TECHNICAL DOCUMENT

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

Influenza virus characterisation

Summary Europe, July 2011

Influenza virus characterisation

Summary

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

- Recently isolated A(H1N1)pdm viruses continue to fall into several genetic groups but all groups show antigenic similarity to the currently recommended vaccine virus A/California/7/2009.
- A(H3N2) viruses fall into distinct genetic groups but there is no consistent correlation of altered antigenicity with any genetic group, compared with the vaccine virus A/Perth/16/2009.
- Influenza B viruses of the B/Victoria/2/87 lineage predominate over those of the B/Yamagata/16/88 lineage. Most of the B/Victoria/2/87 lineage viruses remain genetically and antigenically similar to the currently recommended vaccine virus B/Brisbane/60/2008.
- The majority of influenza B viruses of the B/Yamagata-lineage are in the B/Bangladesh/3333/2007 genetic clade but viruses received from three countries with Baltic coastlines fall within a different genetic clade.

Since the last influenza virus characterisation report, 250 virus specimens (propagated virus isolates or clinical samples) collected from February to June 2011 have been received from EU and EU-affiliated countries at the WHO CC in London (Table 1). These viruses were predominantly 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 have continued to be received. In Table 1, a batch of specimens from France for which analysis has yet to be completed is 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 continued to react well with the panel of post-infection ferret antisera, including antiserum raised against the vaccine virus A/California/7/2009. Only a single virus, A/Umea/1/2011, showed greater than a four-fold reduction in HI titre compared to titre of the vaccine virus with the antiserum raised against the homologous vaccine virus, A/California/7/2009. A/Umea/1/2011 displayed nucleotide polymorphism in the HA gene to encode an amino acid mixture at residue 155 of the HA.

A representative phylogenetic tree of the HA1 coding region of the HA gene is shown in Figure 1. The phylogenetic tree highlights residues that define genetic groups that have been predominant over the last six months; also marked on the tree are sporadic observations of particular amino acid substitutions or polymorphisms. The polymorphisms K153X, K154X, G155X, N156D and S157L are frequently associated with low reactivity in HI assays and commonly result from propagation of viruses in certain tissue culture cells as they are observed rarely in clinical samples. A sequence change at D222G, or a polymorphism D222X, is postulated to be detected more often in viruses recovered from patients suffering with severe disease. The amino acid substitution Q223R is associated with the isolation and passage of virus in hens' eggs.

We have described six genetic groups for the circulating H1N1 viruses which can be defined by the amino acid substitutions:

i) N125D, observed originally as an emerging genetic group in the southern hemisphere and subsequently widespread in the northern hemisphere and exemplified by the reference virus A/Christchurch/16/2010;

ii) D97N and S185T, e.g. A/St Petersburg/27/2011;

iii) D97N, S143G, S185T and A197T, e.g. A/St Petersburg/100/2011;

iv) A134T and S183P, e.g. A/Hong Kong/3934/2011;

v) D97N, R205K, I216V and V249L, e.g. A/Astrakhan/1/2011;

vi) N31D, S162N (adding a glycosylation site) and A186T, e.g. A/Czech Republic/32/2011

The viruses highlighted in Table 2, as included in the HA gene phylogenetic analysis, belong to genetic groups (ii), (iv) and (v), but viruses from each of the genetic groups have been collected in EU and EU-affiliated countries. Four new reference viruses against which ferret antisera have been raised are indicated in Figure 1.

Influenza A(H3N2) virus analysis

Since February 2011, influenza A(H3N2) viruses have been successfully isolated and propagated from 13 EU and ECDC-affiliated countries. The problems with antigenic characterisation of recent H3N2 viruses have been described previously. Shown in Table 3 are the results of HI assays using guinea pig red blood cells in the presence of oseltamivir to reduce any effect of the virus neuraminidase on the agglutination of the red blood cells (Lin et al. 2010). The results showed that none of the viruses tested yielded an eight-fold or greater reduction in titre with the post-infection ferret antiserum raised against the vaccine virus A/Perth/16/2009 compared with the homologous reaction between the antiserum and the vaccine virus, although three of the four viruses showed four-fold reductions. This proportion of viruses showing a reduced activity with the antiserum is lower than the figure that was observed in earlier reports and described in our WHO CC report for the WHO Consultation on the Composition of Influenza vaccines for the Northern Hemisphere 2011/2012. However, all the test viruses in Table 3 reacted well with a ferret antiserum raised against A/Wisconsin/15/2009, a virus genetically and antigenically closely related to A/Perth/16/2009. Many viruses show low reactivity in HI tests with antisera raised against A/Victoria/208/2009 and A/Victoria/210/2009, but this pair of viruses often shows 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. Seven reference viruses and the vaccine virus A/Perth/16/2009 used in the HI test are highlighted, and four new reference viruses have been selected (A/Alaska/5/2010, A/Arizona/13/2010, A/Iowa/19/2010 and A/Guadeloupe/202/2010). The majority of viruses from ECDC-affiliated countries fall within the A/Victoria/208/2009 genetic clade and a minority in the A/Perth/16/2009 clade. Distinct genetic groups within the HA gene are seen within both genetic clades.

Five genetic groups have been previously defined in circulating H3N2 viruses. Within the A/Perth/16/2009 genetic clade there are two genetic groups (i and ii) defined by amino acid substitutions:

(i) I260M, R261Q, e.g. A/Victoria/210/2009 with some viruses also carrying P162S and substitutions of either E50K, e.g. A/Stockholm/7/2011, or N81D, e.g. A/Niigata/510/2011;

(ii) N133D (resulting in the loss of a glycosylation site), R142G, T212A and V213A, e.g. A/Norway/1330/2010.

There are at least three genetic groups in the A/Victoria/208/2009 genetic clade of H3N2 viruses. These were defined by additional amino acid substitutions compared with the substitutions that define viruses of the A/Victoria/208/2009 clade compared with the A/Perth/16/2009 clade (K62E, K144N and T212A):

(iii) N145S and V223I, e.g. A/Cote d'Ivoire/GR1678/2010 with some viruses having the substitution N144D that results in the loss of a glycosylation site, e.g. A/Paris/2120/2010;

(iv) N312S, e.g. A/England/270/2010, with many viruses also carrying T48A and K92R, e.g. A/Rhode Island/01/2010;

(v) D53N, Y94H, I230V and E280A, e.g. the reference virus A/Alabama/05/2010, with some viruses also carrying the substitution S199A, e.g. A/Rheinland-Pfalz/7/2010, and some encoding an additional glycosylation site from as a result of S45N substitution, e.g. A/Hong Kong/3951/2011.

It is possible to describe two new genetic groups in the A/Victoria/208/2009 clade, groups (vi to vii). These can be defined by the following substitutions:

(vi) S45N, which results in an additional glycosylation site, e.g. A/Norway/685/2011;

(vii) S45N (+CHO), T48I, A198S and N321S, e.g. A/Slovenia/507/2011.

The virus highlighted in Table 3 falls into genetic group (v). Viruses from all genetic groups have been collected in EU and EU-affiliated countries since January 2011.

Analysis of viruses from each of the emerging genetic groups has indicated that no group shows consistent marked change in antigenicity compared with the vaccine virus.

Influenza B virus analyses

Similar to our previous report, influenza B viruses of the B/Victoria/2/87 lineage (80%) have continued to predominate over those of the B/Yamagata/16/88 lineage (20%).

B/Victoria-lineage viruses

Antigenic analysis of influenza B/Victoria viruses recently received and propagated is shown in Table 4. In the HI assay all but one test virus showed low reactivity with antisera raised against B/Brisbane/60/2008: the egg-propagated vaccine virus. As we have described previously, HI assays for influenza B viruses propagated only in cells frequently show reduced HI titres when tested with antisera raised against egg-propagated reference viruses, including vaccine viruses (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, hence they are considered to be antigenically similar to the vaccine virus. B/Lyon/CHU/12.88/2011 showed a different pattern of reactivity, reacting well with sera raised against egg-propagated viruses.

Figure 3 shows a phylogenetic tree based on the HA1-coding region of the HA gene of selected viruses of the B/Victoria-lineage. Amino acid substitutions N75K, N165K and S172P define the B/Brisbane/60/2008 genetic clade. All recently collected influenza B viruses of the B/Victoria-lineage from EU and EU-affiliated countries fall into the B/Brisbane/60/2008 genetic clade. As described previously, 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 effect on antigenicity of the viruses. The HA gene of B/Lyon/CHU/12.88/2011 carried a sequence polymorphism that encoded a mixed amino acid sequence at residue 197 of the HA gene, the site at which a glycosylation site is lost in egg-propagated viruses.

B/Yamagata-lineage viruses

Table 5 shows an antigenic analysis of two viruses of the B/Yamagata-lineage, collected in EU and EU-affiliated countries, carried out since our last report. Both viruses showed good reactivity with antisera raised against the three reference viruses falling within the B/Bangladesh/3333/2007 genetic clade (B/Wisconsin/1/2010, B/England/145/2008 and B/Bangladesh/3333/2007). One of the two viruses, B/Athens/9784/2011, showed an increased reactivity with antiserum raised against B/Brisbane/3/2007, a virus whose HA gene falls into a distinct genetic clade within the B/Yamagata-lineage.

Figure 4 shows a phylogenetic tree based on the HA1-coding region of the HA gene of selected viruses of the B/Yamagata-lineage. Amino acid substitutions S150I, N165Y, G229D define the B/Bangladesh/3333/2007 genetic clade. The majority of viruses are in the B/Bangladesh/3333/2007 genetic clade, falling into several genetic sub-groups. HI analyses do not differentiate these genetic sub-groups. A cluster of viruses from Sweden, Finland and Estonia fell into a distinct genetic clade represented by B/Brisbane/3/2007 as the prototype virus of the clade. The HA gene of the virus that showed a distinct pattern in the HI assay, B/Athens/9784/2011, fell into the B/Bangladesh/3333/2007 genetic clade but encoded the substitutions V29A, L172Qand M251V compared with the reference viruses. Viruses of the B/Yamagata-lineage that showed unusual HI results in our previous report all fell into the B/Bangladesh/3333/2007 genetic clade: one (B/Stockholm/12/2011) clustered with B/Athens/9784/2011 and fell into the same genetic sub-group; another fell into a genetic sub-group of the B/Bangladesh/3333/2007 clade defined by the substitutions T181A and K253R.

Note on 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 February 2011 and received by the end of July 2011

MONTH	H1N1pdm		H3N2		B Yamagata lineage		B Victoria lineage	
Country	Number received	Number isolated	Number received	Number isolated	Number received	Number isolated	Number received	Number isolated
FEBRUARY								
Czech Republic	1	1					3	3
Estonia	31	29			1	1		
France	11	in process	2	in process	3	in process	5	in process
Greece	9	7						
Ireland	2	2	2	2			1	1
Italy	1	1						
Latvia	1	1			1	1	3	2
Malta	5	1						
Norway	4	3	5	5				
Portugal	12	4						
Slovakia	1	1					2	2
Slovenia	6	6					6	in process
Spain	5	4	2	1			5	5
Sweden	2	2	4	4	1	1	3	3
MARCH								
Estonia	6	4			5	5	1	1
France	4	in process	2	in process	3	in process	9	in process
Greece	5	4			1	1	2	2
Ireland							1	1
Latvia							2	2
Malta							5	5
Norway	5	3	3	3	1	0	6	6
Romania			1	1				
Slovenia	1							
Spain							1	1
Sweden	5	5	1	1	2	2	9	9
APRIL								
France			1	in process			7	7
Ireland			1	1				
Malta							1	1
Slovenia	1	1						
Sweden	2	2	3	3			2	2
MAY								
France			5	5			1	0
Sweden	1	1	2	2			1	1
JUNE								
France			1	1				
Total Received = 250	121	82	35	29	18	11	76	54

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

			Haemagglutination inhibition titre						
			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 F29/10	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/34/09	A/HK 2212/10 F21/10 Egg	A/C'church 16/10 F30/10
REFERENCE VIRUSES									
A/California/7/2009	2009-04-09	E2/E2	2560	1280	1280	1280	1280	1280	1280
A/England/195/2009	2009-04-28	MDCK7	1280	1280	1280	640	640	1280	1280
A/Auckland/3/2009	2009-04-25	Ex/E4	1280	1280	2560	640	1280	1280	640
A/Bayern/69/2009	2009-07-01	MDCK5	160	40	40	640	160	80	80
A/Lviv/N6/2009	2009-10-27	M4/S2	320	80	80	1280	640	320	160
A/Hong Kong/2212/2010	2010-07-16	E3	2560	2560	2560	1280	2560	2560	2560
A/Christchurch/16/2010	2010-07-12	E2/E1	2560	1280	2560	2560	2560	1280	5120
TEST VIRUSES									
A/Slovenia/772/2011	2011-02-03	MDCK1/MDCK1	1280	1280	1280	640	640	1280	320
A/Slovenia/892/2011	2011-02-07	MDCK1/MDCK2	1280	1280	1280	640	640	1280	640
A/Stockholm/3/2011	2011-02-08	MDCK1/MDCK2	640	320	320	640	640	320	320
A/Stockholm/4/2011	2011-02-08	MDCK1/MDCK2	640	160	320	1280	640	320	320
A/Lisbon/SU429/2011	2011-02-10	MDCK2	1280	5120	1280	320	640	640	640
A/Lisbon/SU468/2011	2011-02-11	MDCK4	1280	1280	1280	640	1280	1280	640
A/Slovenia/1039/2011	2011-02-16	MDCK1/MDCK2	1280	1280	1280	1280	1280	1280	640
A/Lyon/1067/2011	2011-02-21	MDCK2/MDCK1	1280	1280	2560	640	1280	1280	640
A/Toulon/1173/2011	2011-02-22	MDCK2/MDCK1	1280	1280	1280	320	640	1280	640
A/Norway/924/2011	2011-02-22	MDCK1/MDCK2	640	80	160	1280	640	160	160
A/Slovenia/1273/2011	2011-03-11	MDCK1/MDCK2	1280	1280	1280	640	1280	1280	1280
A/Stockholm/11/2011	2011-03-14	MDCK3/MDCK2	1280	640	1280	640	640	640	640
A/Slovenia/1552/2011	2011-04-30	MDCKx/MDCK1	1280	1280	1280	640	1280	1280	1280
A/Umea/1/2011	2011-05-19	MDCK3/MDCK2	320	160	80	640	320	320	160

Sequences included in HA phylogeny Vaccine virus

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

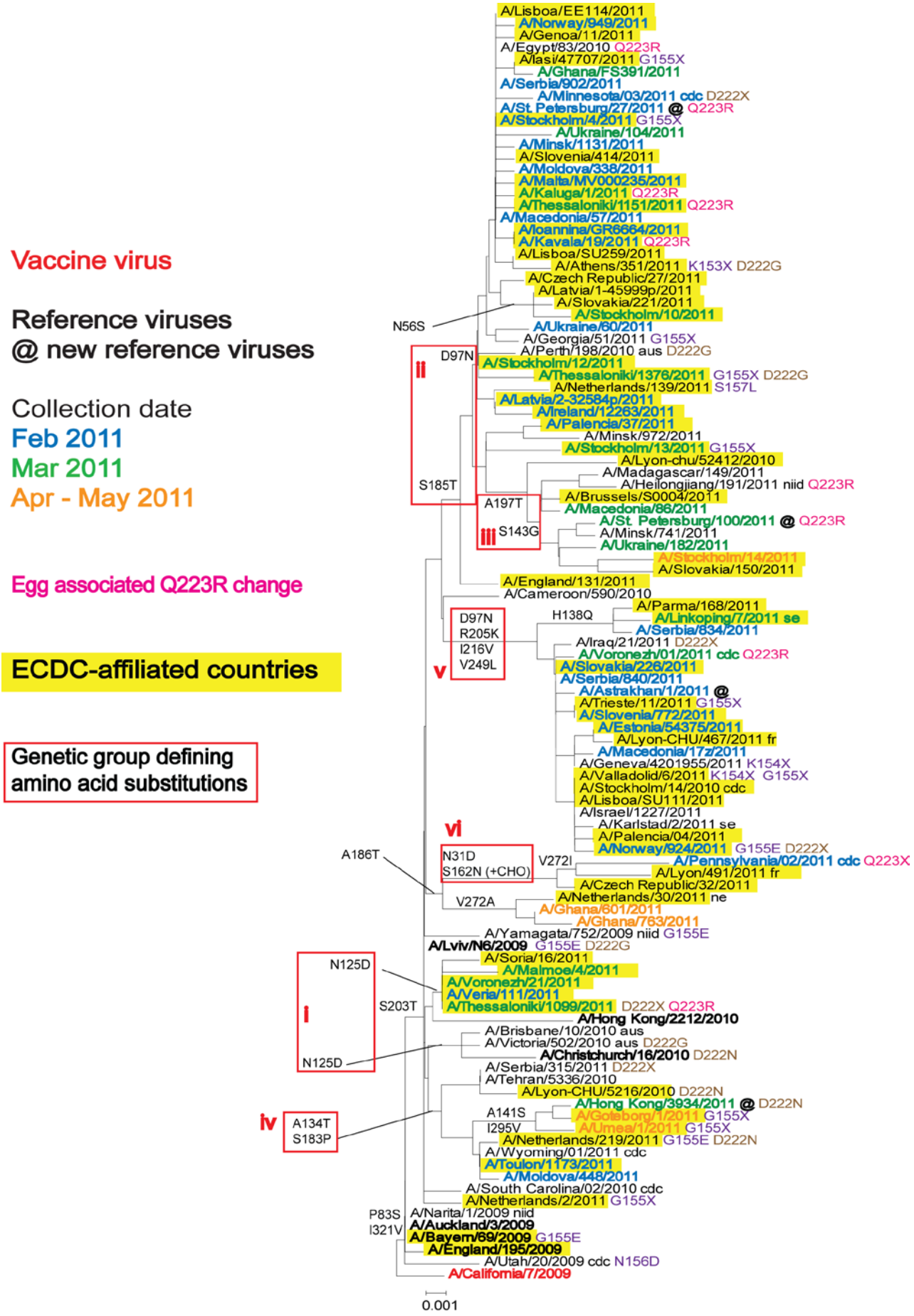

Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)

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/E9	640	640	<	<	80	<	<	40
A/Brisbane/10/2007	2007-02-06	E2/E4	640	2560	40	<	160	160	<	80
A/Perth/16/2009	2009-07-04	E3/E4	<	80	640	640	640	1280	320	1280
A/Wisconsin/15/2009	2009-07-06	E2/E3	<	<	320	160	40	160	1280	320
A/Victoria/208/2009	2009-06-02	E3/E1	80	160	640	640	1280	2560	320	1280
A/Victoria/210/2009	2009-06-02	E2/E3	160	320	640	640	2560	5120	1280	5120
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT4	<	<	160	80	40	40	160	320
A/Perth/10/2010	2010-05-25	E2/E1	<	<	320	160	160	320	320	640
TEST VIRUSES										
A/Lyon/CHU/6.614/2011	2011-02-09	MDCK2/MDCK2	<	40	640	320	80	160	160	320
A/Lyon/CHU/7.496/2011	2011-02-16	MDCK2/SIAT2	<	80	160	160	160	320	640	640
A/St Etienne/1130/2011	2011-03-01	MDCK2/SIAT2	<	40	160	160	80	160	320	320
A/Clermont-Ferrand/1472/2011	2011-05-18	MDCK2/SIAT2	<	40	160	160	80	160	320	320

1. < = <40

Vaccine strain

Sequences included in the HA phylogeny

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

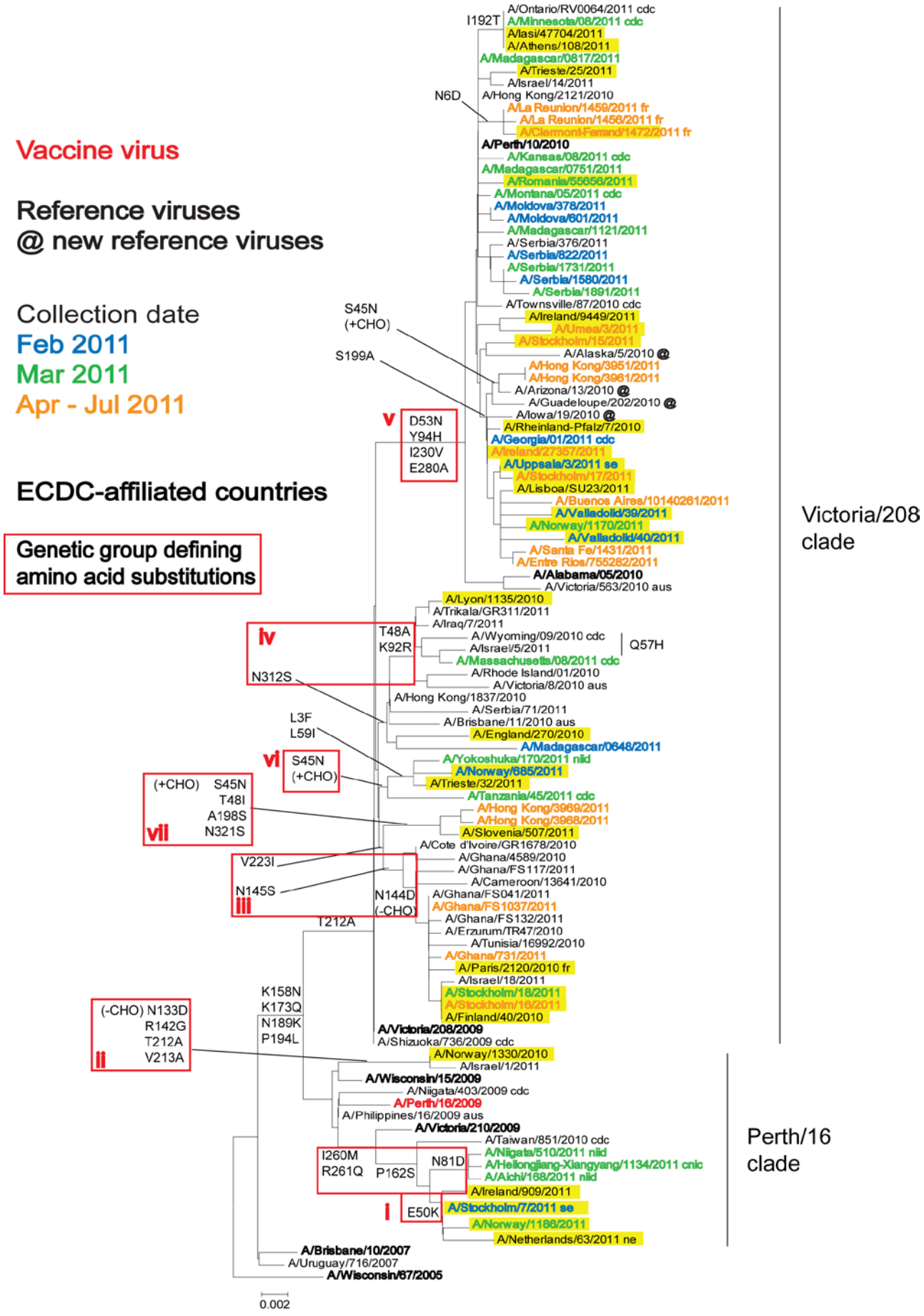

Table 4. Antigenic analysis of influenza B/Victoria-lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]						
				Post infection ferret sera					
			B/Bris 60/08 Sh 523	B/Mal 2506/04 F28/05	B/England 393/08 F05/11	B/Bris 60/08 F06/11	B/Paris 1762/09 F07/11	B/HK 514/09 F3/10	B/Odessa 3886/10 F17/10
REFERENCE VIRUSES									
B/Malaysia/2506/2004	2004-12-06	E3/E3	2560	640	80	160	10	<	10
B/England/393/2008	2008-08-29	E1/E6	2560	320	640	640	80	40	80
B/Brisbane/60/2008	2008-08-04	E4/E4	2560	160	640	1280	80	40	80
B/Paris/1762/2009	2009-02-09	C2/MDCK4	5120	40	80	160	320	160	160
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK2	5120	20	40	80	160	160	160
B/Odessa/3886/2010	2010-03-19	C2/MDCK3	5120	40	80	160	320	320	320
TEST VIRUSES									
B/Slovenia/819/2011	2011-02-03	MDCK1/MDCK1	640	80	40	80	80	80	80
B/Slovenia/1010/2011	2011-02-15	MDCK1/MDCK1	640	80	80	80	160	80	160
B/Slovenia/1042/2011	2011-02-16	MDCKx/MDCK1	5120	40	40	80	160	160	160
B/Slovenia/1088/2011	2011-02-19	MDCKx/MDCK1	5120	40	80	160	320	320	320
B/Slovenia/1123/2011	2011-02-22	MDCKx/MDCK1	5120	40	80	160	320	160	160
B/Slovenia/1141/2011	2011-02-24	MDCKx/MDCK1	5120	40	80	160	160	160	160
B/Lyon/CHU/12.88/2011	2011-03-21	MDCK2/MDCK1	2560	320	640	640	160	80	160
B/Lyon/CHU/12.1127/2011	2011-03-27	MDCK2/MDCK1	640	20	10	80	80	80	160
B/Lyon/CHU/13.584/2011	2011-03-31	MDCK2/MDCK1	640	20	10	80	80	80	160
B/Lyon/CHU/14.411/2011	2011-04-06	MDCK2/MDCK1	640	20	10	40	80	80	80
B/Lyon/CHU/14.767/2011	2011-04-08	MDCK2/MDCK1	640	20	10	40	80	80	80
B/Lyon/CHU/15.216/2011	2011-04-12	MDCK2/MDCK1	640	20	10	40	80	80	160
B/Lyon/CHU/15.489/2011	2011-04-13	MDCK2/MDCK1	640	20	10	40	80	80	80
B/Lyon/CHU/15.731/2011	2011-04-15	MDCK2/MDCK1	640	20	10	40	80	80	80
B/Lyon/CHU/16.167/2011	2011-04-18	MDCK2/MDCK1	640	20	10	40	80	80	80
B/Lyon/CHU/16.432/2011	2011-04-20	MDCK2/MDCK1	640	20	20	40	160	80	160

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

Sequences included in the HA phylogeny

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

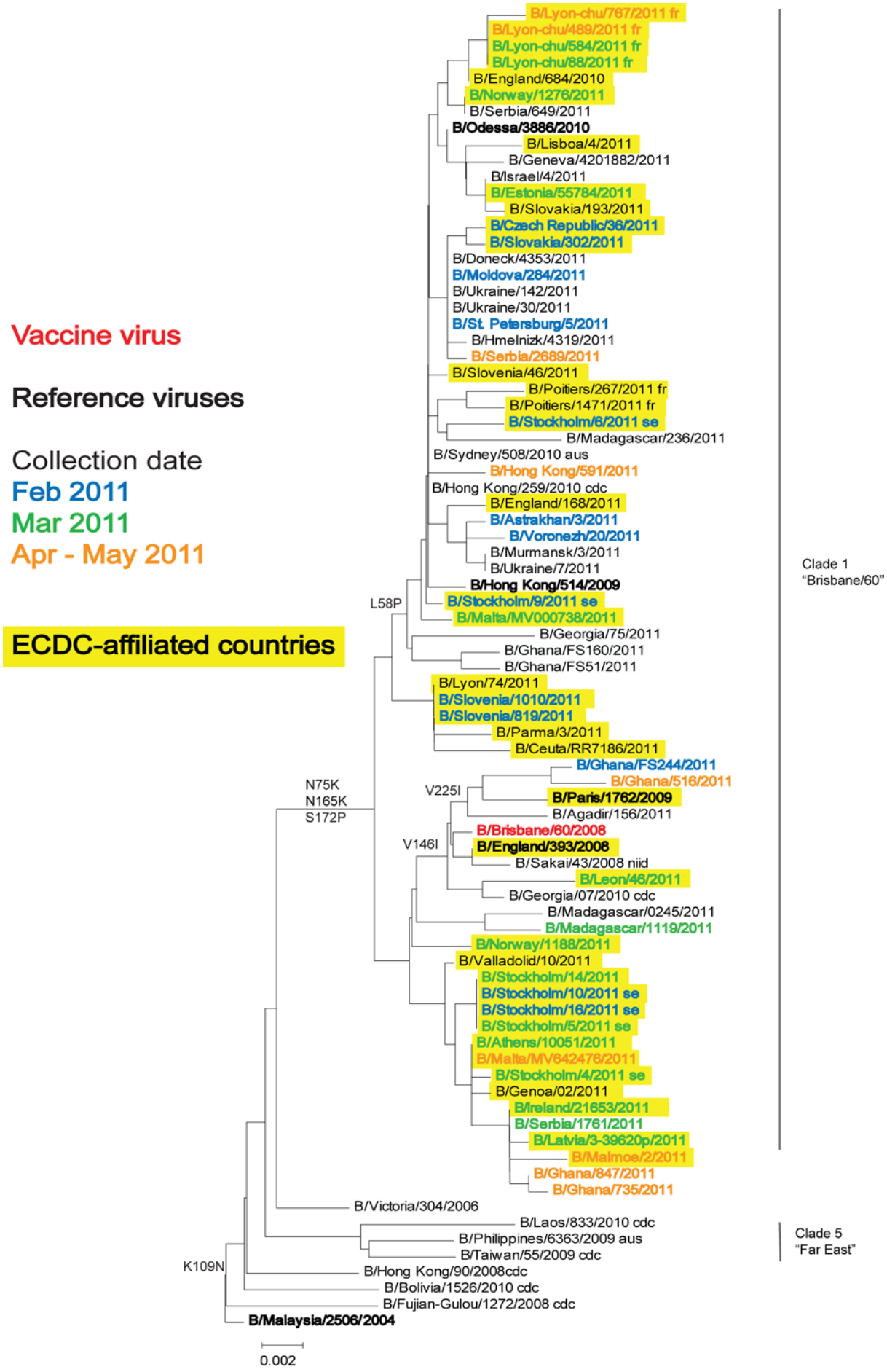

Table 5. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre						
				Post infection ferret sera					
			B/Fl[3] 4/06 SH479	B/Eg[1] 144/05 F7/05	B/Fl[1] 4/06 F20/07	B/Bris[2] 3/07 F24/07	B/Eng[2] 145/08 F12/11	B/Bang[2] 3333/07 F25/08	B/Wis[2] 1/10 F26/10
REFERENCE VIRUSES									
B/Egypt/144/2005	2005-05-01	E3/E6	2560	640	1280	640	160	160	160
B/Florida/4/2006	2006-12-15	E3/E4	5120	640	1280	640	320	160	320
B/Brisbane/3/2007	2007-09-03	E2/E4	2560	640	1280	1280	160	320	320
B/England/145/2008		Ex/E5	640	80	160	40	160	40	20
B/Bangladesh/3333/2007	2007-08-07	E3/E4	2560	320	1280	160	160	320	320
B/Wisconsin/1/2010	2010-02-20	E3/E2	1280	160	640	160	80	160	320
TEST VIRUSES									
B/Lyon/1179/2011	2011-03-01	MDCK2/MDCK1	2560	160	320	40	160	80	80
B/Athens/9784/2011	2011-03-13	MDCK2	5120	640	1280	640	320	320	160

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

Sequences included in the HA phylogeny

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