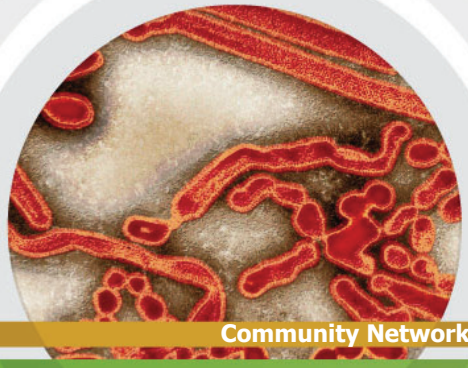

TECHNICAL DOCUMENT

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

Influenza virus characterisation

Summary Europe, May–June 2011

Influenza virus characterisation

Summary

Influenza A(H1N1)pdm, influenza A(H3N2), and influenza B/Victoria/2/87 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 also continue to fall into distinct genetic groups with some viruses showing antigenic difference from the currently used vaccine virus A/Perth/16/2009, but there is no consistent correlation of altered antigenicity with any genetic group.
- Influenza B viruses of the B/Victoria/2/87 lineage have predominated 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 450 virus specimens (propagated virus isolates or clinical samples) collected from January to May 2011 have been received from EU and EU-affiliated countries at the WHO CC in London (Table 1). As in the previous report, 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 continued to be received. In Table 1, certain batches of specimens (from Norway, Sweden and Portugal 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. Approximately 15% of the viruses showed greater than a four-fold reduction in HI titre, as compared with the vaccine virus, when tested with serum raised against the vaccine virus.

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 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, and amino acid substitutions associated with the isolation and passage of virus in hens' eggs.

Six genetic groups can be defined for the circulating H1N1 viruses. These groups 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/England/676/2010;

iii) S143G, S185T and A197T, e.g. A/Baden-Wurtemburg/14/2010 or A/Brussels/S0004/2011;

iv) A134T and S183P, e.g. A/Alborz/5607/2010;

v) D97N, R205K, I216V and V249L, e.g. A/Trieste/11/2011;

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 (i) and (ii) but viruses from each of the genetic groups have been collected in EU and EU-affiliated countries.

As previously, encoded periodic amino acid substitutions or polymorphisms at amino acid residues 153-157, 222 and 223 are marked on the phylogenetic tree. Substitutions at residues 153-157 in the HA are commonly associated with reduced titres in HI assays and often the substitution or polymorphism was not seen when the nucleotide sequence of the corresponding clinical specimen has been analysed. This indicates that polymorphism at residues 153-157 is often 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 and propagation of virus in hens' eggs.

Influenza A(H3N2) virus analysis

Since January 2011, influenza A(H3N2) viruses have been successfully isolated and propagated from nine ECDC-affiliated countries. The problems with antigenic characterisation of recent H3N2 viruses have been described previously. 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 of the virus neuraminidase on the agglutination of the red blood cells (Lin et al. 2010). The results show that close to 50% of the viruses tested gave a reduction in titre of 8-fold 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. The figure of 50% of viruses showing a reduced activity with the antiserum is similar to the figure that was observed earlier and reported in the WHO CC report for the WHO Consultation on the Composition of Influenza vaccines for the Northern Hemisphere 2011/2012. 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. As described before, many viruses in the test showed low reactivity 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. The five reference viruses and the vaccine virus A/Perth/16/2009 used in the HI test are highlighted. The majority of viruses from ECDC-affiliated countries fall within the A/Victoria/208/2009 genetic clade and a small minority fall into the A/Perth/16/2009 clade. Distinct genetic groups within the HA gene are seen within both genetic clades.

Five genetic subgroups can be 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 the substitutions E50K and P162S, e.g. A/Hessen/5/2010;

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

There are three genetic groups (iii), (iv) and (v) in the A/Victoria/208/2009 genetic clade of H3N2 viruses. These can be 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 A212T):

(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. the reference virus A/Rhode Island/01/2010;

(v) D53N, Y94H, I230V, 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.

The viruses highlighted in Table 3 and in Figure 2 fall into genetic groups (i) and (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 there is no 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

Representative results of antigenic analysis of influenza B/Victoria viruses are shown in Table 4. In the HI assay the vast majority of 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 but one of 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. The exception was B/Malmoe/2/2011 and this virus is under further investigation.

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. 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 affect on antigenicity of the viruses.

B/Yamagata lineage viruses

Table 5 shows antigenic analysis of viruses of the B/Yamagata-lineage, collected in EU and EU-affiliated countries, that have been carried out since the last report. All 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). Three of the five viruses analysed, 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. Genetic analysis of these viruses is underway.

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 January 2011 and received by the end of May 2011

MONTH	A	H1N1pdm		H3N2		B	B Yamagata lineage		B Victoria lineage	
Country		Number received	Number isolated	Number received	Number isolated		Number received	Number isolated	Number received	Number isolated
JANUARY										
Belgium	1	1	1						2	1
Czech Republic		13	13						1	0
Estonia		9	5							
France							1	1	3	3
Germany									2	2
Greece	1	30	16	2	1	3				
Ireland				7	5					
Italy		52	50	3	1	1	2	2	23	23
Latvia		6	6							
Malta		2	2							
Netherlands		6	6	1	1		1	1		
Norway		2	in process	2	2		4	1	4	4
Portugal		15	in process	2	1				8	6
Romania		2	2	1	1					
Slovakia		9	7						1	1
Slovenia		14	13						3	3
Spain		31	21						5	5
Sweden		4	3	5	3					
United Kingdom		4	4				2	2	3	3
FEBRUARY										
Czech Republic		1	1						3	3
Estonia		31	29			1	1	1		
Greece	1	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		6	in process							
Slovakia		1	1						2	2
Spain		5	4	2	1				5	5
Sweden		2	in process	4	4		1	1	3	3
MARCH										
Estonia		6	4				5	5	1	1
Greece	4	5	4			5	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					
Spain									1	1
Sweden		5	5	1	1		2	2	9	9
APRIL										
Ireland				1	1					
Malta									1	1
Sweden		2	2	3	3				2	2
MAY										
Sweden		1	1	2	2				1	1
Total Received = 481	7	292	218	47	38	10	22	18	103	98

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 NIB F18/09	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 Egg	A/C'church 16/2010 F30/10
REFERENCE VIRUSES									
A/California/7/2009	2009-04-09	E2/E4	2560	1280	2560	640	1280	1280	1280
A/England/195/2009	2009-04-28	MDCK1/MDCK4	2560	1280	1280	640	1280	1280	640
A/Auckland/3/2009	2009-04-25	Ex/E3	5120	2560	2560	640	1280	1280	1280
A/Bayern/69/2009	2009-07-01	MDCK4/MDCK2	320	80	80	320	640	160	160
A/Lviv/N6/2009	2009-10-27	MDCK5	640	80	80	640	640	320	160
A/Hong Kong/2212/2010	2010-07-16	E4	2560	2560	2560	1280	2560	2560	1280
A/Christchurch/16/2010	2010-07-12	E2/E1	1280	1280	1280	1280	1280	1280	5120
TEST VIRUSES									
A/England/132/2011	2011-01-01	SIAT2/MDCK1	640	1280	1280	320	640	640	640
A/England/213/2011	2011-01-03	MDCK4/MDCK1	1280	2560	2560	640	1280	1280	1280
A/England/131/2011	2011-01-06	MDCK2/MDCK1	320	640	640	320	640	640	320
A/Slovakia/150/2011	2011-01-24	MDCKx/MDCK1	320	160	160	160	160	160	80
A/Slovakia/152/2011	2011-01-24	MDCKx/MDCK1	1280	1280	640	320	640	640	640
A/Slovakia/156/2011	2011-01-25	MDCKx/MDCK1	1280	1280	2560	640	1280	1280	5120
A/Slovakia/181/2011	2011-01-26	MDCKx/MDCK1	1280	1280	1280	640	1280	1280	640
A/Stockholm/19/2010	2011-01-27	MDCK0/MDCK1	1280	1280	640	640	640	1280	5120
A/Slovakia/200/2011	2011-01-28	MDCKx/MDCK1	1280	640	640	640	640	640	640
A/Slovakia/217/2011	2011-01-31	MDCKx/MDCK1	2560	2560	2560	640	2560	1280	1280
A/Slovakia/221/2011	2011-01-31	MDCKx/MDCK1	320	320	320	640	320	320	320
A/Ireland/11542/2011	2011-02-01	MDCKP2/MDCK1	640	1280	1280	640	1280	1280	1280
A/Slovakia/226/2011	2011-02-01	MDCKx/MDCK1	2560	1280	2560	640	1280	1280	1280
A/Ireland/12263/2011	2011-02-02	MDCKP3/MDCK1	640	640	640	640	640	640	640
A/Thessaloniki/15/2011	2011-02-07	MDCK3/MDCK1	1280	1280	1280	640	1280	2560	1280
A/Thessaloniki/80/2011	2011-02-07	MDCK3/MDCK1	1280	2560	2560	640	1280	2560	5120
A/Kavala/19/2011	2011-02-10	E2/E1	1280	1280	640	640	640	640	640
A/Norway/949/2011	2011-02-17	MDCK2/MDCK4	1280	640	640	640	640	640	320
A/Norway/950/2011	2011-02-21	MDCK2/MDCK3	1280	640	640	640	320	640	320
A/Norway/1043/2011	2011-03-01	MDCK2/MDCK2	2560	2560	2560	640	1280	2560	1280
A/Norway/1047/2011	2011-03-01	MDCK2/MDCK3	2560	2560	2560	640	1280	1280	1280
A/Norway/1058/2011	2011-03-03	MDCK2/MDCK2	1280	1280	1280	640	640	1280	640
A/Thessaloniki/1099/2011	2011-03-08	E2/E1	640	320	640	640	640	640	2560
A/Thessaloniki/1078/2011	2011-03-08	E2/E1	640	320	640	640	640	640	1280
A/Thessaloniki/1376/2011	2011-03-08	E1/E1	640	80	80	640	1280	320	160
A/Thessaloniki/1151/2011	2011-03-08	E1/E1	640	640	640	640	640	640	320
A/Stockholm/12/2011	2011-03-14	MDCK1/MDCK1	2560	1280	1280	640	1280	1280	1280
A/Stockholm/10/2011	2011-03-14	MDCK2/MDCK1	2560	1280	1280	640	1280	1280	1280
A/Stockholm/13/2011	2011-03-28	MDCK2/MDCK1	640	160	160	640	640	320	160
A/Malmoe/4/2011	2011-03-28	MDCK1/MDCK1	1280	1280	1280	640	1280	1280	5120
A/Stockholm/14/2011	2011-04-12	MDCK1/MDCK1	320	160	320	640	320	160	160
A/Göteborg/1/2011	2011-04-12	MDCK1/MDCK1	320	80	160	640	320	160	160

Sequences included in HA phylogeny

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

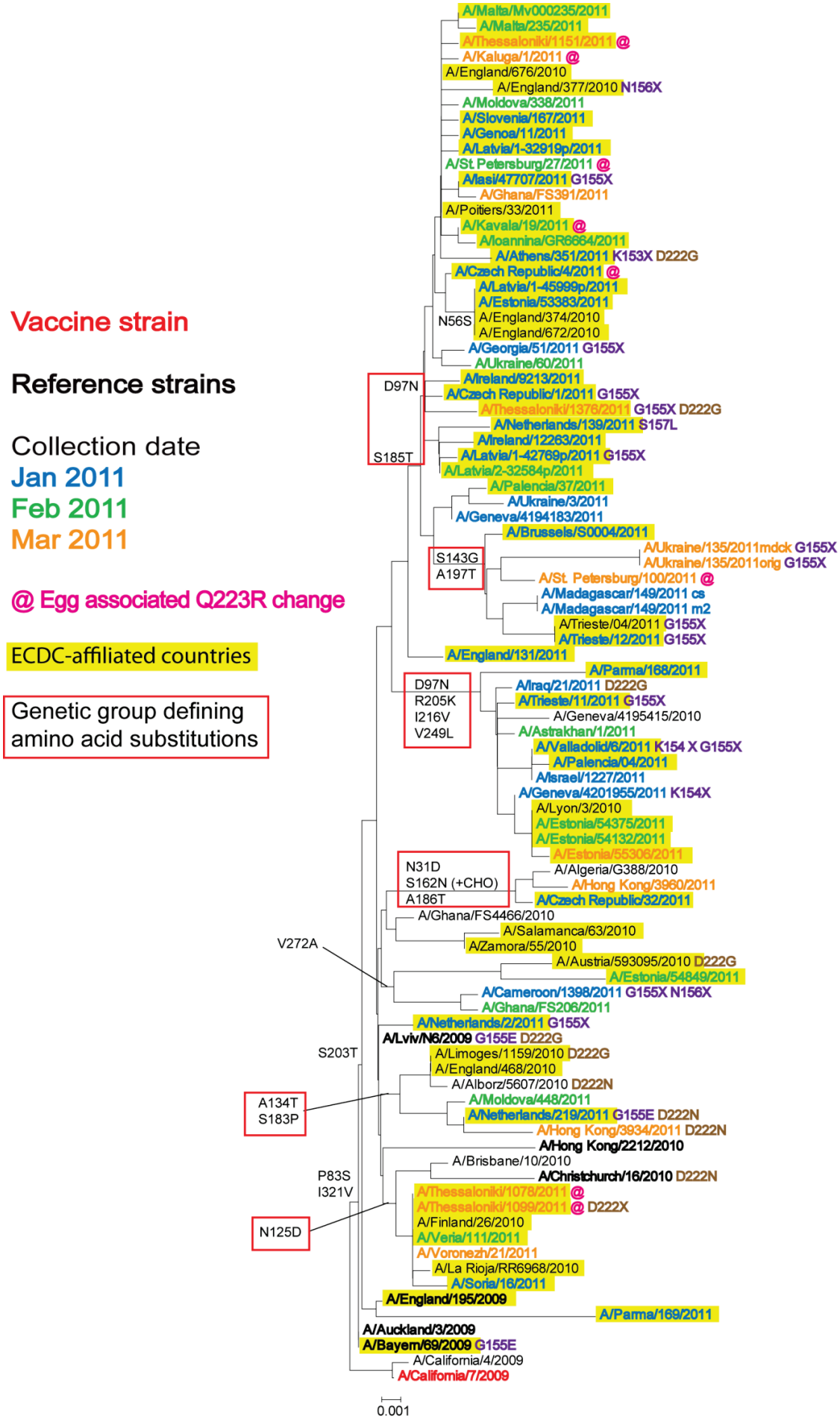

Table 3 Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBCs with 20nM oseltamivir)

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1]							
			Post infection ferret sera							
			A/Wis 67/05 F18/08	A/Bris 10/07 F09/11	A/Perth 16/09 F30/09	A/Wis 15/09 F24/09	A/Vic 208/09 F7/10	A/Vic 210/09 F10/11	A/Ala 5/10 F27/10	A/Perth 10/2010 F03/11
REFERENCE VIRUSES										
A/Wisconsin/67/2005	2005-08-31	SpfCk3E3/E9	640	320	<	<	80	<	<	40
A/Brisbane/10/2007	2007-02-06	E2/E4	2560	2560	<	<	320	160	<	160
A/Perth/16/2009	2009-07-04	E3/E4	<	40	640	320	160	320	320	640
A/Wisconsin/15/2009	2009-07-06	E2/E3	<	<	320	160	40	80	640	320
A/Victoria/208/2009	2009-06-02	E3/E1	160	160	640	640	1280	2560	640	1280
A/Victoria/210/2009	2009-06-02	E2/E3	160	160	1280	640	1280	2560	320	2560
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT4	<	<	80	80	40	40	160	320
A/Perth/10/2010	2010-05-25	E2/E1	<	<	320	160	40	80	320	640
TEST VIRUSES										
A/Stockholm/1/2011	2011-01-03	C0/SIAT1	<	40	160	160	160	80	320	320
A/Ireland/4185/2011	2011-01-12	MDCK3/SIAT1	40	80	320	160	320	320	320	1280
A/Ireland/558/2011	2011-01-17	MDCK2/SIAT1	<	40	160	160	320	160	320	640
A/Ireland/7408/2011	2011-01-21	MDCK2/SIAT1	<	<	320	320	160	160	160	640
A/Norway/600/2011	2011-01-26	MDCK1/SIAT3	<	<	40	40	40	40	40	320
A/Ireland/909/2011	2011-01-27	MDCK2/SIAT1	<	<	320	320	160	160	160	640
A/Umea/2/2010	2011-01-27	C1/SIAT1	<	40	640	320	80	640	320	320
A/Ireland/9449/2011	2011-01-28	MDCK2/SIAT1	40	80	640	320	2560	1280	1280	2560
A/Norway/846/2011	2011-01-31	MDCK2/SIAT1	40	80	80	160	160	160	320	640
A/Norway/685/2011	2011-02-04	MDCK2/SIAT1	40	80	160	160	160	160	320	640
A/Norway/842/2011	2011-02-06	MDCK2/SIAT3	<	<	40	40	40	40	80	160
A/Norway/845/2011	2011-02-07	MDCK2/SIAT1	<	80	80	80	80	80	160	320
A/Ireland/13527/2011	2011-02-09	MDCK2/SIAT1	<	40	160	160	320	160	320	640
A/Stockholm/6/2011	2011-02-09	C2/SIAT1	40	80	320	640	160	640	320	640
A/Stockholm/5/2011	2011-02-09	C1/SIAT1	<	40	160	80	80	40	160	320
A/Ireland/13334/2011	2011-02-10	MDCK2/SIAT1	<	40	320	320	640	320	640	1280
A/Norway/865/2011	2011-02-22	MDCK2/SIAT3	40	40	80	160	80	160	320	640
A/Norway/884/2011	2011-02-22	MDCK2/SIAT1	40	40	80	160	80	80	160	640
A/Stockholm/7/2011	2011-02-24	C2/SIAT1	40	40	320	320	160	640	160	320
A/Uppsala/3/2011	2011-02-24	C2/SIAT1	<	40	160	320	160	160	320	320
A/Norway/1138/2011	2011-03-09	MDCK2/SIAT2	<	40	80	80	80	80	160	320
A/Norway/1186/2011	2011-03-16	MDCK2/SIAT1	<	<	160	80	40	320	80	160
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT1	<	40	80	40	40	40	160	160
A/Ireland/11M27357/201	2011-04-06	SIAT2	<	40	80	160	80	160	320	640
A/Stockholm/16/2011	2011-04-06	MDCK2/SIAT1	40	80	80	80	80	80	160	320
A/Stockholm/15/2011	2011-04-06	MDCK0/SIAT1	<	80	160	320	160	160	320	640
A/Stockholm/17/2011	2011-04-12	MDCK1/SIAT1	<	<	80	80	<	40	160	160
A/Umea/2/2011	2011-05-05	MDCK2/SIAT1	<	40	80	80	80	80	160	320
A/Umea/3/2011	2011-05-23	MDCK3/SIAT1	<	<	80	80	40	80	160	320

1. < = <40

Sequences included in HA phylogeny

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

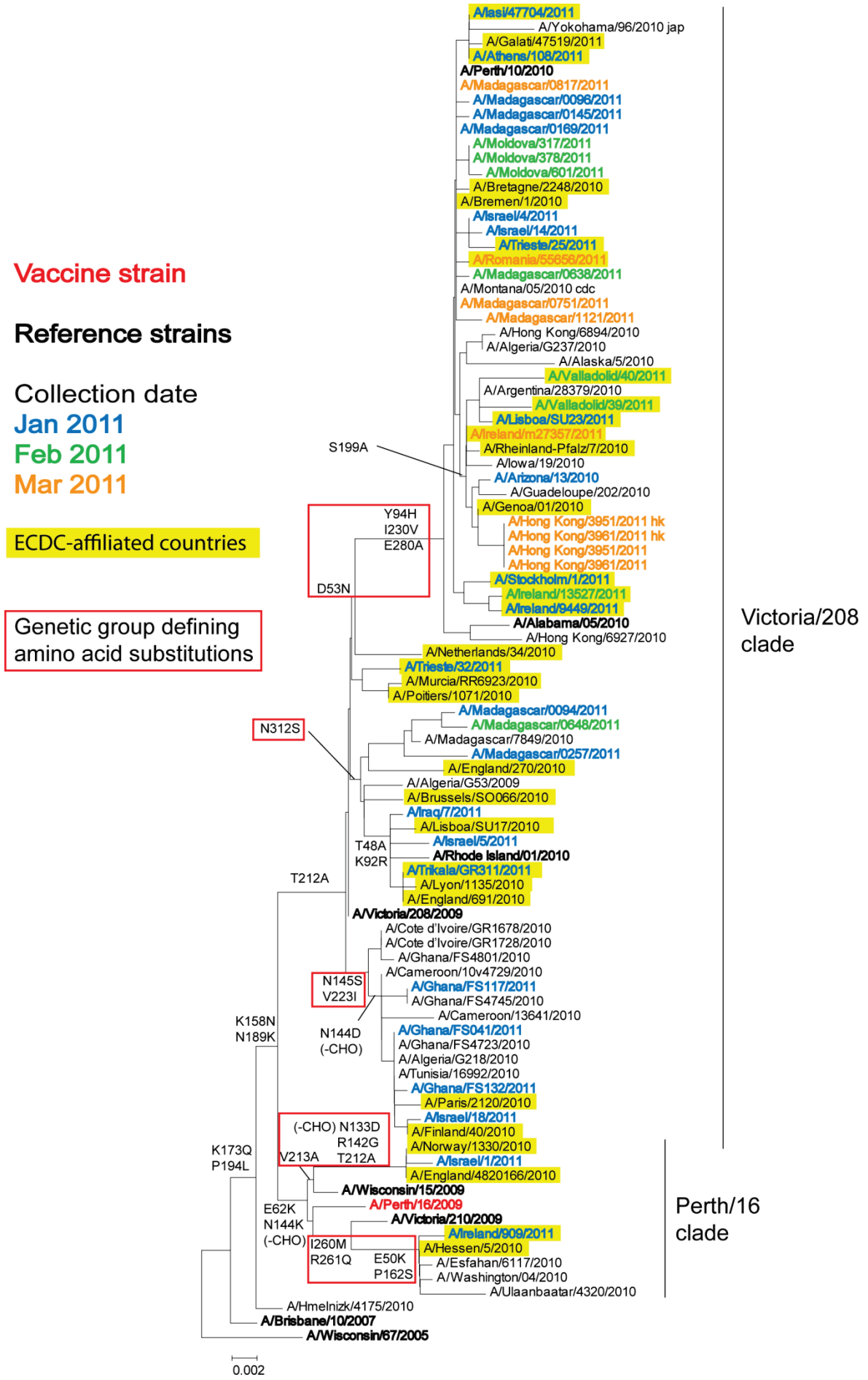

Table 4 Antigenic analysis of influenza B/Victoria lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]						
				Post infection ferret sera					
			B/Bris 60/08 Sh 524	B/Mal 2506/04 F28/05	B/England 393/08 F05/11	B/Bris 60/08 F06/11	B/Paris 1762/08 F7/11	B/HK 514/09 F3/10	B/Odessa 3886/10 F17/10
REFERENCE VIRUSES									
B/Malaysia/2506/2004	2004-12-06	E3/E3	1280	640	80	160	10	<	10
B/England/393/2008	2008-08-29	E1/E6	1280	320	640	1280	80	80	80
B/Brisbane/60/2008	2008-08-04	E4/E4	640	160	320	640	80	40	80
B/Paris/1762/2008	2009-02-09	C2/MDCK4	1280	20	80	80	160	160	160
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK1	1280	20	40	80	160	160	160
B/Odessa/3886/2010	2010-03-19	C2/MDCK3	2560	20	80	160	160	320	320
TEST VIRUSES									
B/England/171/2011	2011-01-01	SIAT2/MDCK1	640	10	10	40	80	80	80
B/England/170/2011	2011-01-02	SIAT2/MDCK1	640	10	20	40	80	80	80
B/England/168/2011	2011-01-04	MDCK2/MDCK1	640	10	20	40	80	160	160
B/Norway/1292/2011	2011-01-04	MDCK1/MDCK1	640	10	20	40	80	80	80
B/Norway/294/2011	2011-01-11	MDCK1/MDCK1	640	10	20	40	80	80	80
B/Norway/296/2011	2011-01-15	MDCK1/MDCK4	1280	20	80	80	160	160	160
B/Slovakia/193/2011	2011-01-27	MDCKx/MDCK1	1280	20	40	80	160	160	160
B/Slovakia/249/2011	2011-02-02	MDCKx/MDCK1	1280	20	40	20	80	80	320
B/Ireland/11497/2011	2011-02-03	MDCK2/MDCK1	1280	20	40	80	80	160	160
B/Slovakia/302/2011	2011-02-07	MDCKx/MDCK1	1280	20	40	40	80	160	160
B/Stockholm/1/2011	2011-02-09	MDCK1/MDCK1	1280	20	40	40	80	160	160
B/Stockholm/6/2011	2011-02-09	MDCK1/MDCK1	1280	20	80	40	160	320	320
B/Stockholm/3/2011	2011-02-09	MDCK1/MDCK2	1280	20	40	80	80	80	80
B/Malta/MV636793/2011	2011-03-05	MDCK2	1280	80	80	80	160	320	320
B/Malta/MV636714/2011	2011-03-07	MDCK2	1280	40	40	80	160	160	320
B/Ireland/21653/2011	2011-03-14	MDCKP1/MDCK1	1280	10	20	40	80	160	80
B/Stockholm/7/2011	2011-03-14	MDCK2/MDCK1	1280	20	80	80	160	160	320
B/Stockholm/16/2011	2011-03-14	MDCK2/MDCK1	1280	10	80	80	80	80	160
B/Stockholm/8/2011	2011-03-14	MDCK2/MDCK2	640	10	20	80	80	80	160
B/Stockholm/15/2011	2011-03-14	MDCK0/MDCK1	640	10	40	40	80	160	160
B/Norway/1188/2011	2011-03-17	MDCK1/MDCK3	640	10	40	40	80	80	80
B/Norway/1249/2011	2011-03-18	MDCK1/MDCK1	640	10	20	40	80	160	80
B/Norway/1227/2011	2011-03-19	MDCK1/MDCK4	1280	20	40	40	80	160	160
B/Malta/MV639140/2011	2011-03-22	MDCK2	1280	20	40	80	80	80	160
B/Malta/MV639141/2011	2011-03-22	MDCK2	1280	20	40	80	160	80	160
B/Norway/1248/2011	2011-03-22	MDCK1/MDCK1	640	10	20	40	80	80	80
B/Malta/MV000738/2011	2011-03-25	MDCK2	1280	40	80	160	160	80	320
B/Stockholm/9/2011	2011-03-28	MDCK2/MDCK2	640	10	20	40	80	160	160
B/Stockholm/10/2011	2011-03-28	MDCK0/MDCK2	640	10	20	80	80	80	160
B/Stockholm/14/2011	2011-03-28	MDCK0/MDCK1	1280	20	80	80	160	160	320
B/Stockholm/13/2011	2011-03-28	MDCK0/MDCK1	1280	20	40	80	160	160	160
B/Stockholm/11/2011	2011-03-28	MDCK0/MDCK2	640	10	20	20	80	80	160
B/Norway/1276/2011	2011-03-29	MDCK1/MDCK1	640	10	20	40	160	160	80
B/Norway/1326/2011	2011-03-31	MDCK1/MDCK4	1280	20	80	80	160	160	160
B/Malta/MV642476/2011	2011-04-02	MDCK2	1280	80	40	80	160	160	320
B/Stockholm/4/2011	2011-04-06	MDCK1/MDCK1	2560	20	160	80	80	80	160
B/Stockholm/5/2011	2011-04-06	MDCK1/MDCK2	640	10	20	20	40	80	80
B/Malmoe/2/2011	2011-05-04	MDCK1/MDCK1	1280	320	80	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)

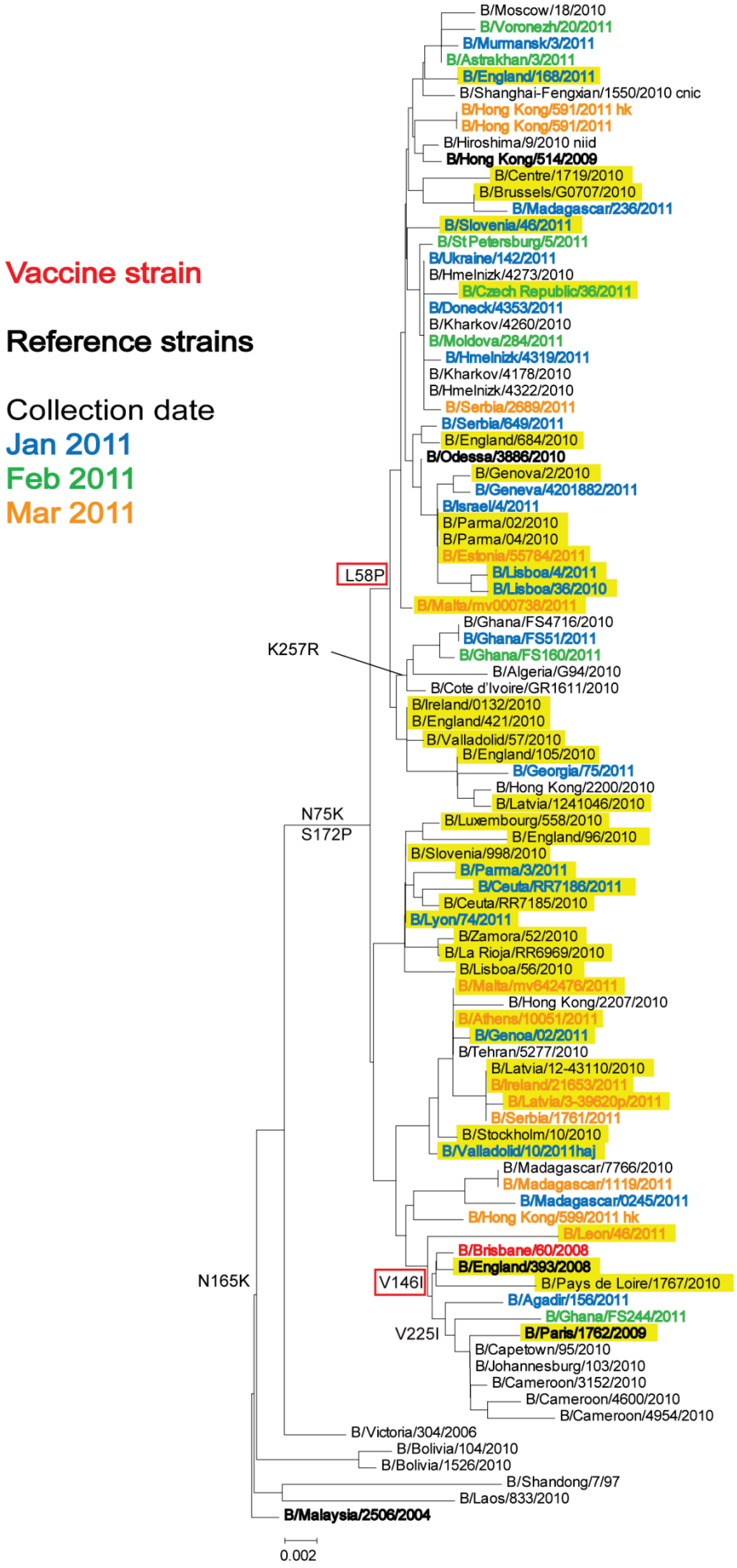

Table 5 Antigenic analysis of influenza B/Yamagata lineage viruses by HI (turkey RBCs)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre						
				Post infection ferret sera					
			B/Fl[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 F09/08	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	5120	640	1280	1280	40	320	160
B/Florida/4/2006	2006-12-15	E3/E4	5120	640	1280	640	80	320	320
B/Brisbane/3/2007	2007-09-03	E2/E4	2560	640	2560	640	80	320	160
B/England/145/2008		Ex/E5	640	40	160	40	80	20	10
B/Bangladesh/3333/2007	2007-08-07	E3/E4	2560	320	640	160	40	320	320
B/Wisconsin/1/2010	2010-02-20	E3/E2	1280	160	640	160	20	160	320
TEST VIRUSES									
B/England/172/2011	2011-01-04	MDCK2/MDCK1	2560	640	640	<	320	320	160
B/England/169/2011	2011-01-06	SIAT2/MDCK3	5120	1280	1280	<	640	320	320
B/Norway/490/2011	2011-01-24	MDCK1/MDCK3	5120	1280	1280	640	320	320	160
B/Stockholm/2/2011	2011-02-09	MDCK1/MDCK1	5120	1280	1280	640	320	640	320
B/Stockholm/12/2011	2011-03-28	MDCK0/MDCK1	5120	1280	1280	320	320	320	160

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