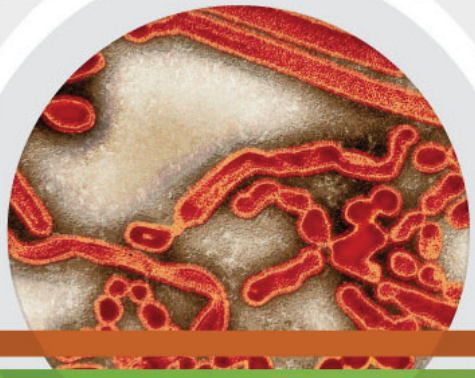

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, September 2012

Summary

Since 01 January 2012, influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B/Victoria and B/Yamagata lineage viruses have been detected in ECDC-affiliated countries.

- Type A viruses have predominated over type B.
- A(H3N2) viruses have predominated over A(H1N1)pdm09 viruses.
- A(H1N1)pdm09 viruses continue to show genetic drift from the vaccine virus, A/California/07/2009, but the vast majority remain antigenically similar to it.
- During the last nine months, all European A(H3N2) viruses sequenced fell within five genetic clusters. Test viruses isolated in mammalian cells show low titres with post-infection ferret antisera raised against egg-propagated viruses, including the new vaccine virus A/Victoria/361/2011. They react well with post-infection ferret antisera raised against A/Victoria/361/2011 and other current reference viruses propagated exclusively in tissue culture.
- Recent B/Victoria lineage viruses fell within the B/Brisbane/60/2008 genetic clade and were antigenically similar to reference cell-propagated viruses of the B/Brisbane/60/2008 genetic clade.
- Recent B/Yamagata-lineage viruses fell into two genetic clades, represented by the recommended vaccine component for the 2012/2013 influenza season, B/Wisconsin/1/2010 (clade 3), or B/Estonia/55669/2012 (clade 2); viruses in these clades are antigenically distinguishable.
- Antigenic analyses of A(H3N2)v viruses, the cause of zoonotic infections in the USA, indicate that these viruses are antigenically distinct from seasonal A(H3N2) viruses.

A summary of viruses received by the WHO Collaborating Centre for Reference and Research on Influenza at MRC National Institute for Medical Research from EU and EEA countries since 01 January 2012 is shown in Table 1.

Viruses and/or clinical samples were received from 21 EU/EEA countries. Table 1 is an update of the table published in the previous report (July 2012). The majority (69%) of viruses received were influenza A(H3N2) viruses; among influenza B receipts, viruses of the B/Yamagata and B/Victoria lineages were received in approximately equal proportions; influenza A(H1N1)pdm09 viruses were received in low numbers and from eight countries only, with Norway providing nearly 50% (18/37).

This report was prepared by Rod Daniels, Vicki Gregory and John McCauley on behalf of the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL), under contract to the European Centre for Disease Prevention and Control (ECDC).

Influenza A(H1N1)pdm09 virus analyses

Only four A(H1N1)pdm09 influenza viruses have been analysed by HI assay since the previous report (Table 2).

Phylogenetic analyses of the HA genes of viruses collected throughout 2012 (Figure 1) show that the most recently detected H1N1 viruses from EU/EEA countries have HAs that cluster within two of eight genetic groups that have been described previously. These viruses fall into genetic groups 6 and 7, which are defined by amino acid substitutions in HA1:

- Group 6: **D97N** and **S185T,** e.g. A/St Petersburg/27/2011.
- Group 7: **S143G, S185T** and **A197T,** e.g. A/St Petersburg/100/2011.

Influenza A(H3N2) virus analyses

As described before, A(H3N2) viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pigs, turkeys and humans. Approximately 70% of viruses gave sufficient titre in HA assays, in the presence of 20nM oseltamivir, to be analysed by HI assay using guinea pig red blood cells in the presence of 20nM oseltamivir, added to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010).

The results of HI assays carried out since the July report are shown in Table 3. HI assays using post-infection ferret antiserum raised against the virus recommended for the 2011/2012 northern hemisphere influenza vaccine, A/Perth/16/2009, showed all 35 test viruses to have ≥8-fold reductions in HI titre compared with the titre for the homologous virus.

Using post-infection ferret antiserum raised against the newly recommended, egg-propagated vaccine virus for the northern hemisphere 2012/2013 influenza season, A/Victoria/361/2011, only 8/35 test viruses gave an HI titre within fourfold of that of the homologous virus. In contrast, with antiserum raised against the cell-culture propagated A/Victoria/361/2011 only 1/35 test viruses showed ≥8-fold reduced reactivity compared with the titre of the homologous cell-propagated virus (Table 3). The test viruses also showed good reactivity with post-infection ferret antisera raised against other reference viruses propagated exclusively in cell culture, regardless of HA genetic group: A/Alabama/5/2010 (group 5), A/Stockholm/18/2011 (subgroup 3A), A/Athens/GR112/2012 (subgroup 3B), A/Hong Kong/3969/2011 (subgroup 3C) and A/Berlin/93/2011 (subgroup 3C). However, test viruses reacted poorly with post-infection ferret antisera raised against other egg-propagated reference viruses, A/Victoria/208/2009 and A/Iowa/19/2010, compared to the HI reactivity of the homologous egg-propagated viruses. The low reactivity of test viruses with antisera raised against each of the egg-adapted viruses, importantly including the new vaccine virus A/Victoria/361/2011, suggests that egg adaptation of the H3N2 reference viruses influences the immune response of the ferret and consequently the HI assay. In light of these observations, results of HI tests and other serological assays with currently circulating A(H3N2) viruses continue to warrant careful consideration.

Phylogenetic analysis of the HA gene sequences of representative viruses has been carried out (Figure 2). Viruses from the EU/EEA collected since January have HA genes that fall into HA genetic groups 5 and 6, and subgroups 3A, 3B and 3C.

The amino acid substitutions that are associated with each of these groupings are:

- Subgroup 3A: **V223I ,N144D** (resulting in the loss of a glycosylation site), and **N145S,** e.g. A/Stockholm/18/2011;
- Subgroup 3B: **V223I, N145S, A198S** and **N312S,** e.g. A/Athens/GR112/2012;
- Subgroup 3C: **V223I, S45N, T48I, A198S** and **N312S,** e.g. A/Hong Kong/3969/2011 and the prototype vaccine virus A/Victoria/361/2011, with some viruses also carrying the substitutions **Q33R** and **N278K**;
- Group 5: **D53N, Y94H, I230V** and **E280A,** e.g. A/Alabama/5/2010;
- Group 6: **D53N, Y94H, S199A, I230V** and **E280A,** e.g. A/Iowa/19/2010.

Influenza B virus analyses

B/Victoria lineage viruses

B/Victoria lineage viruses were received from 14 EU/EEA Member States. The results of HI analyses of influenza B viruses of the B/Victoria lineage performed since the last report are shown in Table 4. All eight test viruses showed reduced reactivity (≥4-fold reduction in titre compared with the homologous titre) with post-infection ferret antiserum raised against the egg-propagated vaccine virus recommended for the northern hemisphere 2011/2012 season, B/Brisbane/60/2008. In contrast, they reacted well with antisera raised against viruses genetically closely related to the vaccine virus but propagated in cells. These antisera were raised against B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010, which are surrogate cell-propagated antigens for the egg-propagated vaccine virus. The reactivity of test viruses with antiserum raised against B/Malta/MV636714/2011, another egg isolate, was low and similar to their reactivities with antiserum raised against B/Brisbane/60/2008.

Phylogenetic analysis of the HA genes of representative B/Victoria lineage viruses is shown in Figure 3. All recently collected viruses received from EU and EEA laboratories carried HA genes that fell into clade 1, the B/Brisbane/60 clade.

B/Yamagata lineage viruses

Influenza B viruses of the B/Yamagata lineage have also been received from 14 EU/EEA Member States. Table 5 shows the results of HI assays for the propagated viruses examined since the last report. Seven of the eleven test viruses reacted within a fourfold titre of that of the homologous virus with the post-infection ferret antiserum raised against the recommended egg-propagated vaccine virus for the northern hemisphere 2012/2013 influenza season, B/Wisconsin/1/2010. Generally, the test virus HI reaction profiles fell into two sets with the five viruses from Norway reacting well with the majority of the antisera, including that raised against B/Florida/4/2006, while five other viruses, two of which have been sequenced and shown to fall within clade 2, react best with post-infection ferret antiserum raised against B/Estonia/55669/2011. A virus from France, B/Lorraine/1220/2012, reacted relatively poorly (≥4-fold reduced) with all antisera. This again indicates the co-circulation of two antigenically distinguishable clades within the B/Yamagata lineage.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata lineage viruses. The HA genes of these viruses fall into two genetic clades, one defined as clade 3, represented by the reference virus B/Bangladesh/3333/2007 and including B/Wisconsin/1/2010 and B/Stockholm/12/2011), with the other defined as clade 2 represented by the reference viruses B/Brisbane/3/2007 and B/Estonia/55669/2011. Viruses falling within these two clades are antigenically distinguishable.

The two clades are differentiated by substitutions at HA1 residues 48, 108, 150, 165, 181 and 229. The HA gene of viruses of clade 2 encodes **K48**, **A108**, **S150**, **N165**, **A181** and **G229**; the HA gene of viruses in clade 3 encodes **R48**, **P108**, **I150**, **Y165**, **T181** and **D229**.

Clade 2 appears to be genetically homogenous but clade 3 can be sub-divided into four genetic groups:

- a group defined by the amino acid substitution **N202S** similar to B/Wisconsin/1/2010;
- a group defined by the substitution **T181K** (e.g. B/Ireland/M1522/2012);
- a group defined by the substitution **M251V** with the substitutions **T181A** and **K253R** (e.g. B/Serbia/1894/2011);
- a group defined by the substitution **M251V** with the substitutions **V29A** and **L172Q** (e.g. B/Stockholm/12/2011).

Influenza A(H3N2)v virus

On 3 August 2012, the United States CDC issued a Health Advisory describing an increase in the number of influenza A(H3N2)v infections in three US states; CDC prepared further background information and provided regular updates. Antigenic and genetic characterisation of H3N2v viruses has been described by Lindstrom et al., 2012. The virus was characterised as being antigenically distinct from currently circulating human seasonal influenza viruses and to be a reassortant virus with seven genes from swine influenza 'triple reassortant' H3N2 viruses and the M gene from an influenza A(H1N1)pdm09 virus.

The prevalence of antibodies in human sera cross-reactive with H3N2v virus has been examined in Norway, the USA and Canada, and the authors concluded that there was a high level of immunity in young adults but less so in other groups. Active surveillance of swine influenza has shown that influenza A(H3N2)v viruses have not circulated in pigs in Europe. Antigenic analysis of two H3N2v viruses, three viruses from humans infected with triple reassortant swine influenza viruses (TRV) and two human seasonal H3N2 viruses, performed at the WHO CC in London (Table 6), support the conclusion that both triple reassortant H3N2 and H3N2v influenza viruses are antigenically distinct from recent human seasonal influenza viruses.

Risk assessments for these A(H3N2)v viruses as a risk to public health have been posted by the United States CDC and ECDC.

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza, based at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva from 20 to 22 February 2012 and Beijing, China, from 17 to 19 September 2012 can be found at:

http://www.nimr.mrc.ac.uk/documents/about/interim-report-feb-2012.pdf

http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_September_2012_2.pdf

Note on the figures

The phylogenetic trees were constructed using RAxML and drawn using FigTree. The bars indicate the proportion of nucleotide changes in the sequence. Reference strains are viruses to which post-infection ferret antisera have been raised. The colours indicate the month of sample collection. Isolates from WHO NICs in ECDC countries are highlighted within boxes. Sequences for some of the viruses from non-EU/EEA countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, collection dates since 2012-01-01

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
		Not subtyped					Lineage unknown				
Country			Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]
UNKNOWN											
Czech Republic[3]	26				10	10		14	10	2	2
JANUARY											
Austria	6				4	4		1	1	1	1
Bulgaria	4				4	3					
Denmark	2				2	1					
Estonia	1				1	0					
Finland	3				3	2					
France	4				4	4					
Germany	15				14	13				1	1
Greece	18				8	7	5	1	1	4	4
Iceland	9				9	7					
Ireland	9				7	2		1	1	1	1
Italy	14				13	12				1	1
Latvia	7				6	5		1	1		
Netherlands	2				2	2					
Norway	16		5	2	11	11					
Portugal	7				7	4					
Romania	3				3	3					
Slovenia	3				3	3					
Spain	21		1	0	18	10		2	2		
Sweden	11		2	2	8	7		1	1		
United Kingdom	4				3	3		1	1		
FEBRUARY											
Bulgaria	8				8	8					
Denmark	11				6	4		2	2	3	3
Estonia	18				18	2					
Finland	5				4	2		1	1		
France	5		2	2	3	3					
Greece	24				14	13	4	2	2	4	4
Iceland	11				11	11					
Ireland	3		1	1	1	1		1	1		
Italy	12				6	5		1	1	5	5
Norway	29		10	6	15	15		1	1	3	3
Portugal	3				1	1				2	1
Slovenia	12		1	1	9	7		1	0	1	0
Sweden	5		3	3				1	1	1	1
United Kingdom	7				5	4		1	1	1	1
MARCH											
Denmark	13		3	0	8	7		1	0	1	0
Estonia	11				11	7					
Finland	1				1	1					
France	19		2	2	11	10		4	4	2	2
Iceland	3				3	2					
Ireland	4				4	4					
Italy	11				4	1		1	0	6	6
Norway	14		3	0	6	6		2	1	3	3
Portugal	10				6	4		1	1	3	2
Slovenia	16		1	0	9	7		4	1	2	0
Sweden	2				1	1				1	1
United Kingdom	10		1	1	7	4		1	1	1	1
APRIL											
Denmark	6				4	3		1	1	1	0
Estonia	8				7	3				1	1
France	6							3	3	3	3
Iceland	1				1	1					
Ireland	10				10	2					
Norway	11				8	8		1	1	2	2
Slovenia	2				1	1	1				
Sweden	5		1	1	3	2				1	1
United Kingdom	10				7	6		2	2	1	1
MAY											
Finland	1							1	1		
Norway	2				2	2					
United Kingdom	3				2	2		1	1		
JUNE											
United Kingdom	1									1	1
AUGUST											
Ireland	1		1	0							
United Kingdom	2				2	2					
	521	0	37	21	359	275	10	56	45	59	52
				7.1%		68.9%			10.7%		11.3%

1. Propagated to sufficient titre to perform HI assay
2. Propagated to sufficient titre to perform HI assay in presence of 20nM oseltamivir
3. Package received 2012-05-10

Table 2. Antigenic analysis of A(H1N1)pdm09 viruses by HI (turkey RBCs)

				Haemagglutination inhibition titre[1]							
				Post infection ferret sera							
Viruses	Genetic group	Collection date	Passage History	A/Cal 7/09 F29/11	A/Bayern 69/09 F11/11	A/Lviv N6/2009 C4/34/09	A/C'church 16/2010 F30/10 Group 4	A/HK 3934/2011 F21/11 Group 3	A/Astrak 1/2011 F22/11 Group 5	A/St. P'burg 27/11 F23/11 Group 6	A/St. P'burg 100/11 F24/11 Group 7
REFERENCE VIRUSES											
A/California/7/2009		2009-04-09	E1/E2	1280	1280	1280	640	640	640	320	640
A/Bayern/69/2009		2009-07-01	MDCK4/MDCK1	80	320	160	80	40	80	80	40
A/Lviv/N6/2009		2009-10-27	M4/S1/M2	640	1280	640	160	80	160	160	160
A/Christchurch/16/2010		2010-07-12	E2/E2	2560	1280	2560	5120	1280	2560	1280	5120
A/Hong Kong/3934/2011		2011-03-29	M2/M2	640	160	320	320	1280	1280	640	1280
A/Astrakhan/1/2011		2011-02-28	M1/M5	1280	640	1280	1280	2560	2560	2560	5120
A/St. Petersburg/27/2011		2011-02-14	E1/E2	2560	1280	1280	1280	2560	2560	2560	5120
A/St. Petersburg/100/2011		2011-03-14	E1/E1	320	320	320	320	320	640	640	2560
TEST VIRUSES											
A/Caen/635/2012	6	2012-02-20	MDCK2/MDCK1	1280	320	1280	1280	2560	2560	1280	5120
A/Paris/1054/2012	7	2012-02-21	MDCK1/MDCK1	1280	320	640	640	1280	1280	1280	2560
A/Paris/1119/2012	7	2012-03-21	MDCK2/MDCK1	1280	640	1280	1280	2560	2560	2560	5120
A/Pays de Loire/1224/2012	7	2012-03-31	MDCK2/MDCK1	1280	640	1280	1280	2560	2560	2560	5120

1. < = <40

Vaccine

Sequence in phylogenetic tree

Figure 1. Phylogenetic comparison of influenza A(H1N1)pdm HA genes

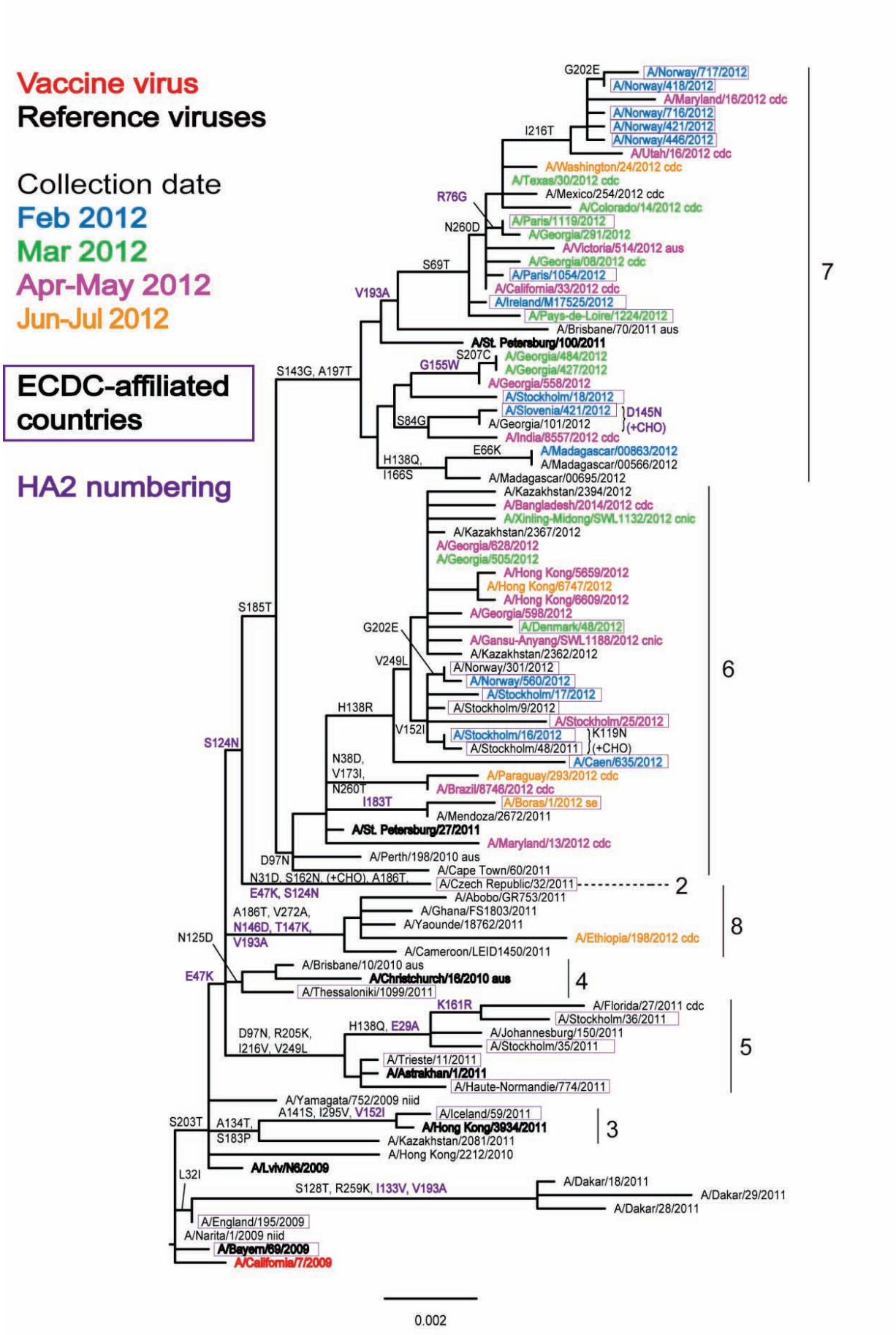

Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1]									
				Post-infection ferret antisera									
				A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Ala 5/10 F27/10 group 5	A/HK 3969/11 F27/11 group 3C	A/Stock 18/11 F28/11 group 3A	A/Iowa 19/10 F15/11 group 6	A/Vic 361/11 Egg F05/12 group 3C	A/Berlin 93/11 T/C F11/12 group 3C	A/Vic 361/11 T/C F15/12 group 3C	A/Athens GR112/12 F16/12 group 3B
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E2	1280	40	160	640	160	160	160	320	320	640
A/Victoria/208/2009		2009-06-02	E3/E1	1280	5120	1280	5120	2560	5120	5120	5120	2560	5120
A/Alabama/5/2010		2010-07-13	MK1/C2/SIAT2	<	<	160	320	80	80	40	160	160	320
A/Hong Kong/3969/2011		2011-05-19	MDCK2/SIAT5	80	80	160	1280	320	160	320	640	640	640
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT6	40	40	80	320	320	80	160	320	160	640
A/Iowa/19/2010		2010-12-30	E3/E2	320	1280	640	1280	1280	5120	1280	1280	1280	1280
A/Victoria/361/2011		2011-10-24	E3/E2	320	640	320	1280	160	640	1280	640	320	160
A/Berlin/93/2011		2011-12-07	NVD3/S3	80	80	160	640	160	160	320	1280	320	640
A/Victoria/361/2011		2011-10-24	M2/S2	80	80	160	640	320	320	320	640	640	640
A/Athens/GR112/2012		2012-02-01	SIAT4	80	160	160	640	320	160	320	640	320	1280
TEST VIRUSES													
A/Lorraine/329/2012		2012-02-06	MDCK2/SIAT3	80	80	160	320	320	160	320	640	320	640
A/Paris/340/2012		2012-02-06	MDCK2/SIAT3	80	160	160	640	320	160	320	640	320	640
A/Caen/539/2012	3B	2012-02-07	MDCK2/SIAT3	80	160	160	640	320	160	320	640	320	640
A/Norway/878/2012		2012-02-27	SIAT1/SIAT2	80	80	160	640	320	160	80	320	320	640
A/Centre/848/2012	3B	2012-03-01	MDCK2/SIAT1	40	80	160	320	320	160	160	320	320	640
A/Bourgogne/850/2012	3B	2012-03-01	MDCK2/SIAT1	80	160	160	320	320	160	160	320	320	640
A/Caen/900/2012	6	2012-03-01	MDCK2/SIAT1	80	160	320	320	320	320	160	320	640	640
A/Lorraine/883/2012	3B	2012-03-02	MDCK2/SIAT3	80	160	160	640	320	160	320	640	320	1280
A/Pays de Loire/882/2012	3C	2012-03-02	MDCK1/SIAT1	80	80	160	640	160	160	160	640	320	640
A/Alsace/889/2012	3A	2012-03-04	MDCK2/SIAT3	40	80	80	320	320	160	160	320	320	640
A/Norway/876/2012		2012-03-06	SIAT1/SIAT2	40	80	80	640	160	160	80	320	320	320
A/Paris/919/2012	3B	2012-03-06	MDCK2/SIAT1	40	80	80	320	160	160	160	320	320	320
A/Centre/929/2012	3C	2012-03-07	MDCK2/SIAT1	80	160	160	640	320	320	160	640	320	640
A/Paris/972/2012	3B	2012-03-08	MDCK2/SIAT1	<	<	<	80	80	40	80	160	80	160
A/Norway/943/2012		2012-03-09	MDCK1/SIAT2	80	160	320	640	320	320	160	640	640	640
A/Norway/962/2012		2012-03-10	SIAT1/SIAT2	160	160	160	640	320	320	160	640	640	1280
A/England/554/2012		2012-03-10	S1/SIAT2	40	40	40	160	80	40	160	160	160	320
A/Centre/1066/2012	3C	2012-03-12	MDCK2/SIAT3	80	160	160	640	320	320	320	640	640	640
A/Norway/996/2012		2012-03-15	SIAT1/SIAT1	80	160	160	640	320	320	160	640	640	1280
A/Norway/1003/2012		2012-03-22	SIAT1/SIAT1	80	80	160	640	320	160	160	640	640	640
A/Norway/1059/2012		2012-03-22	SIAT1/SIAT2	40	80	80	320	160	160	80	320	320	320
A/Norway/1196/2012	3A	2012-04-13	SIAT1/SIAT2	<	40	80	320	160	80	80	320	160	320
A/Norway/1225/2012		2012-04-18	MDCK1/SIAT1	160	320	320	1280	640	640	320	1280	1280	1280
A/Norway/1250/2012	3C	2012-04-20	SIAT1/SIAT1	80	320	160	1280	320	320	160	640	640	1280
A/Norway/1248/2012		2012-04-23	SIAT1/SIAT1	160	320	320	1280	640	640	160	1280	640	1280
A/Norway/1296/2012		2012-04-26	MDCK1/SIAT2	40	80	80	320	640	160	80	320	320	640
A/Norway/1293/2012	3C	2012-04-27	MDCK1/SIAT1	160	320	320	1280	640	640	320	1280	1280	1280
A/Norway/1292/2012		2012-04-29	MDCK1/SIAT1	80	80	160	640	320	320	160	640	640	640
A/Norway/1252/2012	3C	2012-04-30	SIAT1/SIAT1	160	160	160	1280	320	320	160	1280	640	1280
A/Norway/1295/2012	3C	2012-05-02	MDCK1/SIAT2	160	160	320	1280	320	320	160	640	640	640
A/England/555/2012		2012-05-09	SIAT1/SIAT3	80	80	160	320	160	160	160	320	320	320
A/Norway/1359/2012	3C	2012-05-14	MDCK1/SIAT1	160	320	320	1280	640	640	320	1280	640	1280
A/England/565/2012		2012-05-31	SIAT2/SIAT3	<	<	40	160	80	40	80	160	160	320
A/England/569/2012		2012-08-18	SIAT2/SIAT2	<	40	<	160	160	40	80	160	160	320
A/England/568/2012		2012-08-18	SIAT1/SIAT3	<	<	40	160	80	40	80	160	160	160
Sequence in phylogenetic tree				Vaccine 2011-12 2012						Vaccine 2012-13 2013		Vaccine 2012-13 2013	

1. < = <40

Figure 2. Phylogenetic comparison of influenza A(H3N2) HA genes

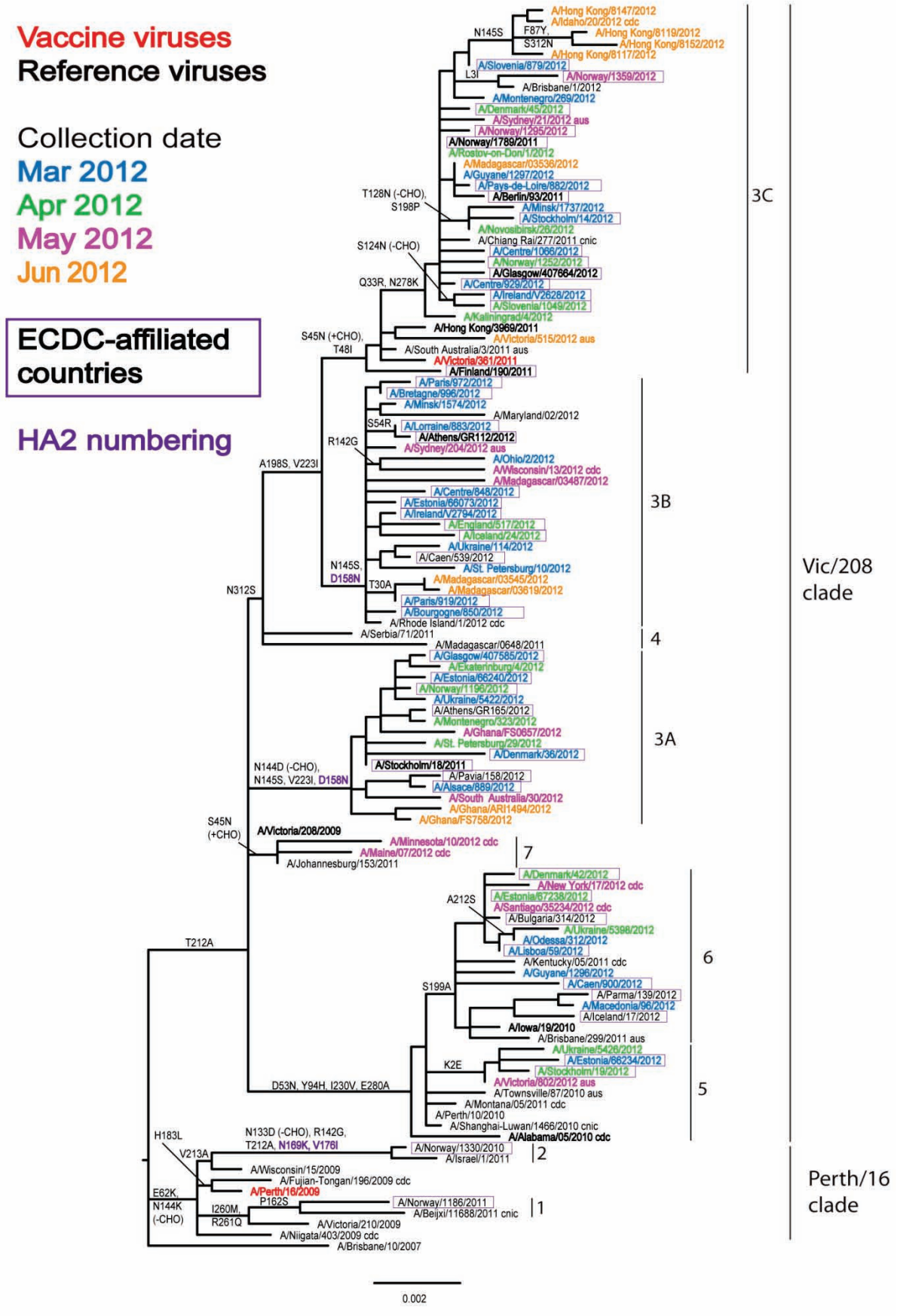

Table 4. Antigenic analysis of Influenza B/Victoria-lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]							
			Post infection ferret sera							
			B/Bris 60/08[2] Sh 523	B/Mal 2506/04 F28/05	B/England 393/08 F05/11	B/Bris 60/08 F22/12	B/Paris 1762/08 F17/11	B/HK 514/09 F13/10	B/Odessa 3886/10 F19/11	B/Malta 636714/11 F33/11
REFERENCE VIRUSES										
B/Malaysia/2506/2004	2004-12-06	E7	640	320	20	80	<	<	<	80
B/England/393/2008	2008-08-29	E1/E1	1280	80	160	640	40	40	80	320
B/Brisbane/60/2008	2008-08-04	E4/E3	1280	160	160	320	40	40	80	320
B/Paris/1762/2008	2009-02-09	C2/MDCK1	2560	<	<	40	40	80	80	20
B/Hong Kong/514/2009	2009-10-11	MDCK4	2560	<	<	40	80	80	320	20
B/Odessa/3886/2010	2010-03-19	MDCK2/MDCK4	2560	<	<	40	80	80	160	20
B/Malta/636714/2011	2011-03-07	E4/E1	1280	80	160	640	40	40	80	320
TEST VIRUSES										
B/Lorraine/1023/2012	2012-03-13	MDCK2/MDCK1	2560	10	10	80	80	80	80	40
B/Paris/1039/2012	2012-03-15	MDCK2/MDCK1	2560	<	10	80	160	80	160	40
B/Paris/1087/2012	2012-03-19	MDCK1/MDCK1	2560	<	10	80	80	80	160	40
B/Paris/1184/2012	2012-03-27	MDCK2/MDCK1	1280	<	<	40	80	40	80	10
B/Pays de Loire/1240/2012	2012-04-03	MDCK1/MDCK1	1280	<	10	40	80	80	80	40
B/Centre/1244/2012	2012-04-05	MDCK1/MDCK1	1280	<	<	40	80	40	80	10
B/Centre/1376/2012	2012-04-20	MDCK1/MDCK1	1280	<	<	40	40	40	80	10
B/England/560/2012	2012-05-17	SIAT1/MDCK1	5120	20	20	40	80	80	160	40
						Vaccine				
Sequence in phylogenetic tree						2011-12				
						2012				

1. < = <10; 2. hyperimmune sheep serum

Table 5. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI (turkey RBCs)

Viruses	Clade	Collection date	Passage History	Haemagglutination inhibition titre										
				Post infection ferret sera										
				B/FI[3] 4/06 SH479	B/Eg[1] 144/05 F3/07	B/FI[1] 4/06 F01/10	B/Bris[2] 3/07 F21/12 2	B/Eng[2] 145/08 F20/12 3	B/Bang[2] 3333/07 F25/08 3	B/Wis[2] 1/10 F23/10 3	B/Stock[2] 12/2011 F12/12 3	B/Estonia[2] 55669/2011 F26/11 2	B/Serbia[2] 1894/2011 F25/11 3	B/Stock[2] 12/2011 T/C F8/12 3
REFERENCE VIRUSES														
B/Egypt/144/2005		2005-05-01	E3/E5	5120	320	1280	1280	320	640	320	640	160	20	640
B/Florida/4/2006		2006-12-15	E3/E3	5120	320	1280	1280	320	640	320	640	160	20	640
B/Brisbane/3/2007		2007-09-03	E2/E1	5120	160	640	640	160	320	160	640	160	10	320
B/England/145/2008			Ex/E4	1280	40	320	80	320	40	20	160	<	<	80
B/Bangladesh/3333/2007		2007-08-07	E4/E4	5120	80	640	320	80	320	80	640	<	20	640
B/Wisconsin/1/2010		2007-08-07	E3/E1	2560	40	640	320	80	320	160	640	10	20	640
B/Stockholm/12/2011		2007-08-07	E4/E1	5120	160	640	320	160	320	160	640	10	40	640
B/Estonia/55669/2011		2011-03-14	MDCK2/MDCK2	5120	10	320	160	160	80	40	160	640	80	80
B/Serbia/1894/2011		2011-03-08	MDCK1/MDCK4	5120	160	320	160	320	160	80	320	160	320	320
B/Stockholm/12/2011		2011-03-28	Cx/MDCK4	5120	80	320	160	160	160	80	320	160	320	320
TEST VIRUSES														
B/Norway/959/2012		2012-03-13	MDCK1/MDCK1	5120	80	320	160	320	160	80	320	640	160	320
B/Norway/1071/2012		2012-03-16	MDCK1/MDCK1	5120	80	320	160	320	160	40	320	640	160	160
B/Norway/1073/2012		2012-03-15	MDCK1/MDCK1	5120	80	320	160	320	160	80	640	1280	320	320
B/Bretagne/1160/2012	2	2012-03-23	MDCK2/MDCK1	2560	<	80	80	40	20	20	80	320	20	20
B/Nord Pas de Calais/1175/2012		2012-03-26	MDCK2/MDCK1	1280	<	160	80	80	10	20	80	320	10	20
B/Lorraine/1220/2012		2012-04-01	MDCK2/MDCK1	2560	<	80	40	80	40	20	160	20	40	80
B/Norway/1165/2012		2012-04-06	MDCK1/MDCK1	5120	40	320	80	160	160	80	640	160	320	160
B/Centre/1363/2012		2012-04-19	MDCK1/MDCK1	1280	<	80	40	40	10	10	80	320	20	20
B/Norway/1249/2012		2012-04-26	MDCK1/MDCK1	5120	80	320	160	320	80	40	320	1280	320	160
B/Lorraine/1408/2012	2	2012-04-28	MDCK1/MDCK1	1280	<	80	40	40	10	40	80	320	20	20
B/England/567/2012		2012-06-18	SIAT1/MDCK2	2560	40	160	80	80	20	80	160	640	20	40
										Vaccine				
Sequence in phylogenetic tree										2012-13				
										2013				

1. < = <40; 2. < =<10 ; 3. hyperimmune sheep serum

Figure 3. Phylogenetic comparison of influenza B/Victoria-lineage HA genes

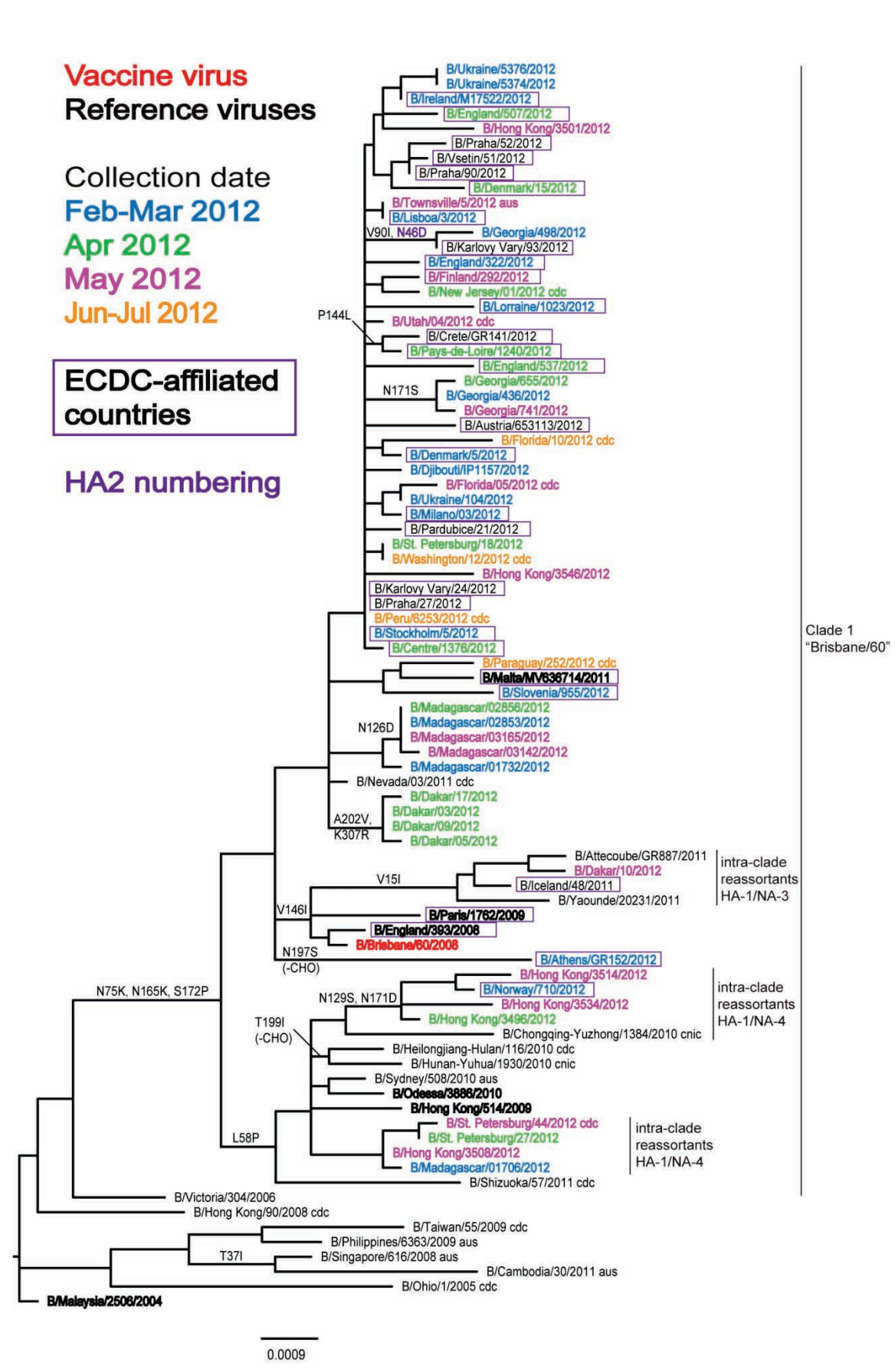

Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes

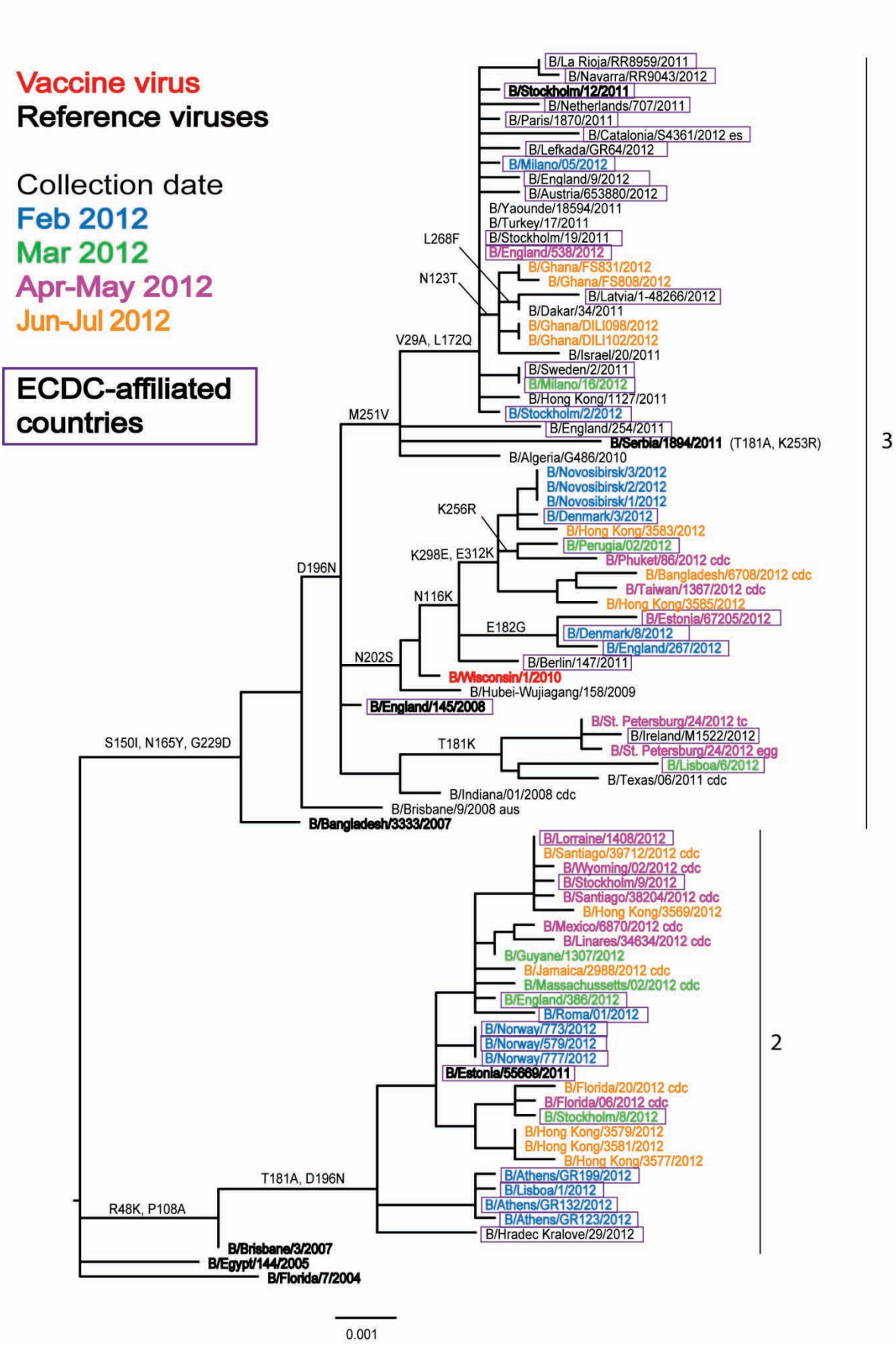

Table 6. Antigenic analysis of triple reassortant (TRV) influenza A(H3N2)v and human H3N2 viruses by HI (turkey RBCs)

Viruses		Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera A/Pan 2007/99 F03/06	A/Perth 16/09 F35/11	A/Wis 12/10 F36/11	A/Pen 14/10 F39/11	A/Ind 8/11 F38/11
VIRUSES							
A/Panama/2007/99	Seasonal	Ex/1	**2560**	<	<	<	<
A/Perth16/2009	Seasonal	E3/E1	<	**1280**	<	<	<
A/Wisconsin/12/2010	TRV	M1/C1/MDCK2	<	<	**640**	40	<
A/Minnesota/11/2010	TRV	E2/E1	<	<	320	160	80
A/Pennsylvania/14/2010	TRV	E2/E2	<	<	1280	**1280**	1280
A/Indiana/8/2011	H3N2v	C2/MDCK2	<	<	1280	640	**640**
A/Indiana/10/2011	H3N2v	E2/E1	<	<	640	160	320

1. < = <40