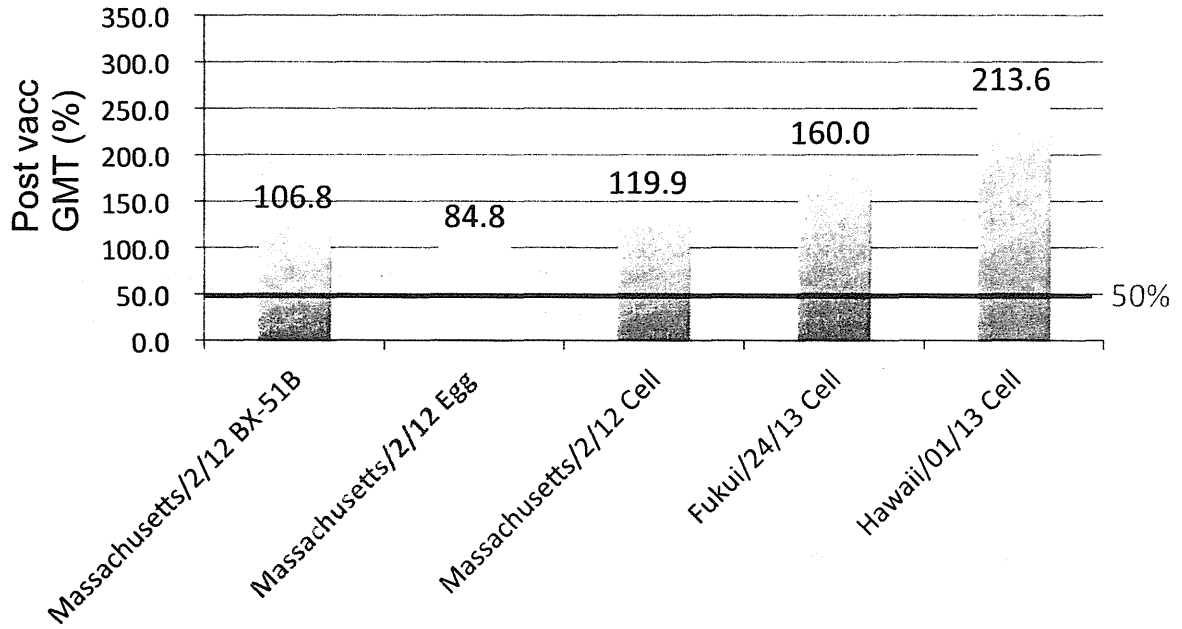
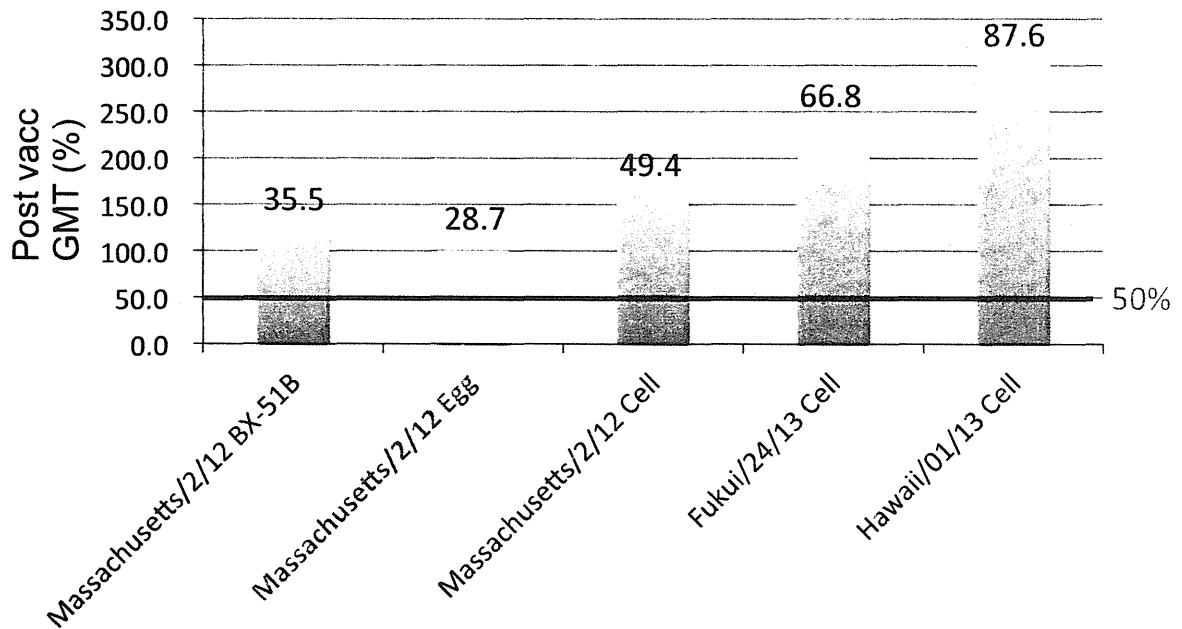


## HI antibody response to Byamagata viruses (NIBSC Sera) : Mass2 Egg

### Adult



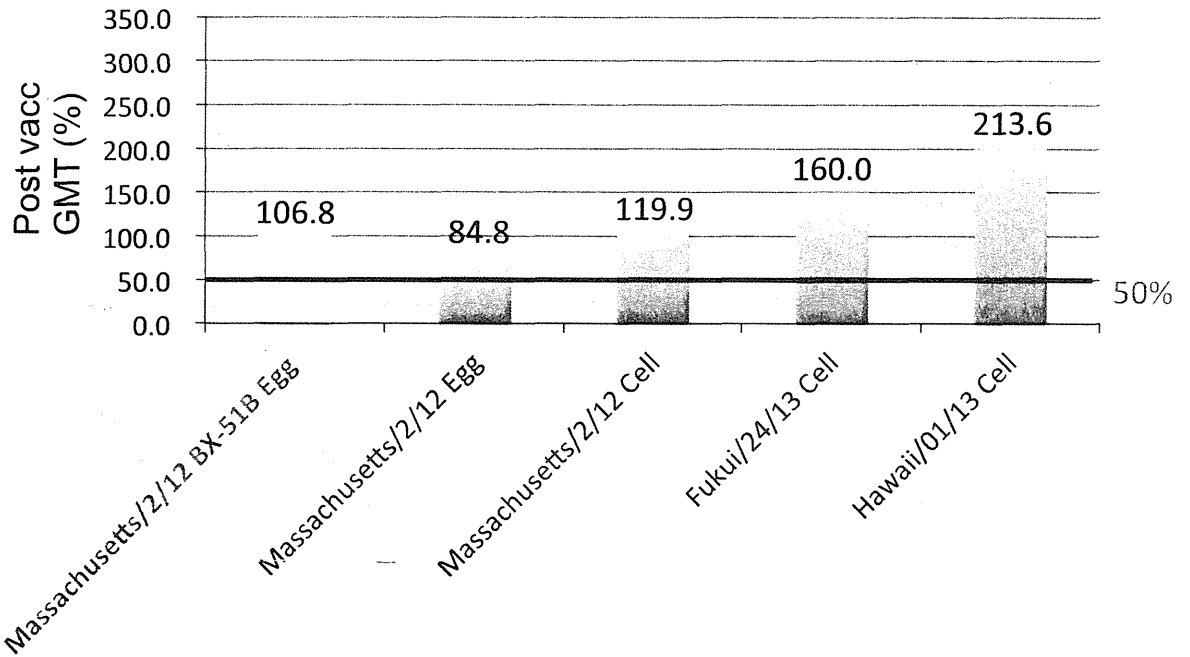
### Elderly



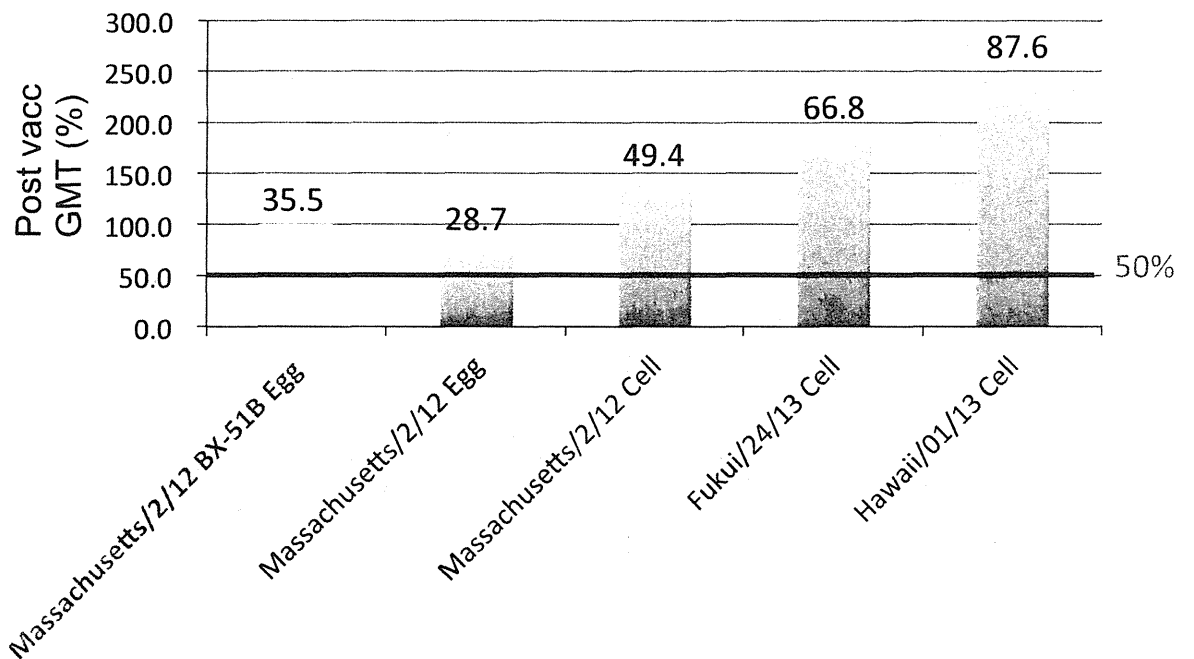
Numbers above the columns indicate the HI GMTs of viruses.

## HI antibody response to Byamagata viruses (NIBSC Sera) : BX-51B

### Adult



### Elderly



Numbers above the columns indicate the HI GMTs of viruses.

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

February 17-21, 2014, Geneva, Switzerland



WHO Collaborating Center for Reference and Research on Influenza at Laboratory of  
Influenza Virus Surveillance, Center for Influenza Virus Research,  
National Institute of Infectious Diseases, Tokyo, Japan

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**Mai Miura**

**Acknowledgement**

Epidemiological data were provided from Infectious Diseases Surveillance Center at NIID (Drs T. Sunagawa and K. Oishi).

We acknowledge NICs in Laos, Taiwan CDC, Myanmar, and Viet Nam (HCM), and 76 Local Public Health Laboratories in Japan for sharing clinical specimens and virus isolates.

Nucleotide sequence data of viruses for phylogenetic analyses of HA and NA genes are used from GISAID.

## **Epidemiology of the 2013/14 season (since September, 2013)**

### ***Influenza activity in Japan from September 2013 to February 7 2014***

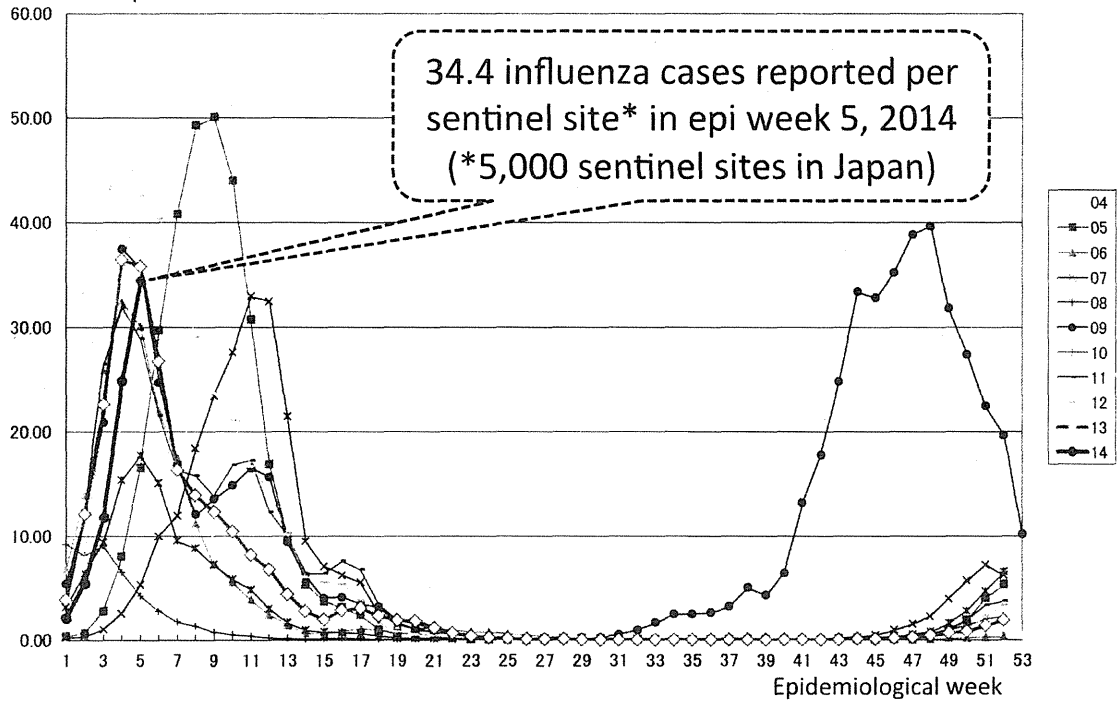
- Influenza activity based on the weekly case number per sentinel hospital in the 2013/14 season started later than previous seasons, but sharply increased after new year' holiday and has been increasing greatly at the moment.
- Estimated influenza case numbers in weeks 4 and 5 were 1,320,000 and 1,870,000, respectively, and cumulative number was 4,620,000 (as of week 5). Age distribution of influenza cases per week since week 46, 2013 exhibited that 5-9 year-old group was predominant and followed by 10-14 and 0-4 year-old groups.
- As of February 7, 2014, total 1667 viruses were isolated/detected since week 36, 2013. Of those viruses, A(H1N1)pdm09 (39%) and A(H3N2) virus (35%) were co-circulated with almost similar proportion. Notably, greatly increased numbers of A(H1N1)pdm09 virus were observed since week 2, 2014 and the virus predominated (52%) in recent 5 weeks.
- Both B/Victoria-lineage and B/Yamagata-lineage viruses were co-circulated, but B/Yamagata-lineage viruses (63%) predominated over B/Victoria-lineage viruses (37%).

### ***Viruses provided by NICs***

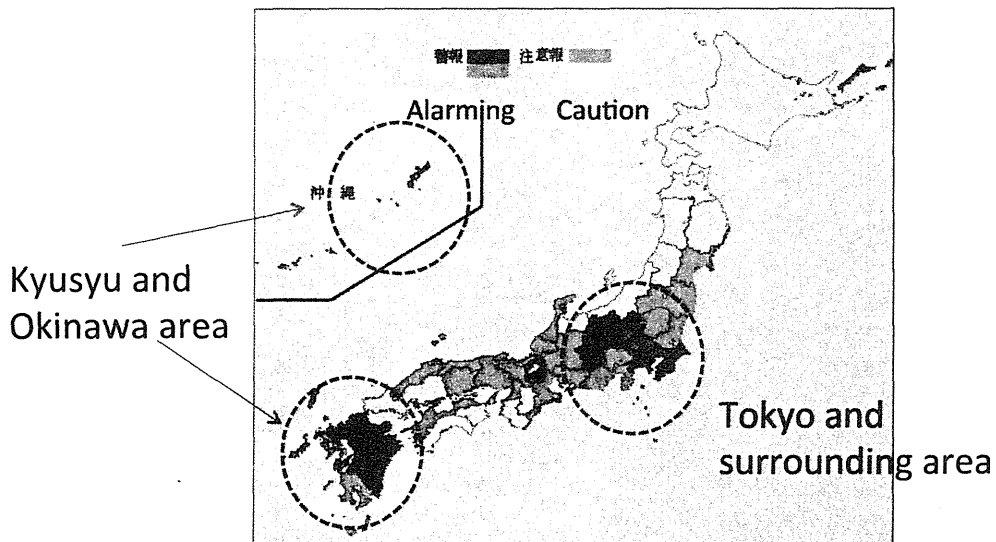
- Viruses provided by NICs are summarized in a table in page 9.
- Antigenic and genetic characterizations of the viruses provided by NICs were performed by WHO CC Tokyo and the data were included in either data package distributed for 1<sup>st</sup> teleconference (December 19, 2013), 2<sup>nd</sup> teleconference (January 23, 2014) or vaccine composition meeting held in WHO-HQ (February 17-20, 2014).

# Weekly influenza cases reported per sentinel site in Japan from 2013 to week 5, 2014 (27 Jan – 2 Feb)

No. of cases per sentinel site

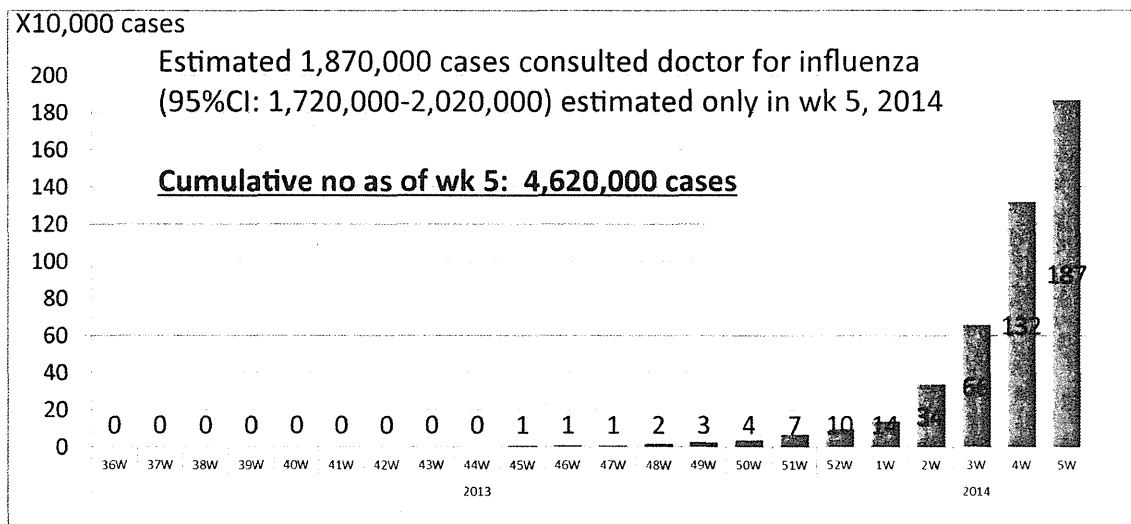


## Service indicating epidemic level for Influenza per prefecture in week 5, 2014

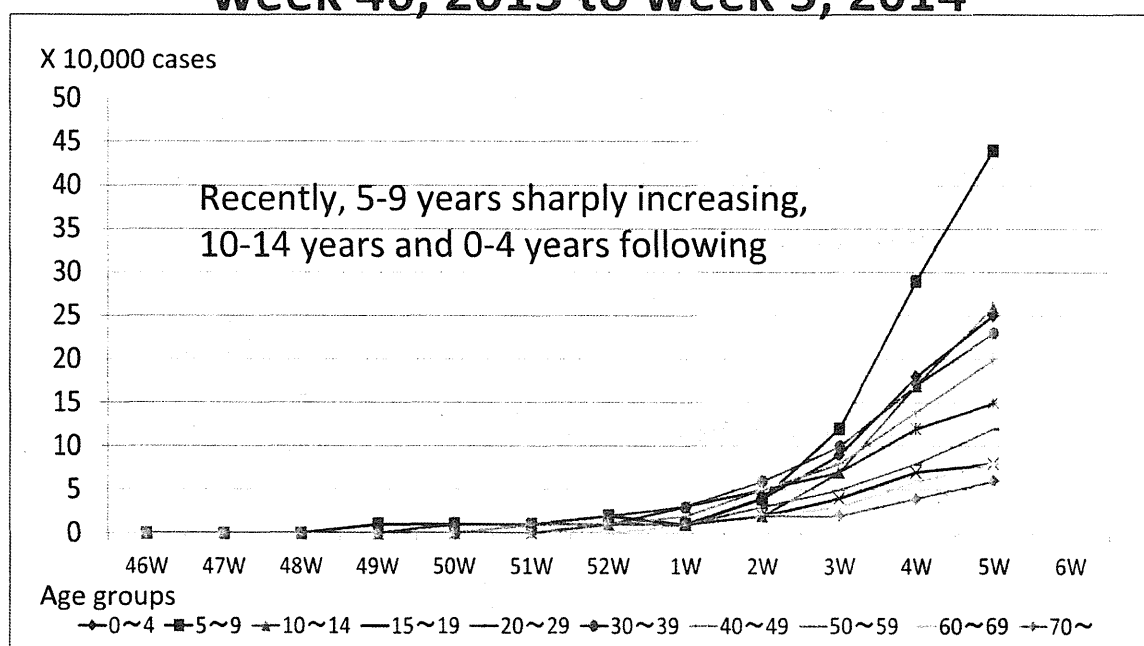


[https://nesid3g.mhlw.go.jp/Hasseidoko/Levelmap/flu/new\\_jmap.html](https://nesid3g.mhlw.go.jp/Hasseidoko/Levelmap/flu/new_jmap.html)

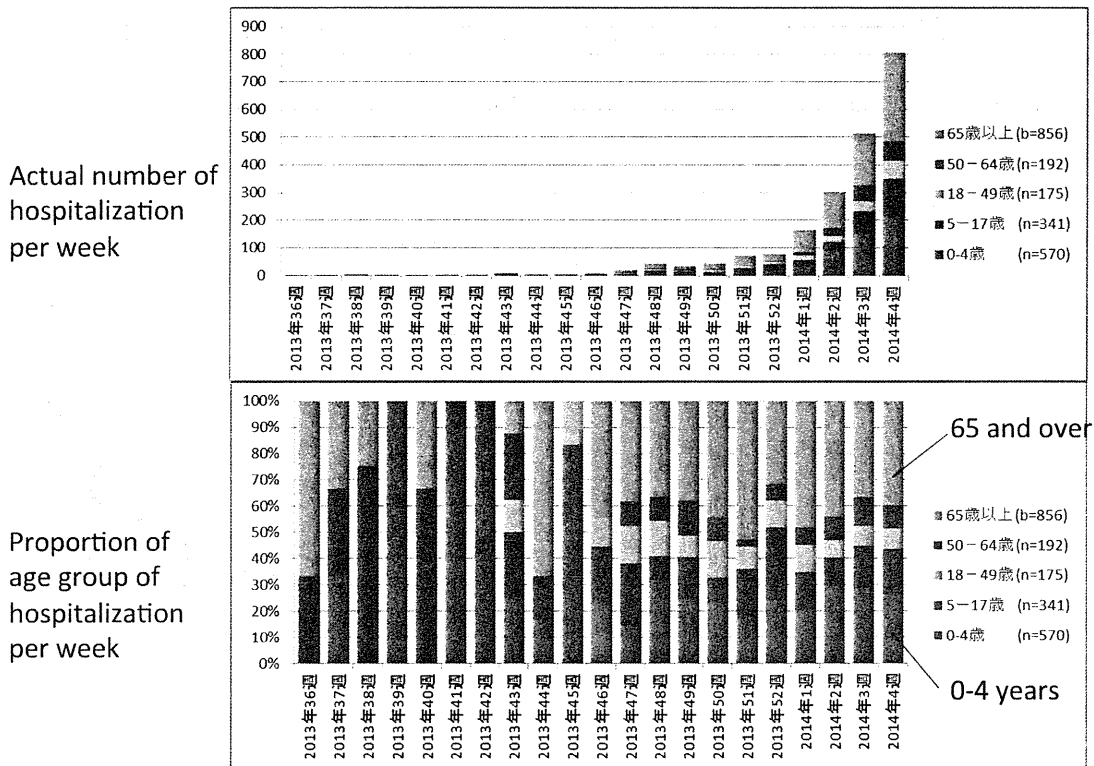
## Weekly number of estimated cases of influenza in 2013/14 season nationwide in Japan from week 36, 2013 to week 5, 2014



## Trend of age distribution for estimated number of influenza cases per week from week 46, 2013 to week 5, 2014

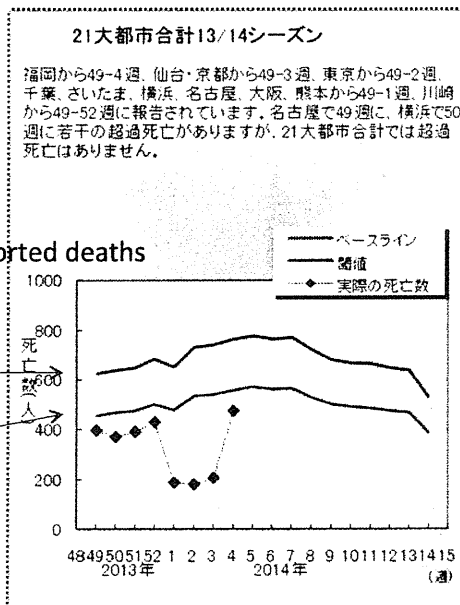


## Hospitalization due to influenza reported from 500 designated hospital nationwide in Japan from week 36, 2013 to week 4, 2014 (n=2,134)



## Excess deaths associated with influenza reported from 21 major cities nationwide in Japan as of 3 February 2014

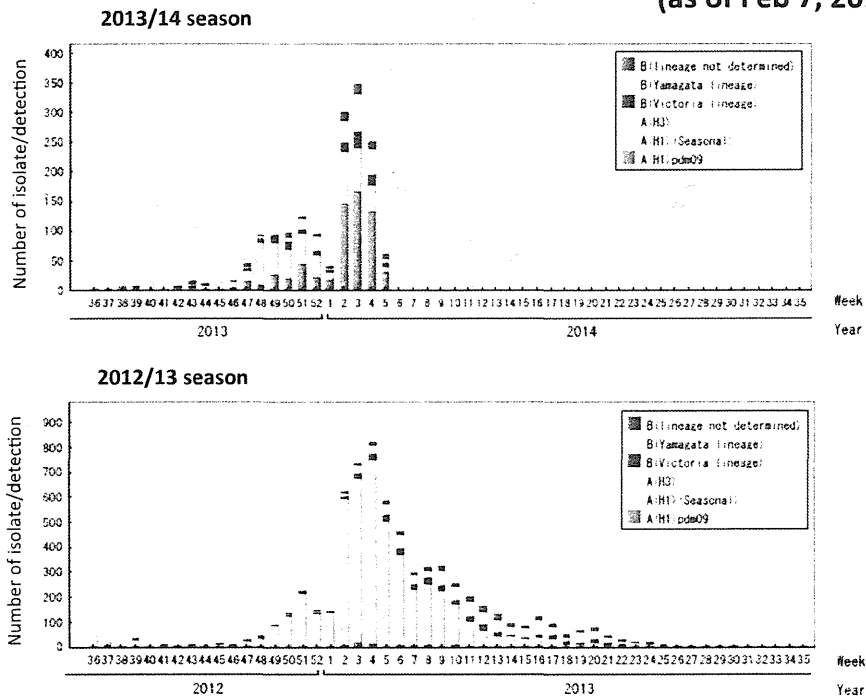
- No excess deaths were reported as of 3 February 2014



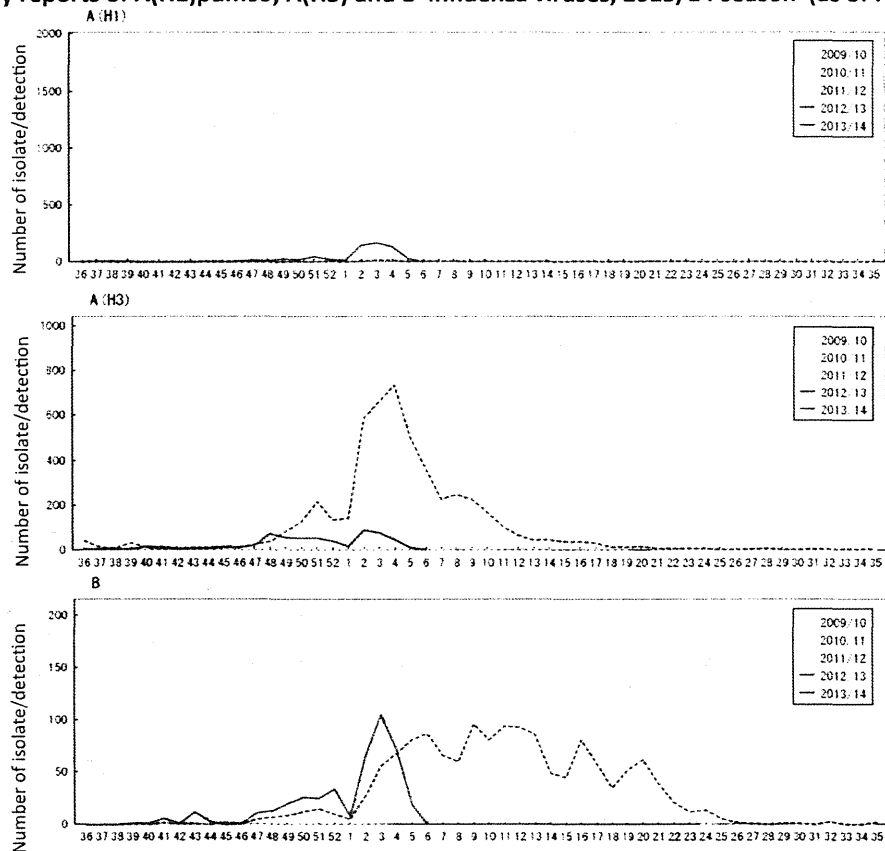
<http://www.nih.go.jp/niid/ja/flu-m/2112-idsc/jinsoku/1847-flu-jinsoku-2.html>



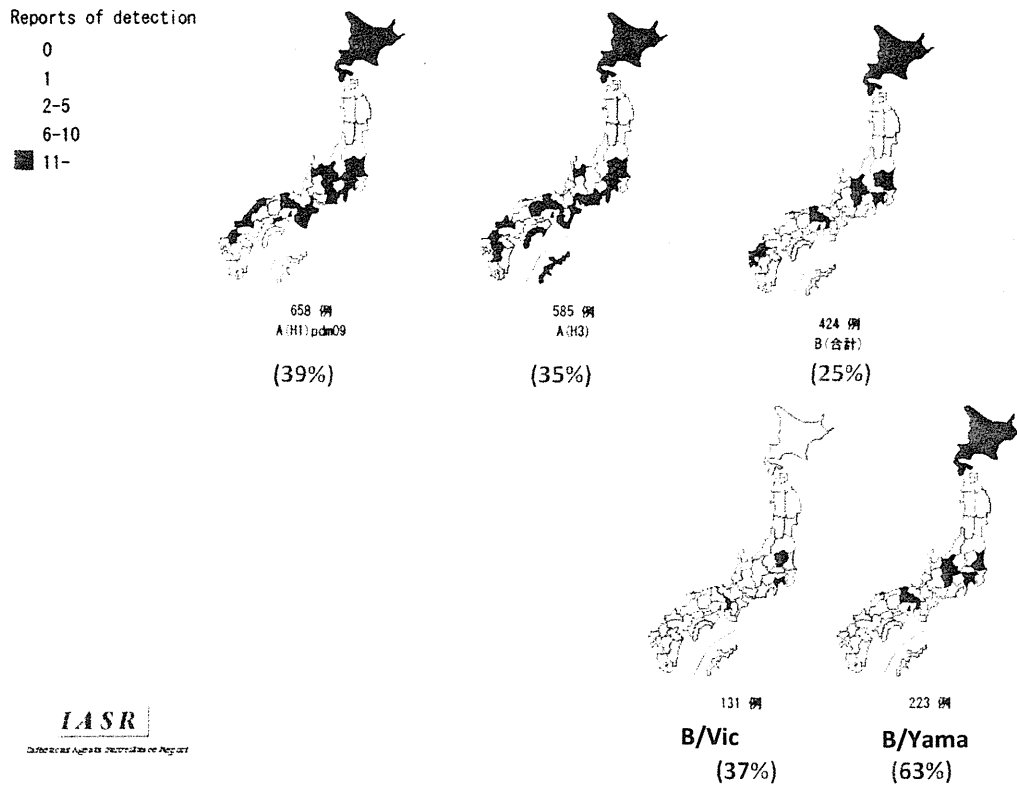
## Weekly reports of influenza virus isolation/detection, 2013/14 season (as of Feb 7, 2014)



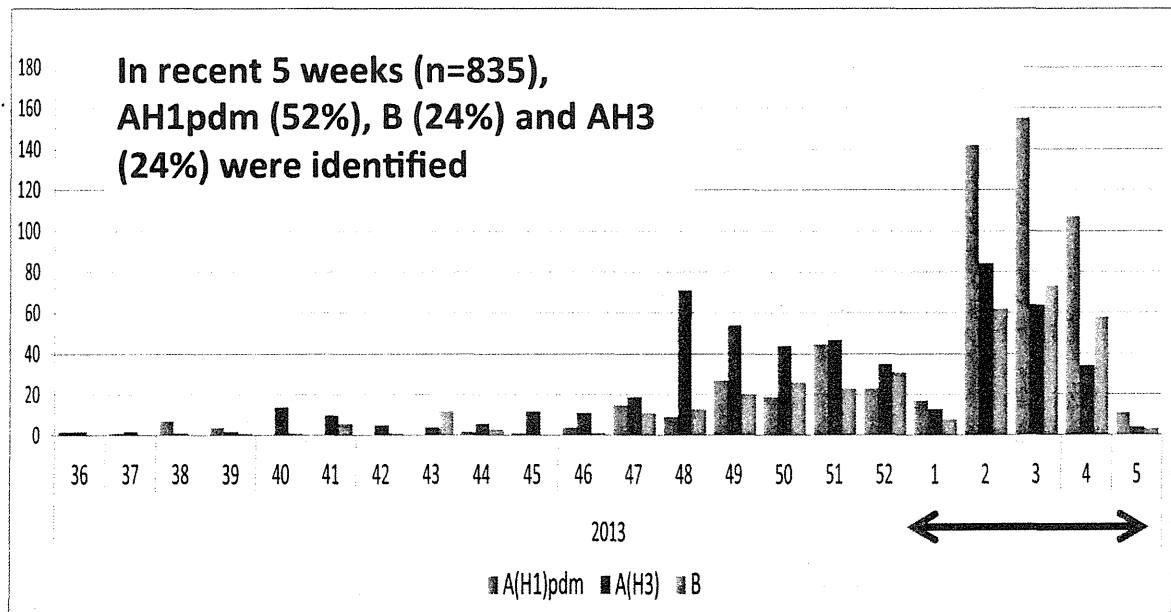
## Weekly reports of A(H1)pdm09, A(H3) and B influenza viruses, 2013/14 season (as of Feb 7, 2014)



**Influenza virus isolation/detection by prefecture in 2013/14 season  
(as of Feb 7, 2014)**



**Weekly trend of isolated/detected influenza virus strain reported from local PHI nationwide in Japan from week 36, 2013 to week 5, 2014**



### Countries of Origin of Isolates Characterized by NIID

Month of Collection	A(H1N1)pdm09	A(H3N2)	B/Victoria	B/Yamagata
2012/2013 season viruses (HI-test after Sept.2013)	Laos 3	Laos 2 Myanmar 12		
September, 2013	Japan 13 Laos 4	Japan 5 Laos 4		
October, 2013	Japan 1 Laos 2 Taiwan 2	Japan 6 Taiwan 3	Japan 4	Japan 5
November, 2013	Japan 19 Taiwan 1	Japan 44 Taiwan 3	Japan 9	Japan 1
Decemder, 2013	Japan 39	Japan 27 Taiwan 1	Japan 21 Taiwan 1	Japan 18 Taiwan 1 Vietnam(HCM)* 1
January, 2014	Japan 52 Mongolia 3	Japan 10 Mongolia 1 Vietnam(HCM)* 3	Japan 4	Japan 11 Vietnam(HCM)* 4
<b>Total</b>	<b>139</b>	<b>121</b>	<b>39</b>	<b>41</b>

\*Vietnam(HCM) lineage not determined.

## **A(H1N1)pdm09 virus**

### ***Antigenic and Phylogenetic analyses:***

- Total 52 A(H1N1)pdm09 viruses were subjected to antigenic analysis by hemagglutination inhibition (HI) tests. The majority of A(H1N1)pdm09 viruses analyzed was isolated between September and December 2013. The genetic group of the HA gene are listed together with HI profiles in the HI table.
- All viruses tested reacted well with antisera raised against egg propagated A/California/7/2009. There were not antigenic variant viruses with mutation(s) in 153-157 region of HA gene at the moment.
- Similarly, the vast majority of test viruses were well covered with antiserum raised against cell-propagated A/Narita/1/2009 and only 2% of test viruses showed 8-fold reduced HI titer to the homologous titer.
- Ferret antiserum raised against a high growth reassortant (HGR) A/California/7/2009 X-179A also covered well all test viruses, but one virus each from Japan and Laos showed 8-fold reduced HI titer to A/California/7/2009 X-179A antiserum.
- Forty-three (12%) oseltamivir/permivir resistant viruses with H275Y substitution in the NA protein have been detected in Japan. A community cluster occurred in Sapporo and its neighboring cities (see detail in the section of antiviral-resistance viruses). Those resistant viruses were antigenically indistinguishable from the sensitive viruses and were antigenically similar to A/California/7/2009.
- Phylogenetic analysis of the HA and the NA genes were correlated well each other. All recent test viruses fell into group 6 with amino acid substitution of D97N, K283E, E499K in the HA protein. Group 6 was further subgrouped 6B with K163Q, A256T and 6C with V234I. Viruses belonging to subgroup 6B (79%) were predominant than those belonging to subgroup 6C (21%). Those subgroups were antigenically indistinguishable from each other.
- In phylogenetic tree of NA gene, all antiviral resistant viruses detected in Japan as well as in China belonged to subgroup with N386K in group 6B, whereas the resistant viruses detected in USA belonged to another subgroup with N397K+S82P in group 6B.

### ***Conclusions***

Vast majority of recent viruses isolated since September 2013 were antigenically closely related to A/California/7/2009 virus and a vaccine production virus A/California/7/2009 X-179A. Antiviral resistant viruses to oseltamivir and peramivir have increased since January 2014 in Sapporo city area. Those resistant viruses were antigenically similar to A/California/7/2009 virus

### ***Antigen viruses for serology tests.***

A/California/7/2009 X-179A vaccine virus

A/New Hampshire/04/2013 (recent egg-grown group 6B)

A/Wakayama/153/2013 (recent cell-grown group 6B)

A/Cameroon/3234/2013 (recent cell-grown group 6C)

## Influenza A/H1pdm09 isolates characterized by NIID

		Japan	Taiwan	Laos	Nepal	Vietnam	Total	
March 2013 - August 2013							n	%
A/California/7/2009 -like	Egg	23	2	3	9	1	38	71.7
A/California/7/2009 -like*	Egg	8	0	0	0	0	8	15.1
A/California/7/2009 (Low)**	Egg	6	0	0	0	1	7	13.2
Total		37	2	3	9	2	53	
A/Narita/1/2009 -like	Cell	24	2	3	9	1	39	73.6
A/Narita/1/2009 -like*	Cell	6	0	0	0	0	6	11.3
A/Narita/1/2009 (Low)**	Cell	7	0	0	0	1	8	15.1
Total		37	2	3	9	2	53	

		Japan	Taiwan	Laos	Nepal	Vietnam	Total	
September 2013 - December 2013							n	%
A/California/7/2009 X179A -like		34	2	2	0	0	38	73.1
A/California/7/2009 X179A -like*		8	1	3	0	0	12	23.1
A/California/7/2009 X179A (Low)**		1	0	1	0	0	2	3.8
Total		43	3	6	0	0	52	
A/California/7/2009 -like	Egg	42	2	5	0	0	49	94.2
A/California/7/2009 -like*	Egg	1	1	1	0	0	3	5.8
A/California/7/2009 (Low)**	Egg	0	0	0	0	0	0	0.0
Total		43	3	6	0	0	52	
A/Narita/1/2009 -like	Cell	42	2	6	0	0	50	96.2
A/Narita/1/2009 -like*	Cell	0	1	0	0	0	1	1.9
A/Narita/1/2009 (Low)**	Cell	1	0	0	0	0	1	1.9
Total		43	3	6	0	0	52	

\* 4-fold low to homologous titer

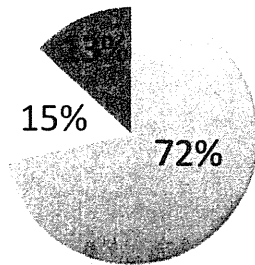
\*\* 8-fold or greater low to homologous titer

# A(H1N1)pdm09

California/07/09 (Egg)

2012/13 season

Mar - Aug

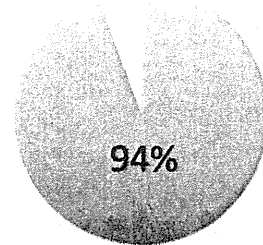


N=53

2013/14 season

Sep - Dec

6%



N=52

California/07/2009-like

California/07/2009-like\*

Antigenic variants\*\*

\*4-fold lower than the homologous titers

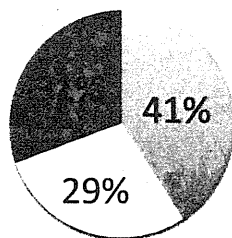
\*\*≥8-fold lower than the homologous titers

# A(H1N1)pdm09

California/07/09 (X-179A) (Egg)

2012/13 season

Mar - Aug

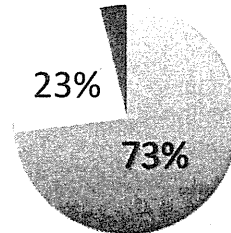


N=49

2013/14 season

Sep - Dec

4%



N=52

California/07/2009 (X-179A)-like

California/07/2009 (X-179A)-like\*

Antigenic variants\*\*

\*4-fold lower than the homologous titers

\*\*≥8-fold lower than the homologous titers

## Hemagglutination inhibition tests of influenza A/H1pdm09 viruses-0.5%TRBC

Rabbit serum										HI test date:2014/1/23
Strains	Passage History	Sample date	Wisconsin/ 10/98 Cell&Egg No.9930-2	California/ 07/09 pdm Egg NIID No.1	California/ 07/09 pdm X-179A Egg NIID No.8	Narita/ 1/09 pdm Egg NIID No.4	Narita/ 1/09 pdm Cell NIID No.5	Sapporo/ 163/11 pdm Cell NIID No.1	HA group	Amino acid substitution in HA
REF. Ag.										
A/Wisconsin/10/1998	C3/C3E2 +2		1280	640	320	1280	1280	80	-	
A/California/07/2009pdm	E2 +3	2009/04/09	160	640	320	640	640	160	-	
A/California/07/2009pdm X-179A	Ex/Ex +1		320	1280	640	2560	2560	320	-	
A/Narita/1/2009pdm	E2 +1	2009/05/08	160	1280	320	1280	1280	160	-	
A/Narita/1/2009pdm	MDCK 1 +2	2009/05/08	160	640	320	1280	1280	160	-	
A/SAPPORO/163/2011pdm	MDCK 2 +2	2011/03/04	40	20	40	< 10	< 10	320	7	R102R>>>S, K154E*, S185I*, S190R*
TEST Ag.										
A/Taiwan/874/2013	E2 +1	2013/10/03	80	640	320	1280	1280	160	CL 6B	
A/YOKOHAMA/161/2013	MDCK 2 +1	2013/12/10	160	640	320	1280	1280	160	CL 6B	
A/TOCHIGI/39/2013	MDCK 1 +1	2013/12/02	80	640	320	1280	1280	320	CL 6B	
A/TOKYO/32434/2013	MDCK 1 +1	2013/11/28	160	640	320	1280	1280	160	CL 6B	
A/SAKAI/23/2013	MDCK 1 +1	2013/12/15	160	640	320	1280	1280	160	CL 6B	
A/SAITAMA/142/2013	MDCK 2 +1	2013/12/06	160	640	320	1280	1280	160	CL 6B	
A/SAPPORO/116/2013	MDCK 1 +1	2013/11/26	160	640	320	640	1280	160	CL 6B	Oseltamivir-resistant
A/SAITAMA/145/2013	MDCK 1 +1	2013/12/06	160	640	320	640	1280	160	CL 6B	
A/MIE/27/2013	MDCK 1 +1	2013/12/25	160	320	320	1280	1280	160	CL 6B	Oseltamivir-resistant
A/Taiwan/1024/2013	MDCK 3 +1	2013/10/25	80	320	320	640	1280	160	CL 6B	
A/SAPPORO/114/2013	MDCK 1 +1	2013/11/24	160	320	320	640	1280	160	CL 6B	Oseltamivir-resistant
A/SAPPORO/119/2013	MDCK 1 +1	2013/12/07	160	320	320	640	1280	160	CL 6B	Oseltamivir-resistant
A/SAPPORO/120/2013	MDCK 1 +1	2013/12/09	160	320	320	640	1280	160	CL 6B	Oseltamivir-resistant
A/YAMAGATA/157/2013	MDCK 2 +1	2013/11/14	160	320	320	640	1280	160	CL 6B	Oseltamivir-resistant
A/SAKAI/18/2013	MDCK 1 +1	2013/11/30	80	320	320	640	640	160	CL 6C	
A/YAMAGATA/158/2013	MDCK 2 +1	2013/11/14	80	320	320	640	640	80	CL 6B	Oseltamivir-resistant
A/YOKOHAMA/163/2013	MDCK 1 +1	2013/12/10	160	320	160	640	1280	160	CL 6B	
A/KAWASAKI/62/2013	MDCK 1 +1	2013/12/06	80	320	160	640	1280	160	CL 6B	
A/OSAKA/40/2013	MDCK 2 +1	2013/11/28	80	320	160	640	640	160	CL 6B	
A/SAITAMA/134/2013	MDCK 2 +1	2013/10/28	80	320	160	640	640	160	CL 6B	
A/SAITAMA/135/2013	MDCK 1 +1	2013/11/25	80	320	160	640	640	80	CL 6B	
A/YOKOHAMA/162/2013	MDCK 2 +1	2013/12/10	80	320	160	640	640	160	CL 6B	
A/KANAGAWA/146/2013	MDCK 2 +1	2013/11/11	80	320	160	640	640	80	CL 6B	Oseltamivir-resistant
A/Taiwan/1026/2013	MDCK 3 +1	2013/11/03	40	160	160	320	320	80	CL 6B	
A/OSAKA/39/2013	MDCK 3 +1	2013/11/18	20	160	80	160	160	40	CL 6C	

\*Antigenic sites

### Hemagglutination inhibition tests of influenza A/H1pdm09 viruses-0.5%TRBC

Rabbit serum

HI test date:2013/12/26

Strains	Passage History	Sample date	Rabbit serum								HA group	Amino acid substitution in HA
			Wisconsin/ 10/98 Cell&Egg No.9930-2	California/ 07/09 pdm Egg NIID No.1	California/ 07/09 pdm X-179A Egg NIID No.8	Narita/ 1/09 pdm Egg NIID No.4	Narita/ 1/09 pdm Cell NIID No.5	Yamagata/ 752/09 pdm Cell NIID No.1	Sapporo/ 163/11 pdm Cell NIID No.1			
REF. Ag.												
A/Wisconsin/10/1998	C3/C3E2 +2		2560	1280	640	1280	1280	80	80	-		
A/California/07/2009pdm	E2 +3	2009/04/09	320	1280	640	1280	1280	640	320	-		
A/California/07/2009pdm X-179A	Ex/Ex +1		640	5120	2560	5120	5120	1280	640	-		
A/Narita/1/2009pdm	E2 +1	2009/05/08	320	1280	640	2560	2560	640	320	-		
A/Narita/1/2009pdm	MDCK 1 +2	2009/05/08	320	1280	640	2560	2560	320	160	-		
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	20	160	160	160	160	1280	320	-	G155E*	
A/SAPPORO/163/2011pdm	MDCK 2 +2	2011/03/04	80	40	40	40	40	80	640	7	R102R>S, K154E*, S185I*, S190R*	
TEST Ag.												
A/YAMAGUCHI/43/2013	MDCK 1 +1	2013/12/04	640	2560	2560	5120	10240	1280	1280	6B		
A/YAMAGUCHI/42/2013	MDCK 1 +1	2013/12/02	640	2560	2560	5120	5120	2560	1280	6B		
A/Laos/I812/2013	MDCK 1 +1	2013/09/10	320	2560	1280	5120	5120	640	640	6C		
A/TOKYO/32432/2013	MDCK 2 +1	2013/11/28	640	2560	1280	5120	5120	1280	640	6B		
A/TOKYO/33409/2013	MDCK 1 +1	2013/11/19	320	2560	1280	5120	5120	640	320	6B		
A/SAKAI/16/2013	MDCK 1 +1	2013/11/24	320	2560	1280	2560	5120	640	640	6B		
A/Laos/O032/2013	MDCK 1 +1	2013/10/01	320	1280	1280	2560	5120	640	640	6C		
A/TOKYO/32417/2013	MDCK 1 +1	2013/11/20	320	1280	1280	2560	5120	640	640	6B		
A/Laos/SA333/2013	MDCK 1 +1	2013/09/28	640	1280	640	5120	5120	320	320	6C		
A/Laos/I829/2013	MDCK 1 +1	2013/09/16	160	1280	640	1280	2560	640	320	6C		
A/Laos/O031/2013	MDCK 1 +1	2013/10/01	160	640	640	2560	2560	640	320	6C		
A/Laos/I894/2013	MDCK 1 +1	2013/09/25	320	320	320	1280	1280	320	320	6C	F200F>L, G202G>E*	

\*Antigenic sites



### Hemagglutination inhibition tests of influenza A/H1pdm09 viruses-0.5%TRBC

Rabbit serum

HI test date:2013/12/19

Strains	Passage History	Sample date	Wisconsin/ 10/98 Cell&Egg No.9930-2	California/ 07/09 pdm Egg NIID No.1	California/ 07/09 pdm X-179A Egg NIID No.8	Narita/ 1/09 pdm Egg NIID No.4	Narita/ 1/09 pdm Cell NIID No.5	Yamagata/ 752/09 pdm Cell NIID No.1	Sapporo/ 163/11 pdm Cell NIID No.1	HA group	Amino acid substitution in HA
REF. Ag.											
A/Wisconsin/10/1998	C3/C3E2 +2		2560	640	320	2560	2560	80	80	-	
A/California/07/2009pdm	E2 +3	2009/04/09	320	1280	640	1280	1280	640	320	-	
A/California/07/2009pdm X-179A	Ex/Ex +1		1280	2560	1280	5120	5120	1280	640	-	
A/Narita/1/2009pdm	E2 +1	2009/05/08	320	640	320	1280	1280	640	160	-	
A/Narita/1/2009pdm	MDCK 1 +3	2009/05/08	320	1280	640	2560	2560	640	320	-	
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	20	160	80	80	160	1280	320	-	G155E*
A/SAPPORO/163/2011pdm	MDCK 2 +2	2011/03/04	80	40	< 10	< 10	< 10	160	640	7	R120R>S, K154E*, S185I*, S190R*
TEST Ag.											
A/MIE/22/2013	MDCK 1 +1	2013/09/05	640	2560	1280	5120	5120	1280	640	6B	
A/MIE/23/2013	MDCK 1 +1	2013/09/05	640	1280	1280	2560	2560	640	320	6B	
A/MIE/24/2013	MDCK 1 +1	2013/09/26	640	1280	1280	2560	2560	640	320	6B	
<b>A/WAKAYAMA/153/2013 \$</b>	<b>MDCK 1 +1</b>	<b>2013/11/05</b>	<b>320</b>	<b>1280</b>	<b>640</b>	<b>2560</b>	<b>2560</b>	<b>640</b>	<b>320</b>	<b>6B</b>	
A/OSAKA-C/2006/2013	MDCK 1 +1	2013/09/17	320	1280	640	2560	2560	640	320	6B	
A/WAKAYAMA-C/32/2013	MDCK 1 +1	2013/09/17	160	640	640	1280	2560	320	160	6C	
A/WAKAYAMA-C/31/2013	MDCK 1 +1	2013/09/17	160	640	640	1280	1280	320	160	6C	
A/SAPPORO/107/2013	MDCK 1 +1	2013/11/15	160	640	320	1280	1280	160	160	6B	Oseltamivir-resistant

\*Antigenic sites

\$: Serology antigen

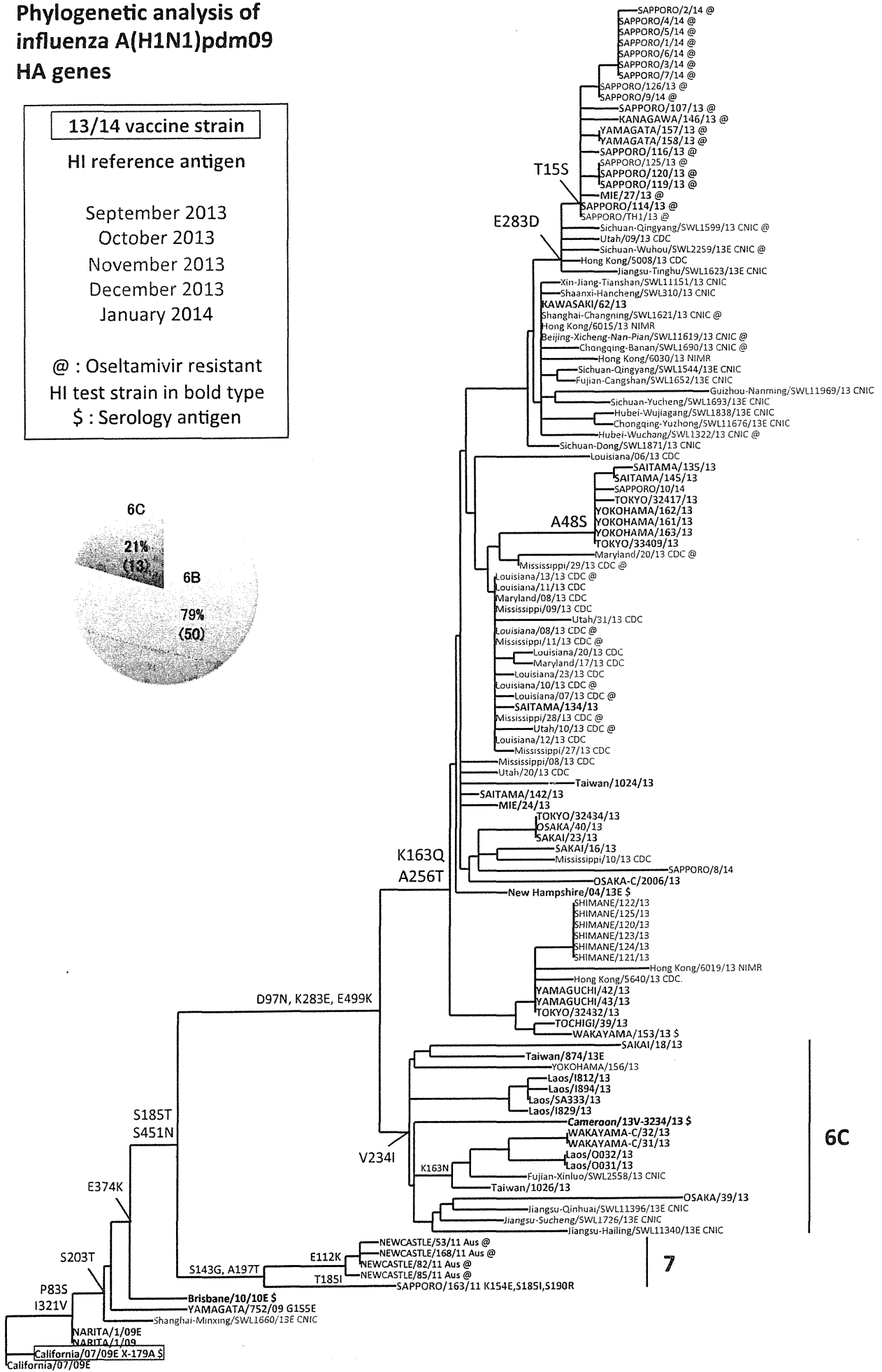
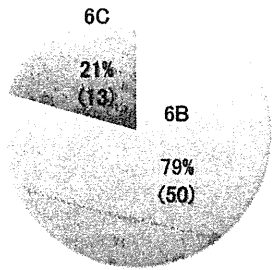
# Phylogenetic analysis of influenza A(H1N1)pdm09 HA genes

**13/14 vaccine strain**

HI reference antigen

September 2013  
October 2013  
November 2013  
December 2013  
January 2014

@ : Oseltamivir resistant  
HI test strain in bold type  
\$ : Serology antigen



6B

6C

7



## **A(H3N2) viruses**

### ***Antigenic and Phylogenetic analyses:***

- Total 62 and 45 viruses isolated since September 2013 were available for HI tests using ferret antisera raised against cell- and egg-propagated A/Texas/50/2012, respectively.
- All test viruses reacted within 4-fold different HI titer from the homologous titer of cell-propagated A/Texas/50/2012 antiserum and no virus with 8-fold reduced HI titer was observed, whereas 31% of test viruses showed over 8-fold reduced HI titer to egg-propagated A/Texas/50/2012 antiserum.
- Of those viruses, 74% exhibited 8-fold reduced HI titer from the homologous titer of X-223 (A/Texas/50-HGR) antiserum.
- All cell-propagated test viruses reacted within 4-fold different HI titer from the homologous titers of both antisera raised against cell- and egg-propagated A/New York/39/2012 viruses.
- A small number of test viruses (18 viruses) were subjected to HI tests using antisera of A/New York/39/2012 X-233 and A/New York/39/2012 X-233A. The A/New York/39/2012 X-233A antiserum well covered all test viruses, whereas 28% and 67% of tests viruses showed 8-fold and 4-fold reduced HI titers, respectively, against A/New York/39/2012 X-233 antiserum.
- The same test viruses were also subjected to HI tests using antisera raised against egg-propagated A/Almaty/2958/2013 wild type and NIB-85 (A/Almaty/2958/2013-HGR), which were kindly provided by NIMR. Fifty-six percent and 89 % of test viruses showed 8-fold reduced HI titer to A/Almaty/2958/2013 and NIB-85 antisera, respectively.
- In phylogenetic tree of HA gene, all test viruses fell into either recent major subgroup 3C.3 (51%) with T128A, R142G or subgroup 3C.2 (49%) with D489N. Phylogenetic tree of NA gene exhibited similar to that of HA gene.

### ***Conclusions***

- The majority of A(H3N2) viruses isolated since September 2013 was antigenically closely related to cell-grown A/Texas/50/2012virus, but they reacted poorly with A/Texas/50/2012 X-233 antiserum. Antisera against reference cell- and egg-propagated A/New York/39/2012 viruses covered recent test viruses well, while antisera against another egg-propagated reference virus A/Almaty/2958/2013 reacted poorly with recent viruses. No antiviral resistant virus to any of 4 NA inhibitors is found.

### ***Antigen viruses for serology tests.***

A/Texas/50/2012 X-223

A/Texas/50/2012 cell

A/New York/39/2012 cell (group 3C.3)

A/Louisiana/09/2013 cell (group 3C.3)

A/Tokyo/31512/2013 cell (group 3C.3)