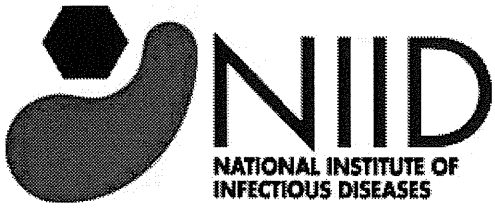
Information for WHO Annual Consultation on the Composition of Influenza Vaccine in the Southern Hemisphere

September 17-19, 2012, Beijing, China



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Acknowledgement

Genetic analyses were supported by collaboration with National Institute of Technology and Evaluation, Tokyo, Japan (Dr A. Oguchi, Dr S. Yamazaki and Dr N. Fujita). Epidemiological data were provided from Infectious Diseas Surveillance Center at NIID (Dr K. Yamashita, Dr S. Yasui and Dr Oishi).
We acknowledge NICs in Myanmar, Lao, Mongolia, China, Korea , Nepal and Taiwan CDC, and 76 Local Public Health Laboratories in Japan for sharing clinical specimens and virus isolates. Specimens and HPAI-A(H5N1) isolates were shared from NIC of National Institute of Health Research and Development, Jakarta, Indonesia.
Nucletide sequence data of viruses for phylogenetic analyses of HA and NA genes are used from GISAID.

Epidemiology

Summary of 2011/12 season

Influenza activity from September 2011 to August 2012 in Japan

- Influenza activity based on the case number per sentinel hospital at every week in 2011/12 season was relatively higher than usual seasons. The activity peaked at weeks 5~6, 2012 with 43 patients per week in a sentinel. This was the second large activity in previous 10 seasons.
- Cumulative number of patients was estimated 16.7 millions. Of those, 0-9 years old age group was 43% in total and 10-14 years old age group was 16%.
- As of September 6, 2012, total 5,427 virus isolates and 1,742 positive cases by PCR (5 for H1pdm09, 1412 for H3, and 325 for B) were reported in Japan. Of those isolates, the majority was A(H3N2) viruses (68% in total) and B was 32% in total isolates. A(H1N1)pdm09 viruses were only sporadically detected in the entire region of Japan (9 isolates).
- Of B virus, both B/Victoria- and B/Yamagata-lineage viruses were cocirculated with the relative proportion of 2:1.

Detection of antiviral resistant viruses:

- As of September 2012, 11 A(H1N1)pdm09, 362 A(H3N2) and 387 B viruses isolated in Japan, Lao PDR, Taiwan, China, Korea, Nepal and Mongolia were subjected to the susceptibility assay of 4 neuraminidase inhibitors (NAIs: oseltamivir, zanamivir, peramivir, and laninamivir).
- Criteria of the susceptibility to NAIs are adapted to the recommendations issued by WHO Expert Working Group on Surveillance of Antiviral Susceptibility for the GISRS (June 28-29, WHO-HQ, Geneva).
- All A(H1N1)pdm09 viruses tested exhibited sensitive (terms as "normal inhibition") to 4 NAIs.
- One A(H3N2) virus isolated in June 2012 in Japan showed highly resistant to oseltamivir, peramivir and zanamivir. The virus and its original clinical specimen possessed R292K substitution in the NA protein. The clinical specimen was collected from a hospitalized patient just before prescription of antiviral(s); however, her precise prescription history at initial clinic before hospitalization was unknown. Clinical information of the patient after treatment of antiviral(s) is investigated.
- One Mongolian B virus showed reduced susceptibility (terms as "reduced inhibition") to peramivir. The virus possessed H273Y substitution in the NA protein.

A(H1N1)pdm09 virus

Antigenic analyses:

- Only 7 A(H1N1)pdm09 viruses isolated from December 2011 to April 2012 were available for hemagglutination inhibition (HI) tests. One virus, A/Kumamoto-C/39/2011, possessed I152V and G155E/G substitutions in the antigenic site Sa of HA protein and showed 8~16-fold reduced HI titer against homologous titers of the reference antisera.
- Other viruses, particularly isolates after March, were well inhibited with A/California/7/2009 and A/Narita/1/2009 ferret antisera.
- In summary, most viruses tested are antigenically similar to current vaccine strain A/California/7/2009.

Phylogenetic analysis:

HA and NA genes:

- Phylogenetic tree of A(H1N1)pdm09 virus HA gene indicates genetic diversity with 7 different clades. Recent viruses isolated after March 2012 tended to form clade 6 (S185T) and 7 (S185T+A197T).
- Phylogenetic tree of the NA gene is correlated well with that of HA gene and majority of recent viruses fell into clade shared with T241I+N369K.

A(H3N2) viruses

Antigenic analysis:

- All ferret antisera in our reference serum panel are raised against MDCK-grown reference viruses for precise evaluation of antigenic variation of epidemic isolates, because in our previous HI assay ferret antisera raised against egg-grown viruses poorly reacted with viruses isolated in MDCK cells.
- The antisera used are; antisera of A/Mie/31/2011 (CL3B), A/Victoria/361/2011 (CL3C), A/Yamaguchi/72/2011 (CL3C), and A/Yamaguchi/77/2011 (CL6). To compare HI profile of previous vaccine-like (A/Victoria/210/2009) group, A/Niigata/403/2009 antiserum was included in the reference antiserum panel. For antigenic cartography, A/Shizuoka/736/2009 and A/Hunan-beihu/1313/2009 antisera were used.
- Low reactors with 8-fold reduced HI titer to anti- A/Niigata/403/2009 antiserum were increased in the period of Mar-Aug 2012 than the period of Sept 2011-Feb 2012, indicating that recent viruses are antigenically distinguished from previous vaccine viruses A/Perth/16/2009 and A/Victoria/201/2009.
- Most recent viruses tested (isolation from February to June 2012) were well inhibited with A/Victoria/361/2011 and other A/Victoria/361/2011-like antisera,

although isolates with 4-fold reduced HI titer against A/Victoria/361/2011 antiserum slightly increased after Mach 2012. A half of test viruses showed 8-fold reduced HI titer to homologous titer of A/Yamaguchi/72/2011 (CL3C) antiserum.

- In summary, the majority of recent viruses analyzed are antigenically closely related to A/Victoria/361/2011.

Phylogenetic analysis

HA and NA genes:

- All test viruses fell into Victoria/208 clade in phylogenetic tree of HA gene. Recent viruses isolated after March 2012 belonged either clade 3B (N145S) or 3C (S45N, T48I) or 5 and 6 (D53N, Y94H, I230V, E280A). Of those, the majority was CL3B and 3C.
- Phylogenetic tree of NA gene was correlated well with the HA gene.

B viruses

Antigenic and Phylogenetic analysis:

(Victoria-lineage)

- All test viruses are mostly isolated from April to June 2012 and some isolates in the early period of this season (Feb-Mar) are also included.
- The majority of test viruses reacted well with MDCK-grown B/Brisbane/60/2008 and our B/Brisbane/60/2008-like reference (B/Sakai/43/2008 and B/Shizuoka/57/2011) ferret antisera. There were not antigenic variants with 8-fold reduced HI titer to B/Brisbane/60/2008.
- In summary, the recent viruses still retained similar antigenicity to vaccine virus B/Brisbane/60/2008 in this lineage.

- Clade 1 (N75K+N165K+S172P) in phylogenetic tree of HA gene can be redesignated as subclade **1a** and **1b**. The majority of recent B/Victoria-lineage viruses isolated after April 2012 tended to form subclade 1a.
- Phylogenetic tree of NA gene was correlated well with the HA gene.

(Yamagata-lineage)

- Ferret reference antiserum panel of B/Wisconsin/1/2010-like group (CL3) consists of antisera against egg-grown (B/Wisconsin/1/2010, B/Wisconsin/1/2010 BX-41A) and MDCK-grown (B/Wisconsin/1/2010, B/Sakai/68/2009 and B/Sakai/36/2011) viruses. Antisera from CL2 (B/Kanagawa/37/2011) and old vaccine virus (B/Florida/4/2006) are also included.
- Since MDCK-grown B/Wisconsin/1/2010 virus (passage history: C1/C1+MDCK2) used for production of the antiserum possessed 196(N>>>S) and 198(T=I) mixtures in the HA, it is likely that its HA partially loses the

glycosylation site at 196-198. The glycosylation site of egg-grown B/Wisconsin/1/2010 (E3+1) was 196(D>N) and 198(T>>I) mixtures, suggesting loss of glycans at the site. B/Wisconsin/1/2010 BX-41A (EX/E1+1) possessed N196D and completely lost the glycans.

- Most tested viruses reacted well with both MDCK-and egg-grown B/Wisconsin/1/2010 antisera including B/Wisconsin/1/2010 BX-41A antiserum. No different HI profile between MDCK-and egg-grown B/Wisconsin/1/2010 antisera was observed. This suggests that the presence and/or absence of glycans at residues 196-198 of B/Wisconsin/1/2010 virus may not influence to the HI reaction with MDCK-grown isolates.
- Some of test viruses exhibited 4~8-fold reduced HI titers to homologous titer of MDCK-grown B/Sakai/68/2009 and B/Sakai/36/2011 antisera, but those reacted well with MDCK-grown B/Wisconsin/1/2010 antiserum within 2~4-fold differences of HI titer.
- In summary, the majority of recent isolates are antigenically similar to upcoming vaccine strain B/Wisconsin/1/2010 for 2012-13 Northern Hemisphere and antiserum against vaccine production virus BX-41A inhibited well recent isolates of B/Yamagata-lineage.

- Phylogenetic tree of the HA and NA genes of B/Yamagata-lineage viruses was quite similar and the majority of recent viruses fell into either clade 2 or 3. The clade 2 and 3 viruses were cocirculated.

Information for WHO Annual Consultation on the Composition of Influenza Vaccine in the Northern Hemisphere

February 18-21, 2013, Geneva, Switzerland

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We acknowledge NICs in China, Laos and Taiwan CDC, and 76 Local Public Health Laboratories in Japan for sharing clinical specimens and virus isolates.
Nucletide sequence data of viruses for phylogenetic analyses of HA and NA genes are used from GISAID.

Epidemiology of the 2012/13 season (until January, 2013)

Influenza activity from September 2012 to January 2013 in Japan

- Influenza activity based on the weekly case number per sentinel hospital in the 2012/13 season substantially started since the first week in 2013. This was few weeks later than the last season, but the case numbers per week increased sharply between the weeks 2 and 4 up to the level of 36.4 per sentinel. Cumulative total case number until the week 4 was estimated 5.18 million.
- Unlike usual age distribution of patients, 45% of total patients was in adult group (between 20 and 59 year-old). The highest percentage was at the age group of 5-9 (13.8%) and 30-39 years old (13.8%), followed by 20-29 (11.8%) and 40-49 (11.8%) years old.
- As of Feb. 7, 2013, total 1643 influenza A and B viruses were isolated and/or detected by PCR. Of those viruses, the majority was A(H3N2) (91.2%) and a small number of A(H1N1)pdm09 (2.9%) and B (5.9%) were detected.
- Of B virus, both B/Victoria- and B/Yamagata-lineage viruses cocirculated in a similar proportion, 2.6% and 3.3%, respectively.

Detection of antiviral resistant viruses in 2012/13 season:

- During September 2012－February 2013, 12 A(H1N1)pdm09, 86 A(H3N2) and 28 B viruses isolated in Japan, China, and Laos were tested for susceptibility to 4 neuraminidase (NA) inhibitors (oseltamivir, zanamivir, peramivir, and laninamivir). Those viruses were also subjected to real-time RT-PCR allelic determination (for A(H1N1)pdm09) and to NA gene sequencing for detection of mutations which correlate to the phenotype of antiviral resistance.
- All viruses tested were sensitive (normal) to all 4 antivirals.

A(H1N1)pdm09 virus

Antigenic and Phylogenetic analyses:

- Total 10 A(H1N1)pdm09 viruses collected in Japan and China between September 2012 and January 2013 were subjected to hemagglutination inhibition (HI) tests for antigenic analysis.
- The majority of test viruses (9 out of 10 viruses tested) reacted well with A/California/7/2009 and an A/California/7-like reference (A/Narita/1/2009) ferret antisera.
- One virus, A/Shimane/72/2012, exhibited 4-fold lower HI titers to A/California/7/2009 ferret antiserum. The virus contained mixed amino acid sequences of K154E>K and G155G>E in the hemagglutinin (HA) protein.
- Another virus, A/Saitama/88/2012, exhibited 16-fold lower HI titers to egg-grown A/California/7/2009 and egg-grown A/Narita/1/2009 ferret antisera. The virus acquired an amino acid replacement of G155E in the HA.
- Phylogenetic analysis of the HA gene indicated genetic diversity into 8 clades. The majority of recent viruses fell into either clade 6 or 7. A clade 6 reference

virus A/Bangladesh/2021/2012, which was provided by US-CDC, was used for serology study.

- An antigenic variant virus, A/Saitama/88/2012, fell into clade 8.
- Phylogenetic tree of the NA gene was correlated well with that of HA gene, but viruses in clade 6 divided into two groups with I106V+N200S signature amino acids and with no characteristic amino acids in NA gene.
- Recent viruses fell into clades 6 and 7.

Conclusions:

- The majority of A(H1N1)pdm09 viruses isolated since September 2012 is antigenically closely related to current vaccine virus A/California/7/2009 and belongs to either clade 6 or clade 7 in phylogenetic tree. There was not different from the data for the VCM in September 2012.

A(H3N2) viruses

Antigenic and Phylogenetic analyses:

- It was clear from the previous HI data provided by NIID and other WHO CCs that ferret antisera raised against egg-grown A/Victoria/361/2011 vaccine virus poorly reacted with most epidemic viruses isolated in MDCK cells. Based on these observations well consistent among all WHO CCs, we omitted ferret antisera raised against egg-grown reference viruses from our reference antiserum panel of HI tests and used antisera against MDCK-grown A/Victoria/361/2011 and recent representative viruses (A/Sapporo/125/2012 and A/Yamaguchi/30/2012) of Clade 3C.
- Ferret antisera against MDCK cell-isolate of A/Victoria/361/2011 reacted well with the majority (97.8%) of recent A(H3N2) viruses isolated in MDCK cells at similar HI titers to the homologous virus, despite an increasing tendency of proportion of viruses showing 4-fold lower HI titer to A/Victoria/361/2011 ferret antiserum, i.e., 20% during Mach – August 2012 vs 51% during September 2012- February 2013.
- To confirm whether proportion of viruses showing 4-fold lower HI titer to A/Victoria/361/2011 ferret antiserum has actually increased or is in the deviation due to the reactivity of ferret antiserum prepared by NIID, the ferret antiserum of NIID was distributed to all WHO CCs to compare the HI profiles in their HI tests. The results reported by CDC were indicated that HI reaction profiles using NIID ferret antiserum were quite similar to those using CDC ferret antiserum, suggesting that an increasing tendency of proportion of viruses showing 4-fold lower HI titer to A/Victoria/361/2011 was not precisely confirmed.
- The viruses with 8-fold lower HI titers to A/Victoria/361/2011 ferret antiserum was 2.2% in total viruses tested.
- All recent Japanese viruses tested possessed N145S substitution in HA protein exclusively and fell into clade 3C. Some of viruses in the N145S subclade formed a cluster with R142G+T128A substitutions and they lost a potential glycosylation site. Antigenic difference was not observed among viruses belonging to the different subclades.

- Serology antigen viruses were chosen from the N145S subclade viruses A/Hawaii/22/2012, A/Texus/50/2012, A/Sapporo/125/2012 in addition to A/Victoria/361/2011, while A/Yamaguchi/30/2012 was chosen for the subclade R142G+T128A antigen reference virus.
- Phylogenetic tree of NA gene correlated well with that of HA gene and all test viruses fell into D93G clade (corresponds to clade 3C of HA tree).

Conclusions:

- Most viruses tested were antigenically related to current vaccine virus A/Victoria/361/2011, and therefore there is essentially no difference from the data for the VCM in September 2012.
- All viruses tested belonged to the N145S subclade in 3C.

B viruses

Antigenic and Phylogenetic analyses:

B/Victoria-lineage

- All recent B/Victoria-lineage viruses tested were inhibited well with ferret antisera raised against MDCK-grown B/Brisbane/60/2008 and our B/Brisbane/60-like reference virus B/Sakai/43/2008.
- Viruses tested belonged to clade 1A, but were distinguishable from a subclade V146I of clade 1A, to which B/Brisbane/60/2008 and B/Sakai/43/2008 belonged. Phylogenetic tree of NA gene was similar to that of HA gene.

Conclusion:

- All viruses tested were antigenically similar to B/Brisbane/60/2008 and fell into clade 1A.

B/Yamagata-lineage

- All B/Yamagata-lineage viruses tested were inhibited well with both ferret antisera raised against egg- and MDCK-grown B/Wisconsin/1/2010 within 2-fold difference in HI titers to the homologous titers. Moreover, the ferret antisera raised against the vaccine virus B/Wisconsin/1/2010 BX-41A also reacted well with viruses isolated in MDCK cell. From the HI data in our hand, adaptation of vaccine virus B/Wisconsin/1/2010 to eggs did not affect the antigenic property in HI test, despite the fact that a potential glycosylation site in the 196-198 region of HA protein was lost.
- Recent B/Yamagata-lineage viruses isolated in Japan belonged to both clades 2 and 3 with an almost similar proportion. Phylogenetic tree of NA gene was similar to that of HA gene

Conclusion:

- All viruses tested were antigenically similar to B/Wisconsin/1/2010 and fell into clades 2 and 3.

HB-01-P01

試験計画書

HB-01 を免疫したマウスに対するインフルエンザウイルスインドネシア株
（A/Indonesia/5/2005 (H5N1) 強毒型野生株）攻撃試験

（試験番号：HB-01-P01）

2012 年 8 月 10 日

国立感染症研究所

目次

1. 試験実施概要

1.1 表題

HB-01 を免疫したマウスに対するインフルエンザウイルスインドネシア株（A/Indonesia/5/2005 (H5N1) 強毒型野生株）攻撃試験

1.2 試験番号

HB-01-P01

1.3 試験目的

HB-01 のインドネシア株（A/Indonesia/5/2005 (H5N1) 強毒型野生株）に対する感染防御効果について、HB-01 を免疫したマウスにインドネシア株（A/Indonesia/5/2005 (H5N1) 強毒型野生株）ウイルスを攻撃接種し、マウスの生存率及び体重減少を評価する。また、ウイルス攻撃前のマウスより採血し、インドネシア株（A/Indonesia/5/2005 (H5N1) 強毒型野生株）及びワクチン株（A/Indonesia/5/2005 (H5N1)-PR8-IBCDC-RG2、弱毒型ワクチン株）に対する HI 及び中和抗体価をそれぞれ評価する。

1.4 適用ガイドライン

なし

1.5 試験施設

「被験物質の免疫、ウイルス攻撃、インドネシア株（A/Indonesia/5/2005 (H5N1) 強毒型野生株）に対する HI 及び中和抗体価測定」
国立感染症研究所
東京都武蔵村山市学園 4-7-1

「ワクチン株（A/Indonesia/5/2005 (H5N1)-PR8-IBCDC-RG2、弱毒型ワクチン株）に対する HI 及び中和抗体価測定」
一般財団法人 阪大微生物病研究会
香川県観音寺市瀬戸町四丁目 1 番 70 号

1.6 試験責任者

国立感染症研究所 インフルエンザウイルス研究センター
第５室長　山本典生

1.7　試験担当者

国立感染症研究所 インフルエンザウイルス研究センター
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主任研究官　中村一哉
主任研究官　原田勇一
研究員　浜本いつき
研究員　相内　章

1.8　試験日程

試験開始日		2012 年	8 月	10 日				
被験物質の受領日		2012 年	8 月	24 日				
免疫予定日	1 次免疫	2012 年	9 月	29 日	～	2012 年	9 月	18 日
	2 次免疫	2012 年	9 月	19 日	～	2012 年	10 月	2 日
採血日		2012 年	10 月	2 日				
ウイルス攻撃日		2012 年	10 月	3 日				
生存率観察		2012 年	10 月	3 日	～	2012 年	10 月	17 日
HI 抗体価測定		2012 年	10 月	4 日	～	2012 年	10 月	10 日
中和抗体価測定		2012 年	10 月	4 日	～	2012 年	10 月	19 日
一般財団法人阪大微生物病研究会への標本発送日		2012 年	10 月	15 日				
一般財団法人阪大微生物病研究会で実施した試験の報告書受領日		2012 年	12 月	7 日				
試験終了日		2012 年	12 月	21 日				

1.9　試験計画書の変更

試験計画書を変更する場合は、変更内容及びその理由を記載した試験計画書変更書を作成し、試験責任者が日付を記し署名する。

1.10　資料の保存場所

インフルエンザウイルス研究センター　第３室居室及び第５室居室

2. 被験物質、対照物質及び使用した動物

2.1 被験物質

名称　　：HB-01
ロット番号　：IFM1103
製造元　　：一般財団法人 阪大微生物病研究会
HA 含量　　：1 mL 中に 30 μg の HA タンパク質および 300 μg のアルミニウムを含む。
性状　　：振り混ぜるとき，均等に白濁した液剤
受領日　　：2012 年 8 月 24 日
入手量　　：5 本（表示量：1 mL/本）
保存条件　　：遮光して，10°C 以下に凍結を避けて保存する。
調製濃度　　：0.03、0.003 及び 0.0003 μg HA/100 μL となるよう PBS で段階希釈する。用事調製する。
残余物質　　：すべて廃棄する。

2.2 対照物質

名称　　：水酸化アルミニウムゲル
ロット番号　：AL120822
製造元　　：一般財団法人 阪大微生物病研究会
HA 含量　　：1 mL 中に 300 μg のアルミニウムを含む。
性状　　：振り混ぜるとき，均等に白濁した液剤
受領日　　：2012 年 8 月 24 日
入手量　　：2 本（表示量：10 mL/本）
保存条件　　：遮光して，10°C 以下に凍結を避けて保存する。
調製濃度　　：0.3 μg /100 μL となるよう PBS で段階希釈する。用事調製する。
残余物質　　：すべて廃棄する。

2.3 使用した動物

種　　：マウス
系統　　：BALB/cCr Slc
入手元　　：日本エスエルシー株式会社
性　　：雌
免疫開始時　：5 週齢
入手日　　：2012 年 8 月 28 日

3. 方法

3.1 免疫及び採血

マウスの筋肉内に調製した被験物質 0.1 mL を群 1～6 に、調製した対照物質 0.1 mL を群 7 と 8 にそれぞれ 3 週間隔で 2 回投与する。2 回目投与の 2 週間後に、ウイルス攻撃・臨床観察群（群 2、4、6、8）は、3.3 ウイルス接種の項に従い、ウイルスを経鼻接種し、抗体価測定群（群 1、3、5、7）は全採血を行い、血清を分離し標本とする。血清は使用するまで-20℃以下で保存する。また、分離した血清の一部は、ドライアイスを梱包し一般財団法人阪大微生物病研究会に送付する。

3.2 群構成

	免疫		測定項目	マウス (匹)
	抗原投与量 (μg HA/mouse)	アルミニウムゲル投与量 (μg Alum./mouse)		
1	0.03	0.3	抗体価測定	10
2			ウイルス接種・臨床観察	10
3	0.003	0.03	抗体価測定	10
4			ウイルス接種・臨床観察	10
5	0.0003	0.003	抗体価測定	10
6			ウイルス接種・臨床観察	10
7	0	3	抗体価測定	10
8			ウイルス接種・臨床観察	10

3.3　ウイルス接種、生存率及び体重推移

名称　　：A/Indonesia/5/2005 (H5N1)
ロット番号　　：E1E1
ウイルス原液の力価　　：$10^{8.3}$ $TCID_{50}$ / 50　μL
調製後の力価(20MLD_{50})　　：$10^{4.0}$ $TCID_{50}$ / 10　μL
保存条件　　：冷凍

ソムノペンチル（共立製薬㈱）の麻酔下にて，A/Indonesia/5/2005 (H5N1)を 0.2% BSA-MEM で 20 MLD_{50}/0.01mL に希釈したウイルス液を、片鼻に 0.01 mL 接種し感染させる。もう片鼻に接種しない。

体重測定は、ウイルス接種直後に 1 度（接種する日）、接種後は 1 回/日で接種 14 日後まで行う。死亡の判定は以下のどちらかが当てはまった個体とし、接種 14 日後まで観察する。

・生物学的に死亡と認められた個体
・ウイルス接種前の体重と比べ、体重が 30%以上減少した個体（多数の感染実験の経験から、マウス個体死との強い相関が考えられる設定値）