

Phylogenetic analysis of influenza H3N2 NA genes

12/13 Japanese vaccine strain

HI reference strains in Red

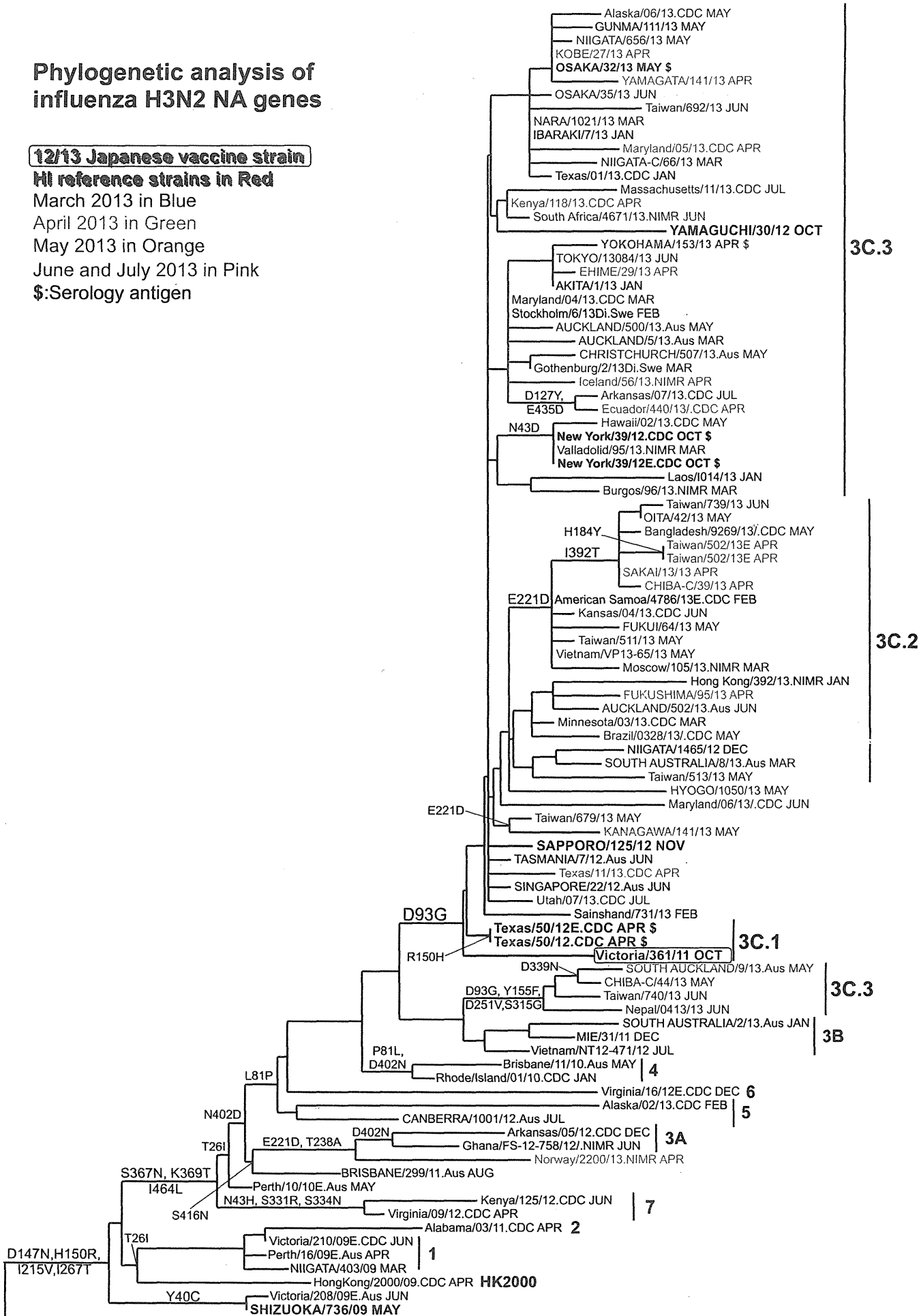
March 2013 in Blue

April 2013 in Green

May 2013 in Orange

June and July 2013 in Pink

\$: Serology antigen



Influenza A (H3N2) HA1 amino acid comparison

	10aa	CL	-----X-----X-----X-----X-----X-----X-----X	
	Consensus (n=277)		1 QKLPGNDNSTATLCLGHVAVPNGTIVKTIITNDRIEVTNATELVQNSSIGEICDSPHQILD	60
Epidemic strains	TOKYO/13084/13_JUN	3C.3	1	60
	Nepal/0413/13_JUN	3C.3	1	60
	Taiwan/692/13_JUN	3C.2	1	60
	Vietnam/VP13-65/13_MAY	3C.2	1	60
	CHIBA-C/39/13_APR	3C.2	1	60
Serology Ag	NewYork/39/12_OCT	3C.3	1	60
	NewYork/39/12E_OCT	3C.3	1	60
	YOKOHAMA/153/13_APR	3C.3	1	60
	OSAKA/32/13_MAY	3C.2	1	60
	Texas/50/12(X-223)_E4E8+1	3C.1	1	60
Reference Strains	Texas/50/12_M1C1+1	3C.1	1	60
	SAPPORO/125/12_NOV	3C.2	1	60
	YAMAGUCHI/30/12_OCT	3C.3	1	60
	Victoria/361/11_OCT	3C.1	1	60
	SHIZUOKA/736/09_MAY	-	1	60
HUNAN-BEIHU/1313/09_MAY	HK	1	60	
	H3_AG_site(Vic361).txt		-----	
	H3_RB_site(Vic361).txt		-----	

	10aa	CL	-----X-----X-----X-----X-----X-----X-----X	
	Consensus (n=277)		61 GENCTLIDALLGDPQCDGFQNKWDLFVRSKAYSNCYPYDVPDYASLRSLVASSGTLEF	120
Epidemic strains	TOKYO/13084/13_JUN	3C.3	61	120
	Nepal/0413/13_JUN	3C.3	61	120
	Taiwan/692/13_JUN	3C.2	61	120
	Vietnam/VP13-65/13_MAY	3C.2	61	120
	CHIBA-C/39/13_APR	3C.2	61	120
Serology Ag	NewYork/39/12_OCT	3C.3	61	120
	NewYork/39/12E_OCT	3C.3	61	120
	YOKOHAMA/153/13_APR	3C.3	61	120
	OSAKA/32/13_MAY	3C.2	61	120
	Texas/50/12(X-223)_E4E8+1	3C.1	61	120
Reference Strains	Texas/50/12_M1C1+1	3C.1	61	120
	SAPPORO/125/12_NOV	3C.2	61	120
	YAMAGUCHI/30/12_OCT	3C.3	61	120
	Victoria/361/11_OCT	3C.1	61	120
	SHIZUOKA/736/09_MAY	-	61	120
HUNAN-BEIHU/1313/09_MAY	HK	61	120	
	H3_AG_site(Vic361).txt		-----	
	H3_RB_site(Vic361).txt		-----	

	10aa	CL	----- ¹²⁸ X----- ^{142 145} X-----X-----X-----X-----X	
	Consensus (n=277)		121 NNESFNWAGVTQNGTSSACIRGSNSSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIW	180
Epidemic strains	TOKYO/13084/13_JUN	3C.3	121	180
	Nepal/0413/13_JUN	3C.3	121	180
	Taiwan/692/13_JUN	3C.2	121	180
	Vietnam/VP13-65/13_MAY	3C.2	121	180
	CHIBA-C/39/13_APR	3C.2	121	180
Serology Ag	NewYork/39/12_OCT	3C.3	121	180
	NewYork/39/12E_OCT	3C.3	121	180
	YOKOHAMA/153/13_APR	3C.3	121	180
	OSAKA/32/13_MAY	3C.2	121	180
	Texas/50/12(X-223)_E4E8+1	3C.1	121	180
Reference Strains	Texas/50/12_M1C1+1	3C.1	121	180
	SAPPORO/125/12_NOV	3C.2	121	180
	YAMAGUCHI/30/12_OCT	3C.3	121	180
	Victoria/361/11_OCT	3C.1	121	180
	SHIZUOKA/736/09_MAY	-	121	180
HUNAN-BEIHU/1313/09_MAY	HK	121	180	
	H3_AG_site(Vic361).txt		-----	
	H3_RB_site(Vic361).txt		-----	

	10aa	CL		-----X-----X-----X-----X-----X-----X-----X	
	Consensus (n=277)		181	GVHHPGTDKQIFLYAQSSGRITVSTKRSQQAVIPNIGSRPRIRNIPSRISYWTIVKPG	24C
Epidemic strains	TOKYO/13084/13_JUN	3C.3	181B.....	24C
	Nepal/0413/13_JUN	3C.3	181	24C
	Taiwan/692/13_JUN	3C.2	181X.....	24C
	Vietnam/VP13-65/13_MAY	3C.2	181B.....	24C
	CHIBA-C/39/13_APR	3C.2	181	24C
	Texas/50/12(X-223)_E4E8+1	3C.3	181V.....P.....F.....N.....	24C
Serology Ag	Texas/50/12_M1C1+1	3C.3	181P.....	24C
	NewYork/39/12_OCT	3C.3	181X.....	24C
	NewYork/39/12E_OCT	3C.2	181	..X..X.....X.....	24C
	YOKOHAMA/153/13_APR	3C.1	181	24C
	OSAKA/32/13_MAY	3C.1	181X.....	24C
	Victoria/361/11_OCT	3C.2	181	24C
Reference Strains	SAPPORO/125/12_NOV	3C.3	181	24C
	YAMAGUCHI/30/12_OCT	3C.1	181	24C
	SHIZUOKA/736/09_MAY	-	181A.....V.....	24C
	HUNAN-BEIHU/1313/09_MAY	HK	181N.....A.....T.....X.....D.....	24C
	H3_AG_site(Vic361).txt			-----	
	H3_RB_site(Vic361).txt			-----	
	10aa	CL		-----X-----X-----X-----X-----X-----X-----X	
	Consensus (n=277)		241	DILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCKSECITPNGSIPNDKPFQNVNRI	30C
Epidemic strains	TOKYO/13084/13_JUN	3C.3	241V.....	30C
	Nepal/0413/13_JUN	3C.3	241	30C
	Taiwan/692/13_JUN	3C.2	241	30C
	Vietnam/VP13-65/13_MAY	3C.2	241	30C
	CHIBA-C/39/13_APR	3C.2	241G.....	30C
	Texas/50/12(X-223)_E4E8+1	3C.3	241	30C
Serology Ag	Texas/50/12_M1C1+1	3C.3	241	30C
	NewYork/39/12_OCT	3C.3	241	30C
	NewYork/39/12E_OCT	3C.2	241	30C
	YOKOHAMA/153/13_APR	3C.1	241	30C
	OSAKA/32/13_MAY	3C.1	241	30C
	Victoria/361/11_OCT	3C.2	241N.....	30C
Reference Strains	SAPPORO/125/12_NOV	3C.3	241	30C
	YAMAGUCHI/30/12_OCT	3C.1	241	30C
	SHIZUOKA/736/09_MAY	-	241N.....	30C
	HUNAN-BEIHU/1313/09_MAY	HK	241X.....K.....N.....	30C
	H3_AG_site(Vic361).txt			-----N-----	
	H3_RB_site(Vic361).txt			-----	
	10aa	CL		-----X-----X-----X	
	Consensus (n=277)		301	TYGACPRYVKQSTLKLATGMRNVPEKQTRG	33C
Epidemic strains	TOKYO/13084/13_JUN	3C.3	301	33C
	Nepal/0413/13_JUN	3C.3	301	33C
	Taiwan/692/13_JUN	3C.2	301	33C
	Vietnam/VP13-65/13_MAY	3C.2	301	33C
	CHIBA-C/39/13_APR	3C.2	301	33C
	Texas/50/12(X-223)_E4E8+1	3C.3	301	33C
Serology Ag	Texas/50/12_M1C1+1	3C.3	301	33C
	NewYork/39/12_OCT	3C.3	301	33C
	NewYork/39/12E_OCT	3C.2	301	33C
	YOKOHAMA/153/13_APR	3C.1	301	33C
	OSAKA/32/13_MAY	3C.1	301	33C
	Victoria/361/11_OCT	3C.2	301	33C
Reference Strains	SAPPORO/125/12_NOV	3C.3	301	33C
	YAMAGUCHI/30/12_OCT	3C.1	301	33C
	SHIZUOKA/736/09_MAY	-	301N.....	32E
	HUNAN-BEIHU/1313/09_MAY	HK	301N.....	32E
	H3_AG_site(Vic361).txt			-----	
	H3_RB_site(Vic361).txt			-----	

B viruses

Antigenic and Phylogenetic analyses:

B/Yamagata-lineage

- Total 120 viruses belonging to B/Yamagata-lineage were subjected to HI tests. As mentioned in A(H3N2) section, we similarly omitted antisera against egg-grown vaccine and reference viruses from our ferret antiserum panel of HI tests of B viruses, except for B/Massachusetts/2/2012 BX-51B antiserum to evaluate reactivity.
- Recent viruses of this lineage is phylogenetically classified into two clades, clade 2 represented by B/Massachusetts/2/2012 virus and clade 3 represented by B/Wisconsin/1/2010 virus. These two major clades were not antigenically distinguishable from each other by our ferret antisera.
- All recent test viruses were inhibited well by both cell-grown B/Massachusetts/2/2012 antiserum and cell-grown B/Wisconsin/1/2010 antiserum within 2-fold different HI titers to each homologous titer
- Antiserum to B/Massachusetts/2/2012 BX-51B covered well most test viruses and overall only 7% of test viruses exhibited 8-fold lower HI titer to B/Massachusetts/2/2012 BX-51B antiserum.
- All Japanese test viruses, except one isolated after March, fell into clade 2. Recent viruses further formed a certain group without particular marker amino acid substitution, tentatively designated subclade 2A.
- A few Laos isolates (December, 2012, and Jan-Feb, 2013) fell into clade 3.

Conclusions

- Most recent viruses are antigenically closely related to B/Massachusetts/2/2012 and B/Wisconsin/1/2010 viruses. Although vaccine production virus B/Massachusetts/2/2012 BX-51B has lost a glycosylation site at 196-198 region, the B/Massachusetts/2/2012 BX-51B ferret antiserum covers well most (93%) of recent epidemic viruses isolated in MDCK cells.
- No antiviral resistant virus to any of 4 NA inhibitors is found in this lineage.

Antigen viruses for serology tests.

B/Massachusetts/2/2012 egg-grown wild type

B/Massachusetts/2/2012 BX-51B

B/Fukui/24/2013 (recent clade 2, cell-grown B/Massachusetts/2/2012-like)

B/Hawaii/1/2013 (recent clade 2, cell-grown virus)

B/Victoria-lineage

- All recent B/Victoria-lineage viruses tested were antigenically similar to cell-grown B/Brisbane/60/2008. They showed the same HI titers to the homologous titer of B/Brisbane/60/2008 antiserum.
- All viruses tested belonged to clade 1A in phylogenetic tree of the HA. The majority of test viruses further formed K209N subclade.
- In NA gene, all test viruses but one belonged to clade 1A, and some recent viruses tended to form a subclade with K343E or K343E+K107N.

Conclusions

- All viruses tested are antigenically and phylogenetically homogeneous and closely related to B/Brisbane/60/2008 virus. A few viruses bearing both K343E and K107N substitutions in the NA protein exhibited reduced sensitivity to oseltamivir and peramivir. These substitutions were detected with viruses in clinical specimens.

Influenza B isolates characterized by NIID

		Japan	China	Taiwan	Laos	Myanmar	Nepal	Vietnam	Total	
September 2012 - February 2013									n	%
B/Brisbane/60/2008 -like	Cell	44	1	0	18	0	0	0	63	98.4
B/Brisbane/60/2008 -like*	Cell	1	0	0	0	0	0	0	1	1.6
B/Brisbane/60/2008 -(Low)**	Cell	0	0	0	0	0	0	0	0	0.0
B/Wisconsin/1/2010 -like	Cell	58	1	0	4	3	6	1	73	93.6
B/Wisconsin/1/2010 -like*	Cell	2	0	0	2	0	1	0	5	6.4
B/Wisconsin/1/2010 -(Low)**	Cell	0	0	0	0	0	0	0	0	0.0
B/Massachusetts/02/2012 -like	Cell	38	0	0	5	3	7	1	54	69.2
B/Massachusetts/02/2012 -like*	Cell	6	0	0	0	0	0	0	6	7.7
B/Massachusetts/02/2012 -(Low)**	Cell	1	0	0	0	0	0	0	1	1.3
Vic Total		45	1	0	18	0	0	0	64	45.1
Yam Total		60	1	0	6	3	7	1	78	54.9
Total		105	2	0	24	3	7	1	142	

		Japan	China	Taiwan	Laos	Myanmar	Nepal	Vietnam	Total	
March 2013 - August 2013									n	%
B/Brisbane/60/2008 -like	Cell	27	0	3	1	0	0	0	31	100.0
B/Brisbane/60/2008 -like*	Cell	0	0	0	0	0	0	0	0	0.0
B/Brisbane/60/2008 -(Low)**	Cell	0	0	0	0	0	0	0	0	0.0
B/Wisconsin/1/2010 -like	Cell	40	0	0	2	0	0	0	42	100.0
B/Wisconsin/1/2010 -like*	Cell	0	0	0	0	0	0	0	0	0.0
B/Wisconsin/1/2010 -(Low)**	Cell	0	0	0	0	0	0	0	0	0.0
B/Massachusetts/02/2012 -like	Cell	40	0	0	2	0	0	0	42	100.0
B/Massachusetts/02/2012 -like*	Cell	0	0	0	0	0	0	0	0	0.0
B/Massachusetts/02/2012 -(Low)**	Cell	0	0	0	0	0	0	0	0	0.0
Vic Total		27	0	3	1	0	0	0	31	42.5
Yam Total		40	0	0	2	0	0	0	42	57.5
Total		67	0	3	3	0	0	0	73	

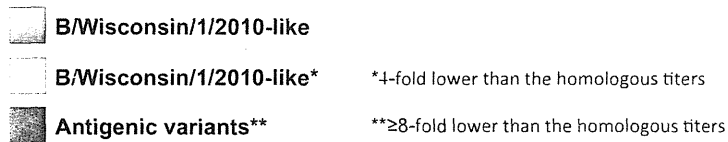
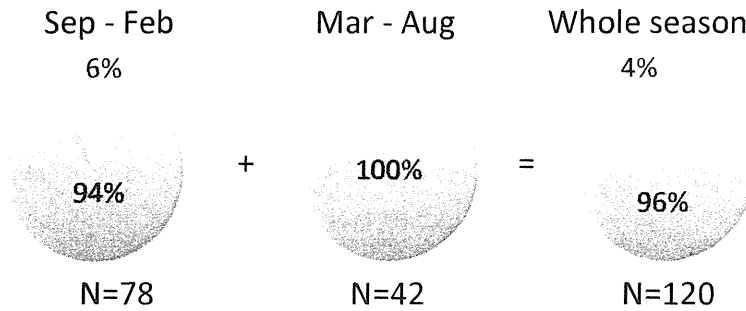
* 4-fold low to homologous titer

** 8-fold low to homologous titer

B Yamagata

B/Wisconsin/1/2010 (Cell)

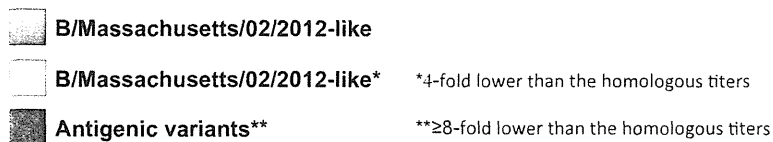
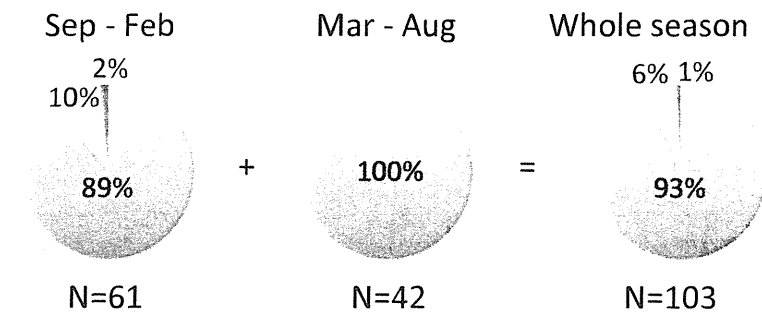
2012/13 season



B Yamagata

B/Massachusetts/02/2012 (Cell)

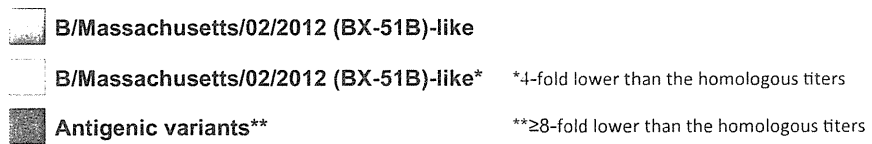
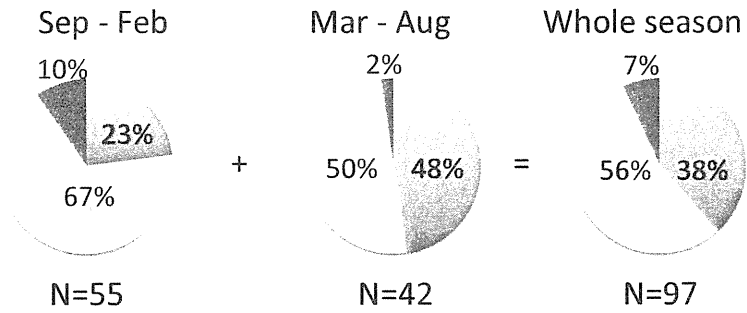
2012/13 season



B Yamagata

B/Massachusetts/02/2012 (BX-51B) (Egg)

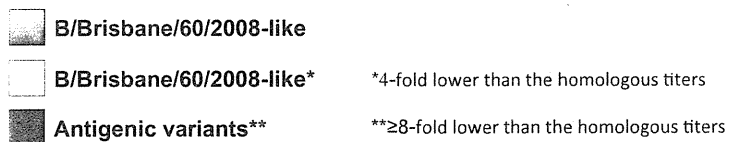
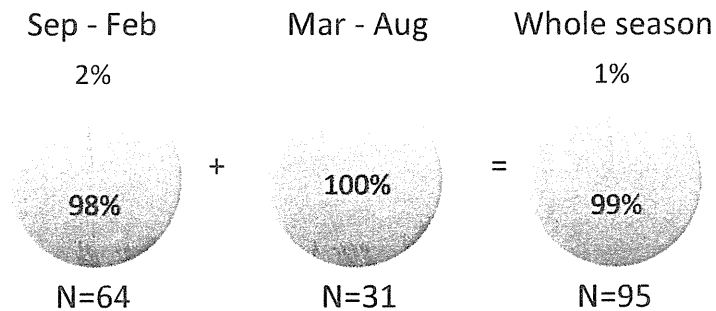
2012/13 season



B Victoria

B/Brisbane/60/2008 (Cell)

2012/13 season



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

Strains	Passage History	Sample date	CL1	CL2		CL3		Genetic information	
			Florida/ 04/06 Egg No.08-1	Kanagawa/ 37/11 Cell No.1	Massachusetts/ 02/12 Cell NIH No.2	Massachusetts/ 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	1280	320	320	1280	320	320	CL 1
B/KANAGAWA/37/2011	MDCK 1 +2	2011/10/21	160	320	640	160	160	320	CL 2
B/Massachusetts/02/2012	M 1/C 2 +2		80	160	320	160	80	80	CL 2
B/Massachusetts/02/2012 (BX-51B)	E3/E7 +1		320	80	160	640	80	40	CL 2, N196D*
B/Wisconsin/01/2010	C 1/C 1 +2		80	80	80	160	160	160	CL 3, N202S*, N196N>S*, T198T>I*
B/SAKAI/36/2011	MDCK 1 -2	2011/11/01	160	320	320	160	320	320	CL 3, N202S*
TEST.Ag									
B/Nepal/0534/2012	MDCK 1 +1	2012/09/22	160	640	640	160	160	320	ND
B/Nepal/0544/2012	MDCK 1 +1	2012/09/11	160	320	640	160	160	320	ND
B/NAGANO/2292/2013	MDCK 1 +1	2013/05/27	160	320	640	160	80	320	CL 2, #
B/SHIGA/42/2013	MDCK 1 +1	2013/05/14	160	320	640	160	80	320	CL 2A, #
B/Nepal/0491/2012	MDCK 1 +1	2012/08/29	160	320	640	160	80	320	ND
B/Nepal/0559/2012	MDCK 1 +1	2012/09/12	160	320	640	160	80	320	ND
B/Nepal/0588/2012	MDCK 1 +1	2012/09/13	160	320	640	160	80	320	ND
B/NIIGATA-C/13/2013	MDCK 2 +1	2013/06/10	160	320	640	160	80	320	ND
B/Nepal/0469/2012	MDCK 1 +1	2012/08/16	160	320	640	160	80	160	ND
B/NIIGATA-C/6/2013	MDCK 2 +1	2013/04/27	80	320	640	160	80	160	CL 2, #
B/FUKUSHIMA/31/2013	MDCK 2 +1	2013/05/29	80	320	640	160	80	160	CL 2, #
B/TOKYO/13094/2013	MDCK 1 +1	2013/06/03	160	160	640	160	80	160	CL 2A, #
B/NIIGATA-C/12/2013	MDCK 2 +1	2013/06/02	160	320	320	320	160	320	ND
B/Nepal/0586/2012	MDCK 1 +1	2012/09/14	160	320	320	160	80	320	ND
B/Nepal/00988/2012	MDCK 1 +1	2012/08/20	160	320	320	160	80	320	ND
B/Laos/1319/2013	MDCK 2 +1	2013/4/22	160	320	320	160	80	320	CL 2
B/OSAKA/17/2013	MDCK 2 +1	2013/06/12	80	320	320	160	80	320	CL 2, #
B/Nepal/0518/2012	MDCK 1 +1	2012/09/11	160	320	320	160	80	160	ND
B/Laos/1435/2013	MDCK 1 +1	2013/05/27	160	320	320	160	80	160	CL 2
B/Nepal/0496/2012	MDCK 1 +1	2012/09/03	80	160	320	80	40	160	ND

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

Strains	Passage History	Sample date	CL1	CL2		CL3		Genetic information	
			Florida/ 04/06 Egg No.08-1	Kanagawa/ 37/11 Cell No.1	Massachusetts/ 02/12 Cell NIID No.2	Massachusetts/ 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	1280	320	320	1280	320	320	CL 1
B/KANAGAWA/37/2011	MDCK 1 +2	2011/10/21	160	320	640	160	160	320	CL 2
B/Massachusetts/02/2012	M 1/C 2 +2		80	160	320	160	80	80	CL 2
B/Massachusetts/02/2012 (BX-51B)	E3/E7 +1		320	80	160	640	80	40	CL 2, N196D*
B/Wisconsin/01/2010	C 1/C 1 +2		80	80	80	160	160	160	CL 3, N202S*, N196N>S*, T198T>I*
B/SAKAI/36/2011	MDCK 1 +2	2011/11/01	160	320	320	160	320	320	CL 3, N202S*
TEST.Ag									
B/TOCHIGI/6/2013	MDCK 1 +1	2013/05/26	320	640	640	160	320	640	CL 2, #
B/VN/VP12-98/2012	MDCK 2 +2	2012/10/30	160	640	640	160	160	320	CL 2
B/FUKUI/28/2013	MDCK 1 +1	2013/06/04	160	320	640	160	80	320	CL 2A, #
B/ISHIKAWA/75/2013	MDCK 1 +1	2013/04/19	160	320	640	160	80	320	ND
B/TOCHIGI/4/2013	MDCK 2 +1	2013/05/13	160	320	640	160	80	320	ND
B/FUKUSHIMA/11/2013	MDCK 2 +1	2013/04/27	160	320	640	160	80	160	CL 2A, #
B/SHIZUOKA/118/2013	MDCK 1 +1	2013/05/14	160	320	640	80	80	320	ND
B/SHIZUOKA/121/2013	MDCK 1 +1	2013/05/27	320	640	320	160	320	640	CL 3, #
B/SENDAI/18/2013	MDCK 1 +1	2013/06/05	160	320	320	160	160	320	CL 2A, #
B/SAKAI/15/2013	MDCK 2 +1	2013/05/10	160	320	320	160	160	320	CL 2A, #
B/AICHI/58/2013	MDCK 2 +1	2013/05/22	80	320	320	160	80	160	ND
B/GUNMA/110/2013	MDCK 1 +1	2013/05/07	80	320	320	160	80	160	CL 2, #
B/IWATE/5/2013	MDCK 1 +1	2013/05/21	80	320	320	160	80	160	CL 2A, #
B/SAITAMA/15/2013	MDCK 1 +1	2013/06/04	80	320	320	160	80	160	CL 2, #

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

Strains	Passage History	Sample date	CL1	CL2			CL3		HI test date:2013/06/27
			Florida/ 04/06 Egg No.08-1	Kanagawa/ 37/11 Cell No.1	Massachusetts/ 02/12 Cell NIID No.2	Massachusetts/ 02/12 BX-51B Egg No.1	Wisconsin/ 01/10 Cell No.1	Sakai/ 36/11 Cell No.1	Genetic information
REF.Ag									
B/Florida/04/2006	E2 /E1+1	2006/12/15	640	320	320	640	160	320	CL 1
B/KANAGAWA/37/2011	MDCK 1 +2	2011/10/21	320	640	640	320	160	320	CL 2
B/Massachusetts/02/2012	M 1/C 2 +2		80	160	320	160	40	80	CL 2
B/Massachusetts/02/2012 (BX-51B)	E3/E7 +1		320	80	160	320	40	40	CL 2, N196D*
B/Wisconsin/01/2010	C 1/C 1 +2		40	40	80	80	160	80	CL 3, N202S*, N196N>S*, T198T>I*
B/SAKAI/36/2011	MDCK 1 +2	2011/11/01	160	320	320	160	320	640	CL 3, N202S*
TEST.Ag									
B/NAGANO/2272/2013	MDCK 1 +1	2013/04/26	160	320	640	160	160	320	ND
B/SENDAI/13/2013	MDCK 1 +1	2013/05/01	160	640	640	160	160	320	CL 2, #
B/FUKUI/24/2013	MDCK 1 +1	2013/05/07	160	640	640	160	160	320	CL 2A, #
B/TOCHIGI/3/2013	MDCK 1 +1	2013/05/02	160	320	640	160	160	320	CL 2A, #
B/OITA/5/2013	MDCK 1 +1	2013/06/03	160	320	640	160	160	320	CL 2A, #
B/YAMAGATA/135/2013	MDCK 1 +1	2013/04/08	160	320	640	160	80	320	ND
B/YAMAGATA/143/2013	MDCK 1 +1	2013/04/24	160	320	640	160	80	320	CL 2, #
B/KOBE/17/2013	MDCK 1 +1	2013/04/12	160	320	640	160	80	320	ND
B/NAGANO/2274/2013	MDCK 1 +1	2013/05/02	160	320	640	160	80	320	CL 2A, #
B/WAKAYAMA/145/2013	MDCK 1 +1	2013/05/07	160	320	640	160	80	320	CL 2A, #
B/TOKYO/30008/2013	MDCK 1 +1	2013/05/07	160	320	640	160	80	320	CL 2, #
B/SAPPORO/20/2013	MDCK 1 +1	2013/05/07	80	320	640	160	80	320	ND
B/SAPPORO/21/2013	MDCK 1 +1	2013/05/07	80	320	640	160	80	320	CL 2, #
B/KITAKYUSYU/3/2013	MDCK 2 +1	2013/05/17	80	320	320	160	80	320	CL 2A, #

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

Phylogenetic analysis of influenza B (Yamagata-lineage) HA genes (HA1)

12/13 Japanese vaccine strain

HI reference strains in Red

March 2013 in Blue

April 2013 in Green

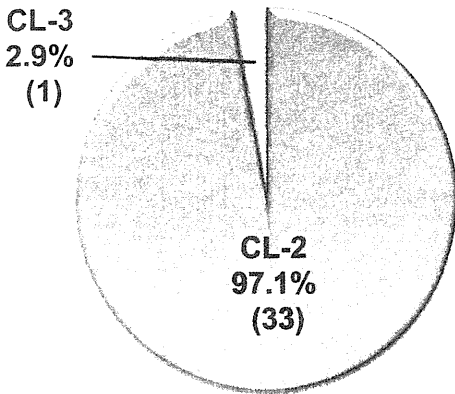
May 2013 in Orange

June and July 2013 in Pink

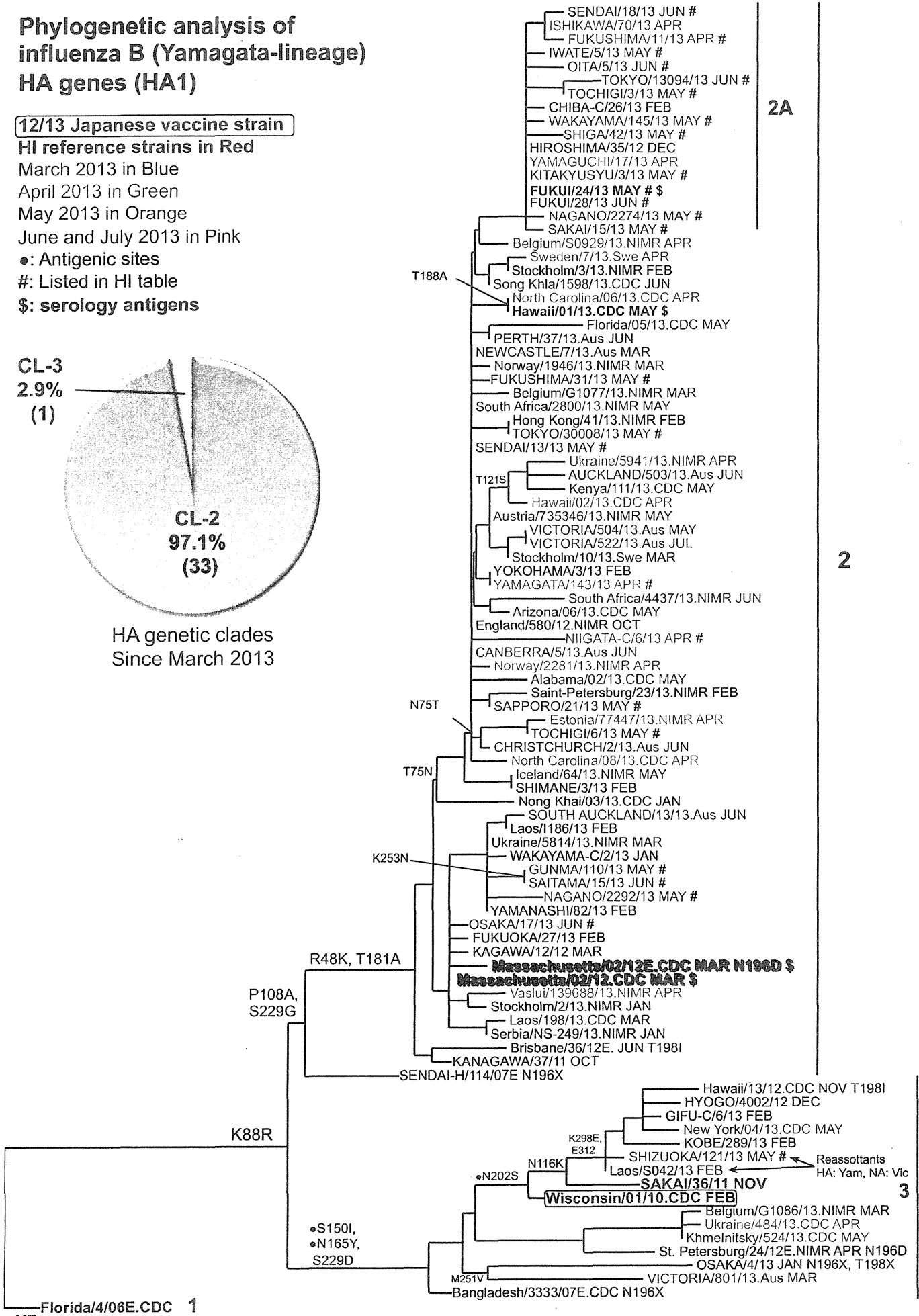
●: Antigenic sites

#: Listed in HI table

§: serology antigens



HA genetic clades Since March 2013



2A

2

3

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

12/13 Japanese vaccine strain

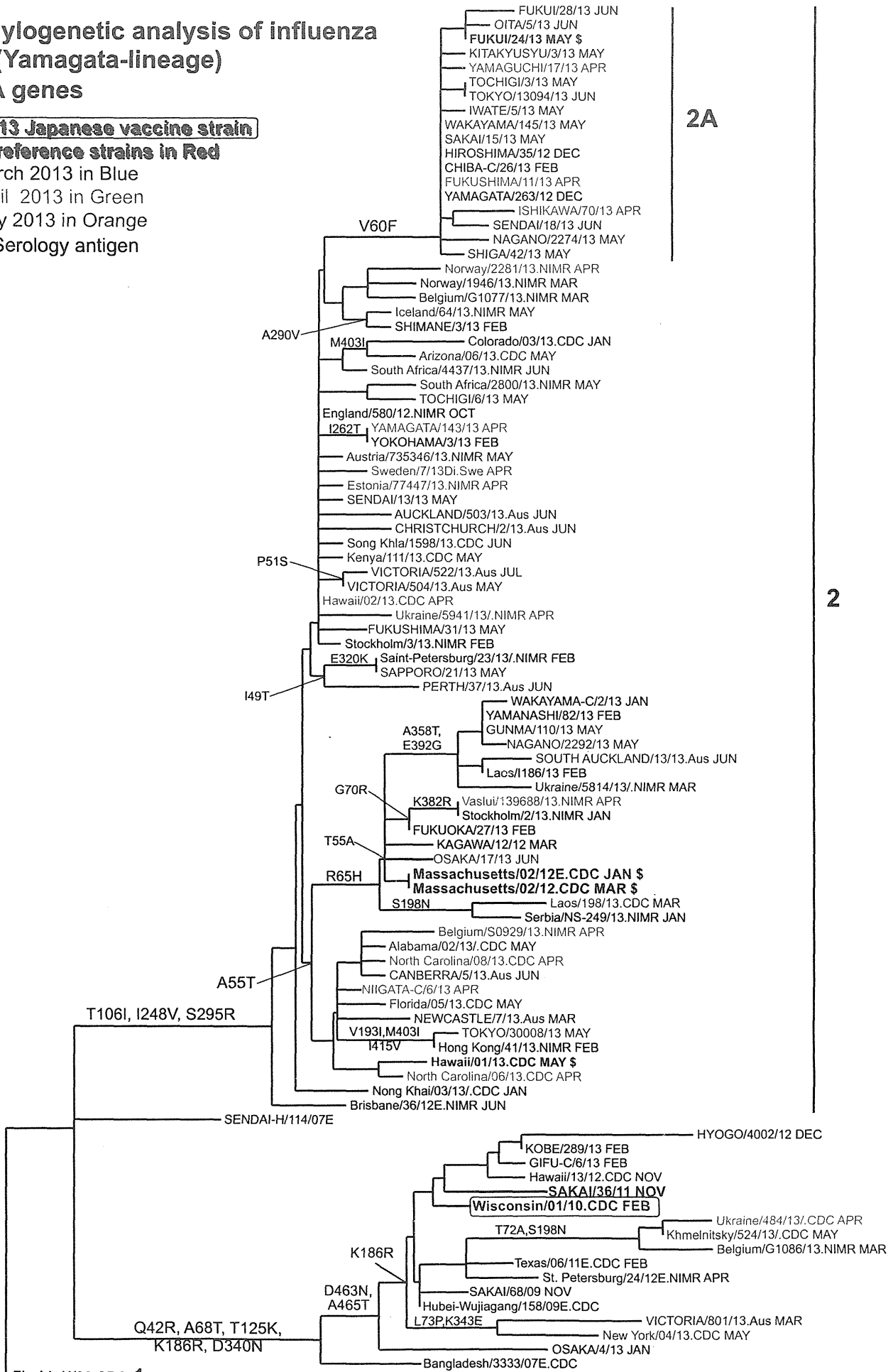
HI reference strains in Red

March 2013 in Blue

April 2013 in Green

May 2013 in Orange

\$. Serology antigen



2A

2

3

Influenza B (Yamagata-lineage) HA1 amino acid comparison

10aa	CL	-----X-----X-----X-----X-----X-----X-----X	
Consensus (n=400)	1	DRICTGITSSNSPHVVKTATQGEVNVTVIPLTTPTKSYFANLKGTKTRGKLCPCDCLNC	60
Epidemic strains			
TOCHIGI/6/13_MAY	2	1	60
SAITAMA/15/13_JUN	2	1	60
IWATE/5/13_MAY	2A	1	60
KITAKYUSYU/3/13_MAY	2A	1	60
Serology Ag			
Massachusetts/02/12-M1C2+2	2	1	60
Massachusetts/02/12 (BX51B)-E3E7+1	2	1	60
FUKUI/24/13_MAY	2A	1	60
Hawaii/01/13_MAY	2	1	60
Reference Strains			
KANAGAWA/37/11_OCT	2	1	60
WISCONSIN/1/10_C1+2	3	1	60
SAKAI/36/11_NOV	3	1	60
Florida/4/06E.CDC	1	1	60
BYam_AG_site(Wis01).txt			

10aa	CL	-----X-----X-----X-----X-----X-----X-----X	
Consensus (n=400)	61	TDLVVALGRPMCVGTTPSAKASILHEVRPVTSGCFPIIMHDRTKIRQLANLLRGYENIRLS	120
Epidemic strains			
TOCHIGI/6/13_MAY	2	61	120
SAITAMA/15/13_JUN	2	61	120
IWATE/5/13_MAY	2A	61	120
KITAKYUSYU/3/13_MAY	2A	61	120
Serology Ag			
Massachusetts/02/12-M1C2+2	2	61	120
Massachusetts/02/12 (BX51B)-E3E7+1	2	61	120
FUKUI/24/13_MAY	2A	61	120
Hawaii/01/13_MAY	2	61	120
Reference Strains			
KANAGAWA/37/11_OCT	2	61	120
WISCONSIN/1/10_C1+2	3	61	120
SAKAI/36/11_NOV	3	61	120
Florida/4/06E.CDC	1	61	120
BYam_AG_site(Wis01).txt			

10aa	CL	-----X-----X-----X-----X-----X-----X-----X	
Consensus (n=400)	121	TQNVIDAEKAPGGPYRLGTSGSCP NATSKSGFFATMAWAVPKDNNKNATNPLTVEVPYIC	180
Epidemic strains			
TOCHIGI/6/13_MAY	2	121	180
SAITAMA/15/13_JUN	2	121	180
IWATE/5/13_MAY	2A	121	180
KITAKYUSYU/3/13_MAY	2A	121	180
Serology Ag			
Massachusetts/02/12-M1C2+2	2	121	180
Massachusetts/02/12 (BX51B)-E3E7+1	2	121	180
FUKUI/24/13_MAY	2A	121	180
Hawaii/01/13_MAY	2	121	180
Reference Strains			
KANAGAWA/37/11_OCT	2	121	180
WISCONSIN/1/10_C1+2	3	121	180
SAKAI/36/11_NOV	3	121	180
Florida/4/06E.CDC	1	121	180
BYam_AG_site(Wis01).txt			

		196 198		
10aa	CL		-----X-----X-----X-----X-----X-----X	
Consensus (n=400)		181	AEGEDQITVWGFHSDNKTOMKNLYGDSNPQKFTSSANGVTTTHYVSQIGGFPPDQTEDGGLP	240
Epidemic strains	TOCHIGI/6/13_MAY	2	181	240
	SAITAMA/15/13_JUN	2	181	240
	IWATE/5/13_MAY	2A	181	240
	KITAKYUSYU/3/13_MAY	2A	181	240
	Massachusetts/02/12-M1C2+2	2	181	240
Serology Ag	Massachusetts/02/12 (BX51B) -E3E7+1	2	181	240
	FUKUI/24/13_MAY	2A	181	240
	Hawaii/01/13_MAY	2	181	240
Reference Strains	KANAGAWA/37/11_OCT	2	181	240
	WISCONSIN/1/10_C1+2	3	181	240
	SAKAI/36/11_NOV	3	181	240
	Florida/4/06E.CDC	1	181	240
	BYam_AG_site(Wis01).txt			

		196 198		
10aa	CL		-----X-----X-----X-----X-----X-----X	
Consensus (n=400)		241	QSGRIVVDYMMQKPKGTGTIVYQRGVLLPQKVCASGRSKVIRGSLPLIGEADCLHEKYG	300
Epidemic strains	TOCHIGI/6/13_MAY	2	241	300
	SAITAMA/15/13_JUN	2	241	300
	IWATE/5/13_MAY	2A	241	300
	KITAKYUSYU/3/13_MAY	2A	241	300
	Massachusetts/02/12-M1C2+2	2	241	300
Serology Ag	Massachusetts/02/12 (BX51B) -E3E7+1	2	241	300
	FUKUI/24/13_MAY	2A	241	300
	Hawaii/01/13_MAY	2	241	300
Reference Strains	KANAGAWA/37/11_OCT	2	241	300
	WISCONSIN/1/10_C1+2	3	241	300
	SAKAI/36/11_NOV	3	241	300
	Florida/4/06E.CDC	1	241	300
BYam_AG_site(Wis01).txt				

		196 198		
10aa	CL		-----X-----X-----X-----X-----X-----X	
Consensus (n=400)		301	GLNKSFPYYTGEHAKAIGNCPIWVKTPKLANGTKYRPPAKLLKER	346
Epidemic strains	TOCHIGI/6/13_MAY	2	301	346
	SAITAMA/15/13_JUN	2	301	346
	IWATE/5/13_MAY	2A	301	346
	KITAKYUSYU/3/13_MAY	2A	301	346
	Massachusetts/02/12-M1C2+2	2	301	346
Serology Ag	Massachusetts/02/12 (BX51B) -E3E7+1	2	301	346
	FUKUI/24/13_MAY	2A	301	346
	Hawaii/01/13_MAY	2	301	346
Reference Strains	KANAGAWA/37/11_OCT	2	301	346
	WISCONSIN/1/10_C1+2	3	301	346
	SAKAI/36/11_NOV	3	301	346
	Florida/4/06E.CDC	1	301	346
BYam_AG_site(Wis01).txt				

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

Strains	Passage History	Sample date	CL1a		CL1b	CL5	HI test date:2013/09/12
			Brisbane/60/08 Cell NIID No.4	Sakai/43/08 Cell No.2 Boosted	Shizuoka/57/11 Cell No.3 Boosted	Taiwan/55/09 Cell No.10281-2 Rabbit serum	Genetic information
REF.Ag							
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	160	160	1280	160	CL1A, V146I
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	160	160	640	160	CL1A, V146I
B/SHIZUOKA/57/2011	MDCK 1 +1	2011/03/14	40	80	2560	20	CL1B
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	40	40	40	640	CL5
TEST.Ag							
B/Taiwan/13/2013	MDCK 3 +1	2013/05/10	160	160	2560	160	CL1A, #
B/Taiwan/11/2013	MDCK 3 +2	2013/05/05	160	160	1280	160	CL1A, K209N
B/Laos/1341/2013	MDCK 2 +1	2013/04/29	160	160	1280	160	CL1A

ND: not determined

#: used in phylogenetic tree

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

Strains	Passage History	Sample date	CL1a		CL1b	CL5	HI test date:2013/8/22
			Brisbane/60/08 Cell NIID No.4	Sakai/43/08 Cell No.2 Boosted	Shizuoka/57/11 Cell No.3 Boosted	Taiwan/55/09 Cell No.10281-2 Rabbit serum	Genetic information
REF.Ag							
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	160	160	1280	160	CL1A, V146I
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	160	160	640	320	CL1A, V146I
B/SHIZUOKA/57/2011	MDCK 1 +1	2011/03/14	80	80	1280	40	CL1B
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	80	80	40	640	CL5
TEST.Ag							
B/NIIGATA/672/2013	MDCK 1 +1	2013/04/26	160	160	1280	320	CL1A, K209N, #
B/CHIBA-C/46/2013	MDCK 2 +1	2013/06/12	160	160	1280	160	CL1A, #
B/NIIGATA-C/4/2013	MDCK 2 +1	2013/04/08	160	160	1280	160	CL1A, K209N, #
B/Taiwan/12/2013	MDCK 2 +1	2013/04/24	160	160	1280	160	CL1A, #
B/NAGANO/2313/2013	MDCK 1 +1	2013/05/30	160	160	1280	160	CL1A, K209N, #
B/TOKYO/13134/2013	MDCK 1 +1	2013/06/19	160	160	1280	160	CL1A, K209N, #
B/TOKYO/13037/2013	MDCK 1 +1	2013/05/11	160	160	1280	160	ND

ND: not determined

#: used in phylogenetic tree

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

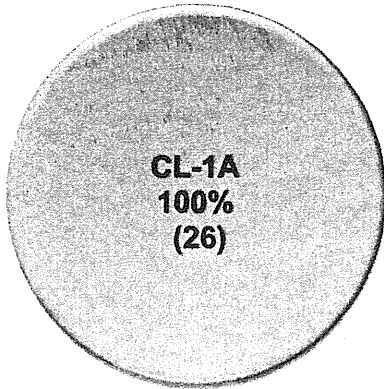
Strains	Passage History	Sample date	CL1a		CL1b	CL5	HI test date:2013/7/25
			Brisbane/60/08 Cell NIID No.4	Sakai/43/08 Cell No.2 Boosted	Shizuoka/57/11 Cell No.3 Boosted	Taiwan/55/09 Cell No.10281-2 Rabbit serum	Genetic information
REF.Ag							
B/Brisbane/60/2008	MDCKx/1 +2	2008/08/04	160	160	1280	80	CL1A, V146I
B/SAKAI/43/2008	MDCK 1 +2	2008/11/24	160	160	640	160	CL1A, V146I
B/SHIZUOKA/57/2011	MDCK 1 +1	2011/03/14	40	80	1280	20	CL1B
B/Taiwan/55/2009	MDCK 2 +2	2009/11/15	20	20	40	640	CL5
TEST.Ag							
B/YOKOHAMA/23/201	MDCK 1 +1	2013/05/13	160	160	1280	160	ND
B/YOKOHAMA/24/201	MDCK 1 +1	2013/05/14	160	160	1280	160	ND
B/FUKUI/26/2013	MDCK 1 +1	2013/05/13	160	160	1280	160	CL1A, #
B/SAITAMA/11/2013	MDCK 1 +1	2013/04/16	160	160	1280	160	CL1A, K209N, #
B/EHIME/5/2013	MDCK 1 +1	2013/04/25	160	160	1280	160	ND
B/TOKYO/30018/2013	MDCK 1 +1	2013/05/01	160	160	1280	160	CL1A, K209N, #
B/KUMAMOTO/24/201	MDCK 1 +1	2013/05/28	160	160	1280	160	CL1A, K209N, #
B/EHIME/6/2013	MDCK 1 +1	2013/05/09	160	160	1280	160	CL1A, K209N, #
B/ISHIKAWA/74/2013	MDCK 1 +1	2013/04/19	160	160	1280	160	ND
B/ISHIKAWA/76/2013	MDCK 1 +1	2013/04/25	160	160	1280	160	CL1A, K209N, #
B/TOCHIGI/5/2013	MDCK 2 +1	2013/05/28	160	160	1280	160	CL1A, K209N, #
B/SHIZUOKA/120/2013	MDCK 1 +1	2013/05/19	160	160	1280	160	ND
B/SENDAI/10/2013	MDCK 2 +1	2013/04/23	160	160	1280	80	CL1A, K209N, #
B/FUKUI/20/2013	MDCK 1 +1	2013/04/17	160	160	1280	80	ND
B/SAPPORO/24/2013	MDCK 1 +1	2013/05/08	160	160	1280	80	CL1A, #
B/VN/VP12-48/2012	MDCK 2 +2	2012/04/19	160	160	640	320	CL1A, K154E, #
B/AICHI/55/2013	MDCK 1 +1	2013/05/13	160	160	640	160	CL1A, K209N, #

ND: not determined

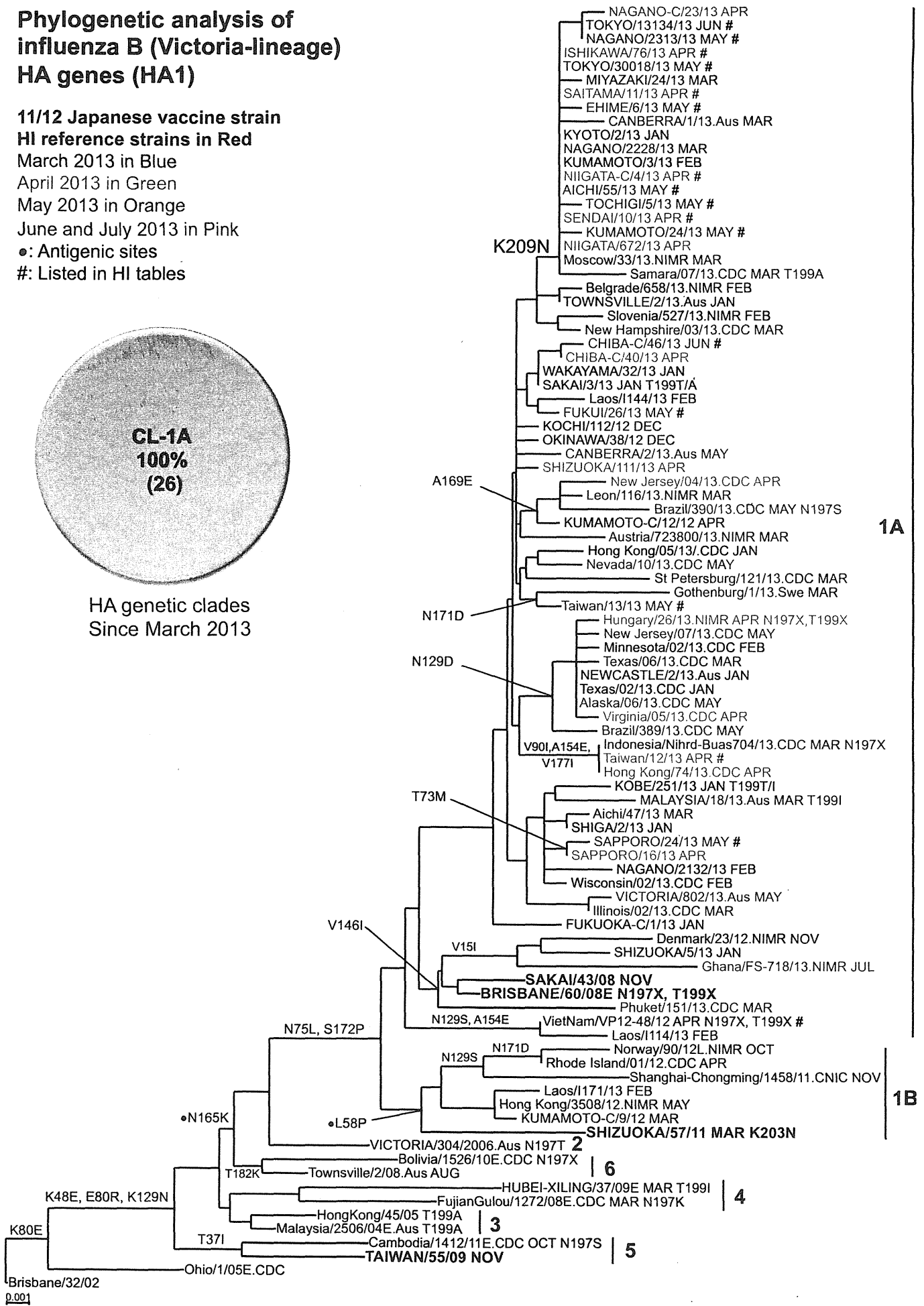
#: used in phylogenetic tree

Phylogenetic analysis of influenza B (Victoria-lineage) HA genes (HA1)

11/12 Japanese vaccine strain
 HI reference strains in Red
 March 2013 in Blue
 April 2013 in Green
 May 2013 in Orange
 June and July 2013 in Pink
 ●: Antigenic sites
 #: Listed in HI tables



HA genetic clades Since March 2013



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

11/12 Japanese vaccine strain
 HI reference strains in Red
 March 2013 in Blue
 April 2013 in Green
 May 2013 in Orange
 June and July 2013 in Pink

