

Influenza A/H3 isolates characterized by NIID

		Japan	Taiwan	Laos	Myanmar	Nepal	Vietnam	Total	
March 2013- August 2013								n	%
A/Texas/50/2012 -like	Cell	41	4	3	7	1	1	57	83.8
A/Texas/50/2012 -like*	Cell	5	6	0	0	0	0	11	16.2
A/Texas/50/2012 (Low)**	Cell	0	0	0	0	0	0	0	0.0
Total		46	10	3	7	1	1	68	
A/Victoria/361/2011 -like								52	76.5
A/Victoria/361/2011 -like*	Cell	12	3	1	0	0	0	16	23.5
A/Victoria/361/2011 (Low)**	Cell	0	0	0	0	0	0	0	0.0
Total		46	10	3	7	1	1	68	

		Japan	Taiwan	Laos	Myanmar	Nepal	Vietnam	Total	
September 2013- December 2013								n	%
A/Texas/50/2012 X-223 -like		0	0	0	0	0	0	0	0.0
A/Texas/50/2012 X-223 -like*		12	3	1	0	0	0	16	25.8
A/Texas/50/2012 X-223 (Low)**		39	4	3	0	0	0	46	74.2
Total		51	7	4	0	0	0	62	
A/Texas/50/2012 -like								3	6.7
A/Texas/50/2012 -like*	Egg	21	6	1	0	0	0	28	62.2
A/Texas/50/2012 (Low)**	Egg	11	0	3	0	0	0	14	31.1
Total		35	6	4	0	0	0	45	
A/Texas/50/2012 -like								54	87.1
A/Texas/50/2012 -like*	Cell	7	1	0	0	0	0	8	12.9
A/Texas/50/2012 (Low)**	Cell	0	0	0	0	0	0	0	0.0
Total		51	7	4	0	0	0	62	

* 4-fold low to homologous titer

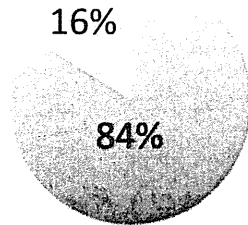
** 8-fold or greater low to homologous titer

A(H3N2)

Texas/50/12 (Cell)

2012/13 season

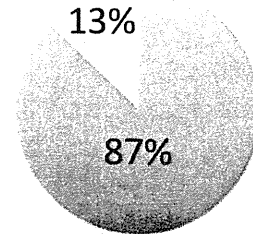
Mar - Aug



N=68

2013/14 season

Sep - Dec



N=62



Texas/50/2012-like



Texas/50/2012-like*



Antigenic variants**

*4-fold lower than the homologous titers

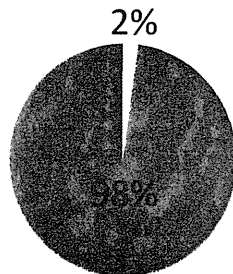
**≥8-fold lower than the homologous titers

A(H3N2)

Texas/50/12 (X-223) (Egg)

2012/13 season

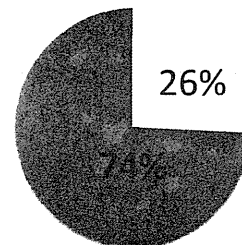
Mar - Aug



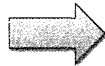
N=49

2013/14 season

Sep - Dec



N=62



Texas/50/2012-like



Texas/50/2012-like*



Antigenic variants**

*4-fold lower than the homologous titers

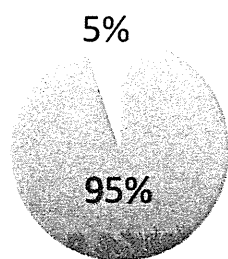
**≥8-fold lower than the homologous titers

A(H3N2)

2013/14 season

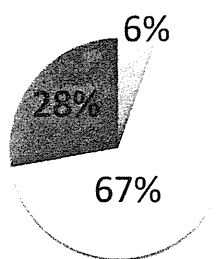
Sep - Dec

NY/39/12 (Cell)



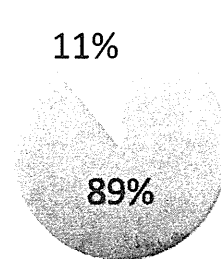
N=58

NY/39/12 (X-233) (Egg)



N=18

NY/39/12 (X-233A) (Egg)



N=18

NY/39/2012 Cell or X-233 or X-233A -like

NY/39/2012 Cell or X-233 or X233A -like*

Antigenic variants**

*4-fold lower than the homologous titers

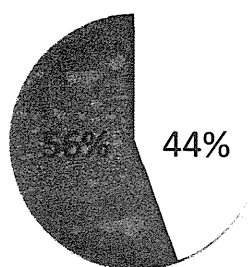
**≥8-fold lower than the homologous titers

A(H3N2)

2013/14 season

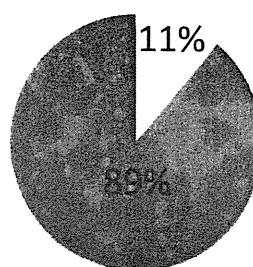
Sep - Dec

Almaty/2958/13 (Egg)



N=18

Almaty/2958/13 (NIB-85) (Egg)



N=18

* Anti-Almaty ferret sera for HI were used the sera from NIMR.

Almaty/2958/2013 Egg or NIB-85 -like

Almaty/2958/2013 Egg or NIB-85 -like*

Antigenic variants**

*4-fold lower than the homologous titers

**≥8-fold lower than the homologous titers

Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

Strains	Passage History	Sample date	CL3C								HI test date:2014/2/5	
			Texas/ 50/12 Cell No.2	Texas/ 50/12 (X-223) Egg No.1	New York/ 39/12 Cell No.2	New York/ 39/12 (X-233) Egg No.1	New York/ 39/12 (X-233A) Egg No.1	Almaty/ 2958/13 Egg from NIMR	Almaty/ 2958/13 (NIB-85) Egg from NIMR	Victoria/ 361/11 Cell No.1	HA group	Amino acid substitution in HA
REF. Ag.												
A/Texas/50/2012	M 1/C 2 +1		320	160	160	160	160	160	160	320	3C.1	
A/Texas/50/2012 (X-223)	E4/E8 +1		640	1280	640	320	160	640	640	640	3C.1	G186V*
A/New York/39/2012	C2 +1		80	80	160	80	80	80	80	160	3C.1	G186V*
A/New York/39/2012 (X-233)	E4E8/E1 +1		160	160	320	320	160	80	80	320	3C.3	
A/New York/39/2012 (X-233A)	E4E8/E1 +1		320	80	320	320	160	80	80	320	3C.3	G186V>G*
A/Almaty/2958/2013	E5 +1		160	640	320	320	160	640	640	320	3C.1	
A/Almaty/2958/2013 (NIB-85)	EX +1		320	640	640	320	160	1280	640	640	-	
A/Victoria/361/2011	MDCK 2 +2		320	160	320	160	160	320	160	320	HK2000	
STANDARD Ag. from AUS												
A/Texas/50/2012			1280	1280	1280	640	320	1280	1280	1280	3C.1	
A/Victoria/361/2011			640	640	640	320	80	640	640	640	3C.1	
A/Victoria/210/2009			640	1280	640	320	160	640	640	640	1	
A/Wisconsin/67/2005			40	40	40	40	80	80	40	40	-	
TEST Ag.												
A/Texas/50/2012 (X-223A) \$	E5/E8 +1		320	640	640	320	80	640	640	640	3C.1	
A/TOKYO/32446/2013	MDCK 3 +1	2013/12/03	320	160	320	160	160	160	160	160	Seq ND	
A/Louisiana/09/2013 \$	C 3 +1		320	80	160	80	160	160	80	160	3C.3	
A/NIIGATA-C/96/2013	MDCK 2 +1	2013/10/27	160	160	160	80	160	160	160	160	3C.3	
A/FUKUOKA/3/2013	MDCK 2 +1	2013/12/09	160	80	160	80	160	160	80	160	Seq ND	
A/Texas/50/2012 \$	M 1/C 2 +2		160	80	160	80	80	160	80	160	3C.1	
A/TOKYO/31512/2013 \$	MDCK 2 +2	2013/10/31	160	80	160	80	80	160	80	160	3C.3	
A/SHIMANE/127/2013	MDCK 2 +1	2013/12/06	160	80	160	80	80	160	80	160	3C.3	
A/HOKKAIDO/54/2013	MDCK 3 +1	2013/11/29	160	80	160	80	80	160	80	160	3C.3	
A/SHIMANE/126/2013	MDCK 2 +1	2013/12/04	160	80	160	80	80	80	80	160	Seq ND	
A/NIIGATA/1199/2013	MDCK 1 +1	2013/12/03	160	80	160	80	80	80	80	160	3C.3	
A/TOCHIGI/38/2013	MDCK 2 +1	2013/11/22	160	80	160	80	80	80	80	80	3C.3	
A/SAKAI/25/2013	MDCK 1 +1	2013/12/14	160	80	160	80	80	80	80	80	3C.3	
A/AICHI/125/2013	MDCK 1 +2	2013/11/28	80	80	80	80	80	80	80	160	3C.3	
A/Taiwan/1019/2013	MDCK 2 +2	2013/10/08	80	80	80	40	80	160	80	160	3C.2	
A/KANAGAWA/148/2013	MDCK 2 +1	2013/11/18	80	80	80	80	80	160	80	80	3C.2	
A/SAITAMA/137/2013	MDCK 2 +1	2013/11/29	80	80	80	80	80	80	80	80	3C.3	
A/FUKUOKA-C/10/2013	MDCK 3 +1	2013/12/03	80	80	80	40	80	80	40	80	3C.2	
A/SAITAMA/138/2013	MDCK 2 +1	2013/12/02	80	80	80	40	80	80	40	80	3C.3	
A/SAITAMA/143/2013	MDCK 2 +1	2013/12/06	80	40	40	40	40	80	40	80	3C.3	
A/KANAGAWA/147/2013	MDCK 2 +1	2013/11/07	80	40	40	40	40	80	40	80	3C.2	

*Antigenic sites

ND: not determined

\$: Serology antigen

Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

Strains	Passage History	Sample date	CL3C						HK2000 group	HI test date:2014/1/16		
			Texas/50/12 Cell No.2	Texas/50/12 Egg No.1	Texas/50/12 (X-223) Egg No.1	New York/39/12 Cell No.2	New York/39/12 Egg No.2	Victoria/361/11 Cell No.1	Shizuoka/736/09 Cell No.2	Hunan-beihu/1313/09 Cell No.1	HA group	Amino acid substitution in HA
REF. Ag.												
A/Texas/50/2012	M 1/C 1 +1		160	80	160	160	160	160	80	80	3C.1	
A/Texas/50/2012	E5 +1		640	320	640	320	640	320	320	80	3C.1	G186V*
A/Texas/50/2012 (X-223)	E4/E8 +1		320	320	640	320	640	320	320	80	3C.1	G186V*
A/New York/39/2012	C2 +1		80	80	80	160	160	80	40	80	3C.3	
A/New York/39/2012	E4 +1		320	320	320	320	320	320	160	40	3C.3	G186V>G*
A/Victoria/361/2011	MDCK 2 +2		320	160	160	320	320	320	160	160	3C.1	
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	160	320	320	320	320	320	-	
A/Hunan-beihu/1313/2009	C3 +1	2009/05/07	40	20	40	20	20	20	20	320	HK2000	
STANDARD Ag. from AUS												
A/Texas/50/2012			320	640	640	640	640	640	320	80	3C.1	
A/Victoria/361/2011			320	320	640	640	640	640	320	80	3C.1	
A/Victoria/210/2009			320	640	640	640	640	640	320	80	1	
A/Wisconsin/67/2005			20	20	40	20	40	40	40	80	-	
TEST Ag.												
A/FUKUOKA/1/2013	MDCK 2 +2	2013/11/27	160	160	160	160	160	160	80	160	3C.2	
A/Taiwan/1023/2013	MDCK 2 +1	2013/11/08	160	80	160	160	160	160	80	160	3C.2	
A/Taiwan/1031/2013	MDCK 3 +1	2013/10/31	160	80	160	160	160	160	80	160	3C.2	
A/Taiwan/1052/2013	MDCK 2 +1	2013/12/03	160	80	160	160	160	160	80	160	3C.2	
A/SAPPORO/112/2013	MDCK 1 +1	2013/11/25	160	80	160	160	160	160	80	160	3C.3	
A/SAPPORO/113/2013	MDCK 1 +1	2013/11/22	160	80	160	160	320	160	80	160	Seq ND	
A/Taiwan/1034/2013	MDCK 3 +1	2013/11/04	160	80	80	160	160	160	80	80	3C.3	
A/Taiwan/1045/2013	MDCK 2 +1	2013/11/16	160	80	80	160	160	160	40	80	3C.2	
A/MIE/26/2013	MDCK 1 +1	2013/12/10	160	80	80	160	160	160	80	160	3C.2	
A/SAPPORO/108/2013	MDCK 2 +1	2013/11/13	160	80	80	160	160	160	40	160	Seq ND	
A/KOCHI/30/2013	MDCK 1 +1	2013/11/30	160	80	80	160	160	80	80	160	Seq ND	
A/KOCHI/31/2013	MDCK 1 +1	2013/12/03	160	80	80	160	160	160	80	160	3C.3	
A/KAWASAKI/61/2013	MDCK 1 +1	2013/12/09	80	80	80	160	160	80	40	80	3C.3	
A/KOCHI/28/2013	MDCK 2 +1	2013/11/25	80	80	80	80	160	80	40	80	Seq ND	
A/SAITAMA/136/2013	MDCK 2 +1	2013/11/25	80	80	80	80	80	80	40	80	3C.3	
A/KOCHI/29/2013	MDCK 1 +1	2013/11/29	80	80	80	80	160	80	40	80	Seq ND	
A/KOCHI/33/2013	MDCK 2 +1	2013/11/28	80	80	80	80	80	80	40	80	Seq ND	
A/Taiwan/1029/2013	MDCK 3 +1	2013/10/25	80	80	40	80	160	80	40	80	3C.2	

*Antigenic sites

ND: not determined

Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

Strains	Passage History	Sample date	CL3C						HK2000 group	HI test date:2013/12/26		
			Texas/ 50/12 Cell No.2	Texas/ 50/12 Egg No.1	Texas/ 50/12 (X-223) Egg No.1	New York/ 39/12 Cell No.2	New York/ 39/12 Egg No.2	Victoria/ 361/11 Cell No.1	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/ 1313/09 Cell No.1	HA group	Amino acid substitution in HA
REF. Ag.												
A/Texas/50/2012	M 1/C 1 +1		160	160	160	160	160	160	80	160	3C.1	
A/Texas/50/2012	E5 +1		640	640	640	320	640	640	320	80	3C.1	G186V*
A/Texas/50/2012 (X-223)	E4/E8 +1		320	320	640	640	640	320	320	80	3C.1	G186V*
A/New York/39/2012	C2 +1		80	80	80	80	160	80	40	80	3C.3	
A/New York/39/2012	E4 +1		320	320	640	320	640	320	320	40	3C.3	G186V>G*
A/Victoria/361/2011	MDCK 2 +2		320	160	160	160	320	320	160	160	3C.1	
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	160	320	320	320	320	160	-	
A/Hunan-beihu/1313/2009	C3 +1	2009/05/07	40	40	40	40	40	40	20	320	HK2000	
STANDARD Ag. from AUS												
A/Texas/50/2012			640	640	1280	640	1280	640	640	160	3C.1	
A/Victoria/361/2011			320	320	640	320	640	320	320	80	3C.1	
A/Victoria/210/2009			320	640	640	640	640	640	320	80	1	
A/Wisconsin/67/2005			40	40	40	40	40	40	40	80	-	
TEST Ag.												
A/TOKYO/33355/2013	MDCK 2 +1	2013/10/25	320	160	160	320	320	160	160	160	3C.3	
A/MIE/25/2013	MDCK 1 +1	2013/11/27	160	160	160	320	320	160	160	80	3C.2	
A/Laos/I856/2013	MDCK 2 +1	2013/09/23	160	160	160	160	320	160	80	80	3C.2	
A/SAPORO/106/2013	MDCK 2 +1	2013/11/04	160	80	160	160	160	160	160	320	3C.3	
A/AICHI/123/2013	MDCK 1 +1	2013/11/28	160	80	160	160	160	160	80	80	3C.3	
A/CHIBA-C/47/2013	MDCK 2 +1	2013/11/22	160	80	160	160	320	160	80	160	3C.3	
A/OSAKA/37/2013	MDCK 2 +2	2013/09/09	160	80	80	160	320	160	80	160	3C.3	
A/Laos/I878/2013	MDCK 2 +1	2013/09/23	160	80	80	160	160	160	40	80	3C.2	
A/AICHI/121/2013	MDCK 1 +1	2013/11/28	160	80	80	160	160	160	40	80	3C.3	
A/AICHI/122/2013	MDCK 1 +1	2013/11/28	160	80	80	160	160	80	40	80	3C.3	
A/TOKYO/33393/2013	MDCK 2 +1	2013/11/08	160	80	80	160	160	160	80	80	3C.2	
A/YAMAGUCHI/41/2013	MDCK 3 +1	2013/11/26	160	80	80	160	160	160	80	80	3C.2	
A/CHIBA-C/48/2013	MDCK 2 +1	2013/11/25	160	80	80	160	160	160	80	160	3C.3	
A/Laos/I898/2013	MDCK 2 +1	2013/09/26	160	80	80	80	160	160	40	80	3C.2	
A/Laos/I918/2013	MDCK 2 +1	2013/09/30	160	80	80	80	160	80	40	80	3C.2	
A/AICHI/124/2013	MDCK 1 +1	2013/11/28	160	80	80	80	160	80	40	80	3C.3	

*Antigenic sites

Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

Strains	Passage History	Sample date	CL3C						HK2000 group	HI test date:2013/12/19		
			Texas/50/12 Cell No.2	Texas/50/12 Egg No.1	Texas/50/12 (X-223) Egg No.1	New York/39/12 Cell No.2	New York/39/12 Egg No.2	Victoria/361/11 Cell No.1	Shizuoka/736/09 Cell No.2	Hunan-beihu/1313/09 Cell No.1	HA group	Amino acid substitution in HA
REF. Ag.												
A/Texas/50/2012	M 1/C 1 +1		160	80	80	160	160	160	80	80	3C.1	
A/Texas/50/2012	E5 +1		320	320	640	320	640	320	320	80	3C.1	G186V*
A/Texas/50/2012 (X-223)	E4/E8 +1		320	320	640	320	320	320	320	80	3C.1	G186V*
A/New York/39/2012	C2 +1		160	80	80	160	160	160	80	80	3C.3	
A/New York/39/2012	E4 +1		160	160	320	320	320	160	160	40	3C.3	G186V>G*
A/Victoria/361/2011	MDCK 2 +2		320	160	160	160	320	320	160	160	3C.1	
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	160	320	320	320	320	160	-	
A/Hunan-beihu/1313/2009	C3 +1	2009/05/07	40	40	40	40	40	40	40	320	HK2000	
STANDARD Ag. from AUS												
A/Texas/50/2012			320	320	640	640	640	320	320	80	3C.1	
A/Victoria/361/2011			320	320	320	320	640	320	160	40	3C.1	
A/Victoria/210/2009			320	320	640	320	320	320	160	40	1	
A/Wisconsin/67/2005			80	80	80	80	80	80	80	160	-	
TEST Ag.												
A/YOKOHAMA/154/2013	MDCK 2 +1	2013/09/02	320	160	160	160	320	160	80	160	3C.3	
A/YOKOHAMA/155/2013	MDCK 2 +1	2013/09/02	160	80	160	160	160	160	80	160	3C.3	
A/TOKYO/31512/2013 \$	MDCK 2	2013/10/31	160	80	160	160	160	160	80	160	3C.3	
A/FUKUOKA-C/9/2013	MDCK 2 +1	2013/11/02	160	80	80	160	320	160	80	160	3C.2	
A/YAMAGUCHI/39/2013	MDCK 2 +1	2013/11/15	160	80	80	160	160	160	80	160	3C.3	
A/YOKOHAMA/160/2013	MDCK 2 +1	2013/11/24	160	80	80	160	160	160	40	160	3C.3	
A/YAMAGUCHI/40/2013	MDCK 3 +1	2013/09/26	80	40	40	40	80	80	20	80	3C.2	

\$: Serology antigen

*Antigenic sites

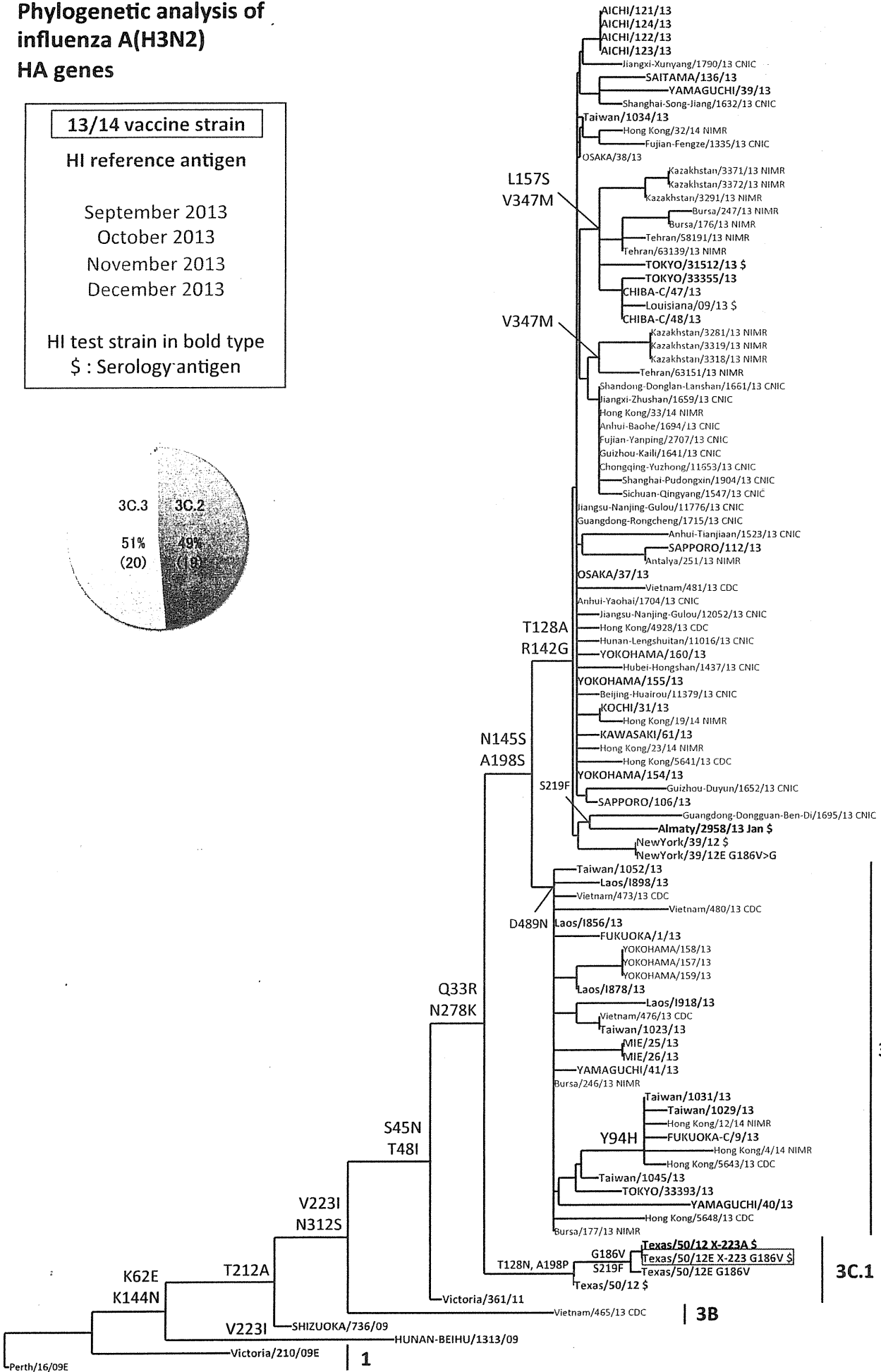
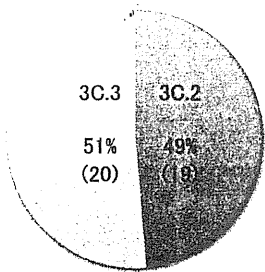
Phylogenetic analysis of influenza A(H3N2) HA genes

13/14 vaccine strain

HI reference antigen

September 2013
October 2013
November 2013
December 2013

HI test strain in bold type
\$: Serology antigen

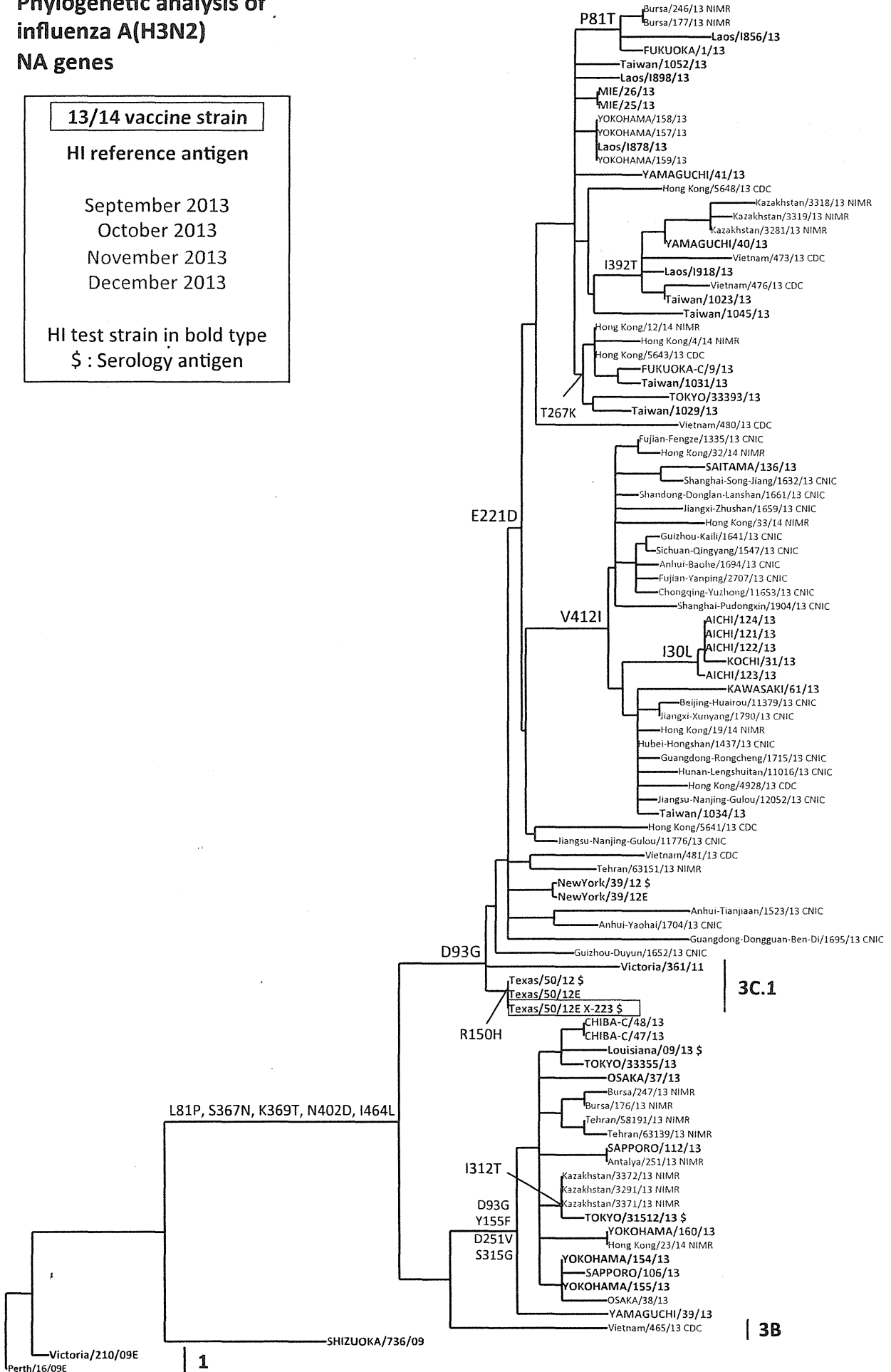


Phylogenetic analysis of influenza A(H3N2) NA genes

13/14 vaccine strain
HI reference antigen

September 2013
 October 2013
 November 2013
 December 2013

HI test strain in bold type
 \$: Serology antigen



B viruses

Antigenic and Phylogenetic analyses:

B/Yamagata-lineage

- Limited number of influenza B viruses is available for HI tests.
- Both antiserum against egg- and cell-propagated B/Massachusetts/2/2012 viruses well covered most cell-propagated test viruses and only 11% of the test viruses showed 8-fold low HI titer from those homologous titers.
- Antiserum against BX-51B (B/Massachusetts/2/2012-HGR) also reacted well with test viruses and 16% of test viruses showed 8-fold reduced HI titer to the homologous titer. However, 68% of test viruses showed 4-fold low HI titer from homologous titer.
- Recent B/Yamagata-lineage viruses fell into phylogenetically two groups of HA gene represented by B/Massachusetts/2/2012 virus (group 2) and by B/Wisconsin/1/2010 virus (group 3). All test viruses isolated in Japan since October 2013 fell into group 3.
- Some inter-lineage B viruses which possessed HA gene of B/Yamagata-lineage and NA gene of B/Victoria-lineage were isolated in Japan since December 2013 and those viruses formed the same cluster of inter-lineage B viruses isolated in China, Taiwan, USA and Canada.

B/Victoria-lineage

- All cell-propagated test viruses isolated since October 2013 reacted well with antisera raised against cell-propagated B/Brisbane/60/2008 and recent reference B/Texas/02/2013, while 89% of test viruses poorly reacted with egg-propagated B/Brisbane/60/2008 antiserum and showed over 8-fold low HI titer from the homologous titer, as well known of loss of glycosylation site at amino acid residues 197-199 in the HA protein.
- Because of low homologous titer of egg-propagated B/Texas/02/2013 antiserum, we could not precisely interpret whether the antiserum covered cell-propagated test viruses.
- All test viruses belonged to group 1A and some inter-lineage B viruses were detected as described above.

Conclusions

- Most recent B/Yamagata-lineage viruses are antigenically closely related to B/Massachusetts/2/2012. They are not antigenically distinguished from B/Wisconsin/1/2010 by our HI test. All test viruses of B/Victoria-lineage are antigenically closely related to cell-propagated B/Brisbane/60/2008 and B/Texas/02/2013 viruses. No antiviral resistant virus to any of 4 NA inhibitors is found in both lineages.

Antigen viruses for serology tests.

(B/Yamagata-lineage)

B/Massachusetts/2/2012 BX-51B

B/Massachusetts/2/201 cell

B/Shangdon-Mudan/1372/2013 cell (group 3)

B/Osaka/18/2013 cell (group 3)

B/Lithuania/6935/2013 cell (group 2)

(B/Victoria-lineage)

B/Brisbane/60/2008 egg

B/Texas/02/2013 egg (recent reference of group 1A)

B/Texas/02/2013 cell (recent reference of group 1A)

Influenza B isolates characterized by NIID

		Japan	Taiwan	Laos	Total	
March 2013 - August 2013					n	%
B/Massachusetts/02/2012 -like	Cell	42	0	5	47	100.0
B/Massachusetts/02/2012 -like*	Cell	0	0	0	0	0.0
B/Massachusetts/02/2012 -(Low)**	Cell	0	0	0	0	0.0
B/Brisbane/60/2008 -like	Cell	30	3	2	35	100.0
B/Brisbane/60/2008 -like*	Cell	0	0	0	0	0.0
B/Brisbane/60/2008 -(Low)**	Cell	0	0	0	0	0.0
Yam Total		42	0	5	47	100.0
Vic Total		30	3	2	35	74.5
Total		42	0	5	47	100

		Japan	Taiwan	Laos	Total	
September 2013 - January 2014					n	%
B/Massachusetts/02/2012 BX-51B -like		3	0	0	3	15.8
B/Massachusetts/02/2012 BX-51B -like*		13	0	0	13	68.4
B/Massachusetts/02/2012 BX-51B -(Low)**		2	1	0	3	15.8
B/Massachusetts/02/2012 -like	Egg	16	0	0	16	84.2
B/Massachusetts/02/2012 -like*	Egg	0	1	0	1	5.3
B/Massachusetts/02/2012 -(Low)**	Egg	2	0	0	2	10.5
B/Massachusetts/02/2012 -like	Cell	16	0	0	16	84.2
B/Massachusetts/02/2012 -like*	Cell	0	1	0	1	5.3
B/Massachusetts/02/2012 -(Low)**	Cell	2	0	0	2	10.5
B/Brisbane/60/2008 -like	Egg	1	0	0	1	5.6
B/Brisbane/60/2008 -like*	Egg	1	0	0	1	5.6
B/Brisbane/60/2008 -(Low)**	Egg	16	0	0	16	88.9
B/Brisbane/60/2008 -like	Cell	18	0	0	18	100.0
B/Brisbane/60/2008 -like*	Cell	0	0	0	0	0.0
B/Brisbane/60/2008 -(Low)**	Cell	0	0	0	0	0.0
Yam Total		18	1	0	19	100.0
Vic Total		18	0	0	18	94.7
Total		18	1	0	19	100.0

* 4-fold low to homologous titer

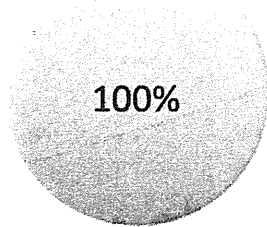
** 8-fold low to homologous titer

B Yamagata

Massachusetts/02/12 (Cell)

2012/13 season

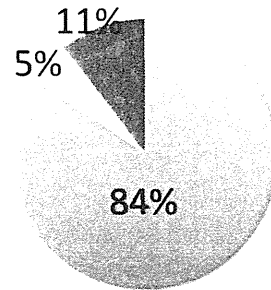
Mar - Aug



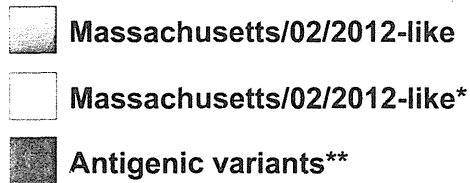
N=47

2013/14 season

Sep - Jan



N=19



*4-fold lower than the homologous titers

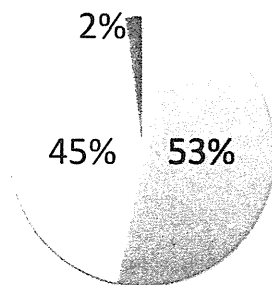
**≥8-fold lower than the homologous titers

B Yamagata

Massachusetts/02/12 (BX-51B) (Egg)

2012/13 season

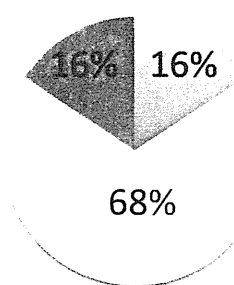
Mar - Aug



N=47

2013/14 season

Sep - Jan



N=19



*4-fold lower than the homologous titers

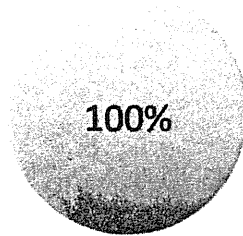
**≥8-fold lower than the homologous titers

B Victoria

Brisbane/60/08 (Cell)

2012/13 season

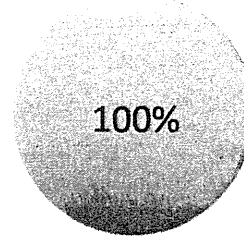
Mar - Aug



N= 35

2013/14 season

Sep - Dec



N= 18



Brisbane/60/2008 Cell-like



Brisbane/60/2008 Cell-like*

*4-fold lower than the homologous titers



Antigenic variants**

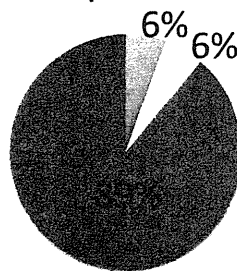
**≥8-fold lower than the homologous titers

B Victoria

Brisbane/60/08 (Egg)

2013/14 season

Sep - Dec



N=18



Brisbane/60/2008 Egg-like



Brisbane/60/2008 Egg-like*

*4-fold lower than the homologous titers



Antigenic variants**

**≥8-fold lower than the homologous titers

Hemagglutination inhibition tests of influenza B viruses (Yamagata lineage)-0.5%CRBC

Strains	Passage History	Sample date	CL2							CL3		HA group	HI test date:2014/1/30	Amino acid Substitution in HA	
			Kanagawa/ 37/11 Cell No.1	Massachu- setts/ 02/12 Cell NIID No.2	Massachu- setts/ 02/12 Egg NIID No.1	Massachu- setts/ 02/12 BX-51B Egg No.1	Malaysia/ 412/12 Cell No.1	Malaysia/ 412/12 Cell No.2	Malaysia/ 412/12 from Aus. F2312-21Day	Wisconsin/ 01/10 Cell No.1	Sakai/ 36/11 Cell No.1				
REF. Ag.															
B/KANAGAWA/37/2011	MDCK 1 +2	2011/10/21	320	640	320	80	320	320	320	80	320	2			
B/Massachusetts/02/2012	M 1/C 2 +2		160	640	160	80	160	160	160	40	80	2			
B/Massachusetts/02/2012	E3 +1		80	160	640	320	80	160	80	40	2	N196D*			
B/Massachusetts/02/2012 (BX-51B)	E3/E7 +1		160	320	640	320	160	160	80	40	2	N196D*			
B/MALAYSIA/412/2012	MDCK X/MDCK 1		160	640	320	160	160	320	160	80	80				
B/Wisconsin/01/2010	C 1/C 1 +2		80	160	160	80	40	80	40	320	160	3	N202S*, N196N>S*, T198T>I*		
B/SAKAI/36/2011	MDCK 1 +2	2011/11/01	160	320	160	80	160	160	160	320	640	3	N202S*		
TEST Ag.															
B/SAKAI/28/2013	MDCK 1 +1	2013/12/23	640	640	640	160	640	320	160	640	640	3			
B/Lithuania/6935/2013 \$	MDCK 4 +1		320	640	320	160	160	320	320	80	160	2			
B/SHIZUOKA/126/2013	MDCK 3 +1	2013/12/16	320	640	320	80	320	320	160	320	640	3			
B/SAPPORO/31/2013	MDCK 1 +1	2013/12/24	640	640	320	80	320	160	160	320	640	3			
B/SAPPORO/33/2013	MDCK 1 +1	2013/12/27	640	640	320	80	320	320	160	320	640	3			
B/OSAKA/18/2013 \$	MDCK 1 +1	2013/10/24	320	320	320	80	320	160	160	320	640	3			
B/SAPPORO/30/2013	MDCK 1 +1	2013/12/05	320	320	320	80	320	160	160	320	640	3			
B/SAKAI/32/2013	MDCK 1 +1	2013/12/22	320	320	320	80	320	160	160	320	640	3			
B/SAITAMA/16/2013	MDCK 1 +1	2013/12/16	320	320	320	80	160	160	160	320	640	3			
B/SHIGA/50/2013	MDCK 2 +1	2013/12/12	320	320	320	80	160	160	160	320	640	3			
B/OSAKA/22/2013	MDCK 2 +1	2013/12/19	320	320	320	80	320	160	160	320	640	3			
B/SAPPORO/36/2013	MDCK 1 +1	2013/12/28	320	320	320	80	320	160	160	320	640	3			
B/SAPPORO/1/2014	MDCK 1 +1	2014/01/06	320	320	320	80	320	160	160	320	640	3			
B/Taiwan/28/2013	MDCK 1 +1	2013/12/13	160	160	160	40	80	40	80	80	320	3			
B/OSAKA/21/2013	MDCK 2 +1	2013/12/16	40	80	80	40	20	40	10	80	80	3	N196N>>>K, T198T/A/N/I/D/V		
B/KAWASAKI/12/2013	MDCK 1 +1	2013/12/05	20	40	80	40	10	20	10	160	80	3	N196N>>>K, T198I>>T		

\$: Serology antigen

*Antigenic sites

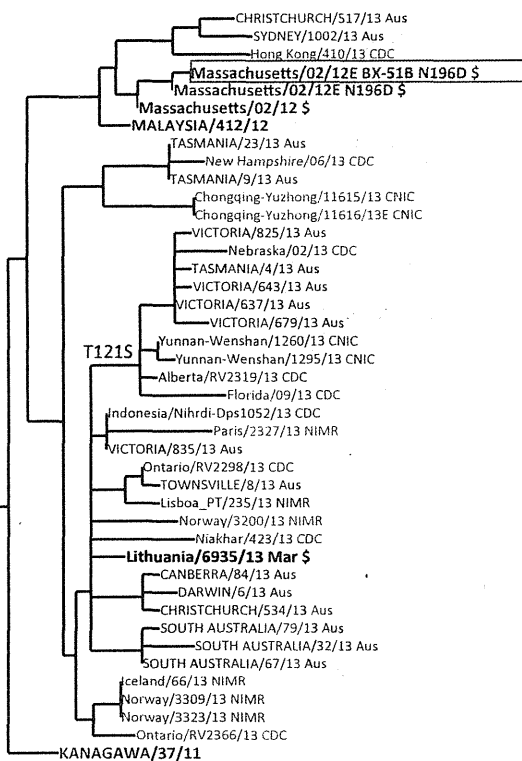
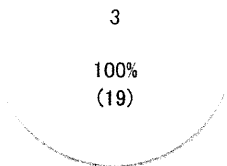
Hemagglutination inhibition tests of influenza B viruses (Yamagata lineage)-0.5%CRBC

Strains	Passage History	Sample date	CL1	CL2			CL3		HA group	Amino acid substitution in HA	
			Florida/ 04/06 Egg No.08-1	Kanagawa/ 37/11 Cell No.1	Massa- chusetts/ 02/12 Cell NIID No.2	Massa- chusetts/ 02/12 Egg NIID No.1	Massa- chusetts/ 02/12 BX-51B Egg No.1	Wisconsin/ 01/10 Cell No.1			Sakai/ 36/11 Cell No.1
REF. Ag.											
B/Florida/04/2006	E2 /E1+1	2006/12/15	640	80	160	640	640	160	160	1	
B/KANAGAWA/37/2011	MDCK 1 +2	2011/10/21	160	320	320	320	160	80	160	2	
B/Massachusetts/02/2012	M 1/C 2 +2		80	80	160	160	80	40	40	2	
B/Massachusetts/02/2012	E3 +1		160	40	80	320	160	20	10	2	N196D*
B/Massachusetts/02/2012 (BX-51B)	E3/E7 +1		160	40	80	320	320	40	20	2	N196D*
B/Wisconsin/01/2010	C 1/C 1 +2		40	40	80	80	80	80	80	3	N202S*, N196N>S*, T198T>I*
B/SAKAI/36/2011	MDCK 1 +2	2011/11/01	80	160	160	160	80	160	320	3	N202S*
TEST Ag.											
B/WAKAYAMA/149/2013	MDCK 1 +1	2013/10/07	160	160	160	160	160	160	160	3	
B/WAKAYAMA/150/2013	MDCK 1 +1	2013/10/08	80	160	160	160	80	160	160	3	
B/WAKAYAMA/151/2013	MDCK 1 +1	2013/10/08	80	160	160	160	80	160	160	3	
B/WAKAYAMA/152/2013	MDCK 1 +1	2013/10/08	80	160	160	160	80	160	160	3	

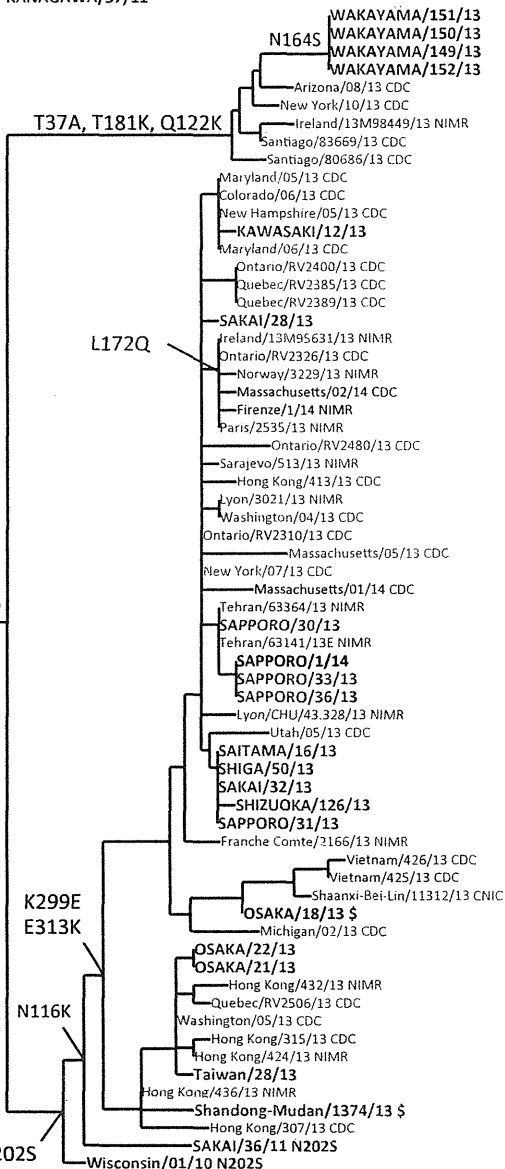
*Antigenic sites

**Phylogenetic analysis of
Influenza B (Yamagata-lineage)
HA genes**

13/14 vaccine strain
HI reference antigen
 September 2013
 October 2013
 November 2013
 December 2013
 January 2014
HI test strain in bold type
§ : Serology antigen



2

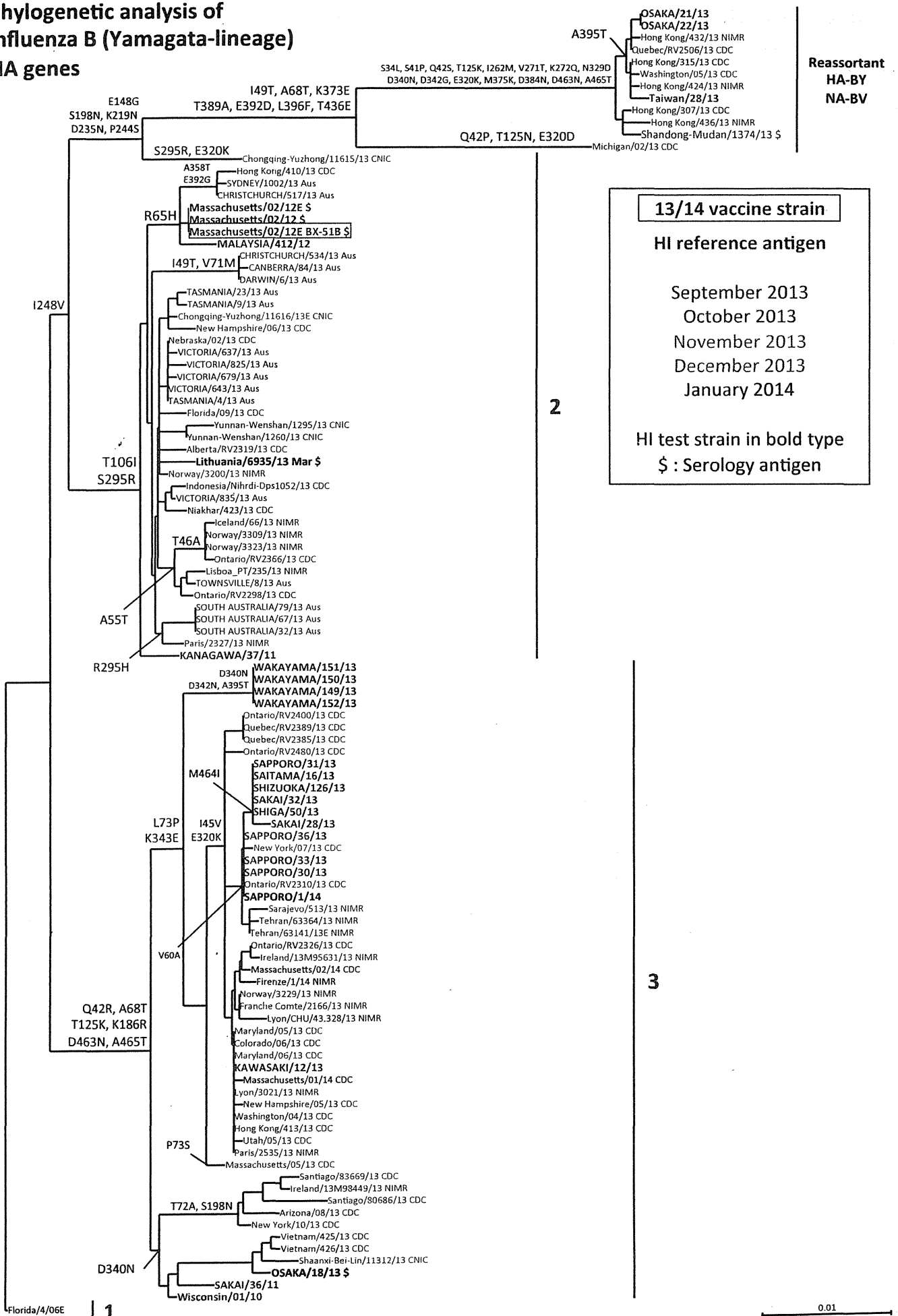


3

K88R, E479D
 S150I, N165Y, S229D
 Florida/4/06E

1

Phylogenetic analysis of Influenza B (Yamagata-lineage) NA genes



Hemagglutination inhibition tests of influenza B viruses (Victoria lineage)-0.5%CRBC

Strains	Passage History	Sample date	CL1a					CL1b	CL5	HA group	Amino acid substitution in HA
			Brisbane/60/08 Cell NIID No.4	Brisbane/60/08 Egg No.2	Texas/02/13 Cell No.1	Texas/02/13 Egg No.1	Sakai/43/08 Cell No.2 Boosted	Shizuoka/57/11 Cell No.3 Boosted	Taiwan/55/09 Cell No.10281-2 Rabbit serum		
REF. Ag.											
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	160	80	320	80	320	2560	160	1A	
B/Brisbane/60/2008	E4 +1	2008/08/04	40	320	320	160	80	640	320	1A	N196N=S, T198T>>A
B/Texas/02/2013	M 1/C 2 +1		80	640	320	320	160	320	320	1A	
B/Texas/02/2013	E5 +1		40	160	160	80	40	160	80	1A	
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	80	80	320	80	160	1280	160	1A	
B/SHIZUOKA/57/2011	MDCK 1 +1	2011/03/14	40	< 10	20	10	80	2560	10	1B	
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	< 10	320	160	40	20	20	640	5	
TEST Ag.											
B/TOKYO/33418/2013	MDCK 1 +1	2013/11/21	160	40	320	80	320	2560	160	1A	
B/TOKYO/33419/2013	MDCK 1 +1	2013/11/22	160	40	320	80	320	2560	160	1A	
B/SAKAI/17/2013	MDCK 1 +1	2013/11/26	160	80	320	80	320	2560	160	1A	
B/SAKAI/19/2013	MDCK 1 +1	2013/12/03	160	40	320	80	320	2560	160	1A	
B/YOKOHAMA/30/2013	MDCK 2 +1	2013/12/12	160	40	320	80	320	2560	160	1A	
B/YOKOHAMA/31/2013	MDCK 1 +1	2013/12/12	160	40	320	80	320	2560	160	1A	
B/YOKOHAMA/32/2013	MDCK 2 +1	2013/12/13	160	40	320	80	320	2560	160	1A	
B/SAKAI/20/2013	MDCK 1 +1	2013/12/08	160	40	320	80	320	2560	160	1A	
B/KAWASAKI/13/2013	MDCK 1 +1	2013/12/11	160	40	320	80	320	2560	160	1A	
B/KAWASAKI/15/2013	MDCK 1 +1	2013/12/11	160	40	320	80	320	2560	160	1A	
B/KAWASAKI/17/2013	MDCK 1 +1	2013/12/11	160	40	320	80	320	2560	160	1A	
B/SENDAI/19/2013	MDCK 1 +1	2013/11/26	160	40	320	80	320	2560	160	1A	
B/TOKYO/31625/2013	MDCK 2 +1	2013/11/27	160	40	320	80	160	1280	160	1A	
B/YOKOHAMA/33/2013	MDCK 2 +1	2013/12/14	160	40	320	80	160	2560	160	1A	

ND: not determined

Hemagglutination inhibition tests of influenza B viruses (Victoria lineage)-0.5%CRBC

Strains	Passage History	Sample date	CL1a			CL1b	CL5	HI test date:2013/12/12	
			Brisbane/ 60/08 Cell NIID No.4	Brisbane/ 60/08 Egg No.2	Sakai/ 43/08 Cell No.2 Boosted	Shizuoka/ 57/11 Cell No.3 Boosted	Taiwan/55/09 Cell No.10281-2 Rabbit serum	HA group	Amino acid substitution in HA
REF. Ag.									
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	80	80	160	640	80	1A	
B/Brisbane/60/2008	E4 +1	2008/08/04	40	320	80	320	160	1A	N196N=S, T198T>>A
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	160	80	160	640	160	1A	
B/SHIZUOKA/57/2011	MDCK 1 +1	2011/03/14	80	20	80	1280	40	1B	
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	80	320	80	40	640	5	
TEST Ag.									
B/SHIZUOKA/122/2013	MDCK 2 +1	2013/10/21	160	40	160	1280	80	1A	
B/SHIZUOKA/123/2013	MDCK 3 +1	2013/10/21	160	40	160	1280	80	1A	
B/AICHI/62/2013	MDCK 1 +1	2013/10/15	160	40	160	640	80	1A	
B/YOKOHAMA/29/2013	MDCK 2 +1	2013/10/09	80	160	80	640	160	1A	