixed with 25μl of a two-fold dilution receptor destroying enzyme (RDE)-treated serum in phosphate buffered saline (PBS) in U- bottomed, 96-well plates. After 60 minutes of incubation at room temperature, 50μl of type O human red blood cells was added to the mixtures. Titer was defined as the highest dilution of

serum able to inhibit hemagglutination. HI titers ≥ 40 were considered to be a protective antibody level.[13] Differences were analyzed by the Chi Square test or Fisher's exact test.

Reactogenicity and safety

After vaccine injection, participants were observed for 30 minutes for local and/or systemic reactogenicity. Body temperature was also measured. Participants received memory aids to record the severity of injection site reactions, reactogenicity events, and general adverse events (AEs). Definitions of events and severity grades were provided with the memory aids. Participants reported immediate reactogenicity symptoms within 30 minutes of immunization before leaving the clinic. Data on local and systemic adverse events were reported by the participants using standardized diaries for 28 consecutive days after immunization. During this period, each participant recorded the appearance, duration, and intensity (coded 1, 2, or 3) of local and systemic reactions. The intensity of local reactions was assessed using a plastic bangle.[14]

Vaccines

The current inactivated, trivalent influenza vaccine contained hemagglutinin (HA) from 3 strains of influenza, A(H1N1), A(H3N2), and B. In this trial, the influenza HA vaccine was formulated from bulk of 3 monovalent, influenza vaccines, A(H1N1), A(H3N2), and B, according to the WHO recommendation for the year. Because of the frequent emergence of new influenza variant strains, the antigenic composition of influenza vaccines needs to be evaluated yearly, and the trivalent inactivated influenza vaccines are reformulated almost every year. Each 0.5 mL dose (Batch #302019) contained 4μg thimerosal and 15 μg of HA from each strain, A/Brisbane/59/2007(H1N1), A/Uruguay/716/2007(H3N2) = A/Brisbane/10/2007-like virus, and B/Brisbane/60/2008.[8]

Results

A total of 60 adolescents and adults enrolled in our study. The antibody titers of two participants were not assessed as they did not provide complete blood specimens. The characteristics of subjects are shown in **Table 1.** The mean age was 26 years (SD 18.5) and the age range was 12 - 62 years.

Following immunization, the percentage of subjects with an anti-influenza titer ≥ 1:40 HI units was 100% for all 3 strains, P=1.000. There were no significant differences between subjects aged 12-18 years and those aged 19-64 years. **(Figure 1)**

Table 1. Demographic characteristics

Description	n (%)
Gender	
Male	40 (67)
Female	20 (33)
Age	
12-18 yr	30 (50)
19-64 yr	30 (50)
Mean (SD)	26 (18.5)
Min - max	12-62
Education completed	
Elementary	13 (23)
Junior High School	23 (38)
Senior High School	17 (28)
Undergraduate	2 (3)
Graduate school	5 (8)
Occupation	
Goverment officer/army	4 (7)
Employee	15 (25)
Labourer	6 (10)
Student	35 (58)

The GMT after immunization of 12-18 year-olds increased from 76.4 to 992.7 for A/Brisbane, 27.6 to 432.1 for A/Uruguay, and 19.9 to 312.7 for B/Brisbane. There were significant differences in GMT between the 12-18 years' group and 19-64 years' group for both A/Brisbane and B/Brisbane (P=0.038 and 0.034, respectively). **(Figure 2)**

The percentages of subjects with a ≥ 4 times increase in antibody titers 28 days after immunization, were 75.8% to A/Brisbane, 84.5% to A/Uruguay, and 77.6% to B/Brisbane. **(Figure 3** shows breakdown by age group.)

We observed that 100% of seronegative subjects transitioned to seropositive for antibodies to all 3 strains of virus.

All vaccines were well-tolerated. There were 11 (18.3%) who reported a local reaction, either pain (7%) or fatigue (7%). There were 4 subjects (6.3%) who reported systemic events, most frequently fatigue (3%). **(Table 2)** There were no serious adverse events reported during the study.

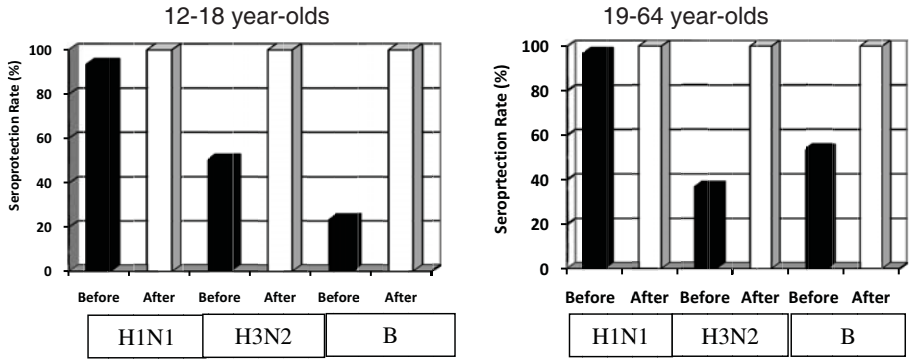

Figure 1. Assessment of seroprotection rates to A (H1N1), A (H3N2), and B virus strain, before and 28 days after immunization.
Differences between 12-18 year-olds and 19-64 year-olds seroprotection rates to A (H1N1), A (H3N2), and B virus strain, before and 28 days after immunization
A /Brisbane pre : P (EF) = 1.0 post : P (EF) = 1.0
A/Uruguay pre : P (EF) = 0.297 post : P (EF) = 1.0
B/Brisbane pre : P(EF) = 0.017 post : P (EF) =1.0

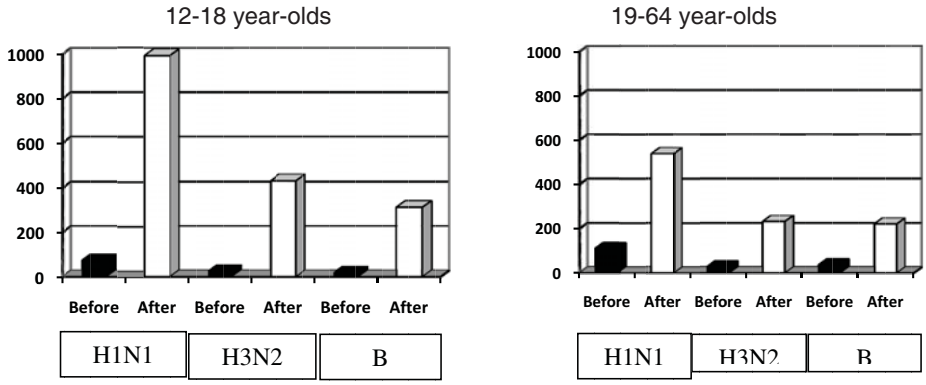

Figure 2. Assessment of GMT to A (H1N1), A (H3N2), and B viral strains, before and 28 days after immunization.
GMT differences between 12-18 years and 19-64 years group:
- A/Brisbane: Z(Mann-Whitney) = 2.072; P = 0.038
- A/Uruguay: Z (Mann-Whitney) = 0.305; P = 0.760
- B/Brisbane: Z (Mann-Whitney) = 2.118; P = 0.034

Table 2. Summary of adverse event

Description	12-18 year-olds		19-64 year-olds		All	
	n	%	n	%	N	%
N =	30	50	30	50	60	100
Any immediate rea ction (from 0 to 30 min after immunization)	2	7	3	10	5	8
Any immediate local reaction	2	7	2	7	4	7
Any immediate systemic event	-		1	3	1	2
Any delayed adverse event: (from 31 min to 72 hours after immunization)	7	23	4	14	11	18
Any delayed local reaction	5	16	2	7	7	12
Any delayed systemic event	2	7	2	7	4	7
Any delayed adverse event (from 72 hours to 28 days after immunization)	-	-	-	-	-	-
		-		-	-	-
Any delayed local reaction	-	-	-	-	-	-
Any delayed systemic event	-	-	-	-	-	-

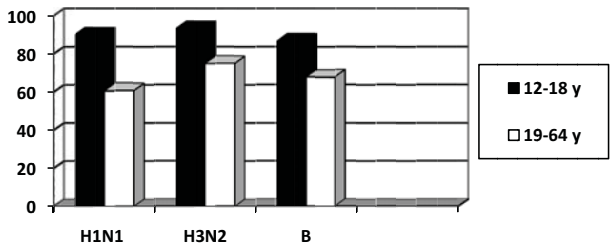

Figure 3. Percentage of subjects with ≥ 4 times increase in antibody titers 28 days after immunization.
Difference in percentage of subjects (12-18 year-olds vs. 19-64 year-olds) with ≥ 4 times increase in antibody titer 28 days after immunization:
- A/Brisbane: EF (exact Fisher Test) : P (EF)=0.009
- A/Uruguay: EF (exact Fisher Test) : P (EF)=0.074
- B/Brisbane: EF (exact Fisher Test) : P (EF)=0.086

Discussion

Influenza vaccination is most effective when circulating viruses are well-matched with vaccine viruses. Influenza viruses are constantly changing, and the WHO Global Influenza Surveillance Network (GISN), a partnership of National Influenza Centres around the world, monitors the influenza viruses circulating among humans. WHO annually recommends a vaccine composition that targets the three most representative strains in circulation.[7,8] The aim of this trial was to assess the immunogenicity and safety of the influenza hemagglutinin (HA) vaccine, FluBio, in adolescents and adults, 28 days after one dose.

We have demonstrated that the influenza HA trivalent vaccine (Bio Farma) is immunogenic and safe when administered to adolescents and adults. The immunogenic parameters we assessed were the percentage of subjects with anti-influenza titers ≥ 1:40 HI units, the GMT, percentage of subjects with a ≥ 4 times increase in antibody titer, and the percentage of subjects transitioning from seronegative to seropositive.[15] The percentages of subjects with anti-influenza titer ≥ 1:40 HI units to A/Brisbane, A/Uruguay, and B/Brisbane strains were all 100% (P=1.000). The GMT after immunization increased as follows: A/Brisbane 76.4 to 992.7, A/Uruguay 27.6 to 432.1, and A/Brisbane 19.9 to 312.7. The percentages of subjects with ≥ 4 times increase in antibody titer after immunization were 73.3% to A/Brisbane, 81.6% to A/Uruguay, and 75.0% to B/Brisbane. According to FDA criteria for evaluation of seasonal influenza vaccine licensure, the percentage of subjects with a ≥ 4 times increase in antibody titer should be > 60%.[15] For all 3 strains, > 73% of our subjects displayed this increase. The percentage of seronegative subjects transitioning to seropositive to A/Brisbane, A/Uruguay, and B/Brisbane were all 100%. In general, immune responses to all strains were higher in the 12-18 year group than in the 19-64 year group. Various lines of evidence suggest that immunological responses and sex steroid hormones are linked at physiological and cellular levels. The increased risk of autoimmunity among pubertal and post-pubertal females (and males to a lesser degree) strongly suggests that sex steroids affect immune function.[17] Macrophages and other cells express intra- and extracellular receptors for oestrogens and androgens, implying a direct effect of these hormones on the immune system.[18] B cells, however, express only intracellular oestrogen and androgen receptors. As a result, sex steroid hormones have many effects on the innate and adaptive immune system.[19]

A previous study, conducted from August to November 2008, used a seasonal influenza HA trivalent (formulated in Bio Farma) on a total of 405 adolescents and adults. The strains used were A/Hiroshima, A/Solomon Island and B/Malaysia. Results showed high, induced, antibody titers against all 3 influenza antigens in adolescents and adults. The percentage of subjects with anti-influenza titer ≥ 1:40 HI units to A/Hiroshima, A/Solomon Island and B/Malaysia strains were similar: 97.8%, 98.2%, and 95.5%, respectively, p=0.025. The GMT after immunization increased as follows: A/Hiroshima 66.16 to 323.37, A/Solomon Islands 41.89 to 554.26, and B/Malaysia 24.02 to 231.83. The percentages of subjects with increased antibody titer ≥ 4 times to A/Hiroshima, A/Solomon, and B/Malaysia were 61.2%, 85.5%, and 81.5%, respectively. The percentages of seronegative subjects transitioning to seropositive to A/Hiroshima, A/Solomon, and B/Malaysia were 93.7%, 95.8%, and 93.9%, respectively.[20]

As a comparison, Jackson *et al*,[21] in 2006-2007 carried out a study in 7658 healthy adults to assess the efficacy, safety, and immunogenicity of a TIV. The study for season 1 showed that the percentage of subjects with anti-influenza titer ≥ 1:40 HI units

to A/New Caledonia (H1N1), A/ New York (H3N2), and B/ Jiangsu (Yamagata) strains were high (97.0%; 94.0%, and 98.0%, respectively). The GMT after immunization increased: A/New Caledonia (H1N1) 35.2 to 385.4, A/New York (H3N2) 16.3 to 258.3, and B/Jiang su (Yamagata) 25.4 to 313.5. The percentages of seronegative subjects who transitioned to seropositive were 68.0% to A/New Caledonia (H1N1), 85.0% to A/New York (H3N2), and 82.0% B/Jiangsu (Yamagata). The same authors' study for season 2 showed that the percentage of subjects with anti-influenza titer ≥ 1:40 HI units to A/New Caledonia (H1N1), A/Wisconsin Malaysia (Victoria) and B/ Malaysia (Victoria) strain were 97.0%, 92.0%, and 97.0%, respectively. The GMT after immunization increased: A/New Caledonia (H1N1) 35.2 to 352.5, A/Wisconsin (H3N2) 14.9 to 157.6, and B/Malaysia (Victoria) 19.6 to 263.9. The percentages of seronegative subjects transitioning to seropositive were 68.0% to A/New Caledonia (H1N1), 72.0% to A/Wisconsin (H3N2), and 74.0% to B/Malaysia (Victoria).

In this study, all vaccines were well-tolerated. There were 11 (18%) who reported local reactions, mostly pain (7%) and fatigue (7%). There were 4 (7%) who reported systemic events, most often fatigue (3%). There were no serious adverse events reported during the study. The reactogenicity events reported were consistent with those commonly reported with TIVs. From a previous study, there were 81 (20%) who reported local reactions and 16.3% who reported systemic reactions. All vaccines were well-tolerated and no serious adverse events occurred during their study.[22] Jackson *et al.* concluded that the reactogenicity events were significant in the TIV versus placebo group. Events observed were injection site pain, injection site redness and swelling, myalgias, arthralgias, fever, and fatigue, but most were of mild (grade 1) severity.[21] There was a slightly higher incidence of spontaneous AEs in the TIV versus placebo group reported up to 21 days post-vaccination and this was primarily due to the persistence of injection site pain and redness. Overall, the results suggest that the safety profile of TIV was acceptable and consistent with the historical performance of similar products. In placebo-controlled, blinded studies, the most frequent side-effect of vaccination is soreness at the vaccination site (affecting 10–64% of patients), which lasts up to two days following administration of influenza vaccine.[23-25] These reactions are generally mild and transient, resolving spontaneously within two to three days. Mild systemic reactions may also occur. Fever, general discomfort and muscle pain can affect those individuals without previous exposure to the antigens in the vaccine (e.g. children). These reactions may occur within 6–12 hours of vaccination and generally persist 1–2 days.[26]

Limitations of this study were the small sample size and the exclusion of children below 12 years of age.

In conclusion, our results support the use of FluBio, a trivalent, inactivated influenza vaccine for adolescents and adults. The vaccine was immunogenic and safe showed no adverse effects. People aged 12–18 years had a higher immune response than those aged 19-60 years.

Acknowledgments

We would like to thank PT Bio Farma, Bandung, and the Department of Child Health Medical School, Padjadjaran University/Hasan Sadikin Hospital for their support of this study.

References

1. Schanzer DL, Langley JM, Tam TW. Hospitalization attributable to influenza and other viral respiratory illnesses in Canadian children**.** Pediatr Infect Dis J. 2006;25:795-800.
2. Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox NJ, et al. Influenza-associated hospitalizations in the United States**.** JAMA. 2004;292:1333-40.
3. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States**.** JAMA. 2003;289:179-86.
4. World Health Organization. WHO factsheet 211: Influenza. 2009 [cited 2009 Oct 15]. Available from: http://who.int/mediacentre/factsheets/2009/fs211/en/print.html
5. Yuwono D, Putranto RH, Sehatman, Subangkit1, Nur K, Susilowati et al. Epidemiological study of influenza in Jakarta and surrounding areas. Bul Penel Kesehatan. 2008;36:71-82.
6. Balkhy HH, Memish ZA, Bafaqeer S, Almuneef MA.

Influenza a common viral infection among Hajj pilgrims: time for routine surveillance and vaccination. J Travel Med. 2004;11:82-6.
7. World Health Organization. WHO global influenza surveillance network. [update 2011, cited 2011 Jan 05]. Available from: http://www.who.int/csr/disease/influenza/surveillance/en/index.html.
8. World Health Organization. Recommended composition of influenza virus vaccines in 2009. Available from: www.who.int/entity/csr/disease/influenza/200809Recommen dation.pdf.
9. Food and Drug Administration, US Department of Health and Human Services. Guidance for Industry: Choice of control group and related issues in clinical trials, May 2001.
10. Food and Drug Administration, US Department of Health and Human Services. Guidance for Industry: Ethnic factors in the acceptability of foreign clinical data, Sep 2006.
11. Food and Drug Administration, US Department of Health and Human Services. Guidance for Industry: Clinical safety data management, definitions and standards for expedited reporting, Mar 1995.
12. Indonesian Food and Drugs Association. Cara uji klinik yang baik, 2000.
13. Hahon N, Booth JA, Eckert HL. Further studies on the anti immunoglobulin G hemagglutination – inhibition test for influenza. Appl Microbiol. 1972;23:485-9.
14. Food and Drug Administration, US Department of Health and Human Services. Guidance for industry: Toxicity grading scale for healthy adult and adolescent volunteers' enrolled in preventive vaccine trials, Apr 2005.
15. Wright P, Webster R. Orthomyxoviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, et al, editors. Fields virology. 4th Ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1534-79.
16. Food and Drug Administration, US Department of Health and Human Services. Guidences for industry: Clinical data needed to support the licensure of seasonal inactivated influenza vaccines, May 2007.
17. Verthelyi D. Sex hormones as immunomodulators in health and disease. Int Immunopharmacol. 2001;1:983-93.
18. Stimson WH. Oestrogen and human T lymphocytes: presence of specific receptors in the T-suppressor/cytotoxic subset. Scand J Immunol. 1998;28:345-50.
19. Benten WPM, Stephan C, Wunderlich F. B cells express intracellular but not surface receptors for testosterone and estradiol. Steroids. 2002;67:647-54.
20. Rusmil K, Fadlyana E, Gunadi R, Dhamayanti M, Boedisetianto E, Bachtiar NS. Protectivity and safety of Influenza HA (Bio Farma) vaccine in adolescents and adults (Bridging Study). Proceedings of the 13th Asian Pacific Congress of Pediatrics, Shanghai, China; 2009 October 14-18.
21. Jackson LA, Gaglani MJ, Keyserling HL, Balser J, Bouveret N, Fries L and Treanor JJ. Safety, efficacy, and immunogenicity of an inactivated influenza vaccine in healthy adults: a randomized, placebo-controlled trial over two influenza seasons. BMC Infect Dis. 2010;10:71.
22. Govaert ME, Dinant GJ, Aretz K, Masurel N, Sprenger MJW, Knottnerus JA. Adverse reactions to influenza vaccine in elderly people: randomized double blind placebo controlled trial. BMJ. 1993;307:988–90.
23. Margolis KL, Nichol KL, Poland GA, Pluhar RE. Frequency of adverse reactions to influenza vaccine in the elderly: a randomized, placebo-controlled trial. JAMA. 1990;264:1139–41.
24. Nichol KL, Margolis KL, Lind A, Murdoch M, McFadden R, Hauge M, et al. Side-effects associated with influenza vaccination in healthy working adults: a randomized, placebo-controlled trial. Arch Internal Med. 1996;156:1546–50.
25. Supplementary information on vaccine safety part 2: Background rates of adverse events following immunization. Geneva: World Health Organization, 2000.
26. Barry DW, Mayner RE, Hochstein HD, Dunlap RC, Rastogi SC, Hannah JE, et al. Comparative trial of influenza vaccines. II. Adverse reactions in children and adults. Am J Epidemiol. 1976;104:47–59.