A(H1N1)pdm09 virus

Antigenic and Phylogenetic analyses:

- Total 94 A(H1N1)pdm09 viruses were subjected to antigenic analysis by hemagglutination inhibition (HI) tests. The majority of A(H1N1)pdm09 viruses analyzed in the present data package was isolated between March and June 2013. The genetic information of the HA gene are listed together with HI profiles in the HI table.
- The hemagglutinin (HA) and the neuraminidase(NA) genes of those viruses were subjected to phylogenetic analysis. The viruses listed in HI tables are marked with # in phylogenetic tree.
- The majority (91%) of test viruses reacted within 4-fold different HI titers to homologous titer of an egg-grown A/California/7/2009 and an A/California/7-like reference (A/Narita/1/2009) ferret antisera.
- Low reactor viruses showing over 8-fold reduced HI titers to A/California/7/2009 and A/Narita/1/2009 ferret antisera were slightly increased in Mar-Aug 2013(18%) than in Sept 2012-Feb 2013 (4%). Those low reactors possessed mixed amino acid substitutions at 153-156 region in the HA protein that were not detected in the original clinical specimens and were known to generate during virus growth in MDCK cells.
- A virus, A/Nagano/231/2013, showing 8-fold reduced HI titer to A/California/7/2009 ferret antiserum possessed both H138R and 119T>>K substitutions, but not substitutions at 153-156 region. The double mutations in and near antigenic site would be responsible for the reduced HI titer.
- Ferret antiserum raised against a high growth reassortant (HGR) A/California/7/2009 X-179A covered well recent viruses (67% of test viruses). Low reactors to the A/California/7/2009 X-179A antiserum possessed amino acid substitutions in the 153-156 region.
- Phylogenetic analysis of the HA and the NA genes were correlated well each other. In phylogenetic tree of HA gene, viruses belonging to clade 6 (76%) were predominant than those belonging to clade 7 (24%), although those two recent major clades were antigenically indistinguishable from each other.

Conclusions

Recent viruses isolated during April-May were antigenically closely related to A/California/7/2009 virus and a vaccine production virus A/California/7/2009 X-179A. There was not tendency to increase antiviral resistant viruses with H275Y mutation to oseltamivir and peramivir. The resistant viruses were sporadically detected (1.7%) throughout the 2012/13 season.

Antigen viruses for serology tests.

A/California/7/2009 egg-grown wild type virus
A/California/7/2009 X-179A vaccine virus
A/Bolivia/559/2013 (recent egg-grown clade 6)

Influenza A/H1pdm09 isolates characterized by NIID

	Japan	China	Taiwan	Mongolia	Laos	Nepal	Vietnam	Total	
September 2012 - February 2013								n	%
A/California/7/2009 -like	42	2	0	4	0	0	0	48	85.7
A/California/7/2009 -like*	5	0	0	0	1	0	0	6	10.7
A/California/7/2009 (Low)**	2	0	0	0	0	0	0	2	3.6
Total	49	2	0	4	1	0	0	56	
A/Narita/1/2009 -like Cell	43	2	0	4	0	0	0	49	87.5
A/Narita/1/2009 -like* Cell	3	0	0	0	0	0	0	3	5.4
A/Narita/1/2009 (Low)** Cell	3	0	0	0	1	0	0	4	7.1
Total	49	2	0	4	1	0	0	56	

	Japan	China	Taiwan	Mongolia	Laos	Nepal	Vietnam	Total	
March 2013 - August 2013								n	%
A/California/7/2009 -like	20	0	2	0	0	0	1	23	60.5
A/California/7/2009 -like*	8	0	0	0	0	0	0	8	21.1
A/California/7/2009 (Low)**	6	0	0	0	0	0	1	7	18.4
Total	34	0	2	0	0	0	2	38	
A/Narita/1/2009 -like Cell	22	0	2	0	0	0	1	25	65.8
A/Narita/1/2009 -like* Cell	5	0	0	0	0	0	0	5	13.2
A/Narita/1/2009 (Low)** Cell	7	0	0	0	0	0	1	8	21.1
Total	34	0	2	0	0	0	2	38	

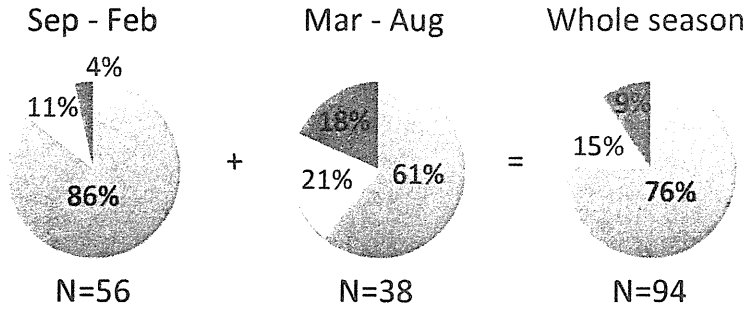
* 4-fold low to homologous titer

** 8-fold or greater low to homologous titer

A(H1N1)pdm09

A/California/07/2009 (Egg)

2012/13 season



A/California/07/2009-like

A/California/07/2009-like*

Antigenic variants*

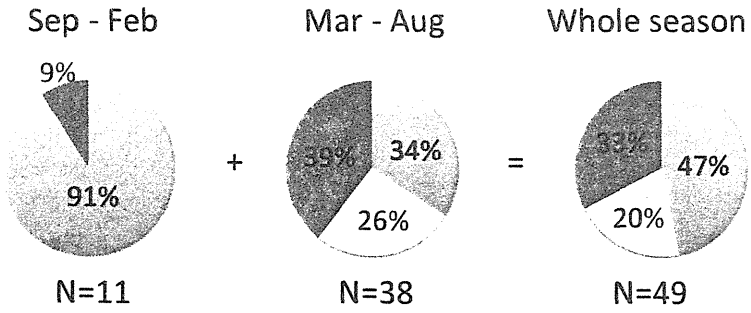
*4-fold lower than the homologous titers

**≥8-fold lower than the homologous titers

A(H1N1)pdm09

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

2012/13 season



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

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

Antigenic variants*

*4-fold lower than the homologous titers

**≥8-fold lower than the homologous titers

1

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

Rabbit serum

HI test date:2013/8/28

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	Genetic information
REF.Ag										
A/Wisconsin/10/1998	C3/C3E2 +2		2560	1280	640	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	5120	1280	5120	5120	1280	320	-
A/Narita/1/2009pdm	E2 +1	2009/05/08	640	2560	640	2560	2560	640	320	-
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	80	80	80	160	640	160	-, G155E*
A/SAPPORO/163/2011pdm	MDCK 2 +1	2011/03/04	80	20	< 10	10	20	80	640	CL7, G154E*, S185I*, S190R*
TEST.Ag										
A/KANAGAWA/142/2013	MDCK 3 +1	2013/05/29	320	1280	640	2560	2560	640	320	CL6, #
A/VN/CD13-114/2013	MDCK 0 +3	2013/04/03	320	640	640	1280	1280	320	320	CL6, H138R*, #
A/SHIGA/38/2013	MDCK 1 +2	2013/04/19	160	640	320	1280	1280	160	160	CL6, #
A/OSAKA/34/2013	MDCK 2 +2	2013/04/30	160	640	320	1280	1280	160	80	CL6, #
A/TOKYO/32378/2013	MDCK 1 +2	2013/06/03	80	320	160	640	640	160	80	CL7, D127D>>>E, H138Q*, V173I, G202G>>W, #
A/NAGANO/2312/2013	MDCK 1 +1	2013/05/30	160	160	160	160	320	320	320	CL6, N38D, K119T>>K, H138R*, #

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

Rabbit serum

HI test date:2013/8/8

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	Genetic information
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	**640**	320	640	640	640	160	-
A/California/07/2009pdm X-179A	Ex/Ex +1		640	2560	**1280**	5120	5120	640	320	-
A/Narita/1/2009pdm	E2 +1	2009/05/08	320	1280	640	**2560**	2560	640	160	-
A/Narita/1/2009pdm	MDCK 1 +3	2009/05/08	320	1280	640	2560	**1280**	320	160	-
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	10	80	80	80	80	**320**	80	-, G155E*
A/SAPPORO/163/2011pdm	MDCK 2 +1	2011/03/04	80	40	40	10	20	80	**320**	CL7, G154E*, S185I*, S190R*
TEST.Ag										
A/SHIZUOKA-C/99/2013	MDCK 1 +1	2013/05/29	320	1280	640	2560	2560	320	320	CL6, #
A/TOKYO/32398/2013	MDCK 2 +1	2013/06/07	320	640	640	2560	1280	320	320	CL6, #
A/EHIME/30/2013	MDCK 1 +1	2013/05/20	160	640	640	1280	1280	640	320	ND
A/OSAKA/33/2013	MDCK 4 +1	2013/04/29	320	640	640	1280	1280	320	160	CL6, #
A/HIROSHIMA/20/2013	MDCK 3 +1	2013/05/29	320	640	320	1280	1280	320	160	ND
A/OSAKA-C/2005/2013	MDCK 1 +2	2013/03/20	160	640	320	1280	1280	160	160	CL7, H138Q*, #
A/Taiwan/691/2013	MDCK 2 +1	2013/05/30	160	640	320	640	1280	160	160	CL6, #
A/NIIGATA/655/2013	MDCK 1 +2	2013/05/07	160	320	320	1280	1280	160	80	ND
A/Taiwan/220/2013	E2 +1	2013/03/23	80	320	320	640	640	640	160	CL7, #
A/AICHI/107/2013	MDCK 1 +3	2013/03/22	160	320	320	640	640	320	80	ND
A/SAITAMA/131/2013	MDCK 2 +1	2013/06/03	20	160	160	320	640	320	160	CL7, R205K*, #
A/SAITAMA-C/13/2013	MDCK 2 +1	2013/05/14	80	160	160	160	320	640	320	CL6, G155G>>E*, #
A/TOKYO/32365/2013	MDCK 3 +1	2013/05/24	40	160	160	80	80	160	160	CL6, G155G>>E*, #
A/VN/NT13-77/2013	MDCK 0 +2	2013/03/14	80	40	80	40	80	640	320	CL6, G155E*, #
A/HYOGO/3177/2013	MDCK 1 +3	2013/03/14	80	40	40	40	80	640	160	CL7, G155E>>>G*, #

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

Rabbit serum

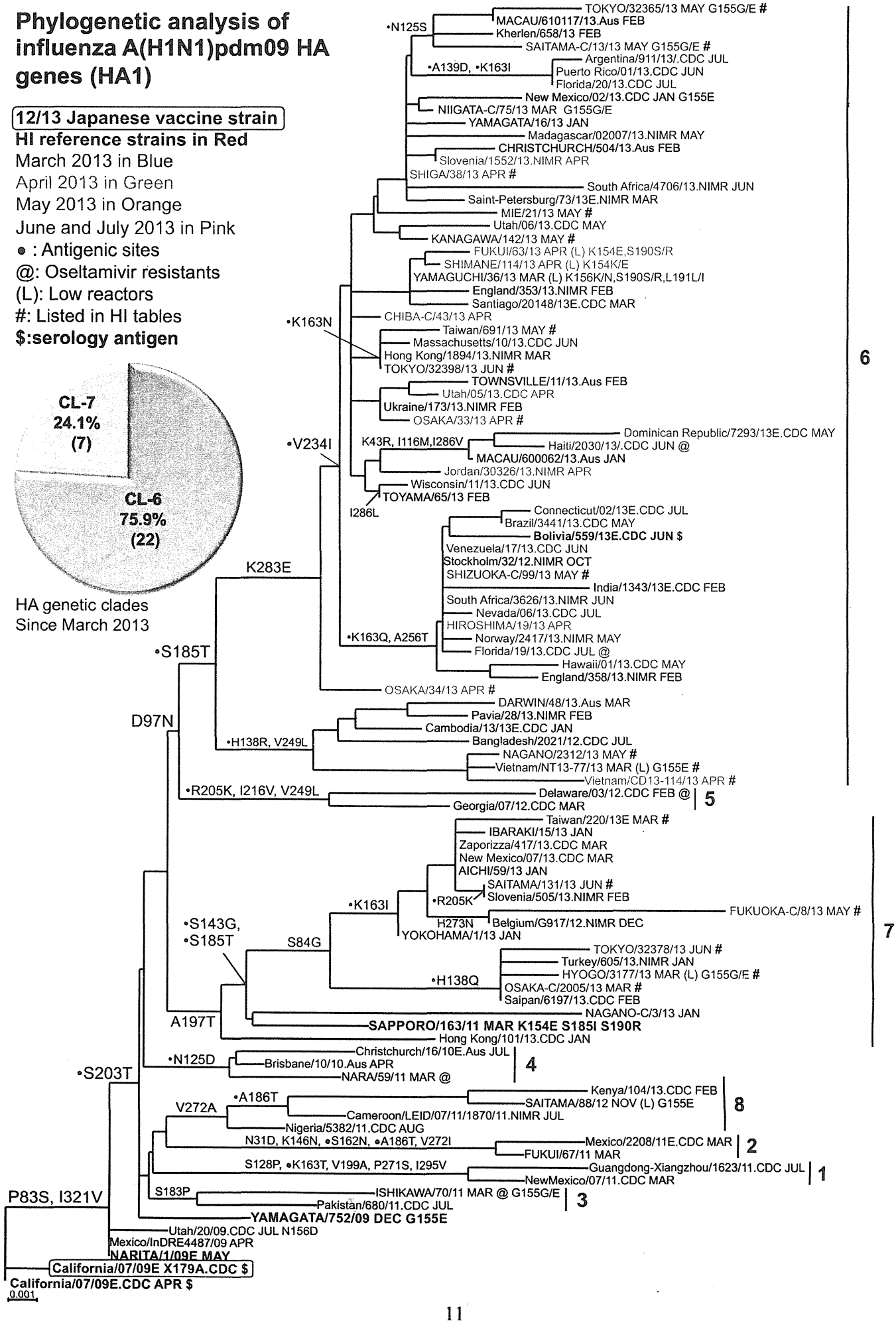
HI test date:2013/07/04

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	Genetic information
REF.Ag										
A/Wisconsin/10/1998	C3/C3E2 +2		2560	1280	640	2560	2560	80	80	-
A/California/07/2009pdm	E2 +3	2009/04/09	320	640	320	640	1280	640	160	-
A/California/07/2009pdm X-179A	Ex/Ex +1		1280	5120	1280	5120	5120	1280	640	-
A/Narita/1/2009pdm	E2 +1	2009/05/08	640	1280	640	2560	2560	640	320	-
A/Narita/1/2009pdm	MDCK 1 +3	2009/05/08	320	1280	640	2560	2560	640	160	-
A/YAMAGATA/752/2009pdm	MDCK 2 +1	2009/12/03	20	80	80	80	160	640	160	-, G155E*
A/SAPPORO/163/2011pdm	MDCK 2 +1	2011/03/04	80	20	40	20	40	80	320	CL7, G154E*, S185I*, S190R*
TEST.Ag										
A/WAKAYAMA/144/2013	MDCK 2 +1	2013/03/04	320	1280	640	2560	2560	640	320	ND
A/SHIMANE/92/2013	MDCK 2 +2	2013/03/04	320	1280	640	2560	2560	320	320	ND
A/MIE/21/2013	MDCK 1 +2	2013/05/13	320	1280	640	2560	2560	320	320	CL6, #
A/FUKUOKA-C/8/2013	MDCK 2 +1	2013/05/08	320	640	640	1280	2560	640	320	CL7, #
A/SAITAMA-C/9/2013	MDCK 3 +1	2013/03/04	160	640	320	1280	1280	320	160	CL7
A/SHIMANE/110/2013	MDCK 2 +2	2013/03/29	640	320	160	640	640	320	160	ND
A/Laos/I067/2013	MDCK 2 +2	2013/01/21	40	160	160	320	320	640	320	CL6, N156N=D*
A/NIIGATA-C/75/2013	MDCK 2 +2	2013/03/16	20	160	80	160	160	1280	320	CL6, G155G=E*, #

*Antigenic sites

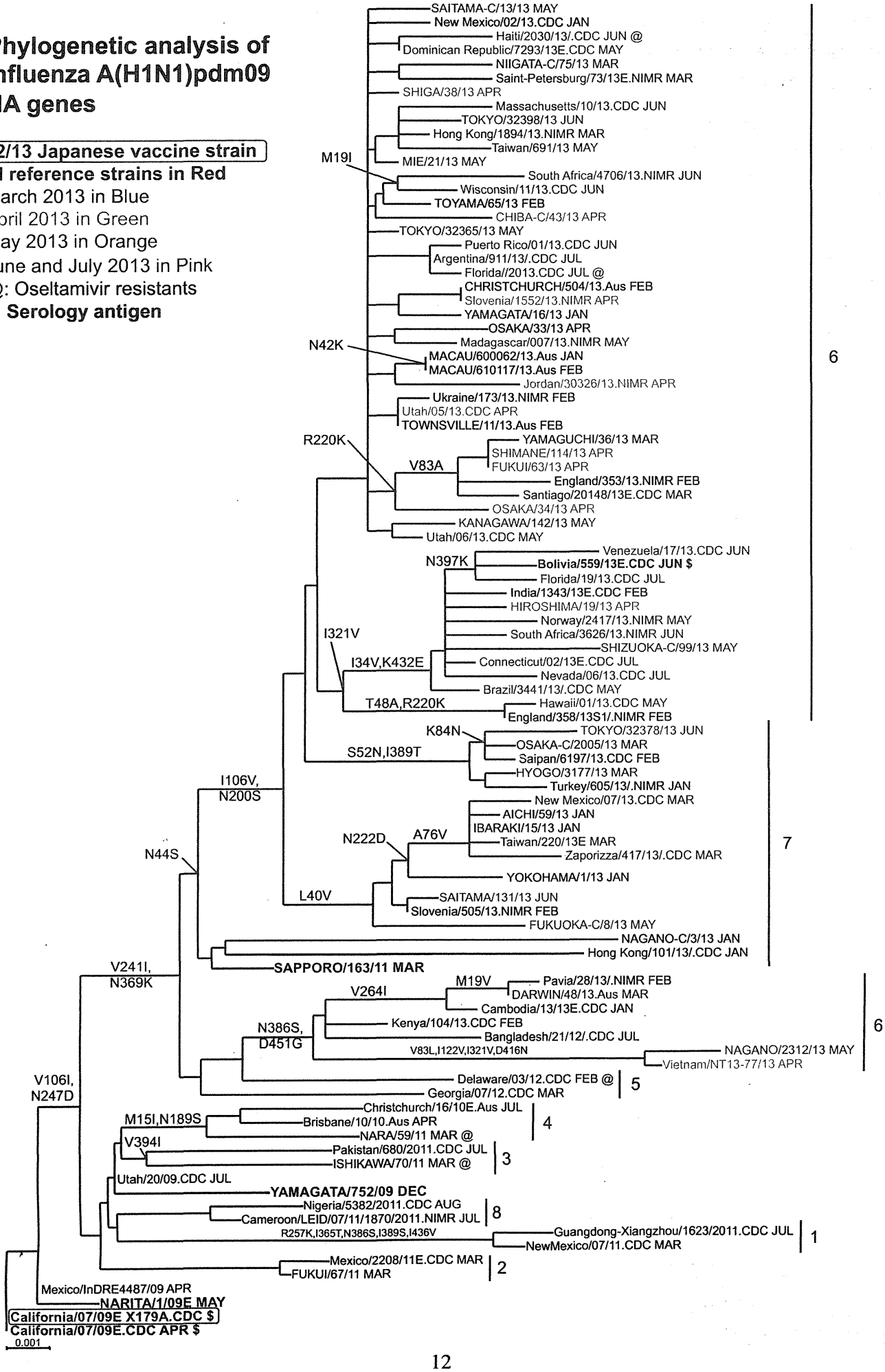
ND: not determined

#: used in phylogenetic tree


Phylogenetic analysis of influenza A(H1N1)pdm09 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
@: Oseltamivir resistants
(L): Low reactors
#: Listed in HI tables
$:serology antigen
CL-7
24.1%
(7)
CL-6
75.9%
(22)
HA genetic clades
Since March 2013
TOKYO/32365/13 MAY G155G/E #
•N125S
MACAU/610117/13.Aus FEB
Kherlen/658/13 FEB
SAITAMA-C/13/13 MAY G155G/E #
Argentina/911/13/.CDC JUL
•A139D, •K163I
Puerto Rico/01/13.CDC JUN
Florida/20/13.CDC JUL
New Mexico/02/13.CDC JAN G155E
NIIGATA-C/75/13 MAR G155G/E
YAMAGATA/16/13 JAN
Madagascar/02007/13.NIMR MAY
CHRISTCHURCH/504/13.Aus FEB
Slovenia/1552/13.NIMR APR
SHIGA/38/13 APR #
South Africa/4706/13.NIMR JUN
Saint-Petersburg/73/13E.NIMR MAR
MIE/21/13 MAY #
Utah/06/13.CDC MAY
KANAGAWA/142/13 MAY #
FUKUI/63/13 APR (L) K154E,S190S/R
SHIMANE/114/13 APR (L) K154K/E
YAMAGUCHI/36/13 MAR (L) K156K/N,S190S/R,L191L/I
England/353/13.NIMR FEB
Santiago/20148/13E.CDC MAR
•K163N
CHIBA-C/43/13 APR
Taiwan/691/13 MAY #
Massachusetts/10/13.CDC JUN
Hong Kong/1894/13.NIMR MAR
TOKYO/32398/13 JUN #
TOWNSVILLE/11/13.Aus FEB
Utah/05/13.CDC APR
Ukraine/173/13.NIMR FEB
OSAKA/33/13 APR #
•V234I
K43R, I116M,I286V
Dominican Republic/7293/13E.CDC MAY
Haiti/2030/13/.CDC JUN @
MACAU/600062/13.Aus JAN
Jordan/30326/13.NIMR APR
Wisconsin/11/13.CDC JUN
TOYAMA/65/13 FEB
I286L
Connecticut/02/13E.CDC JUL
Brazil/3441/13.CDC MAY
Bolivia/559/13E.CDC JUN $
Venezuela/17/13.CDC JUN
Stockholm/32/12.NIMR OCT
SHIZUOKA-C/99/13 MAY #
India/1343/13E.CDC FEB
South Africa/3626/13.NIMR JUN
Nevada/06/13.CDC JUL
HIROSHIMA/19/13 APR
•K163Q, A256T
Norway/2417/13.NIMR MAY
Florida/19/13.CDC JUL @
Hawaii/01/13.CDC MAY
England/358/13.NIMR FEB
OSAKA/34/13 APR #
K283E
•S185T
DARWIN/48/13.Aus MAR
Pavia/28/13.NIMR FEB
D97N
Cambodia/13/13E.CDC JAN
Bangladesh/2021/12.CDC JUL
•H138R, V249L
NAGANO/2312/13 MAY #
Vietnam/NT13-77/13 MAR (L) G155E #
Vietnam/CD13-114/13 APR #
•R205K, I216V, V249L
Delaware/03/12.CDC FEB @
Georgia/07/12.CDC MAR
5
6
Taiwan/220/13E MAR #
IBARAKI/15/13 JAN
Zaporizza/417/13.CDC MAR
New Mexico/07/13.CDC MAR
AICHI/59/13 JAN
SAITAMA/131/13 JUN #
•K163I
•R205K
Slovenia/505/13.NIMR FEB
FUKUOKA-C/8/13 MAY #
H273N
Belgium/G917/12.NIMR DEC
•S143G,
•S185T
YOKOHAMA/1/13 JAN
S84G
TOKYO/32378/13 JUN #
Turkey/605/13.NIMR JAN
HYOGO/3177/13 MAR (L) G155G/E #
•H138Q
OSAKA-C/2005/13 MAR #
Saipan/6197/13.CDC FEB
NAGANO-C/3/13 JAN
A197T
SAPPORO/163/11 MAR K154E S185I S190R
Hong Kong/101/13.CDC JAN
7
•N125D
Christchurch/16/10E.Aus JUL
Brisbane/10/10.Aus APR
NARA/59/11 MAR @
4
•S203T
•A186T
Kenya/104/13.CDC FEB
SAITAMA/88/12 NOV (L) G155E
V272A
Cameroon/LEID/07/11/1870/11.NIMR JUL
8
Nigeria/5382/11.CDC AUG
N31D, K146N, •S162N, •A186T, V272I
Mexico/2208/11E.CDC MAR
FUKUI/67/11 MAR
2
S128P, •K163T, V199A, P271S, I295V
Guangdong-Xiangzhou/1623/11.CDC JUL
NewMexico/07/11.CDC MAR
1
P83S, I321V
S183P
ISHIKAWA/70/11 MAR @ G155G/E
Pakistan/680/11.CDC JUL
3
YAMAGATA/752/09 DEC G155E
Utah/20/09.CDC JUL N156D
Mexico/InDRE4487/09 APR
NARITA/1/09E MAY
California/07/09E X179A.CDC $
California/07/09E.CDC APR $
0.001

Phylogenetic analysis of influenza A(H1N1)pdm09 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
@: Oseltamivir resistants
$: Serology antigen



Influenza A(H1N1)pdm09 HA1 amino acid comparison

	10aa	CL		---------X---------X---------X---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		1	DTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKLRGVAPLHLGKCNIAGW	60
Epidemic strains	OSAKA/33/13_APR	6	1	.....	60
	Taiwan/691/13_MAY	6	1	.....	60
	NIIGATA-C/75/13_MAR	6	1	.....	60
	NAGANO/2312/13_MAY	6	1	.....D.....	60
	TOKYO/32378/13_JUN	7	1	.....	60
	FUKUOKA-C/8/13_MAY	7	1	.....	60
	SAITAMA/131/13_JUN	7	1	.....	60
Serology Ag	California/07/09E_X179A	-	1	.....	60
	Bolivia/559/13E_JUN	6	1	.....	60
Reference Strains	NARITA/1/09E_MAY	-	1	.....	60
	YAMAGATA/752/09_DEC	-	1	.....	60
	SAPPORO/163/11_MAR	7	1	.....	60
	H1pdm_AG_site(Ca17)			----------..-.-.--------.------	
	H1pdm_RB_site(Ca17)			----------	

	10aa	CL		---------X---------X---------X---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		61	ILGNPECESLSTASSWSYIVETSSSDNGTCYPGDFINYEELREQLSSVSSFERFEIFPKT	120
Epidemic strains	OSAKA/33/13_APR	6	61	.....	120
	Taiwan/691/13_MAY	6	61	.....	120
	NIIGATA-C/75/13_MAR	6	61	.....	120
	NAGANO/2312/13_MAY	6	61	.....X.	120
	TOKYO/32378/13_JUN	7	61	.....G.....D.....	120
	FUKUOKA-C/8/13_MAY	7	61	.....G.....D.....	120
	SAITAMA/131/13_JUN	7	61	.....G.....D.X.....	120
Serology Ag	California/07/09E_X179A	-	61	.....P.....D.....	120
	Bolivia/559/13E_JUN	6	61	.....	120
Reference Strains	NARITA/1/09E_MAY	-	61	.....D.....	120
	YAMAGATA/752/09_DEC	-	61	.....D.....	120
	SAPPORO/163/11_MAR	7	61	.....D.....	120
	H1pdm_AG_site(Ca17)			--------.--.--.--------..-----.----------.------	
	H1pdm_RB_site(Ca17)			----------.----------	

	10aa	CL		138 153-157 ---------X---------X---------X---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		121	SSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIH	180
Epidemic strains	OSAKA/33/13_APR	6	121	.....	180
	Taiwan/691/13_MAY	6	121	.....N.....	180
	NIIGATA-C/75/13_MAR	6	121	.....X.....	180
	NAGANO/2312/13_MAY	6	121	.....R.....	180
	TOKYO/32378/13_JUN	7	121	.....X.....Q....G.....I.....	180
	FUKUOKA-C/8/13_MAY	7	121	.....S.....G.....I.....	180
	SAITAMA/131/13_JUN	7	121	.....G.....I.....	180
Serology Ag	California/07/09E_X179A	-	121	.....	180
	Bolivia/559/13E_JUN	6	121	.....Q.....	180
Reference Strains	NARITA/1/09E_MAY	-	121	.....	180
	YAMAGATA/752/09_DEC	-	121	.....E.....R.....	180
	SAPPORO/163/11_MAR	7	121	.....G.....E.....	180
	H1pdm_AG_site(Ca17)			----.---.--.-.--.-....------.-.....-.----------.--------.	
	H1pdm_RB_site(Ca17)			----------.....----------.-------------------------.	

	10aa	CL		---------X---------X---------X---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		181	HPSTTADQQSLYQNADAYVFVGTSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLIEPGDKI	240
Epidemic strains	OSAKA/33/13_APR	6	181	.....	240
	Taiwan/691/13_MAY	6	181	.....	240
	NIIGATA-C/75/13_MAR	6	181	.....X.....	240
	NAGANO/2312/13_MAY	6	181	.....V.....	240
	TOKYO/32378/13_JUN	7	181	.....T....X.....V.....	240
	FUKUOKA-C/8/13_MAY	7	181	..X.....T....X.....R.....V.....	240
	SAITAMA/131/13_JUN	7	181	..X.....T.....K.....V.....	240
Serology Ag	California/07/09E_X179A	-	181	....S.....S.....T.....R.....V.....	240
	Bolivia/559/13E_JUN	6	181	.....X.....X.....V.....	240
Reference Strains	NARITA/1/09E_MAY	-	181	....S.....S.....V.....	240
	YAMAGATA/752/09_DEC	-	181	....S.....T.....V.....	240
	SAPPORO/163/11_MAR	7	181	....I....R.....T.....V.....	240
	H1pdm_AG_site(Ca17)			--..S.....----.---.-..--------..--.-----.----------	
	H1pdm_RB_site(Ca17)			------.---..----------XX..----------	

	10aa	CL		---------X---------X---------X---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		241	TFEATGNLVVPRYAFAMERNAGSGIIISDTPVHDCNTTCQTPEGAINTSLPFQNIHPITI	300
Epidemic strains	OSAKA/33/13_APR	6	241	.....	300
	Taiwan/691/13_MAY	6	241	.....	300
	NIIGATA-C/75/13_MAR	6	241	.....	300
	NAGANO/2312/13_MAY	6	241	.....L.....K.....	300
	TOKYO/32378/13_JUN	7	241	.....K.....	300
	FUKUOKA-C/8/13_MAY	7	241	.....T.....N.....K.....	300
	SAITAMA/131/13_JUN	7	241	.....K.....	300
Serology Ag	California/07/09E_X179A	-	241	.....K.....	300
	Bolivia/559/13E_JUN	6	241	.....T.....	300
Reference Strains	NARITA/1/09E_MAY	-	241	.....K.....	300
	YAMAGATA/752/09_DEC	-	241	.....K.....	300
	SAPPORO/163/11_MAR	7	241	.....K.....	300
	H1pdm_AG_site(Ca17)			----------.--------.--.----------	
	H1pdm_RB_site(Ca17)			----------	

	10aa	CL		---------X---------X---------X	
	AH1pdm-HA-Consensus(n=365)		301	GKCPKYVKSTKLRLATGLRNVPSIQS	326
Epidemic strains	OSAKA/33/13_APR	6	301	.....	326
	Taiwan/691/13_MAY	6	301	.....	326
	NIIGATA-C/75/13_MAR	6	301	.....	326
	NAGANO/2312/13_MAY	6	301	.....	326
	TOKYO/32378/13_JUN	7	301	.....	326
	FUKUOKA-C/8/13_MAY	7	301	.....I.....	326
	SAITAMA/131/13_JUN	7	301	.....	326
Serology Ag	California/07/09E_X179A	-	301	.....I.....	326
	Bolivia/559/13E_JUN	6	301	.....	326
Reference Strains	NARITA/1/09E_MAY	-	301	.....	326
	YAMAGATA/752/09_DEC	-	301	.....	326
	SAPPORO/163/11_MAR	7	301	.....	326
	H1pdm_AG_site(Ca17)			----------	
	H1pdm_RB_site(Ca17)			----------	

A(H3N2) viruses

Antigenic and Phylogenetic analyses:

- Because it was clear from the previous HI data provided by NIID and other WHO CCs that ferret antisera raised against egg-grown vaccine viruses (A/Victoria/361/2011 and A/Texas/50/2012) poorly reacted with most epidemic viruses isolated in MDCK cells, we omitted ferret antisera raised against egg-grown reference viruses for HI tests. However, to evaluate reactivity of A/Texas/50/2012 X-223 antiserum against recent epidemic viruses, we put it in our HI tests.
- Total 236 viruses were subjected to HI tests. Overall, most test viruses collected in 2012/13 season (Sept 2012-Aug 2013) reacted well with antisera against cell-grown A/Texas/50/2012 virus as well as cell-grown A/Victoria/361/2011virus with similar or within 2-4-fold reduced HI titers. Low reactors showing over 8-fold reduced HI titer were only 1% with cell-grown A/Victoria/361/2011 antiserum and 0% with cell-grown A/Texas/50/2012 antiserum.
- Ninety percent of viruses collected in Sept 2012-Feb 2013 and 98% of viruses collected in Mar 2013-Aug 2013 exhibited over 8-fold low HI titers to the homologous titer of A/Texas/50/2012 X-223 ferret antiserum.
- Phylogenetic profiles of HA and NA genes were correlated well each other. In the HA gene, all viruses tested fell into N145S subclade in Clade 3C. More recent viruses tended to form two distinguishable subclade 3C.2 (N145S) and 3C.3 (R142G+T128A). The proportion of these subclades is 71.4% for subclade 3C.2 and 28.6% for subclade 3C.3, but subclade 3C.3 has tended to increase recently.
- Viruses in those subclades were antigenically indistinguishable each other by HI tests.

Conclusions

- The majority of A(H3N2) viruses isolated in the 2012/13 season was antigenically closely related to cell-grown A/Victoria/361/2011 and A/Texas/50/2012 viruses. Vaccine production virus A/Texas/50/2012 X-223 has changed antigenically from cell-grown A/Texas/50/2012 virus by egg-adaptation like that observed with A/Victoria/361/2011 IVR-165. Consequently, the antigenic change of vaccine production virus due to egg-adaptation was not sufficiently improved with A/Texas/50/2012 X-223 virus. The majority of recent A(H3N2) viruses fell into subclade 3C.2. No antiviral resistant viruses to all 4 NA inhibitors was found in the 2012/13 season.

Antigen viruses for serology tests.

A/Texas/50/2012 X-223
A/Texas/50/2012 egg wild type
A/Texas/50/2012 cell wild type
A/Osaka/32/2013 (recent virus in N145S subclade of Clade3C, cell-grown A/Texas/50/2012-like)
A/Yokohama/153/2013 (recent virus in R142G+T128A subclade of Clade3C, 4-fold low to cell-grown A/Texas/50/2012-like)

A/New York/39/2012 egg-grown (subclade 3C.3)
A/New York/39/2012 cell-grown (subclade 3C.3)

Influenza A/H3 isolates characterized by NIID

		Japan	China	Taiwan	Mongolia	Laos	Nepal	Vietnam	Total	
September 2012- February 2013									n	%
A/Victoria/361/2011 -like	Cell	83	5	0	1	6	1	0	96	50.8
A/Victoria/361/2011 -like*	Cell	83	1	0	2	2	3	0	91	48.1
A/Victoria/361/2011 (Low)**	Cell	2	0	0	0	0	0	0	2	1.1
	Total	168	6	0	3	8	4	0	189	
A/Texas/50/2012 -like	Cell	47	0	0	0	3	4	0	54	87.1
A/Texas/50/2012 -like*	Cell	7	0	0	1	0	0	0	8	12.9
A/Texas/50/2012 (Low)**	Cell	0	0	0	0	0	0	0	0	0.0
	Total	54	0	0	1	3	4	0	62	

		Japan	China	Taiwan	Mongolia	Laos	Nepal	Vietnam	Total	
March 2013- August 2013									n	%
A/Victoria/361/2011 -like	Cell	26	0	7	0	0	1	1	35	74.5
A/Victoria/361/2011 -like*	Cell	9	0	3	0	0	0	0	12	25.5
A/Victoria/361/2011 (Low)**	Cell	0	0	0	0	0	0	0	0	0.0
	Total	35	0	10	0	0	1	1	47	
A/Texas/50/2012 -like	Cell	32	0	4	0	0	1	1	38	80.9
A/Texas/50/2012 -like*	Cell	3	0	6	0	0	0	0	9	19.1
A/Texas/50/2012 (Low)**	Cell	0	0	0	0	0	0	0	0	0.0
	Total	35	0	10	0	0	1	1	47	

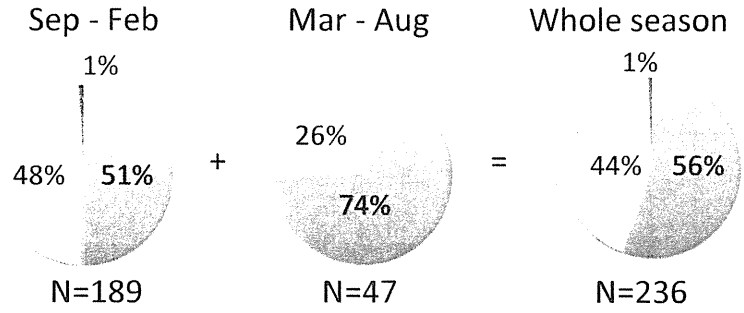
* 4-fold low to homologous titer

** 8-fold or greater low to homologous titer

A(H3N2)

A/Victoria/361/2011 (Cell)

2012/13 season



A/Victoria/361/2011-like

A/Victoria/361/2011-like*

Antigenic variants**

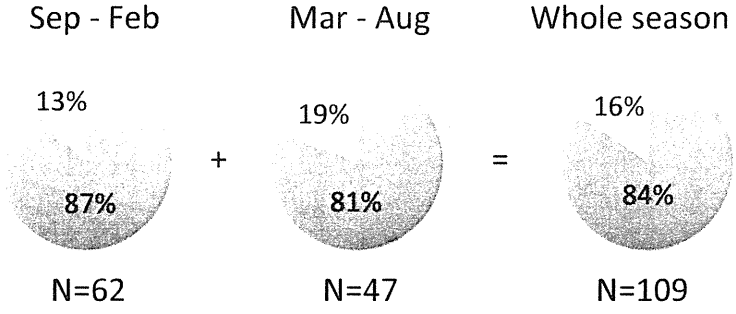
*4-fold lower than the homologous titers

**≥8-fold lower than the homologous titers

A(H3N2)

A/Texas/50/2012 (Cell)

2012/13 season



A/Texas/50/2012-like

A/Texas/50/2012-like*

Antigenic variants**

*4-fold lower than the homologous titers

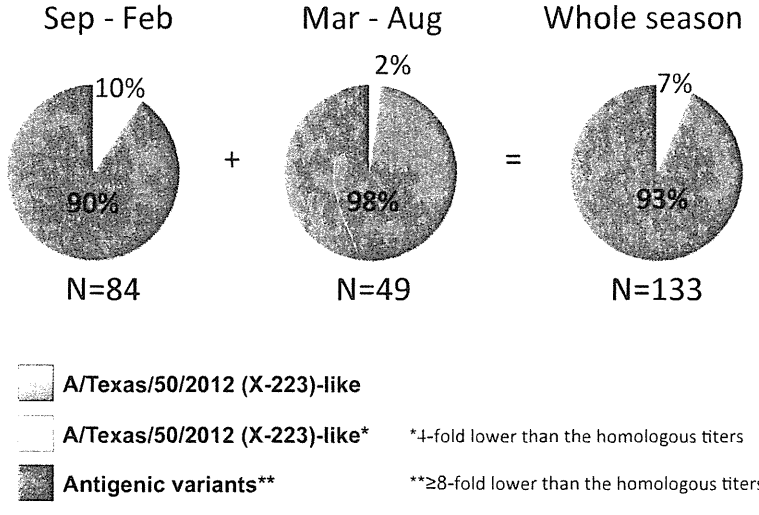
**≥8-fold lower than the homologous titers

2

A(H3N2)

A/Texas/50/2012 (X-223) (Egg)

2012/13 season



3

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

			CL3C						HK2000 group	HI test date:2013/9/5
Strains	Passage History	Sample date	Texas/50/12 Cell No.2	Texas/50/12 (X-223) Egg No.1	Victoria/ 361/11 Cell No.1	Sapporo/ 125/12 Cell No.1	Yamaguchi/ 30/12 Cell No.2	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/ 1313/09 Cell No.1	Genetic information
REF.Ag										
A/Texas/50/2012	M 1/C 1 +1		320	320	320	320	320	160	160	CL 3C.1
A/Texas/50/2012 (X-223)	E4/E8 +1		640	1280	640	320	640	640	80	CL 3C.1, G186V*
A/Victoria/361/2011	MDCK 2 +2		640	320	640	640	640	320	160	CL 3C.1
A/SAPPORO/125/2012	MDCK 1 +2	2012/11/01	160	160	160	160	320	80	80	CL 3C.2
A/YAMAGUCHI/30/2012	MDCK 2 +2	2012/10/10	160	160	160	160	320	80	80	CL 3C.3
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	640	160	320	320	640	640	320	-
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	40	40	40	40	80	40	640	HK2000
TEST.Ag										
A/Nepal/0413/2013	MDCK 1 +1	2013/06/17	640	160	320	320	640	160	160	CL 3C.3, #
A/Nepal/0029/2013	MDCK 1 +1	2013/02/05	320	160	320	320	640	160	320	CL 3C.2
A/TOKYO/13084/2013	MDCK 2 +1	2013/06/03	320	160	320	320	640	160	160	CL 3C.3, #
A/Nepal/0032/2013	MDCK 1 +1	2013/02/10	160	160	160	320	320	80	160	CL 3C.2
A/Nepal/0020/2013	MDCK 1 +1	2013/01/27	160	160	160	160	320	80	160	CL 3C.2
A/OITA/42/2013	MDCK 2 +2	2013/05/07	160	80	160	160	320	80	160	CL 3C.2, #
A/NARA/1021/2013	MDCK 3 +1	2013/03/06	160	80	160	160	320	40	160	CL 3C.2, #
A/GUNMA/111/2013	MDCK 2 +1	2013/05/16	160	80	160	160	160	40	160	CL 3C.2, #
A/Nepal/0023/2013	MDCK 1 +1	2013/01/31	160	80	160	160	160	40	160	CL 3C.3

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

			CL3C						HK2000 group	HI test date:2013/08/15
Strains	Passage History	Sample date	Texas/50/12 Cell No.2	Texas/50/12 (X-223) Egg No.1	Victoria/ 361/11 Cell No.1	Sapporo/ 125/12 Cell No.1	Yamaguchi/ 30/12 Cell No.2	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/ 1313/09 Cell No.1	Genetic information
REF.Ag										
A/Texas/50/2012	M 1/C 1 +1		320	160	160	320	320	160	160	CL 3C.1
A/Texas/50/2012 (X-223)	E4/E8 +1		640	1280	640	160	640	640	80	CL 3C.1, G186V*
A/Victoria/361/2011	MDCK 2 +2		320	160	320	320	320	160	160	CL 3C.1
A/SAPPORO/125/2012	MDCK 1 +2	2012/11/01	160	80	160	160	160	40	80	CL 3C.2
A/YAMAGUCHI/30/2012	MDCK 2 +2	2012/10/10	160	80	160	160	160	40	80	CL 3C.3
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	320	320	640	640	320	-
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	40	40	40	40	40	20	640	HK2000
TEST.Ag										
A/ISHIKAWA/71/2013	MDCK 1 +2	2013/04/05	320	320	320	320	640	320	160	ND
A/VN/NT12-471/2012	MDCK 0 +2	2012/07/16	160	80	160	160	320	80	80	CL 3B, #
A/HYOGO/1050/2013	MDCK 2 +1	2013/05/18	160	80	160	160	160	80	160	CL 3C, #
A/SAITAMA/132/2013	MDCK 2 +1	2013/06/14	160	80	160	160	160	80	160	ND
A/Taiwan/740/2013	MDCK 2 +1	2013/06/20	160	80	160	160	160	80	160	CL 3C.3, #
A/Taiwan/511/2013	MDCK 2 +1	2013/05/02	160	80	160	160	160	80	80	CL 3C.2, #
A/Taiwan/512/2013	MDCK 2 +1	2013/05/13	160	80	160	160	160	80	80	CL 3C.2
A/Taiwan/689/2013	MDCK 2 +1	2013/05/25	160	80	160	160	160	40	80	CL 3C.2
A/Taiwan/690/2013	MDCK 2 +1	2013/05/23	80	80	160	160	160	40	80	CL 3C.2
A/Taiwan/739/2013	MDCK 2 +1	2013/06/17	80	80	160	160	160	40	80	CL 3C.2, #
A/Taiwan/741/2013	MDCK 2 +1	2013/06/21	80	80	160	160	160	40	80	CL 3C.3
A/Taiwan/679/2013	MDCK 3 +1	2013/05/21	80	80	80	160	160	40	80	CL 3C.3, #
A/OSAKA/35/2013	MDCK 2 +1	2013/06/04	80	80	80	160	160	40	80	CL 3C.2, #
A/Taiwan/692/2013	MDCK 2 +1	2013/06/04	80	80	80	80	160	40	80	CL 3C.2, #
A/Taiwan/513/2013	MDCK 2 +1	2013/05/18	80	40	80	80	80	40	80	CL 3C.2, #

*Antigenic sites

ND: not determined

#: used in phylogenetic tree

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

			CL3C						HK2000	HI test date:2013/07/11
Strains	**Passage History**	**Sample date**	**Texas/50/12 Cell No.2**	**Texas/50/12 (X-223) Egg No.1**	**Victoria/ 361/11 Cell No.1**	**Sapporo/ 125/12 Cell No.1**	**Yamaguchi/ 30/12 Cell No.2**	**Shizuoka/ 736/09 Cell No.2**	**Hunan-beihu/ 1313/09 Cell No.1**	**Genetic information**
REF.Ag										
A/Texas/50/2012	M 1/C 1 +1		**320**	320	160	160	320	160	160	CL 3C.1
A/Texas/50/2012 (X-223)	E4/E8 +1		640	**1280**	640	320	640	640	80	CL 3C.1, G186V*
A/Victoria/361/2011	MDCK 2 +2		320	320	**320**	320	640	320	160	CL 3C.1
A/SAPPORO/125/2012	MDCK 1 +2	2012/11/01	160	160	160	**160**	320	80	80	CL 3C.2
A/YAMAGUCHI/30/2012	MDCK 2 +2	2012/10/10	160	160	160	160	**320**	80	80	CL 3C.3
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	320	320	640	**640**	160	-
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	40	40	40	80	80	40	**640**	HK2000
TEST.Ag										
A/OSAKA/30/2013	MDCK 2 +1	2013/04/25	320	160	320	320	640	160	320	ND
A/VN/VP13-65/2013	MDCK 3 +1	2013/05/03	320	160	320	320	320	160	320	CL 3C.2, #
A/NIIGATA/656/2013	MDCK 2 +1	2013/05/07	320	160	160	320	320	160	320	CL 3C.2, #
A/FUKUI/64/2013	MDCK 2 +1	2013/05/27	320	160	160	320	320	80	160	CL 3C.2, #
A/NIIGATA/591/2013	MDCK 2 +1	2013/04/21	320	160	160	160	320	160	160	ND
A/CHIBA-C/44/2013	MDCK 2 +1	2013/05/03	320	80	160	160	320	80	160	CL 3C.3, #
A/SENDAI/36/2013	MDCK 1 +1	2013/04/10	160	160	160	160	320	80	160	ND
A/FUKUI/62/2013	MDCK 2 +1	2013/04/11	160	160	160	160	320	80	160	ND
A/KANAGAWA/141/2013	MDCK 2 +1	2013/05/09	160	160	160	160	320	80	160	CL 3C.3, #
A/OSAKA/32/2013	MDCK 2 +1	2013/05/01	160	160	160	160	320	80	160	CL 3C.2, #
A/FUKUSHIMA/95/2013	MDCK 2 +1	2013/04/17	160	80	160	160	320	80	160	CL 3C.2, #
A/YAMAGATA/151/2013	MDCK 4 +1	2013/04/26	160	80	160	160	160	80	160	ND
A/EHIME/29/2013	MDCK 1 +1	2013/04/26	160	80	160	160	160	40	80	CL 3C.3, #

*Antigenic sites
ND: not determined
#: used in phylogenetic tree

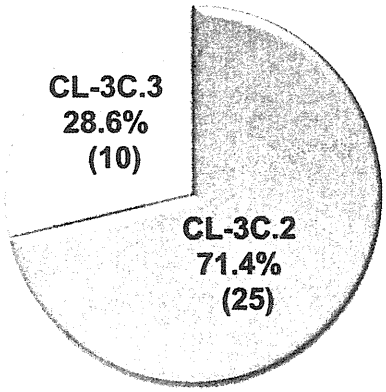
Hemagglutination inhibition tests of influenza A/H3 viruses-1%Guinea Pig RBCs

			CL3C						HK2000	HI test date:2013/06/13
Strains	Passage History	Sample date	Texas/50/12 Cell No.2	Texas/50/12 (X-223) Egg No.1	Victoria/ 361/11 Cell No.1	Sapporo/ 125/12 Cell No.1	Yamaguchi/ 30/12 Cell No.2	Shizuoka/ 736/09 Cell No.2	Hunan-beihu/ 1313/09 Cell No.1	Genetic information
REF.Ag										
A/Texas/50/2012	M 1/C 1 +1		320	160	320	320	320	160	160	CL 3C.1
A/Texas/50/2012 (X-223)	E4/E8 +1		640	1280	640	320	640	640	80	CL 3C.1, G186V*
A/Victoria/361/2011	MDCK 2 +2		320	160	320	320	320	160	160	CL 3C.1
A/SAPPORO/125/2012	MDCK 1 +2	2012/11/01	160	80	160	160	160	80	80	CL 3C.2
A/YAMAGUCHI/30/2012	MDCK 2 +2	2012/10/10	160	160	160	160	320	80	80	CL 3C.3
A/SHIZUOKA/736/2009	MDCK 1 +3	2009/05/23	320	160	320	320	640	640	160	-
A/Hunan-beihu/1313/2009	C 3 +1	2009/05/07	80	40	40	40	40	40	640	HK2000
TEST.Ag										
A/Laos/I014/2013	MDCK 1 +1	2013/01/08	160	80	160	160	320	80	160	CL 3C.3, #
A/SAPPORO/97/2013	MDCK 1 +1	2013/04/08	160	80	160	160	320	80	80	ND
A/KOBE/27/2013	MDCK 1 +1	2013/04/22	160	80	160	160	320	80	80	CL 3C.2, #
A/SHIMANE/118/2013	MDCK 1 +1	2013/04/26	160	80	160	160	320	80	80	ND
A/YAMAGATA/141/2013	MDCK 4 +1	2013/04/11	160	80	160	160	160	40	160	CL 3C.2, #
A/OITA/37/2013	MDCK 2 +1	2013/04/15	160	80	160	160	160	40	80	CL 3C.2
A/SAKAI/13/2013	MDCK 1 +1	2013/04/09	160	80	160	160	160	40	80	CL 3C.2, #
A/OSAKA/29/2013	MDCK 2 +1	2013/04/17	160	80	160	160	160	40	80	CL 3C.3
A/OITA/41/2013	MDCK 2 +1	2013/04/16	160	80	160	160	160	40	80	ND
A/CHIBA-C/42/2013	MDCK 2 +1	2013/04/24	160	80	160	160	160	40	80	ND
A/SAPPORO/103/2013	MDCK 2 +1	2013/04/22	160	80	160	160	160	40	80	ND
A/CHIBA-C/38/2013	MDCK 2 +1	2013/04/09	160	80	80	160	160	40	80	ND
A/AICHI/113/2013	MDCK 1 +1	2013/04/19	160	80	80	160	160	40	80	ND
A/NAGANO/2253/2013	MDCK 2 +1	2013/04/09	160	80	80	160	160	40	80	ND
A/YOKOHAMA/153/2013	MDCK 3 +1	2013/04/12	80	80	80	160	160	40	80	CL 3C.3, #
A/CHIBA-C/39/2013	MDCK 2 +1	2013/04/12	80	40	80	160	80	40	80	CL 3C.2, #

*Antigenic sites
ND: not determined
#: used in phylogenetic tree

Phylogenetic analysis of influenza H3N2 HA genes (HA1)

12/13 Japanese vaccine strain
HI reference strains in Red
March 2013 in Blue
April 2013 in Green
May2013 in Orange
June and July 2013 in Pink
•: Antigenic sites
#: Listed in HI tables
$: serology antigens



HA genetic clades
Since March 2013