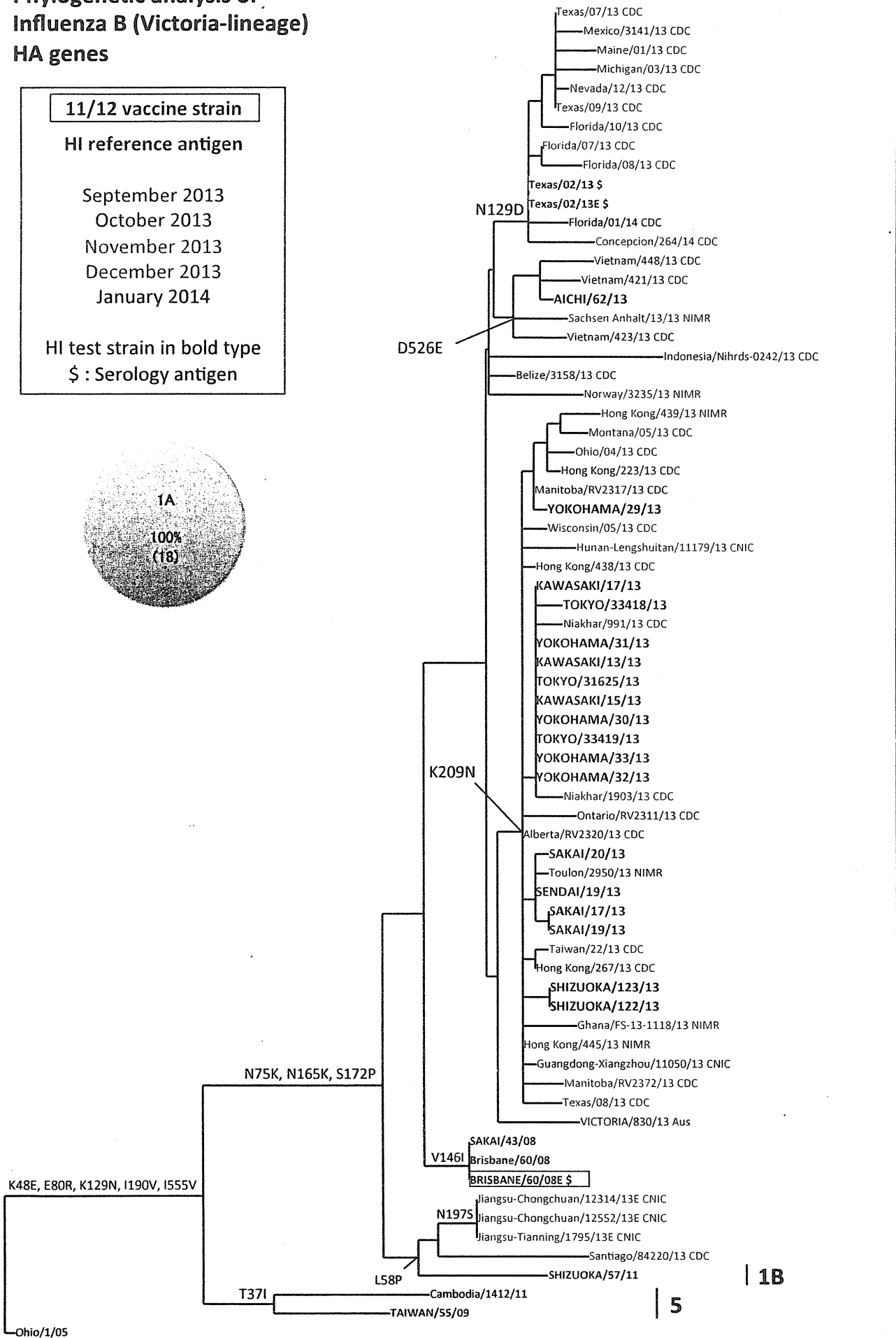
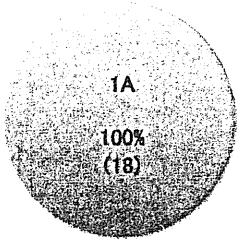


**Phylogenetic analysis of
Influenza B (Victoria-lineage)
HA genes**

11/12 vaccine strain
HI reference antigen
 September 2013
 October 2013
 November 2013
 December 2013
 January 2014
 HI test strain in bold type
 \$: Serology antigen



1A

1B

5

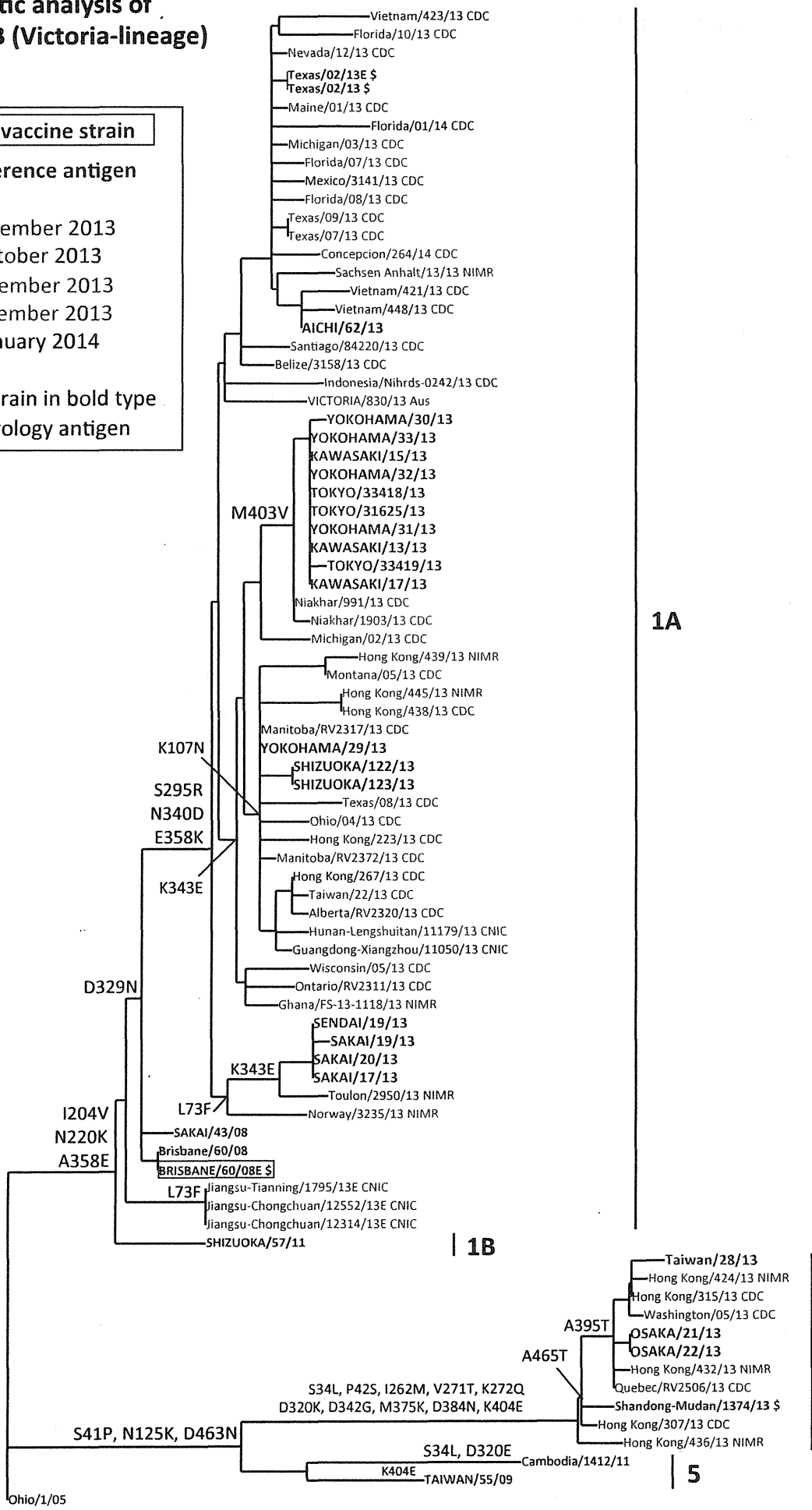
0.001

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

11/12 vaccine strain
HI reference antigen

September 2013
 October 2013
 November 2013
 December 2013
 January 2014

HI test strain in bold type
 \$: Serology antigen



Detection of antiviral resistant viruses in 2013/14 season:

- During September 2013 and February 2014, 400 A(H1N1)pdm09, 66 A(H3N2) and 34 B viruses isolated in Japan were tested for susceptibility to four neuraminidase (NA) inhibitors (oseltamivir, zanamivir, peramivir, and laninamivir). Those viruses were also subjected to real-time RT-PCR allelic determination (for A(H1N1)pdm09) and to NA gene sequencing for detection of mutations which correlate to the phenotype of antiviral resistance.
- Forty-three H275Y A(H1N1)pdm09 mutant viruses were detected and they exhibited highly reduced susceptibility to oseltamivir and peramivir. Of those resistant viruses, 32 viruses (74%) were detected in a community of Sapporo city and its neighboring cities in Hokkaido. Resistant viruses detected in other prefectures were sporadic cases (see Map and table in antiviral resistant section).
- All resistant viruses possessed characteristic amino acid substitution N386K in the NA protein which was different from the resistant viruses detected in USA (S82P) and in Australia (N386S).
- No antiviral resistant virus was detected so far with A(H3N2) and B viruses.

Detection of NA inhibitor-resistant viruses (Sep 2013 - Feb 2014)

Viruses	Oseltamivir			Peramivir			Zanamivir		Laninamivir			
	No. of viruses tested	Normal ^a	Reduced ^a	Highly reduced ^a	No. of viruses tested	Normal ^a	Reduced ^a	Highly reduced ^a	No. of viruses tested	Normal ^a		
A(H1N1)pdm09	400	357	0	43 ^b	400	357	0	43 ^b	64	64	64	64
Japan	400	357	0	43	400	357	0	43	64	64	64	64
A(H3N2)	66	66	0	0	66	66	0	0	66	66	66	66
Japan	66	66	0	0	66	66	0	0	66	66	66	66
B	34	34	0	0	34	34	0	0	34	34	34	34
Japan	34	34	0	0	34	34	0	0	34	34	34	34

Viruses were examined by fluorescent-based NA-Fluor assay, real time RT-PCR allelic discrimination and/or NA sequencing.

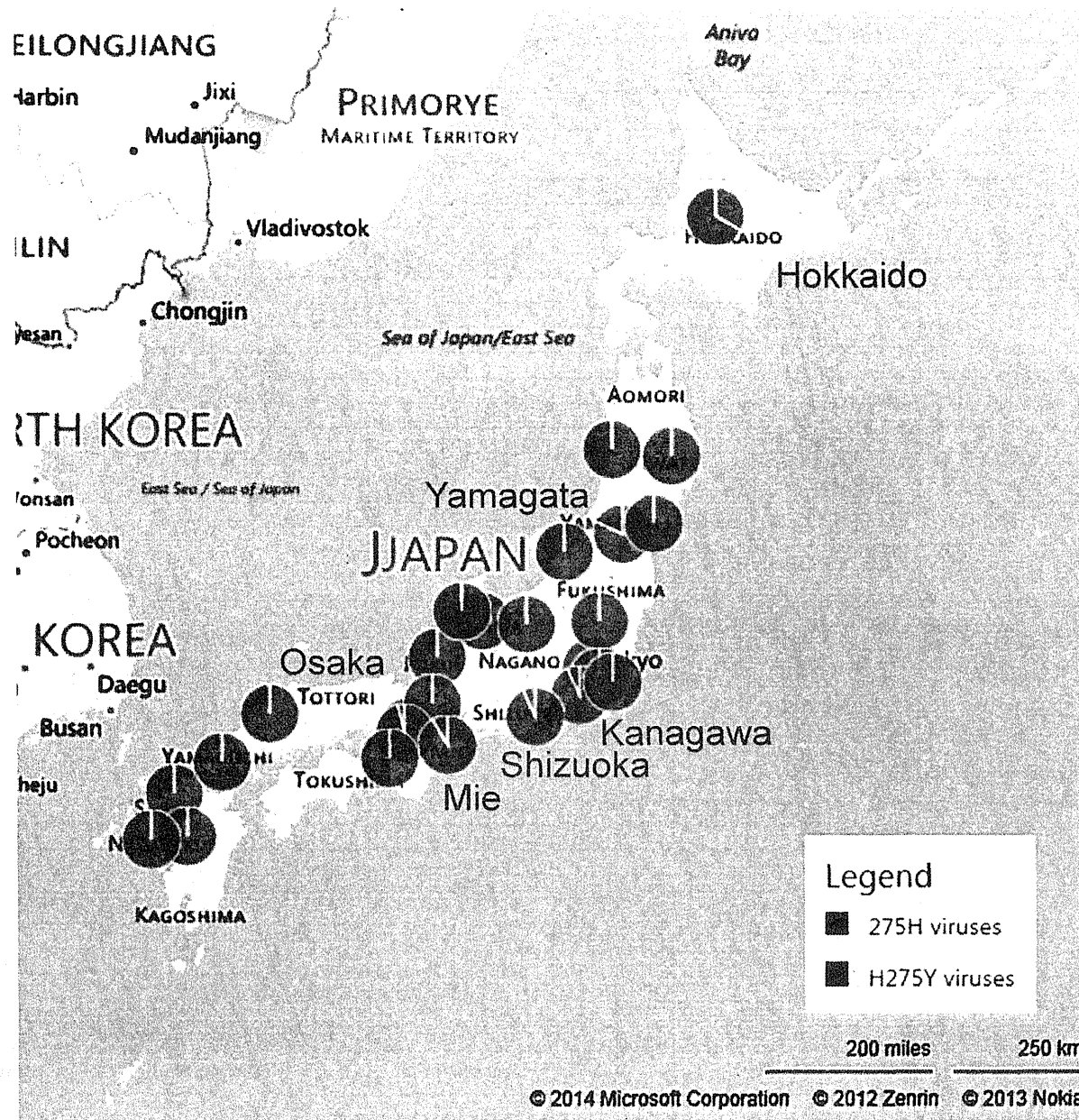
^a Fold difference in IC₅₀ compared to the mean value of sensitive viruses. Influenza A viruses: Normal inhibition: <10-fold; Reduced inhibition: 10-100-fold; Highly reduced inhibition: >100-fold. Influenza B viruses: Normal inhibition: <5-fold; Reduced inhibition: 5-50-fold; Highly reduced inhibition: >50-fold.

^b The viruses possessed an H275Y substitution in NA protein.

H275Y mutant A(H1N1)pdm09 viruses in Japan

No	NIID-ID	Isolate name	Collection date	Patient		City, Prefecture	NA inhibitor exposure	Epidemiological information
				Age	Sex			
1	13/14 - 151	A/KANAGAWA/146/2013	2013/11/11	39	F	Ebina, Kanagawa	Pending	Family cluster
2	13/14 - 139	A/YAMAGATA/157/2013	2013/11/14	9	F	Yamagata, Yamagata	No	Sporadic case
3	13/14 - 140	A/YAMAGATA/158/2013	2013/11/14	11	M	Yamagata, Yamagata	No	Sporadic case
4	13/14 - 46	A/SAPPORO/107/2013	2013/11/15	4	M	Sapporo, Hokkaido	No	Sporadic case
5	-	A/SAPPORO/TH1/2013	2013/11/25	39	F	Sapporo, Hokkaido	No	Sporadic case
6	13/14 - 115	A/SAPPORO/114/2013	2013/11/24	21	M	Sapporo, Hokkaido	No	Sporadic case
7	13/14 - 116	A/SAPPORO/116/2013	2013/11/26	3	M	Sapporo, Hokkaido	No	Sporadic case
8	-	A/KANAGAWA/152/2013	2013/12/06	24	F	Yamato, Kanagawa	Pending	Sporadic case
9	13/14 - 119	A/SAPPORO/119/2013	2013/12/07	10	M	Sapporo, Hokkaido	No	Sporadic case
10	13/14 - 120	A/SAPPORO/120/2013	2013/12/09	5	M	Sapporo, Hokkaido	No	Sporadic case
11	13/14 - 200	A/SAPPORO/125/2013	2013/12/19	3	M	Sapporo, Hokkaido	Pending	Sporadic case
12	13/14 - 201	A/SAPPORO/126/2013	2013/12/25	44	M	Sapporo, Hokkaido	Pending	Sporadic case
13	13/14 - 134	A/MIE/27/2013	2013/12/25	3	M	Kameyama, Mie	No	Family cluster. Visitor to Sapporo
14	-	A/OSAKA/8/2014	2014/01/09	3	M	Neyagawa, Osaka	Peramivir (2014/01/04)	Sporadic case
15	13/14 - 202	A/SAPPORO/1/2014	2014/01/06	2	F	Sapporo, Hokkaido	Pending	Sporadic case
16	13/14 - 256	A/SAPPORO/2/2014	2014/01/07	1	M	Sapporo, Hokkaido	Pending	Sporadic case
17	-	A/HOKKAIDO/2/2014	2014/01/07	28	M	Kuriyama, Hokkaido	No	Vicinity of Sapporo
18	13/14 - 258	A/SAPPORO/4/2014	2014/01/09	29	F	Sapporo, Hokkaido	Pending	Sporadic case
19	13/14 - 257	A/SAPPORO/3/2014	2014/01/08	6	M	Sapporo, Hokkaido	Pending	Sporadic case
20	13/14 - 259	A/SAPPORO/5/2014	2014/01/10	0	F	Sapporo, Hokkaido	Pending	Sporadic case
21	13/14 - 260	A/SAPPORO/6/2014	2014/01/10	48	F	Sapporo, Hokkaido	Pending	Sporadic case
22	13/14 - 263	A/SAPPORO/9/2014	2014/01/10	1	M	Sapporo, Hokkaido	Pending	Sporadic case
23	-	A/HOKKAIDO/3/2014	2014/01/10	11	F	Kuriyama, Hokkaido	No	Family cluster, Vicinity of Sapporo
24	-	A/HOKKAIDO/4/2014	2014/01/10	41	F	Kuriyama, Hokkaido	No	Family cluster, Vicinity of Sapporo
25	13/14 - 261	A/SAPPORO/7/2014	2014/01/14	24	M	Sapporo, Hokkaido	Pending	Sporadic case
26	-	A/SAPPORO/13/2014	2014/01/14	3	M	Sapporo, Hokkaido	Pending	Pending
27	-	A/SAPPORO/18/2014	2014/01/14	13	F	Sapporo, Hokkaido	Pending	Pending
28	-	A/SAPPORO/21/2014	2014/01/17	5	M	Sapporo, Hokkaido	Pending	Pending
29	-	A/SAPPORO/25/2014	2014/01/17	1	F	Sapporo, Hokkaido	Pending	Pending
30	-	A/SAPPORO/22/2014	2014/01/18	9	M	Sapporo, Hokkaido	Pending	Pending
31	-	A/SAPPORO/16/2014	2014/01/20	5	F	Sapporo, Hokkaido	Pending	Pending
32	-	A/SAPPORO/19/2014	2014/01/20	6	F	Sapporo, Hokkaido	Pending	Pending
33	-	A/SAPPORO/23/2014	2014/01/20	5	M	Sapporo, Hokkaido	Pending	Pending
34	-	A/SAPPORO/24/2014	2014/01/20	3	M	Sapporo, Hokkaido	Pending	Pending
35	-	A/YOKOHAMA/18/2014	2014/01/22	7	F	Yokohama, Kanagawa	Laninamivir (2014/01/21)	Family cluster, encephalitis/encephalopathy
36	-	A/HOKKAIDO/	2014/01/23-29			Iwamizawa, Hokkaido	Pending	Pending
37	-	A/YOKOHAMA/24/2014	2014/01/27			Yokohama, Kanagawa	Peramivir (2014/01/24-25)	Pending
38	-	A/MIE/13/2014	2014/02/01	76	F	Tsu, Mie	Oseltamivir (2014/01/27-) Zanamivir (2014/02/01)	Family cluster
39	-	A/HOKKAIDO/	2010/02/04	11		Furano, Hokkaido	Pending	Pending
40	-	A/HOKKAIDO/	2010/02/04	7		Furano, Hokkaido	Pending	Pending
41	-	A/HOKKAIDO/	2010/02/05	10		Furano, Hokkaido	Pending	Pending
42	-	A/SHIZUOKA/	2014/01/24			Shizuoka	Pending	Pending
43	-	A/SHIZUOKA/	2014/02/04			Shizuoka	Oseltamivir (2014/01/30)	Pending

H275Y mutant A(H1N1)pdm09 viruses in Japan



Prefecture	275H viruses	H275Y viruses
HOKKAIDO	16	32
IWATE	3	0
MIYAGI	3	0
AKITA	1	0
YAMAGATA	9	2
NIIGATA	10	0
IBARAKI	8	0
TOCHIGI	6	0
SAITAMA	37	0
CHIBA	4	0
TOKYO	4	0
KANAGAWA	53	4
NAGANO	6	0
SHIZUOKA	28	2
TOYAMA	1	0
ISHIKAWA	6	0
FUKUI	2	0
MIE	22	2
SHIGA	10	0
KYOTO	6	0
OSAKA	26	1
HYOGO	19	0
WAKAYAMA	28	0
SHIMANE	11	0
YAMAGUCHI	18	0
FUKUOKA	4	0
NAGASAKI	7	0
KUMAMOTO	9	0
Total	357	43

Genetic characterizations of A(H5N1) virus

Genetic characterizations of A(H5N1) virus provided by NIC in HCM, Viet Nam, were performed by a team in Laboratory of Molecular Diagnosis and International Training.

Dr Tsutomu Kageyama (Chief)

Dr Ikuyo Takayama

Dr Mina Nakauchi

Dr Hitoshi Takahashi

A/Vietnam/14011801/2014

I. Genetic analysis

The full nucleotide sequences of the following isolates by Egg and MDCK cell were analyzed;

A/Vietnam/14011801/2014 (E1)

A/Vietnam/14011801/2014 (MDCK1)

All eight genes of this virus are originated from H5N1 HPAI virus (clade 2.3.2.1c, formerly HK/6841-like).

HA gene

Cleavage site:

A/Vietnam/14011801/2014 has RERRRK*GLF. (Same as A/Hong Kong/6841/2010 and vaccine candidate strain, A/duck/Vietnam/NCVD-1584/2012)

Receptor-binding pocket: Q222, G224 (avian type receptor SAa2-3Gal)

There is no mutation in the N-glycosylation sites by comparison with A/Hong Kong/6841/2010 and A/duck/Vietnam/NCVD-1584/2012.

NA gene

There is deletion of 20 amino acids in the stalk.

There is no substitution that has been associated with reduced susceptibility or resistance to neuraminidase inhibitors (NAIs) in A/Vietnam/14011801/2014. This virus is sensitive to antivirals from genotypic profile.

PB2 gene

Amino acid position 627 is Glu (Same as A/Goose/Guangdong/1/96, A/Hong Kong/6841/2010 and A/duck/Vietnam/NCVD-1584/2012).

Amino acid position 701 is Asp (Same as A/Goose/Guangdong/1/96, A/Hong Kong/6841/2010 and A/duck/Vietnam/NCVD-1584/2012).

A/Vietnam/14011801/2014 doesn't have both E627K and D701N genetic markers of high virulence for H5N1 viruses in mammals.

PB1 gene

A/Vietnam/14011801/2014 encodes a truncated PB1-F2 protein of 57 amino acids. (Same as A/duck/Vietnam/NCVD-1584/2012).

M gene

A/Vietnam/14011801/2014 has no substitutions associated with amantadine resistance in M2 (Same as A/duck/Vietnam/NCVD-1584/2012).

NS gene

NS1 protein has 5 amino acid deletions (80-84) and Asp at position 92 (Same as A/Hong Kong/6841/2010).

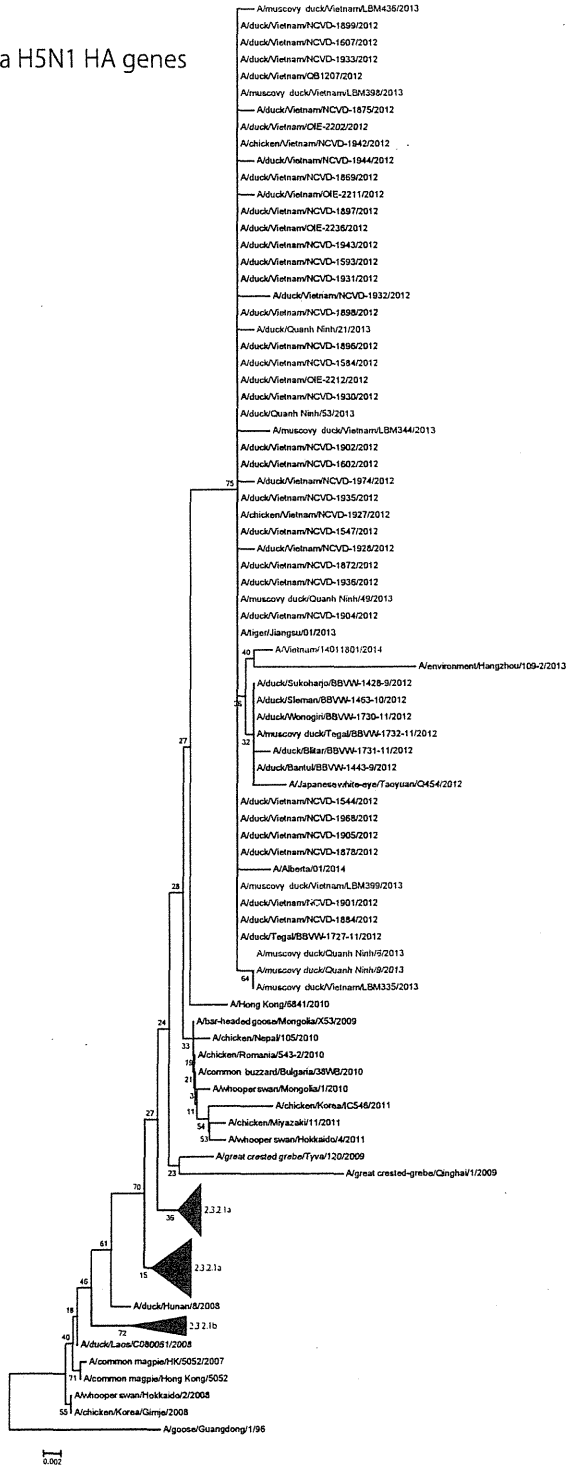
Amino acid differences in HA of the circulating viruses of clade 2.3.2.1c

H5 HA mature	A/duck/Vietnam/NCVD-1584/2012	A/Hubei/1/2010 (clade2.3.2.1a)	A/Hong Kong/6841/2010 (clade2.3.2.1c)	A/barn swallow/Hong Kong/1161/2010(2.3.2.1b)	A/duck/Vietnam/NCVD-1884/2012	A/Alberta/01/2014	A/tiger/Jiangsu01/2013	A/whooper swan/Hokkaido/4/2011	A/duck/Quanh Ninh/53/2013	A/Vietnam/14011801/2014 (clinical specimen)	A/Vietnam/14011801/2014 (E1)	A/Vietnam/14011801/2014 (MDCK1)	Annotation
70	T	I	I	I				I					
154	D									N	N	N	Antigenic site B
174	I	V	V	V				V					
182	N											N>K	increased virus binding to α2-6
186	E									E>>D		D>E	
195	T		I										
238	A									T	T	T	
259	K									T	T	T	
491	Q		H										
524	A		T										

Phylogenetic analysis of H5N1 HA genes

Phylogenetic analysis of
influenza H5N1 HA genes
(clade 2.3.2.1c)

2013 isolates from Vietnam
Reassortant vaccine candidate



Phylogenetic analysis of H5N1 NA genes

Phylogenetic analysis of influenza H5N1 NA genes
(clade 2.3.2.1)

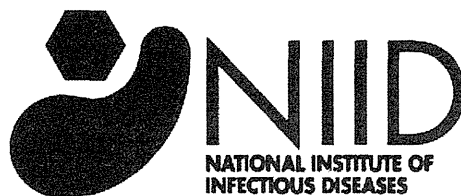
2013 isolates from Vietnam
Reassortant vaccine candidate



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

February 17-21, 2014, Geneva, Switzerland

Serologic Response to Influenza Virus Vaccines



**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

Serum panel for 13/14 Northern hemisphere human serology

Serum provided by	Population details (yrs)		No. of sera	Vaccine components
Niigata Univ., Japan (R. Saito)	<u>Adults</u>		30	A/California/7/2009 X-179A (H1N1)pdm09 A/Texas/50/2012 X-223 (H3N2)
	Age range 21-59	Mean age 41.8		
	<u>Elderly</u>		30	B/Massachusetts/02/2012 BX-51B
	Age range 63-99	Mean age 84.4		
NIBSC, UK (R. Newman)	<u>Adults</u>		24	Cell culture vaccine A/Brisbane/10/2010 (H1N1)pdm09 A/Texas/50/2012 X-223A
	Age range 18-60			
	<u>Elderly</u>		24	B/Massachusetts/02/2012
	Age range 61-			
CBER, USA (Z. Ye)	<u>Adults</u>		24	Quadrivalent vaccine A/California/7/2009 X-179A (H1N1)pdm09 A/Texas/50/2012 X-223A (H3N2)
	Age range 19-64	Mean age 45.3		
	<u>Elderly</u>		24	B/Massachusetts/02/2012 B/Brisbane/60/2008
	Age range 65-83	Mean age 71.9		
CDC, USA (X. Xu)	<u>Pediatric</u>		24	A/California/7/2009 X-179A (H1N1)pdm09 A/Texas/50/2012 X-223A (H3N2) B/Massachusetts/02/2012
	Age range 0-2	Mean age 1.3		

Antigen strains used for serology study by NIID (NIID serum panel)

Type/Subtypes	Viruses	Passage	Remarks
A(H1N1)pdm09	A/California/7/2009 X-179A	Ex/E1+1	Vaccine virus
	A/New Hampshire/04/2013	E4+1	group6B K163Q, A256T, V234I common
	A/Wakayama/153/2013	MDCK1+1	group 6B Vaccine-like
	A/Cameroon/3234/2013	MDCK2+1	group 6C
A(H3N2)	A/Texas/50/2012 X-223	E4/E8+1	Vaccine virus
	A/Almaty/2958/2013	E5+1	group3C.3 common
	A/Texas/50/2012	M 1/C 2 +2	Vaccine wild type
	A/New York/39/2012	C2+1	group 3C.3; T128A, R142G, N145S
	A/Louisiana/09/2013	C3+1	group 3C.3; T128A, R142G, N145S, L157S
	A/Tokyo/31512/2013	MDCK2+2	group 3C.3 Vaccine-like
B-Yam	B/Massachusetts/2/2012 BX-51B	E3/E7+1	Vaccine virus
	B/Massachusetts/02/2012	M 1/C 2 +2	Vaccine wild type common, group 2
	B/Shangdong-Mudan/1374/2013	C4+C1+1	group3 common
	B/Osaka/18/2013	MDCK1+1	group 3 Vaccine-like
	B/Lithuania/6935/2013	MDCK4+1	group 2 Low reactor

Antigen strains used for serology study by NIID (NIBSC serum panel)

Type/Subtypes	Viruses	Passage	Remarks
A(H1N1)pdm09	A/Brisbane/10/2010	E2+1	Vaccine virus
	A/New Hampshire/04/2013	E4+1	group6B K163Q, A256T, V234I common
	A/Wakayama/153/2013	MDCK1+1	group 6B Vaccine-like
	A/Cameroon/3234/2013	MDCK2+1	group 6C
A(H3N2)	A/Texas/50/2012 X-223A	E5/E8+1	Vaccine virus
	A/Almaty/2958/2013	E5+1	group3C.3 common
	A/Texas/50/2012	M 1/C 2 +2	Vaccine wild type
	A/New York/39/2012	C2+1	group 3C.3; T128A, R142G, N145S
	A/Louisiana/09/2013	C3+1	group 3C.3; T128A, R142G, N145S, L157S
	A/Tokyo/31512/2013	MDCK2+2	group 3C.3 Vaccine-like
B-Yam	B/Massachusetts/02/2012	E3+1	Vaccine virus
	B/Massachusetts/02/2012	M 1/C 2 +2	Vaccine wild type common, group 2
	B/Shangdong-Mudan/1374/2013	C4+C1+1	group3 common
	B/Osaka/18/2013	MDCK1+1	group 3 Vaccine-like
	B/Lithuania/6935/2013	MDCK4+1	group 2 Low reactor

Antigen strains used for serology study by NIID (CBER-QIV serum panel)

Type/Subtypes	Viruses	Passage	Remarks
A(H1N1)pdm09	A/California/7/2009 X-179A	Ex/E1+1	Vaccine virus
	A/New Hampshire/04/2013	E4+1	group6B K163Q, A256T, V234I common
	A/Wakayama/153/2013	MDCK1+1	group 6B Vaccine-like
A(H3N2)	A/Texas/50/2012 X-223A	E4/E8+1	Vaccine virus
	A/Almaty/2958/2013	E5+1	group3C.3 common
	A/Texas/50/2012	M 1/C 2 +2	Vaccine wild type
	A/New York/39/2012	C2+1	group 3C.3; T128A, R142G, N145S
	A/Tokyo/31512/2013	MDCK2+2	group 3C.3 Vaccine-like
B-Yam	B/Massachusetts/2/2012	E3+1	Vaccine virus
	B/Massachusetts/02/2012	M 1/C 2 +2	Vaccine wild type common
	B/Shangdong-Mudan/1374/2013	C4+C1+1	group3 common
	B/Osaka/18/2013	MDCK1+1	group 3 Vaccine-like
B-Vic	B/Brisbane/60/2008	E4+1	Vaccine virus
	B/Texas/02/2013	E5+1	group 1A common
	B/Texas/02/2013	M1/C2+1	group 1A

Antigen strains used for serology study by NIID (CDC serum panel)

Type/Subtypes	Viruses	Passage	Remarks
A(H1N1)pdm09	A/California/7/2009 X-179A	Ex/E1+1	Vaccine virus
	A/New Hampshire/04/2013	E4+1	group6B K163Q, A256T, V234I common
	A/Wakayama/153/2013	MDCK1+1	group 6B Vaccine-like
	A/Cameroon/3234/2013	MDCK2+1	group 6C
A(H3N2)	A/Texas/50/2012 X-223A	E5/E8+1	Vaccine virus
	A/Almaty/2958/2013	E5+1	group3C.3 common
	A/Texas/50/2012	M 1/C 2 +2	Vaccine wild type
	A/New York/39/2012	C2+1	group 3C.3; T128A, R142G, N145S
	A/Louisiana/09/2013	C3+1	group 3C.3; T128A, R142G, N145S, L157S
	A/Tokyo/31512/2013	MDCK2+2	group 3C.3 Vaccine-like
B-Yam	B/Massachusetts/2/2012	E3+1	Vaccine virus
	B/Massachusetts/02/2012	M 1/C 2 +2	Vaccine wild type common, group 2
	B/Shangdong-Mudan/1374/2013	C4+C1+1	group3 common
	B/Osaka/18/2013	MDCK1+1	group 3 Vaccine-like
	B/Lithuania/6935/2013	MDCK4+1	group 2 Low reactor

