TECHNICAL DOCUMENT

Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe

Influenza virus characterisation

Summary Europe, April 2011

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Summary

Influenza A(H1N1)pdm, influenza A(H3N2), influenza B/Victoria/2/87 lineage and B/Yamagata/16/88 lineage viruses have been characterised genetically and antigenically.

- Recently isolated H1N1pdm viruses fall into several genetic groups but all groups show antigenic similarity to the currently recommended vaccine virus A/California/7/2009.
- H3N2 viruses also fall into distinct genetic groups but there is no consistent correlation between any group and antigenic differences from the currently used vaccine virus A/Perth/16/2009.
- Influenza B viruses of the B/Victoria/2/87 lineage have continued to predominate over those of the B/Yamagata/2/87 lineage. Most of the B/Victoria/2/87 lineage viruses are genetically and antigenically similar to the currently recommended vaccine virus B/Brisbane/60/2008.

Over 700 virus specimens (propagated virus isolates or clinical samples) collected since December 2010 have been received from EU and affiliated countries at the WHO CC in London (Table 1). Predominantly, these viruses were type A influenza H1N1pdm viruses and type B influenza viruses of the B/Victoria/2/87 lineage, although type A influenza H3N2 viruses and type B influenza viruses of the B/Yamagata/16/88 lineage continued to be detected. In Table 1, batches for which analysis has yet to be completed are shown as in progress.

Influenza A(H1N1)pdm virus analysis

Table 2 shows representative HI results for viruses received since the previous report. The majority of viruses continue to react well with the panel of post-infection ferret antisera, including that raised against the vaccine virus A/California/7/2009. All the viruses in Table 2 show good reactivity with all the antisera.

Nucleotide sequence analysis of the HA1 coding region of the HA gene has been carried out and a representative phylogenetic tree is shown in Figure 1. The phylogenetic tree shows residues that define four genetic groups that have been predominant over the last five months; also marked on the tree are sporadic observations of particular amino acid substitutions or polymorphisms.

As described previously (ibid) four genetic groups can be defined:

i) A134T and S183P, now seen in at least six countries globally;
ii) N125D, observed originally in an emerging genetic group in the Southern Hemisphere and subsequently widespread in the Northern Hemisphere;
iii) D97N, R205K, I216V and V249L, observed in at least 10 countries;
iv) S185T, with many viruses in this group carrying the additional substitution D97N or alternative additional substitutions of S143G and A197T, detected in most countries in Europe and the world.

The viruses highlighted in Table 2 as included in the HA gene phylogenetic analysis belong to genetic groups (iii) and (iv).

The sporadic amino acid substitutions that have been marked on the phylogenetic trees encode substitutions or polymorphisms at amino acid residues 153-157, 222 and 223. Substitutions at residues 153-157 in the HA are often associated with reduced titres in HI assays and often the substitution or polymorphism is not seen when the nucleotide sequence of the corresponding clinical specimen has been analysed. Often polymorphism at residues 153-157 is a result of cell culture.

Substitution and polymorphism at amino acid residue 222 continues to be detected and the change D222G has been postulated to be detected more often in viruses recovered from patients suffering with severe disease.

Substitutions at residue 223 (Q223R) have also been observed and are associated with isolation or propagation of the virus in hens' eggs.

Influenza A(H3N2) virus analysis

Since December 2010 influenza A(H3N2) viruses have been successfully isolated and propagated from 15 countries affiliated with ECDC. The problems with antigenic characterisation of recent H3N2 viruses have been described previously (ibid). Shown in Table 3 are the results of an HI assay using guinea pig red blood cells in the presence of oseltamivir to reduce any effect on the agglutination of the red blood cells by the virus neuraminidase (Lin et al. 2010). The results show that, of the six viruses tested, three have a reduction in titre of eightfold with the post-infection ferret antiserum raised against the vaccine virus A/Perth/16/2009 compared with the homologous reaction between the antiserum and A/Perth/16/2009. However, all the viruses in the test reacted well with a ferret antiserum raised against A/Wisconsin/15/2009, a virus genetically and antigenically closely related to A/Perth/16/2009. All viruses in the test showed low reactivity with antisera raised against A/Victoria/208/2009 and A/Victoria/210/2009, but these viruses show anomalously high titres with their corresponding antisera. All test viruses showed good reactivity with antisera raised against A/Alabama/5/2010 and A/Perth/10/2010.

Nucleotide sequence analysis of the HA1 coding region of the HA gene has been carried out on representative H3N2 viruses and a phylogenetic tree is shown in Figure 2. The five reference viruses and the vaccine virus A/Perth/16/2009 used in the HI test are shown in boldface type and coloured in black and red respectively. Viruses from ECDC-affiliated countries fall into both of the genetic clades of the H3N2 HA gene, the A/Perth/16/2009 clade and the A/Victoria/208/2009 clade, with the majority of viruses falling within the A/Victoria/208/2009 genetic clade. Distinct genetic groups within the HA gene are seen within both genetic clades. As described in the last report (ibid), there are five main genetic groups that are marked on the phylogenetic tree. These groups are characterised by encoded amino acid substitutions at: within the A/Perth/16 clade, i) I260M and R261Q with E50K and P162S, and ii) N133D, R142G A212T and V213A; and, within the A/Victoria/208 clade, iii) N145S and V223I, iv) N312S, and v) D53N, Y94H, I230V, E280A and S312N. Viruses from ECDC-affiliated countries fall into each of these genetic groups.

A correlation of the viruses analysed in the HI assay (Table 3) with the phylogenetic tree of the HA gene (Figure 2) shows that all the viruses fall into genetic group (v), but A/Genoa/01/2010, A/Lisboa/SU23/2011 and A/Valladolid/40/2011 carry the additional amino acid substitution S199A in the HA, and A/Iasi/47704/2011 carries an additional substitution I192T in the HA. Viruses in this genetic group show no consistent reduced reaction in HI assays with antisera raised against the vaccine virus and genetically closely related viruses. Analysis of viruses from each genetic group has indicated that there is no consistent change in antigenicity associated with any emerging genetic group.

Influenza B virus analyses

As in our previous report, influenza B viruses of the B/Victoria/2/87 lineage (~ 85%) have continued to predominate over those of the B/Yamagata/16/88 lineage (~ 15%).

B/Victoria lineage viruses

Representative results of antigenic analysis of influenza B/Victoria viruses are shown in Table 4. In the HI assay some viruses showed low reactivity with antisera raised against B/Brisbane/60/2008, the egg-propagated vaccine virus. It has been known for many years that HI assays for influenza B viruses propagated only in cells frequently show reduced HI titres when tested with antisera raised against egg-propagated reference strains, including vaccine strains (Schild et al. 1983). As a consequence, the antigenic properties of cell propagated viruses are assessed with antisera raised against viruses genetically closely related to the vaccine virus but propagated in cells. In Table 4, the cell-propagated reference viruses B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010 are genetically closely related to the vaccine virus B/Brisbane/60/2008 and post-infection ferret antisera have been raised against these viruses. All the test viruses analysed in Table 4 showed good reactivity with antisera raised against these three viruses and so are considered to be antigenically similar to the vaccine virus.

Figure 3 shows a phylogenetic tree based on the HA1-coding region of the HA gene. Amino acid substitutions N75K, N165K and S172P define the B/Brisbane/60/2008 genetic clade. The vast majority of recently collected viruses from EU and affiliated countries fall into the B/Brisbane/60/2008 genetic clade. The majority of viruses carry the amino acid substitution I146V in the HA compared with the vaccine virus B/Brisbane/60/2008 and many also carry the substitution L58P. Neither substitution has a marked affect on antigenicity of the viruses.

B/Yamagata lineage viruses

Table 5 shows the results of antigenic analysis of representative recently collected influenza B/Yamagata viruses as assessed by HI assay using turkey red blood cells. The majority of the test viruses from EU and affiliated countries reacted well with the panel of antisera, but many showed markedly reduced reactivity when assessed with post-infection antisera raised against the most recently used vaccine virus of the B/Yamagata lineage, B/Florida/4/2006, an egg-propagated vaccine virus. Generally the viruses showed better reactivity with antisera raised against the reference viruses B/Bangladesh/3333/2007 and B/Wisconsin/1/2010, also propagated in hens' eggs, than they did with antisera raised against B/Florida/4/2006, with the best reactivity being seen with the post-infection antiserum raised against B/Wisconsin/1/2010.

Figure 4 shows phylogenetic analysis of the HA1 coding region of the HA gene of representative influenza B/Yamagata lineage viruses. The HA gene of all but four viruses (B/Estonia/55669/2011, B/Estonia/55763/2011, B/Finland/33/2010 and B/Finland/39/2010) fell into the B/Bangaldesh/3333/2007 genetic clade. The B/Bangaldesh/3333/2007 genetic clade is characterised by the amino acid substitutions S150I, N165Y and G229D in the HA. Minor genetic groups can be discerned within this clade, notably one genetic group is defined by the amino acid substitution M251V which is seen in combination with the substitutions G183R or T181A and K253R in some viruses; another group is characterised by the substitution N202S in the HA, with some viruses carrying the additional substitutions of either A146S or N116K; a third genetic group can be characterised by the amino acid substitution T181K. Viruses from EU and affiliated countries fell into two of the three minor genetic groups, those characterised by M251A and by T181K, as well as falling into the main B/Banglasdesh/3333/2007 genetic clade. Antigenic analysis of the two viruses from Finland shown in the phylogenetic tree (B/Finland/33/2010 and B/Finland/39/2010) has been described in a previous report. Table 5 and the results presented in the previous two reports indicate that the large majority of viruses of the B/Bangladesh genetic clade are antigenically closely related to the reference viruses B/Banglasdesh/3333/2007 and B/Wisconsin/1/2010.

Note to the figures

The phylogenetic trees were constructed using neighbour-join in MEGA4. 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 date of sample collection. Isolates from ECDC countries are highlighted in yellow. Sequences for some of the viruses from non-European countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.

Table 1 Summary of specimens collected since December 2010 and received by the end of April 2011

MONTH / Country	A	H1N1pdm		H3N2		B	B Yamagata lineage		B Victoria lineage	
		Number received	A/California/ 7/2009-like	Number received	A/Perth/ 16/2009-like*		Number received	B/Florida/ 4/2006-like	Number received	B/Brisbane/ 60/2008-like
DECEMBER										
Austria		6	6							
Belgium	1	22	16	2	1	10	3	3	17	17
Denmark		5	5							
Finland		2	2	1	1		2	2		
France		20	20	20	12		2	2	9	9
Germany		8	8	3	0		4	4	7	7
Ireland		10	8			1			2	2
Italy		8	6	1	1				9	9
Latvia		5	5						2	2
Luxembourg	5	5	3			8			1	1
Malta		6	4							
Netherlands				1	1					
Norway		1	1	1	1				7	6
Portugal		5	2						44	22
Romania		1	1	2	2					
Slovenia		6	6						2	2
Spain		23	19						7	6
Sweden	1	5	5	1	0		1	1	2	2
United Kingdom		40	in process	2	1		3	3	10	8
JANUARY										
Belgium	1	1	1						2	1
Czech Republic		13	13						1	0
Estonia		9	4							
Germany									2	2
France							1	1	3	3
Greece	1	30	16	2	1	3				
Ireland		2	in process	7	in process					
Italy		54	52	3	1	1	2	2	23	23
Latvia		5	4	1	in process					
Malta		2	2							
Netherlands		5	5	1	1		2	2		
Portugal		1	0	2	1				8	6
Romania		2	2	1	1					
Slovenia		14	13						3	3
Spain		31	21						5	5
Sweden				1	1					
United Kingdom		4	in process				2	in process	3	in process
FEBRUARY										
Czech Republic		1	1						3	3
Estonia		32	26			1	1	1		
Ireland		2	in process	2	in process		1	in process		
Italy		1	1							
Greece	1	9	in process							
Latvia		1	in process				1	1	3	2
Malta		7	in process							
Spain		5	4	2	in process				5	5
MARCH										
Estonia		6	3				5	5	1	1
Greece	4	5	in process			8				
Ireland						1				
Latvia									2	2
Malta						5				
Romania				1	1					
Spain									1	1
APRIL										
Malta						1				
Total Received = 744	**14**	**420**	**285**	**57**	**27**	**39**	**30**	**27**	**184**	**150**

* Although the bulk of these viruses have been isolated, based on NA activity, due to problems related to Oseltamivir sensitivity of red blood cell agglutination, limited HI data has been generated. A portion of the viruses have been assessed by plaque reduction neutralisation and sequencing is ongoing.

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

			Haemagglutination inhibition titre[1]						
			Post infection ferret sera						
Viruses	Collection date	Passage History	A/Cal 7/09 F05/10	A/Eng 195/09 F06/10	A/Auck 3/09 F17/09	A/Bayern 69/09 C4/33/09	A/Lviv N6/2009 C4/34/09	A/HK 2212/2010 F21/10	A/C'church 16/2010 F30/10
REFERENCE VIRUSES									
A/California/7/2009	2009-04-09	E2/E4	5120	5120	5120	1280	2560	2560	2560
A/England/195/2009	2009-04-28	MDCK1/MDCK4	5120	5120	5120	2560	2560	5120	2560
A/Auckland/3/2009	2009-04-25	Ex/E3	5120	5120	5120	2560	2560	5120	2560
A/Bayern/69/2009	2009-07-01	MDCK4/MDCK2	320	160	160	640	640	160	160
A/Lviv/N6/2009	2009-10-27	MDCK5	1280	160	160	1280	1280	640	320
A/Hong Kong/2212/2010	2010-07-16	E4	5120	5120	5120	5120	5120	5120	5120
A/Christchurch/16/2010	2010-07-12	E2/E1	5120	2560	5120	2560	2560	5120	5120
TEST VIRUSES									
A/Czech Republic/1/2011	2011-01-25	MDCK3-E2/E1	2560	2560	1280	640	1280	1280	1280
A/Czech Republic/9/2011	2011-01-11	MDCK3-E3/E1	1280	2560	1280	640	1280	1280	1280
A/Czech Republic/4/2011	2011-01-18	E4/E1	1280	320	320	1280	2560	320	640
A/Czech Republic/30/2011	2011-01-25	MDCK4	2560	2560	2560	1280	1280	2560	1280
A/Estonia/53381/2011	2011-01-28	MDCK1/MDCK1	2560	2560	2560	640	1280	1280	1280
A/Latvia/2-32584p/2011	2011-02-04	MDCK1/MDCK1	5120	5120	5120	2560	2560	2560	2560
A/Estonia/54553/2011	2011-02-15	MDCK2/MDCK1	1280	1280	1280	640	1280	1280	1280
A/Crete/GR6779/2011	2011-02-17	MDCK3	1280	1280	1280	640	1280	1280	1280
A/Arta/GR6666/2011	2011-02-21	MDCK2	5120	2560	5120	1280	2560	2560	2560
A/Ioannina/GR6664/2011	2011-02-21	MDCK3	2560	2560	2560	640	1280	2560	1280
A/Estonia/54922/2011	2011-02-22	MDCK2/MDCK1	1280	2560	2560	640	1280	1280	1280
A/Estonia/55236/2011	2011-03-02	MDCK1/MDCK1	2560	2560	2560	640	2560	2560	1280

1. < = 40

Sequences included in HA phylogeny

Figure 1 Phylogenetic comparison of influenza A(H1N1)pdm HA genes (HA1 coding region)

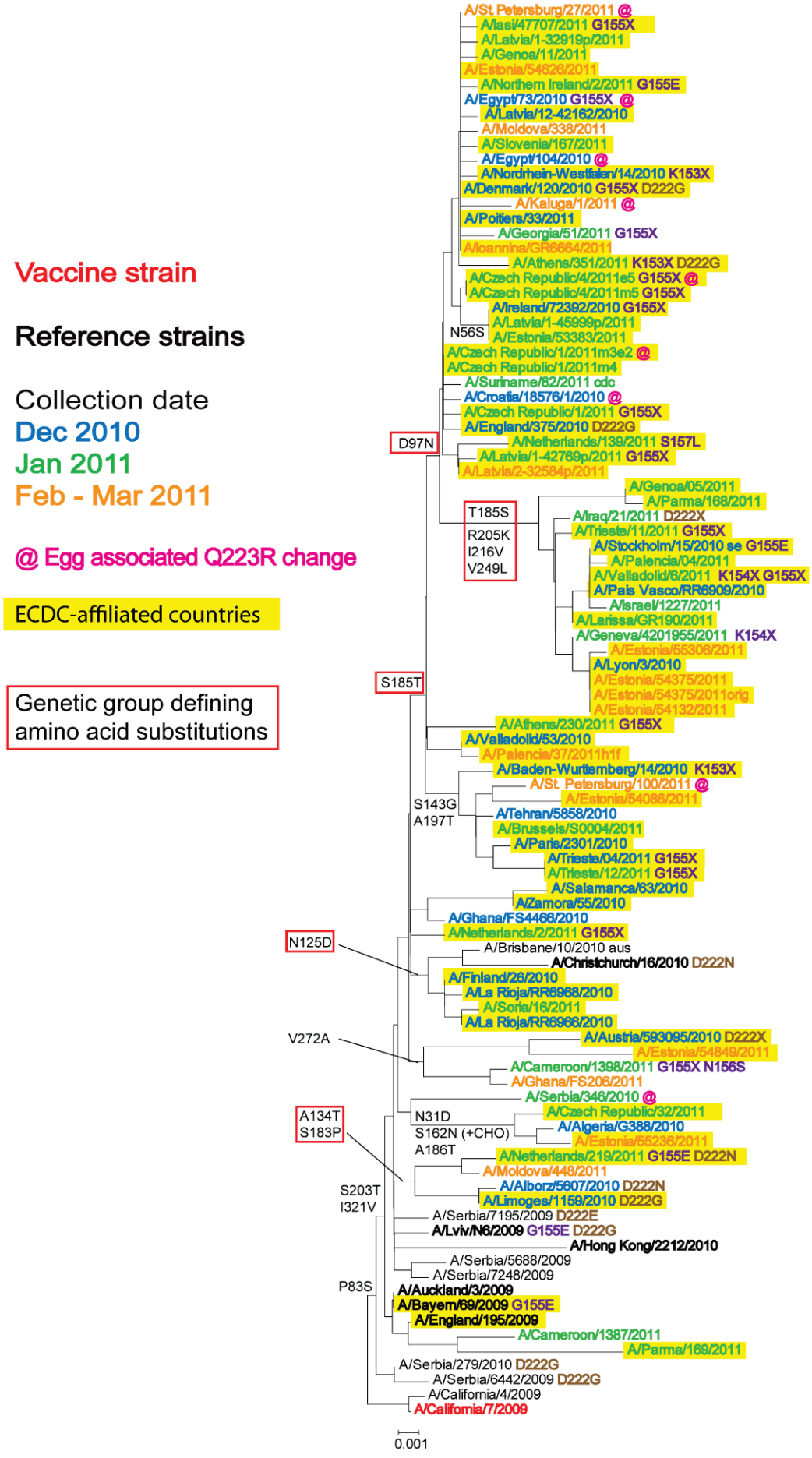

Table 3 Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera							
			A/Wis 67/05 F18/08	A/Bris 10/07 F29/09	A/Perth 16/09 F30/09	A/Wis 15/09 F24/09	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11
REFERENCE VIRUSES										
A/Wisconsin/67/2005	2005-08-31	SpfCk3E3/E11	1280	640	<	<	160	<	<	80
A/Brisbane/10/2007	2007-02-06	E2/E4	5120	5120	40	40	320	160	40	160
A/Perth/16/2009	2009-07-04	E3/E3	40	80	1280	640	1280	1280	640	2560
A/Wisconsin/15/2009	2009-07-06	E2/E3	40	<	640	320	40	160	640	320
A/Victoria/208/2009	2009-06-02	E3/E2	160	160	640	640	5120	5120	1280	2560
A/Victoria/210/2009	2009-06-02	E2/E3	160	320	1280	1280	2560	5120	320	2560
A/Alabama/5/2010	2010-07-13	MK1/M2/S3	<	<	80	40	40	40	160	320
A/Perth/10/2010	2010-05-25	E2/E2	<	<	160	40	40	40	160	320
TEST VIRUSES										
A/Genoa/01/2010	2010-12-31	MDCK2/SIAT4	40	40	160	320	320	160	640	640
A/Lisboa/SU23/2011	2011-01-01	SIAT3	40	80	640	320	320	320	320	1280
A/Iasi/47704/2011	2011-01-03	MDCK2/SIAT1	<	<	80	160	80	80	160	320
A/Trieste/25/2011	2011-01-19	MDCK3/SIAT1	<	<	160	160	160	160	320	640
A/Valladolid/40/2011	2011-02-16	MDCK1/SIAT1	40	40	320	640	320	320	1280	1280
A/Romania/55656/2011	2011-03-10	SIAT2	40	40	320	640	320	320	1280	1280

1. < = <40

Sequences included in HA phylogeny

Figure 2 Phylogenetic comparison of influenza A(H3N2) HA genes (HA1 coding region)

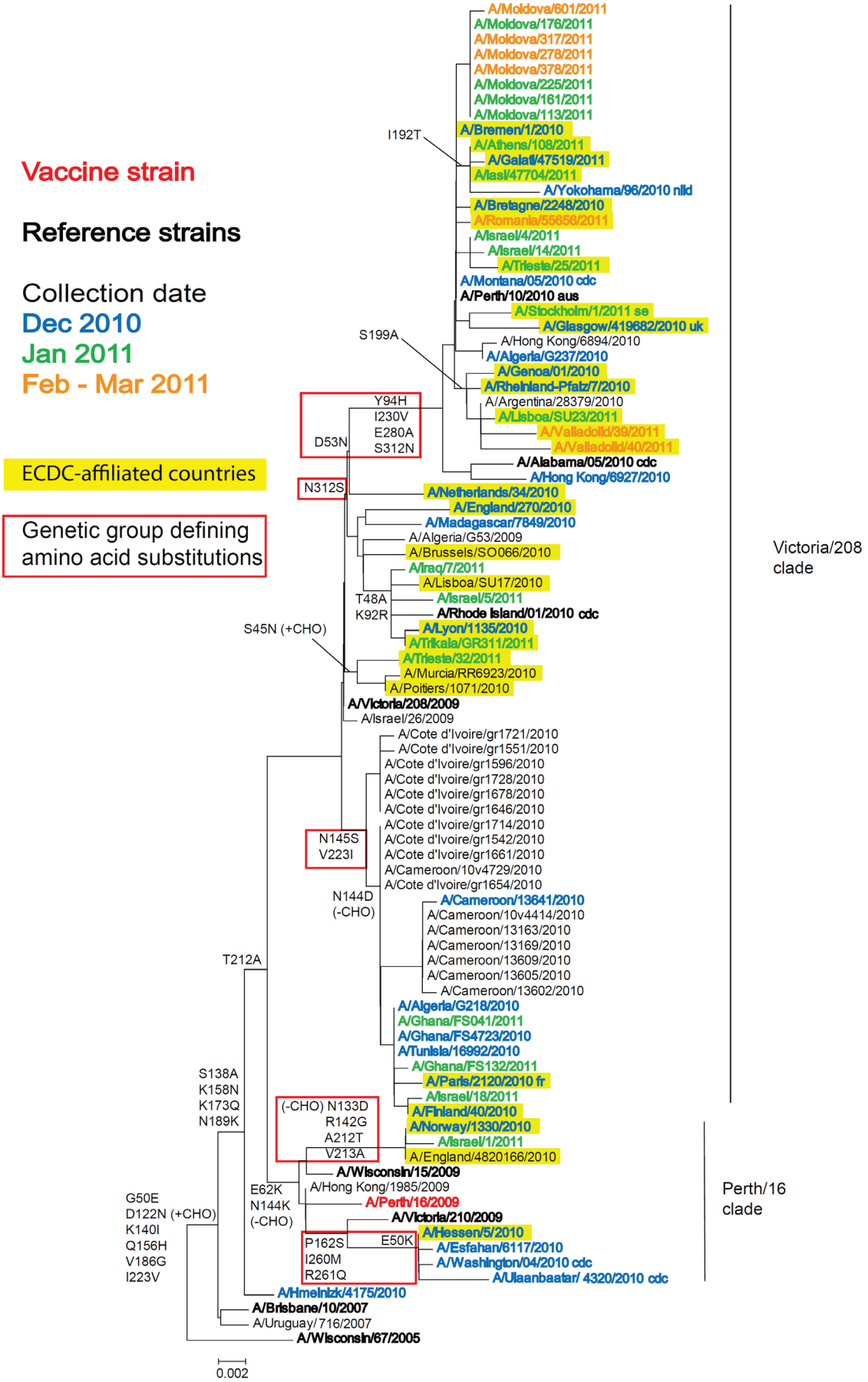

Table 4 Antigenic analysis of influenza B/Victoria lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]						
				Post infection ferret sera					
			B/Bris[2] 60/08 Sh 524	B/Mal 2506/04 F28/05	B/England 393/08 F31/08	B/Bris 60/08 F25/10	B/Paris 1762/08 F11/09	B/HK 514/09 F3/10	B/Odessa 3886/10 F17/10
REFERENCE VIRUSES									
B/Malaysia/2506/2004	2004-12-06	E3/E5	1280	320	80	80	<	<	<
B/England/393/2008	2008-08-29	E1/E6	640	80	160	320	40	20	40
B/Brisbane/60/2008	2008-08-04	E4/E4	640	80	160	320	80	20	40
B/Paris/1762/2008		C2/MDCK4	640	10	20	40	80	160	80
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK1	640	10	10	40	80	160	80
B/Odessa/3886/2010	2010-03-19	C2/MDCK3	1280	10	20	40	80	160	160
TEST VIRUSES									
B/Lisboa/54/2010	2010-11-30	SIAT1/MDCK2	2560	<	80	160	320	640	320
B/Lisboa/56/2010	2010-12-02	SIAT1/MDCK2	5120	640	320	640	160	320	320
B/Lisboa/47/2010	2010-12-20	SIAT1/MDCK2	1280	<	40	80	160	640	320
B/Lisboa/50/2010	2010-12-28	SIAT2/MDCK2	1280	<	40	80	160	640	320
B/Lisboa/53/2010	2010-12-28	SIAT1/MDCK2	1280	<	<	40	160	320	160
B/Lisboa/1/2011	2011-01-05	SIAT1/MDCK1	2560	<	<	80	160	640	320
B/Trieste/01/2011	2011-01-10	IMDCK/MDCK1	640	<	<	80	160	640	160
B/Trieste/02/2011	2011-01-19	IMDCK/MDCK1	640	<	<	80	160	640	320
B/Trieste/06/2011	2011-01-20	IMDCK/MDCK1	640	<	<	80	160	640	160
B/Trieste/07/2011	2011-01-25	IMDCK/MDCK1	640	<	<	80	160	640	320
B/Trieste/08/2011	2011-01-25	IMDCK/MDCK1	640	<	<	80	160	640	320
B/Trieste/10/2011	2011-01-26	IMDCK/MDCK1	640	<	<	80	80	640	160
B/Trieste/11/2011	2011-01-26	IMDCK/MDCK1	640	<	40	80	160	320	160
B/Leon/34/2011	2011-02-02	MDCK1/MDCK2	1280	10	<	80	160	160	160
B/Czech Republic/35/2011	2011-02-03	MDCK2/MDCK1	640	10	10	20	80	160	80
B/Salamanca/35/2011	2011-02-04	MDCK1/MDCK1	640	<	40	80	160	640	320
B/Czech Republic/22/2011	2011-02-08	MDCK2/MDCK1	640	20	10	20	80	160	80
B/Czech Republic/36/2011	2011-02-08	MDCK2/MDCK1	640	20	10	40	80	160	80
B/Segovia/43/2011	2011-02-18	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Latvia/2-43305/2011	2011-02-23	MDCKx/MDCK1	160	10	20	40	80	160	80
B/Leon/45/2011	2011-02-23	MDCK1/MDCK1	640	<	<	40	160	320	160
B/Salamanca/44/2011	2011-02-24	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Latvia/2-45256/2011	2011-02-28	MDCK1/MDCK1	160	10	20	40	80	160	80
B/Leon/46/2011	2011-03-01	MDCK1/MDCK2	640	<	<	20	160	160	160
B/Athens/10051/2011	2011-03-16	MDCK3	640	10	10	20	80	80	80
B/Latvia/3-39525p/2011	2011-03-16	MDCK1/MDCK1	160	10	20	40	80	160	80
B/Latvia/3-39620p/2011	2011-03-16	MDCK1/MDCK1	160	10	20	40	80	160	80
B/Estonia/55784/2011	2011-03-16	MDCK2/MDCK1	640	10	40	80	80	160	80

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

Sequences included in HA phylogeny

Figure 3 Phylogenetic comparison of influenza B/Victoria lineage HA genes (HA1 coding region)

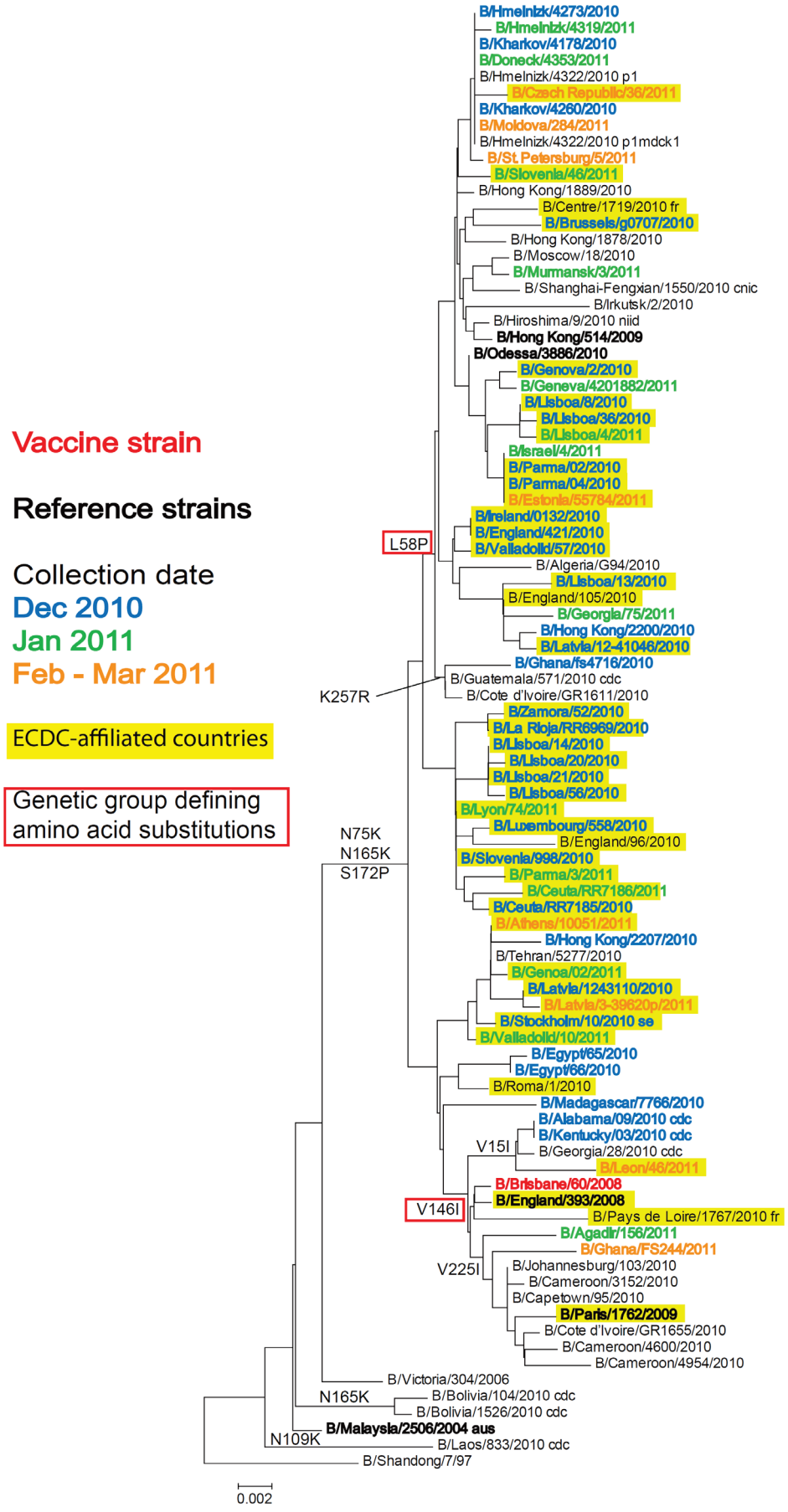

Table 5 Antigenic analysis of influenza B/Yamagata lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre						
				Post infection ferret sera					
			B/Fl[2] 4/06 Sh 479	B/Eg[1] 144/05 F07/05	B/Fl[1] 4/06 F20/07	B/Bris[1] 3/07 F24/07	B/Eng[1] 145/08 F9/08	B/Bang[1] 3333/07 F25/08	B/Wis[1] 1/10 F26/10
REFERENCE VIRUSES									
B/Egypt/144/2005	2005-05-01	E3/E6	5120	160	640	160	40	320	160
B/Florida/4/2006	2006-12-15	E3/E4	5120	640	2560	640	320	1280	640
B/Brisbane/3/2007	2007-09-03	E2/E3	5120	320	1280	320	80	640	320
B/England/145/2008		Ex/E4	640	20	80	10	80	40	20
B/Bangladesh/3333/2007	2007-08-07	E3/E4	5120	80	640	40	40	640	320
B/Wisconsin/1/2010	2010-02-20	E3/E2	1280	40	320	20	20	80	320
TEST VIRUSES									
B/Netherlands/234/2011	2011-01-16	xMDCK2/MDCK1	2560	640	320	160	640	640	160
B/Milano/10/2011	2011-01-17	MDCK1/MDCK1	1280	320	160	40	320	640	160
B/Trieste/05/2011	2011-01-19	MDCK1/MDCK1	1280	320	160	80	80	80	160
B/Latvia/2-70/2011	2011-02-01	MDCK1/MDCK1	5120	160	320	<	80	160	160
B/Estonia/54563/2011	2011-02-17	MDCK2/MDCK1	2560	80	80	<	<	160	80
B/Estonia/55669/2011	2011-03-14	MDCK2/MDCK1	2560	40	80	<	<	80	80
B/Estonia/55760/2011	2011-03-15	MDCK2/MDCK1	2560	160	160	<	80	320	160
B/Estonia/55758/2011	2011-03-15	MDCK2/MDCK1	2560	80	160	<	80	320	160
B/Estonia/55763/2011	2011-03-15	MDCK2/MDCK1	2560	160	160	40	80	640	320
B/Estonia/55786/2011	2011-03-16	MDCK2/MDCK1	2560	80	80	<	<	160	160

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

Sequences included in HA phylogeny

Figure 4 Phylogenetic comparison of influenza B/Yamagata lineage HA genes (HA1 coding region)