SURVEILLANCE REPORT

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

Influenza virus characterisation

Summary Europe, February 2011

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EU and affiliated countries: Summary of NIMR virology results presented to WHO Northern Hemisphere Vaccine Recommendation Meeting for 2011–12 season.

Samples received from EU and affiliated countries (collected from September 2010 until January 2011)

The numbers of viruses and clinical samples received from EU and affiliated countries are listed in **Table 1**; close to 500 samples were received. Batches from countries that had not been completed at the time of the report are shown as in process.

Influenza A(H1N1) virus analysis

Table 2 shows representative HI results for viruses analysed based on isolates derived from specimens collected between November 2010 and January 2011 from EU and affiliated countries. The vast majority of viruses show good reactivity with the panel of post-infection ferret antisera used, including that raised against the current vaccine virus A/California/7/2009. Viruses with reduced activity (eight-fold or greater against the serum raised against the vaccine virus) have been derived from specimens collected in Northern Ireland, France, Sweden, Latvia, England and Germany. These represented approximately 15% of the total of viruses tested by HI at the London WHO CC and approximately 20% of viruses collected from within the EU and affiliated countries. For viruses whose HA genes have been sequenced, the majority of the viruses showing reduced HI titres carry amino acid substitutions at positions 153-155 in the HA1 protein and the HA genes of these viruses do not cluster in phylogenetic analyses (**Figure 1**); it is known that changes in this region are frequently associated with the cell substrate used for isolation and propagation of the virus.

Figure 1 shows a phylogenetic tree for the HA1-coding region of selected H1N1 viruses. Prior to the autumn of 2010 two emerging genetic groups within the HA had been detected: a Southern Hemisphere (SH) group (key changes N125D, some with V250A and others) and a Hong Kong group (key changes S128P, V199A, I295V). Sequence analysis of the viruses characterised in the London WHO CC has revealed only limited geographic spread of viruses in the Hong Kong group but substantial spread of those in the SH group. In addition, three other genetic groups have emerged in recent months characterised by amino acid substitutions:

- (i) A134T, S183P – now seen in at least four countries in the EU and affiliated countries, as well as elsewhere;
- (ii) R205K, I216V, V249L – now seen in at least two countries of the EU and affiliated countries, as well as elsewhere;
- (iii) S185T– the group showing the greatest expansion and wide geographic spread.

The substitution D97N is also associated with viruses in groups (ii) and (iii) and some viruses in group (iii) carry a S143G substitution. A further group characterised by amino acid substitutions N31D, S162N, A186T and V272I and represented

by viruses from Denmark (and South Africa) has not expanded in recent months. To date, none of the genetic groups has shown evidence of significant difference in antigenicity (as assessed by HI results) compared with the current vaccine virus. The location of amino acid positions that define specific genetic groups are shown on an H1-HA structure (**Figure 2**). Some of the substitutions are prominently exposed on the surface of the HA trimer (125, 134, 183, 185, 216) but others are buried.

Considerable heterogeneity has accumulated in the N1 NA gene but HA and NA phylogenetic trees are generally congruent.

Influenza A(H3N2) virus analysis

Polymorphism in the gene sequence encoding position 151 of the N2 neuraminidase within a virus isolate can result in agglutination of red blood cells through the NA. To circumvent the effect of position 151 polymorphism or any other changes in the NA within a virus isolate affecting the agglutination of red blood cells, virus neutralisation (VN) assays, measured by plaque reduction, have been carried out at the London WHO CC to complement the HI test. In addition HI assays have been performed in the presence of oseltamivir to overcome NA-mediated agglutination of erythrocytes.

Over 60 A(H3N2) viruses have been received from countries in the EU and from affiliated countries (**Table 1**)**.** Many viruses have been received recently and, at the time of preparation of this report, had yet to be fully analysed.

The results of virus neutralisation tests are shown in **Table 3**, and the results of HI tests carried out in the presence of oseltamivir in **Tables 4 and 5**. The results of the plaque reduction assays show that the majority of viruses react with antiserum raised against the vaccine virus A/Perth/16/2009 showing titres within four-fold of the homologous titre although two viruses showed reduced titres. HI assay carried out with guinea pig red blood cells in the presence of oseltamivir revealed a greater proportion of test viruses that showed low reactivity with the ferret antiserum raised against the vaccine virus. **Table 4** shows that the reference virus A/Alabama/5/2010 also showed, like many of the test viruses, a low reactivity with the antiserum raised against A/Perth/16/2009 but the antiserum raised against A/Alabama/5/2010 reacted well against the vaccine virus A/Perth/16/2009, as it did with the vast majority of the test viruses. This difference in reactivity represents a one-way antigenic difference. Antisera raised against two reference viruses shown in the **Table 4** show high homologous titres but reduced titres against some closely related viruses and high titres against genetically less closely related viruses. **Table 5** shows the results of HI assays using human type O+ red blood cells carried out in the presence of oseltamivir. Some viruses show a reduction in titre compared with antiserum raised against A/Perth/16/2009, but react well with antiserum raised against the closely related reference virus A/Wisconsin/15/2009.

Genetic analysis of the HA gene of H3N2 viruses is illustrated in **Figure 3**. Several HA genetic groups, observed previously, have been maintained. Viruses still fall into the Perth/16 and Victoria/208 clades with genetic groups emerging within each clade. Within the Perth/16 clade genetic groups can be defined by: key substitutions N133D (loss of glycosylation site), R142G & V213A (group A); and key substitutions I260M & R261Q, combined with P162S and also E50K (group B). Within the Victoria/208 clade genetic groups can be defined by: key substitutions N312S, with subgroups carrying T48A, K92R; a group defined by S45N (gain of glycosylation site); a group defined by N145S, V223I with N144D (loss of glycosylation site) or V323I; and a large group carrying substitutions D53N, Y94H, I230V, E280A with some viruses within this group additionally carrying S199A. These groups are shown on the structures of the HA trimer in **Figure 4**.

For viruses within the Perth/16 clade genetic groups A and B (n=6) all but one virus (A/Hessen/5/2010) showed good inhibition with the antiserum panel. Within the Victoria/208 clade genetic groups a large proportion of viruses showed low reactivity against antiserum raised against A/Perth/16/2009 but fewer did so with antisera raised against A/Wisconsin/15/2009 (a virus genetically closely related to A/Perth/16/2009). Viruses in the Victoria/208 clade within the genetic group defined by D53N, Y94H, I230V and E280A generally showed a lower level of reactivity to antiserum raised against A/Perth/16/2009 but had higher HI titres with the antisera raised against either A/Wisconsin/15/2009 or A/Alabama/5/2010 (a virus that clusters within the Victoria/208 clade).

Several viruses showed low titre reactivity in the HI assay carried out on human red blood cells but sequence data are not currently available.

The amino acid substitutions within each clade (**Figure 4**) and genetic group are located on the surface of the HA trimer in many cases, notably so in the Victoria/208 clade genetic group defined by D53N, Y94H, I230V, E280A and S199A.

The NA gene, like the HA gene, shows evidence of genetic change with the emergence of genetic groups. The NA gene can be divided into three main groups: Perth/16 clade groups A and B, and the Victoria/208 clade.

Influenza B virus analyses

The vast majority of influenza B viruses collected in EU and affiliated countries have been of the B/Victoria lineage (> 85%).

B/Victoria lineage viruses

Representative results of antigenic analysis of influenza B/Victoria lineage viruses by HI assay using turkey red blood cells are shown in **Table 6**. As expected, the majority of viruses showed low reactivity with sera raised against viruses

propagated in hens' eggs. All cell grown test and reference viruses analysed consistently showed low reactivity against the antiserum raised against egg-grown B/Brisbane/60/2009 and better reactivity against those antisera raised against the more recently isolated cell grown reference viruses B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010.

Two viruses were exceptional; B/England/81/2010 and B/England/121/2010 showed low reactivity with all antisera raised against the most recent reference strains.

A phylogenetic tree for influenza B Victoria-lineage HA genes is shown in **Figure 5**. The phylogenetic tree illustrates that the vast majority of viruses belong to the Brisbane/60 clade. Most recently collected viruses carry the amino acid substitution I146V and a significant number also carry the substitution L58P. B/England/81/2010 and B/England/121/2010, the viruses that showed low reactivity with all antisera raised against the most recent reference viruses, can be seen to group into two clades at the bottom of the phylogenetic tree- one in clade 4 and one in clade 5. Viruses in clade 4 and clade 5 are genetically more closely related to the B/Malaysia/2506/2004 prototype virus than they are to viruses of the Brisbane/60 clade.

The vast majority of the NA genes of the B/Victoria lineage viruses belong to the Brisbane/60 clade. There are numerous amino acid substitutions observed in the NA but the significance of these is unclear. No evidence for reassortment between clades of the influenza B/Victoria lineage is apparent.

B/Yamagata lineage viruses

Less than 30 influenza B/Yamagata lineage viruses have been received from the EU and affiliated countries (**Table 1**).

The results of antigenic analysis of influenza B/Yamagata lineage viruses by HI assay using turkey red blood cells are shown in **Table 7**. Most viruses showed good reactivity with antisera raised against viruses of the Bangladesh/3333 clade (these include B/Algeria/G-486/2010 and B/Wisconsin/1/2010).

A phylogenetic tree of influenza B/Yamagata lineage HA genes is shown in **Figure 6**, which illustrates that all viruses received from the EU and affiliated countries fell into the Bangladesh/3333 clade.

Antiviral resistance testing

Antiviral resistance testing has been performed on A(H1N1)2009 viruses using a sialidase inhibition assay with MUNANA as substrate and zanamivir and oseltamivir as inhibitors. Eighty A(H1N1)2009 viruses received from EU and affiliated countries have been propagated to sufficient titre for assay of resistance to zanamivir and oseltamivir. None showed resistance to either antiviral drug.

Full NA gene sequencing for influenza A and B viruses have confirmed the lack/low levels of neuraminidase inhibitor resistance markers among circulating viruses. In terms of resistance to amantadine and rimantadine, M-gene sequencing has shown all pandemic A(H1N1) 2009 and A(H3N2) viruses analysed to be resistant due to S31N substitution in the M-2 ion channel protein.

Summary

Few A(H1N1)2009 viruses show reduced reactivity to post-infection ferret antisera raised against the vaccine virus A/California/7/2009. The majority of these viruses showed good reactivity with sera raised against viruses that have substitutions between 154 and 156 of the HA and many of the viruses with low HI titres showed similar changes that were not present in the clinical sample; the substitutions were most likely associated with the substrate for isolation.

Antigenic analysis of H3N2 viruses is difficult to interpret clearly. Many viruses show reduced or low reactivity with antiserum raised against the prototype virus and the vaccine virus A/Perth/16/2009, and yield better titres with antisera raised against viruses isolated more recently in HI assays. However, this is not a consistent pattern seen within viruses of the same genetic groups.

Amongst the influenza B viruses, B/Victoria lineage viruses have predominated. Genetically the vast majority of viruses of the B/Victoria lineage were from the Brisbane/60 clade. Generally, the viruses from this clade showed good titres with antisera raised against cell-propagated reference viruses genetically closely related to the vaccine strain.

Among recently circulating A(H1N1)2009 viruses, no resistance to oseltamivir and/or zanamivir has been detected.

Table 1 Summary of specimens received and collected since 1 September 2010

MONTH Country	A Untyped	H1N1pdm Number received	 A/California/ 7/2009-like	H3N2 Number received	 A/Perth/ 16/2009-like*	B Untyped	B Yamagata lineage Number received	 B/Florida/ 4/2006-like	B Victoria lineage Number received	 B/Brisbane/ 60/2008-like
SEPTEMBER										
Sweden				2	2					
United Kingdom				2	1					
OCTOBER										
Belgium				2	in process	1				
France		1	1						4	4
Germany				2	in process		1	1		
Greece				1	in process					
Norway		1	1	1	in process					
Portugal									4	0
Spain				1	in process					
United Kingdom		2	2	1	in process				2	2
NOVEMBER										
Belgium	3	1	0			4			2	2
Denmark				1	1					
Finland		1	1				1	1		
France		2	2	6	6		1	1	5	5
Germany		1	1							
Italy	2	3	3						3	2
Norway		1	1				3	3		
Portugal				1	in process				16	2
Slovenia									1	1
Spain		5	5						1	0
Sweden		2	2	2	2		1	1	1	1
United Kingdom		10	10	2	in process		1	1	8	8
DECEMBER										
Austria		6	4							
Belgium	3	20	14	2	1	10	3	3	17	17
Denmark		5	5							
Finland		2	2	1	1		2	2		
France		20	12	19	11		2	2	9	9
Germany		8	8	3	in process		4	4	7	7
Ireland		10	8						2	2
Italy		3	1	1	in process				6	6
Latvia		5	5						2	2
Luxembourg		5	3						1	1
Netherlands				1	1					
Norway		1	1	1	1				7	6
Portugal		1	0						14	8
Romania		1	1	2	2					
Slovenia		6	5						2	2
Spain		14	9						4	3
Sweden	2	6	6						4	3
United Kingdom		14	12	1	1		3	3	8	8
JANUARY										
Belgium	2								2	1
Germany									2	1
France							1	1	3	3
Greece	1	28	in process	2	1	3				
Ireland				2	in process					
Italy		8	8	1	in process				3	2
Latvia	1	3	3							
Netherlands		1	1	1	1	1				
Romania		2	1	1	in process					
Slovenia		14	14						3	3
Spain		14	in process			1				
Sweden				1	1					
United Kingdom		1	1							
UNKNOWN										
Received after 01/09/2010										
Belgium		1	1							
France		1	1							
Reunion		2	2	1	in process				1	1
United Kingdom				2	in process					
Total Received = 499	14	232	157	66	33	20	23	23	144	112

* 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) 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/Chch 16/2010 F30/10
REFERENCE VIRUSES									
A/California/7/2009	2009-04-09	E2/E5	2560	2560	2560	1280	2560	2560	1280
A/England/195/2009	2009-04-28	MDCK1/MDCK4	2560	2560	2560	1280	2560	2560	1280
A/Auckland/3/2009	2009-04-25	Ex/E3	2560	5120	5120	1280	2560	2560	2560
A/Bayern/69/2009	2009-07-01	MDCK4/SIAT1	80	80	80	320	320	160	80
A/Lviv/N6/2009	2009-10-27	MDCK5	160	160	160	1280	2560	640	320
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	2560	5120	2560	5120	2560	5120
TEST VIRUSES									
A/Baden-Wurttemberg/11/2010	2010-11-19	C3/MDCK1	1280	640	640	1280	2560	1280	640
A/Norway/1278/2010	2010-11-22	MDCK1MDCK1	1280	2560	2560	640	1280	2560	640
A/Picardie/1814/2010	2010-11-22	C2/MDCK1	1280	2560	2560	640	1280	2560	640
A/Norway/1326/2010	2010-12-05	MDCK1MDCK1	2560	2560	2560	1280	2560	2560	1280
A/Finland/6/2010	2010-12-09	E12/E1	2560	2560	2560	640	2560	2560	1280
A/Paris/1958/2010	2010-12-10	C2/MDCK1	1280	1280	1280	640	1280	1280	640
A/Stockholm/14/2010	2010-12-12	C1/MDCK1	2560	2560	5120	1280	2560	2560	1280
A/Stockholm/15/2010	2010-12-13	C3/MDCK1	160	160	160	640	1280	320	160
A/Pays de Loire/2059/2010	2010-12-14	C2/MDCK1	2560	2560	2560	640	1280	1280	1280
A/Latvia/12-35825/2010	2010-12-14	MDCK1/MDCK1	640	640	640	640	2560	640	2560
A/Nordrhein-Westfalen/11/2010	2010-12-16	C3/MDCK1	80	160	160	320	320	320	80
A/Austria/592212/2010	2010-12-17	C2/MDCK1	1280	2560	1280	640	1280	1280	1280
A/Paris/2227/2010	2010-12-18	C1/MDCK1	1280	1280	1280	320	640	1280	640
A/Latvia/12-39098/2010	2010-12-19	MDCK1/MDCK1	1280	1280	1280	320	1280	1280	640
A/Baden-Wurttemberg/13/2010	2010-12-20	C2/MDCK1	640	320	640	1280	1280	640	320
A/Nordrhein-Westfalen/12/2010	2010-12-20	C2/MDCK1	1280	1280	1280	640	2560	2560	640
A/Austria/592642/2010	2010-12-21	C2/MDCK1	1280	2560	1280	640	1280	1280	1280
A/Latvia/12-756p/2010	2010-12-22	MDCK1/MDCK2	640	640	1280	640	1280	640	640
A/Nordrhein-Westfalen/14/2010	2010-12-22	C2/MDCK1	160	160	320	640	640	320	320
A/Brest/2244/2010	2010-12-23	C1/MDCK1	1280	2560	2560	640	1280	2560	1280
A/Brussels/S0205/2010	2010-12-24	MDCK2	1280	2560	2560	640	640	1280	640
A/England/537/2010	2010-12-24	SIAT1/MDCK1	2560	2560	2560	640	2560	2560	1280
A/Brussels/S0204/2010	2010-12-26	MDCK3	1280	1280	2560	1280	1280	2560	1280
A/Latvia/12-72162/2010	2010-12-27	MDCK1/MDCK2	2560	640	1280	640	2560	640	640
A/Nordrhein-Westfalen/13/2010	2010-12-27	C2/MDCK1	160	160	160	1280	640	320	160
A/Baden-Wuttermberg/14/2010	2010-12-27	C3/MDCK1	40	80	160	160	320	80	80
A/Centre/2305/2010	2010-12-28	C1/MDCK1	1280	1280	1280	640	1280	2560	5120
A/Nord Pas de Calais/2289/2010	2010-12-28	C1/MDCK1	2560	2560	2560	640	1280	2560	1280
A/Pays de Loire/2308/2010	2010-12-28	C1/MDCK1	1280	2560	2560	640	1280	1280	640
A/Luxembourg/548/2010	2010-12-28	C1/MDCK1	2560	2560	2560	640	1280	2560	1280
A/Zamora/62/2010	2010-12-29	MDCK2/MDCK1	1280	1280	2560	640	1280	1280	640
A/Berlin/12/2010	2010-12-29	C3/MDCK1	80	80	160	640	320	320	160
A/Luxembourg/562/2010	2010-12-29	C1/MDCK1	1280	2560	1280	640	2560	1280	640
A/England/375/2010	2010-12-31	SIAT1/MDCK1	320	320	320	160	320	320	160
A/Slovenia/89/2011	2011-01-07	MDCKx/MDCK1	1280	2560	1280	640	1280	1280	640
A/Latvia/01-33865/2011	2011-01-10	MDCK/MDCK1	1280	2560	2560	640	1280	1280	640
A/Northern Ireland/2/2011	2011-01-10	MDCK1/MDCK1	160	160	160	2560	2560	640	320
A/Slovenia/114/2011	2011-01-10	MDCKx/MDCK1	1280	1280	1280	640	1280	1280	640

Sequence included in HA phylogeny

Vaccine virus

1. = starting serum dilution 1/40

Viruses showing ≥8-fold reduction in HI titre compared to A/California/7/2009 homologous titre

Table 3 Antigenic analysis of A(H3N2) viruses by plaque reduction neutralisation on MDCK-SIAT cells

Viruses	Collection Date	Passage History	Neutralisation titre[1] Post infection ferret sera				
			A/Bris 10/07 F29/09	A/Per 16/09 F30/09	A/Vic 208/09 F07/10	A/Ala 5/10 CDC F38/11	A/Ala 5/10 F27/10
REFERENCE VIRUSES							
A/Brisbane/10/2007	2007-02-06	E2/E4	>5120	80	320	320	80
A/Perth/16/2009	2009-07-04	E3/E4	320	1280	640	640	320
A/Victoria/208/2009	2009-06-02	E3/E2	320	640	2560	1280	640
A/Alabama/5/2010	2010-07-13	MK1/MDCK2/SIAT2	80	160	80	1280	640
TEST VIRUSES							
A/Gothenburg/1/2010	2010-09-10	C2/SIAT1	40	160	80	640	320
A/Stockholm/6/2010	2010-09-29	C2/SIAT1	40	2560	160	1280	640
A/Baleares/RR6848/2010	2010-10-26	SIAT2/SIAT2	80	160	80	320	640
A/Stockholm/10/2010	2010-11-19	C1/SIAT1	40	640	80	320	160
A/Poitiers/1071/2010	2010-11-19	SIAT3	40	320	80	1280	640
A/Denmark/105/2010	2010-11-22	MDCK4/SIAT1	80	640	160	1280	640
A/England/270/2010	2010-12-06	SIAT1/SIAT1	80	320	160	1280	320
A/Lyon/1014/2010	2010-12-06	MDCK2/SIAT1	40	640	160	1280	640
A/Lyon/1058/2010	2010-12-10	MDCK2/SIAT1	80	320	160	1280	640
A/Paris/1992/2010	2010-12-11	C2/SIAT1	80	640	320	2560	640
A/Paris/2005/2010	2010-12-13	C2/SIAT1	80	640	160	2560	640
A/Bremen/1/2010	2010-12-17	C2/SIAT1	80	640	160	2560	640
A/Lyon/1135/2010	2010-12-19	MDCK2/SIAT1	80	320	160	1280	640
A/Bretagne/2248/2010	2010-12-25	C1/SIAT1	40	320	160	1280	640
A/Stockholm/17/2010	2011-01-03	C0/SIAT1	40	320	160	1280	640
A/Athens/108/2010	2011-01-16	SIAT3	80	320	160	1280	640

Sequence included in HA phylogeny

Vaccine Virus

[1] Based on 50% plaque reduction compared to serum negative controls

Viruses showing ≥8-fold reduction in VN titre compared to A/Perth/16/2009 homologous titre

Table 4 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 F1/06	A/Bris 10/07 F29/08	A/Perth 16/09 F25/09	A/Wis 15/09 F24/09	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Alab 5/10 CDC F38/11
REFERENCE VIRUSES									
A/Wisconsin/67/2005	2005-08-31	SpfCk3E3/E10	2560	640	<	<	160	40	<
A/Brisbane/10/2007	2007-02-06	E2/E4	2560	5120	40	40	320	320	160
A/Perth/16/2009	2009-07-04	E3/E4	80	80	1280	640	2560	2560	1280
A/Wisconsin/15/2009	2009-07-06	E2/E3	<	<	640	320	40	160	320
A/Victoria/208/2009	2009-06-02	E3/E1	320	320	1280	1280	5120	5120	5120
A/Victoria/210/2009	2009-06-02	E2/E1	320	320	1280	1280	2560	5120	5120
A/Alabama/5/2010	2010-07-13	MK1/M2/S3	<	40	80	40	<	<	640
TEST VIRUSES									
A/Gothenburg/1/2010	2010-09-10	C2/SIAT1	<	40	80	160	160	160	640
A/Stockholm/6/2010	2010-09-29	C2/SIAT1	40	<	1280	160	160	640	1280
A/Brussels/G0515/2010	2010-10-18	SIAT3	<	<	320	320	160	160	640
A/Brussels/G0550/2010	2010-10-27	SIAT3	<	80	80	80	80	80	640
A/Stockholm/7/2010	2010-11-12	C0/SIAT1	40	<	1280	320	320	640	1280
A/Stockholm10//2010	2010-11-19	C1/SIAT1	<	<	640	80	80	320	640
A/Denmark/105/2010	2010-11-22	MDCK4/SIAT1	<	80	320	160	160	160	640
A/Paris/2031/2010	2010-11-25	C2/SIAT2	<	160	160	320	320	320	1280
A/Paris/1843/2010	2010-11-27	C1/SIAT1	<	40	160	320	320	320	640
A/Paris/1838/2010	2010-11-29	C1/SIAT2	<	160	320	160	320	320	1280
A/Paris/1844/2010	2010-11-29	C1/SIAT1	40	<	640	1280	320	640	640
A/Pays de Loire/1883/2010	2010-12-01	C2/SIAT2	<	80	160	160	160	160	640
A/Paris/1896/2010	2010-12-01	C1/SIAT2	<	80	160	160	160	160	640
A/Norway/1330/2010	2010-12-03	MDCK1/SIAT1	<	<	80	320	80	80	80
A/Paris/2119/2010	2010-12-10	C1/SIAT2	<	160	160	320	320	320	640
A/Paris/2120/2010	2010-12-10	C1/SIAT2	<	80	80	160	160	160	640
A/Hessen/5/2010	2010-12-13	C3/SIAT1	<	<	80	320	80	80	80
A/Paris/2106/2010	2010-12-17	C1/SIAT2	<	160	160	320	320	320	640
A/Paris/2260/2010	2010-12-17	C1/SIAT2	<	80	160	320	320	320	640
A/Brussels/G0801/2010	2010-12-20	SIAT3	<	40	80	80	80	160	640
A/Paris/2174/2010	2010-12-21	C1/SIAT2	<	80	160	320	320	320	640
A/Finland/40/2010	2010-12-22	MDCK2/SIAT1	<	160	640	320	320	320	1280
A/Paris/2291/2010	2010-12-29	SIAT1/SIAT2	<	80	160	320	160	160	640
A/Stockholm/17/2010	2011-01-03	C0/SIAT1	<	40	80	80	80	160	640

Sequences included in HA phylogeny

Vaccine virus

1. < = <40

Viruses showing >8-fold reduction in VN titre compared to A/Perth/16/2009 homologous titre

Table 5 Antigenic analysis of A(H3N2) viruses by HI (human type O+ 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 F29/08	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 CDCF38/11	A/Perth 10/2010 F03/11
REFERENCE VIRUSES										
A/Wisconsin/67/2005	2005-08-31	SpfCk3E3/E10	2560	2560	40	<	320	40	<	160
A/Brisbane/10/2007	2007-02-06	E2/E4	2560	5120	40	40	320	320	160	160
A/Perth/16/2009	2009-07-04	E3/E4	40	160	1280	640	640	1280	1280	1280
A/Wisconsin/15/2009	2009-07-06	E2/E3	<	<	640	320	80	160	320	640
A/Victoria/208/2009	2009-06-02	E3/E1	160	160	1280	1280	5120	5120	2560	5120
A/Victoria/210/2009	2009-06-02	E2/E1	160	160	1280	1280	2560	5120	2560	2560
A/Alabama5/2010	2010-07-13	MK2/M2/S3	<	<	80	40	40	40	640	320
A/Perth/10/2010	2010-05-25	E2/E2	<	<	320	80	80	80	640	320
TEST VIRUSES										
A/Brussels/SO066/2010	2010-11-09	SIAT3	<	<	160	160	160	160	320	160
A/Poitiers/1071/2010	2010-11-19	MDCKx+1/SIAT1	40	80	320	640	320	320	1280	1280
A/Lyon/1014/2010	2010-12-06	MDCK2/SIAT1	40	40	640	1280	640	640	2560	1280
A/England/270/2010	2010-12-06	SIAT1/SIAT1	<	80	160	160	80	80	1280	640
A/Lyon/1058/2010	2010-12-10	MDCK2/SIAT1	<	80	320	640	320	320	1280	1280
A/Lyon/1135/2010	2010-12-19	MDCK2/SIAT1	<	80	320	320	320	320	1280	1280
A/Netherlands/34/2010	2010-12-23	xMDCK2/SIAT1	<	40	160	80	80	80	1280	320
A/Mures/47467/2011	2010-12-27	MDCK1	<	160	640	1280	640	640	1280	2560
A/Galati/47519/2011	2010-12-29	MDCK2/SIAT3	<	160	1280	1280	1280	2560	1280	5120
A/Netherlands/63/2011	2011-01-05	xMDCK2/SIAT1	<	40	640	640	160	640	1280	640
A/Athens/108/2011	2011-01-16	SIAT3	<	80	320	320	320	320	1280	640

1. < = <40

Vaccine virus

Viruses showing ≥8-fold reduction in VN titre compared to A/Perth/16/2009 homologous titre

Table 6 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/Mal[2] 2506/04 SH456	B/Bris[2] 60/08 SH524	B/Mal 2506/04 F28/05	B/Vic 304/06 F16/06	B/Bris 60/08 F5/09	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	2560	1280	1280	320	160	<	<	10
B/Victoria/304/2006	2006-06-06	E2/E4	640	1280	640	640	320	<	<	10
B/England/393/2008	2008-08-29	E1/E6	640	2560	320	320	1280	160	160	160
B/Brisbane/60/2008	2008-08-04	E4/E4	640	2560	320	320	1280	160	160	80
B/Paris/1762/2008[3]	2009-02-09	C2/MDCK4	<	1280	40	<	80	320	320	320
B/Hong Kong/514/2009[3]	2009-10-11	MDCK1/MDCK2	<	1280	40	<	80	320	640	320
B/Odessa/3886/2010[3]	2010-03-19	MDCK2/MDCK1	<	2560	40	<	40	320	640	320
TEST VIRUSES										
B/England/81/2010	2010-10-19	SIAT1/MDCK1	160	40	20	10	<	<	<	<
B/England/121/2010	2010-11-19	SIAT1/MDCK1	320	160	40	40	<	<	<	<
B/Centre/1719/2010	2010-11-04	C1/MDCK1	<	640	10	<	20	40	160	160
B/Pays de Loire/1767/2010	2010-11-15	C1/MDCK1	<	640	20	<	40	40	320	160
B/Bretagne/1775/2010	2010-11-16	C1/MDCK1	<	320	10	<	20	40	160	80
B/Slovenia/961/2010	2010-11-29	MDCK1/MDCK1	<	1280	40	<	40	160	160	320
B/Centre/1858/2010	2010-11-30	C1/MDCK1	<	640	10	<	20	40	160	80
B/Centre/1876/2010	2010-12-01	C1/MDCK1	<	1280	10	<	20	80	160	160
B/Zamora/52/2010	2010-12-01	MDCK1/MDCK2	<	320	10	<	20	40	80	80
B/Lisboa/15/2010	2010-12-03	MDCK1/MDCK2	<	1280	20	<	40	80	320	160
B/Slovenia/998/2010	2010-12-06	MDCK1/MDCK1	<	1280	40	<	40	160	160	320
B/Lisboa/13/2010	2010-12-07	MDCK1/MDCK1	<	640	10	<	20	40	80	160
B/Lisboa/21/2010	2010-12-08	MDCK1/MDCK2	<	1280	20	<	40	80	320	160
B/Norway/1379/2010	2010-12-08	MDCK1/MDCK2	<	1280	20	<	40	40	320	160
B/Norway/1360/2010	2010-12-09	MDCK1/MDCK1	<	640	10	<	20	40	160	160
B/Stockholm/10/2010	2010-12-10	C0/MDCK1	<	640	20	10	40	80	640	160
B/Paris/2087/2010	2010-12-17	C1/MDCK1	<	1280	20	<	40	80	320	160
B/Brussels/S0183/2010	2010-12-18	MDCK2	<	1280	20	<	20	320	320	160
B/Lyon/1140/2010	2010-12-18	MDCK2/MDCK1	<	2560	20	<	40	160	640	160
B/England/511/2010	2010-12-19	MDCK1/MDCK1	<	2560	20	<	40	160	160	320
B/Rheinland-Pfalz/1/2010	2010-12-20	C2/MDCK1	<	640	20	<	40	80	320	80
B/Valladolid/57/2010	2010-12-20	MDCK1/MDCK2	<	1280	20	<	40	160	160	320
B/England/505/2010	2010-12-21	SIAT1/MDCK1	<	2560	40	<	80	320	320	640
B/Poitiers/36/2011	2010-12-21	MDCKx+1/MDCK1	<	2560	40	<	40	160	640	160
B/Latvia/12-41046/2010	2010-12-23	MDCK1/MDCK1	<	640	20	<	40	80	320	160
B/Brussels/S0190/2010	2010-12-24	MDCK2	<	1280	20	<	20	160	320	160
B/Limoges/1160/2010	2010-12-24	MDCK2/MDCK1	<	1280	20	<	40	160	320	160
B/Bremen/4/2010	2010-12-27	C2/MDCK1	<	640	20	<	20	80	320	160
B/Latvia/12-43110/2010	2010-12-27	MDCKx/MDCK2	<	640	10	<	20	40	160	80
B/Nord Pas de Calais/2314/2010	2010-12-28	C1/MDCK1	<	640	10	<	40	80	320	160
B/Luxembourg/558/2010	2010-12-29	C1/MDCK1	<	320	10	<	20	40	40	80
B/Niedersachsen/3/2010	2010-12-29	C2/MDCK1	<	640	20	<	40	80	320	160
B/Nordrhein-Westfalen/5/2010	2010-12-29	C2/MDCK1	<	640	10	<	20	40	160	80
B/Paris/2312/2010	2010-12-30	C1/MDCK1	<	640	10	<	40	80	320	160
B/Lyon/74/2011	2011-01-03	MDCK2/MDCK1	<	1280	20	<	40	160	320	160
B/Nordrhein-Westfalen/1/2011	2011-01-03	C2/MDCK1	<	1280	40	10	80	160	320	320
B/Pays de Loire/2382/2010	2011-01-03	C1/MDCK1	<	1280	10	<	40	80	320	160
B/Slovenia/31/2011	2011-01-03	MDCK2/MDCK1	<	1280	20	<	40	160	640	160
B/Slovenia/46/2011	2011-01-04	MDCK2/MDCK1	<	1280	40	<	80	160	640	160

1. < = <10; 2. hyperimmune sheep serum; 3. B/Brisbane/60/2008-like cell-grown viruses

Vaccine virus

Sequences included in HA phylogeny

Viruses showing >8-fold reduction in HI titre compared to B/Brisbane/60/2008-like cell grown viruses.

Table 7 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 F3/04	B/Fl[1] 4/06 F1/10	B/Bris[1] 3/07 F24/07	B/Eng[2] 145/08 F9/08	B/Bang[2] 3333/07 F25/08	B/Alg[2] G-486/10 F15/10	B/Wis[2] 1/10 F23/10
REFERENCE VIRUSES										
B/Egypt/144/2005	2005-05-01	E3/E6	2560	80	320	320	40	80	20	40
B/Florida/4/2006	2006-12-15	E3/E4	5120	320	1280	1280	160	160	320	320
B/Brisbane/3/2007	2007-09-03	E2/E1	2560	160	640	640	80	80	40	80
B/England/145/2008		Ex/E4	320	<	40	<	80	10	20	10
B/Bangladesh/3333/2007	2007-08-07	E3/E4	1280	80	320	160	40	80	40	40
B/Algeria/G-486/2010	2010-06-06	SIAT0/MDCK3	5120	160	320	320	160	160	1280	80
B/Wisconsin/1/2010	2010-02-20	E3/E2	640	40	160	160	20	40	20	80
TEST VIRUSES										
B/Norway1181//2010	2010-11-02	MDCK1/MDCK1	5120	160	640	320	320	160	640	160
B/Stockholm/6/2010	2010-11-09	C1/MDCK1	5120	320	640	320	320	320	640	160
B/Paris/1850/2010	2010-11-29	C1/MDCK1	2560	80	160	40	80	80	160	40
B/Finland/39/2010	2010-11-30	MDCK1/MDCK1	5120	160	320	160	80	40	160	80
B/England/170/2010	2010-12-01	SIAT1/MDCK1	5120	320	640	80	320	320	ND	320
B/Brussels/G0710/2010	2010-12-13	MDCK2	5120	80	160	80	160	80	320	40
B/England/499/2010	2010-12-14	SIAT1/MDCK1	5120	320	640	320	320	320	ND	320
B/Brussels/G0724/2010	2010-12-15	MDCK3	5120	160	320	80	160	160	ND	160
B/Niedersachsen/2/2010	2010-12-16	C2/MDCK1	1280	40	80	40	80	80	160	40
B/Finland/33/2010	2010-12-16	MDCK1/MDCK1	2560	80	160	80	80	40	80	40
B/Brussels/G0786/2010	2010-12-18	MDCK2/MDCK1	5120	320	640	160	320	160	ND	160
B/England/512/2010	2010-12-20	SIAT1/MDCK1	5120	640	1280	320	320	320	ND	320
B/Nordrhein-Westfalen/1/2010	2010-12-22	C1/MDCK2	5120	160	320	640	320	320	1280	320
B/Berlin/1/2010	2010-12-27	C2/MDCK1	5120	80	160	160	160	160	640	160
B/Bretagne/2278/2010	2010-12-27	C1/MDCK1	1280	80	160	80	160	80	320	80
B/Berlin/2/2010	2010-12-28	C2/MDCK1	5120	160	160	160	320	160	640	160
B/Poitiers/128/2011	2010-12-29	MDCKx+1/MDCK1	5120	320	1280	320	320	320	ND	320
B/Finland/34/2010	2010-12-30	MDCK1/MDCK1	2560	80	320	80	40	20	80	40
B/Lyon/68/2011	2011-01-05	MDCK2/MDCK1	5120	320	640	160	320	320	ND	320

1. < = <40; 2. < =<10 ; 3. hyperimmune sheep serum; ND=not done

Sequences included in the HA phylogeny

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

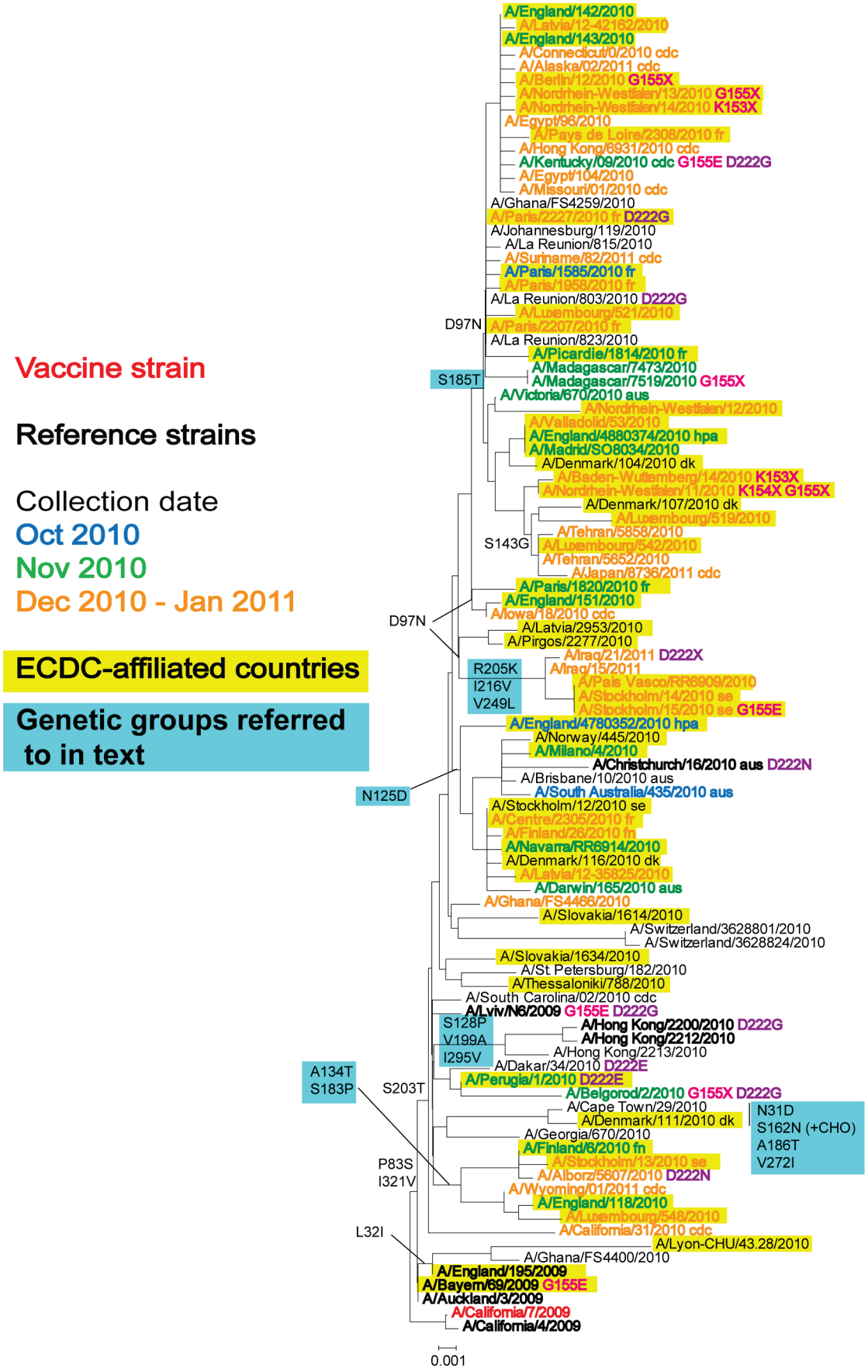


Figure 2 H1 HA location of genetic cluster defining amino acid substitutions

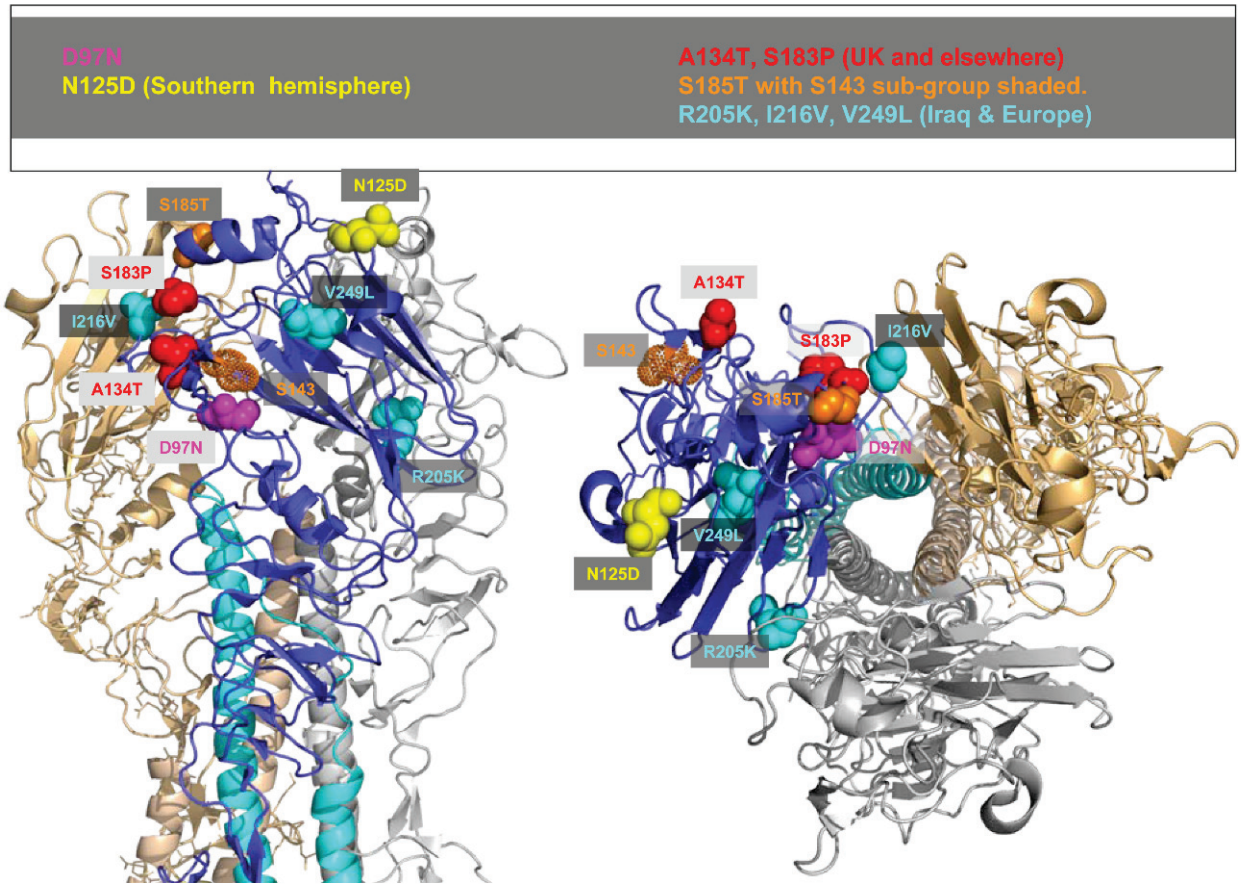


Figure 3 Phylogenetic comparison of influenza A(H3N2) HA genes

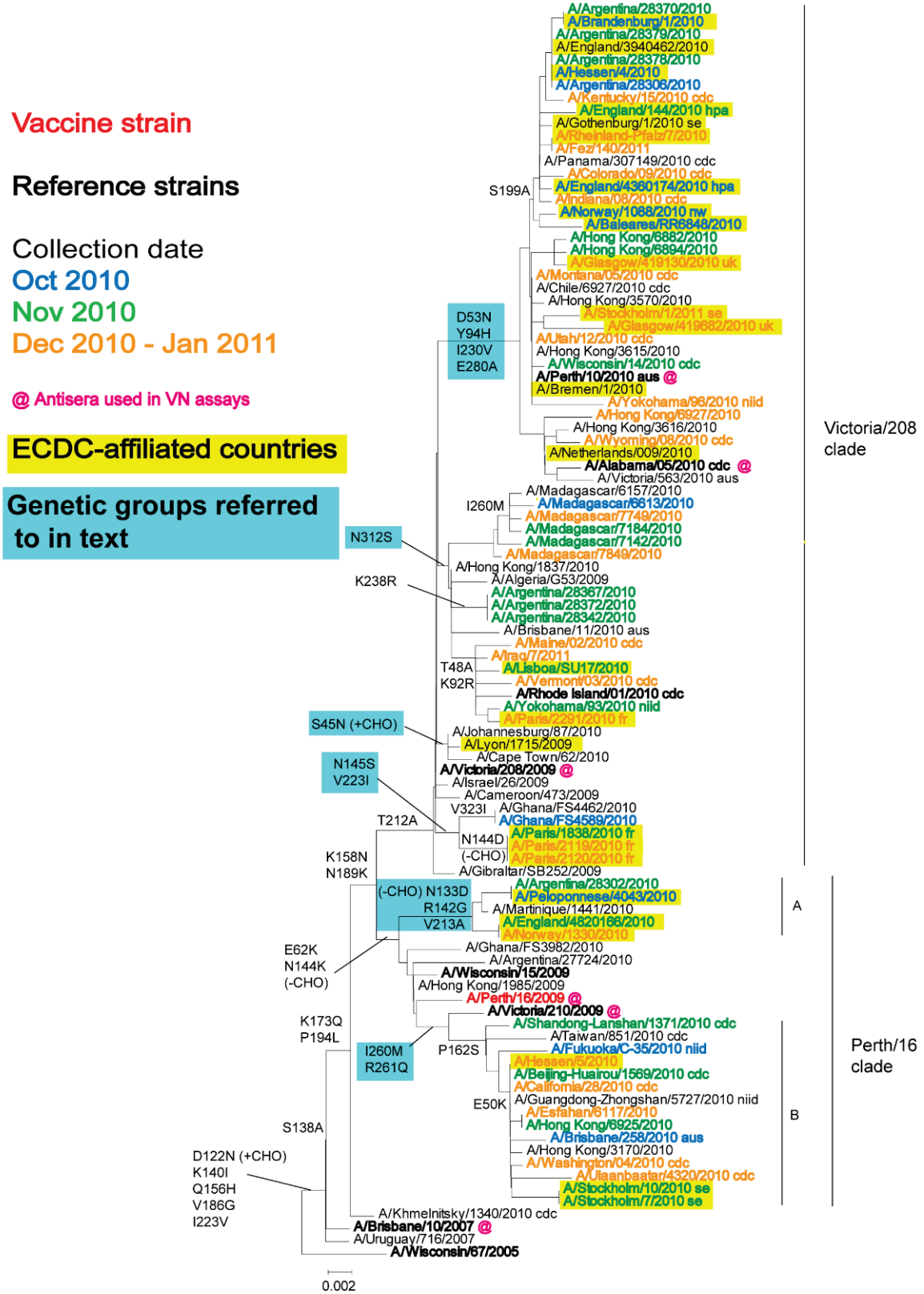


Figure 4 Location of amino acid substitutions observed in H3 HA of the Perth/16 clade (LHS) and the Victoria/208 clade (RHS)

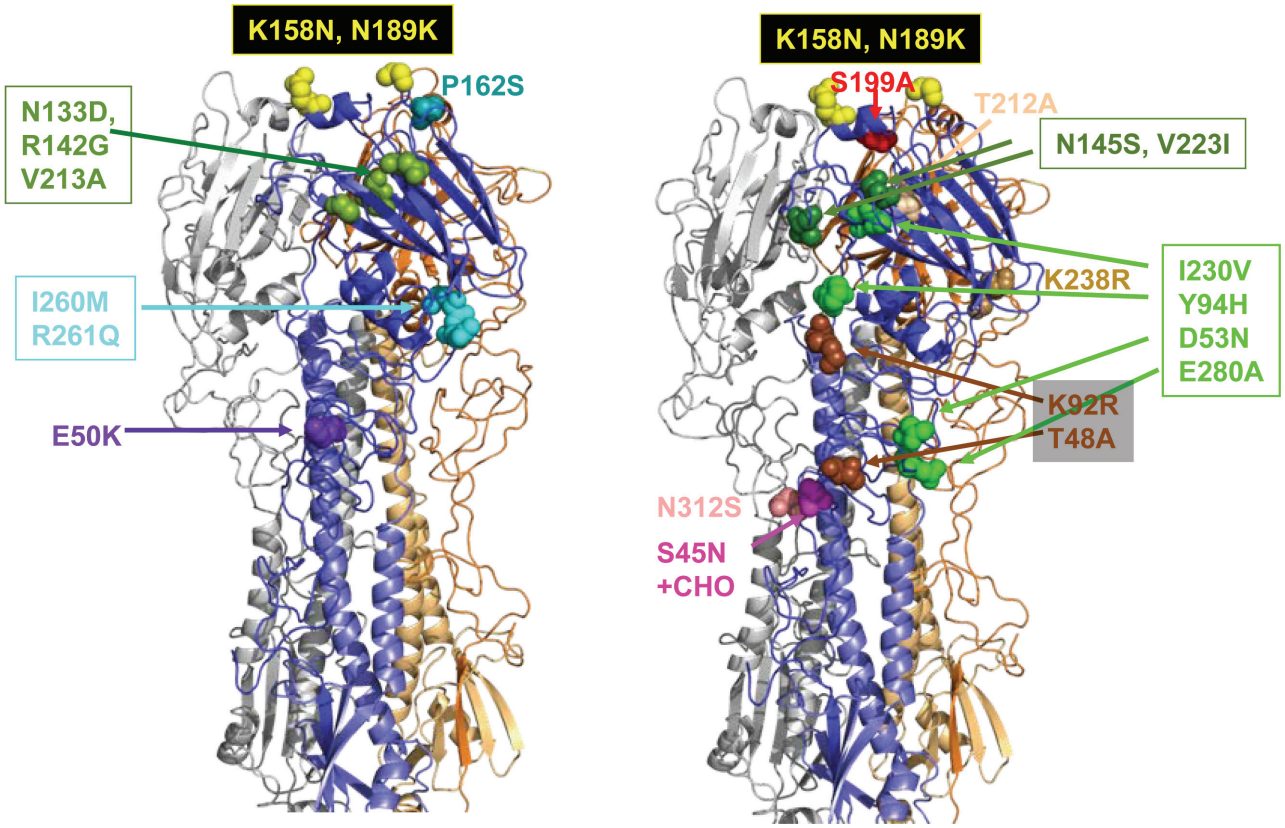


Figure 5 Phylogenetic comparison of influenza B HA1 genes (Victoria-lineage)

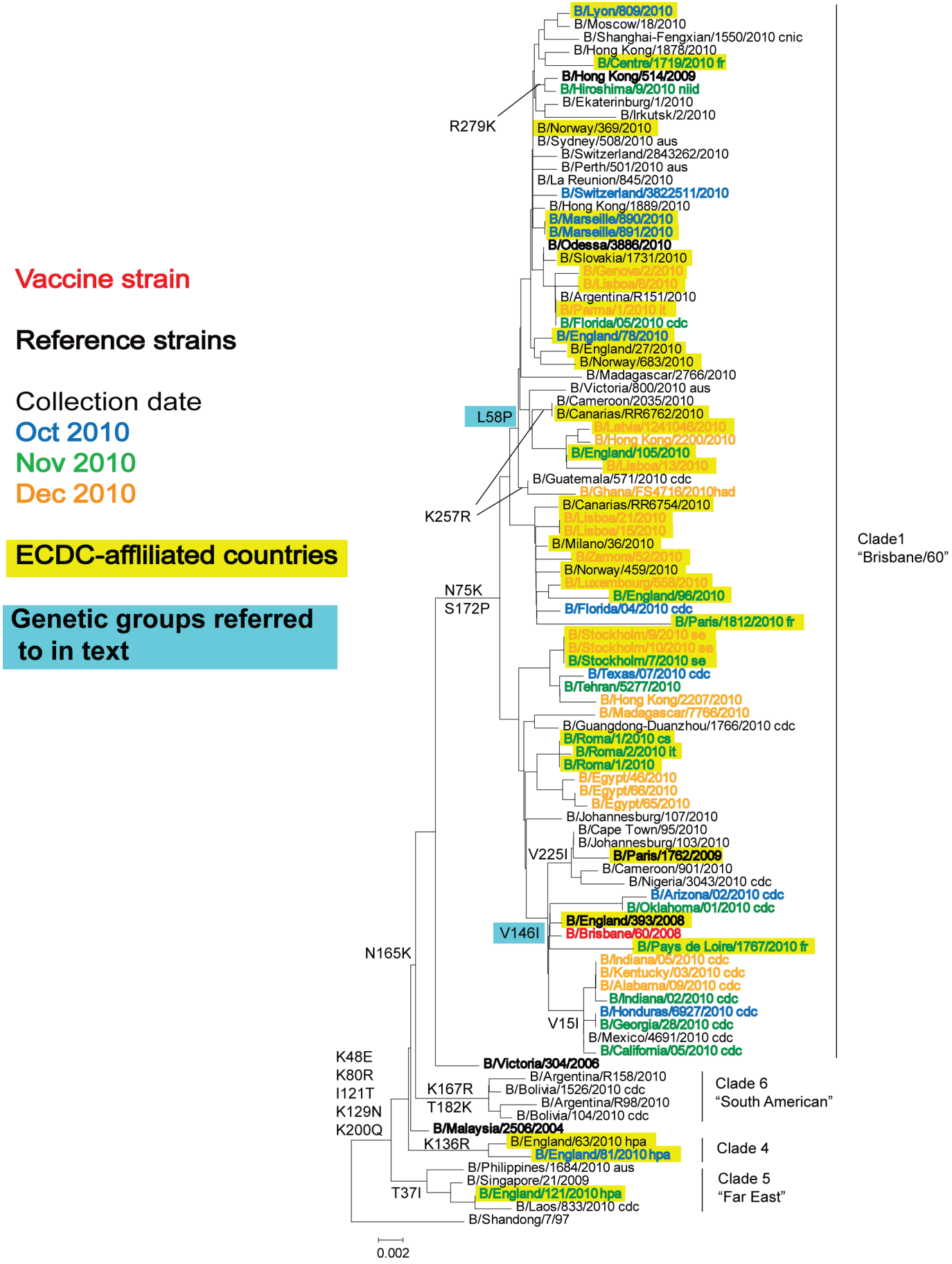
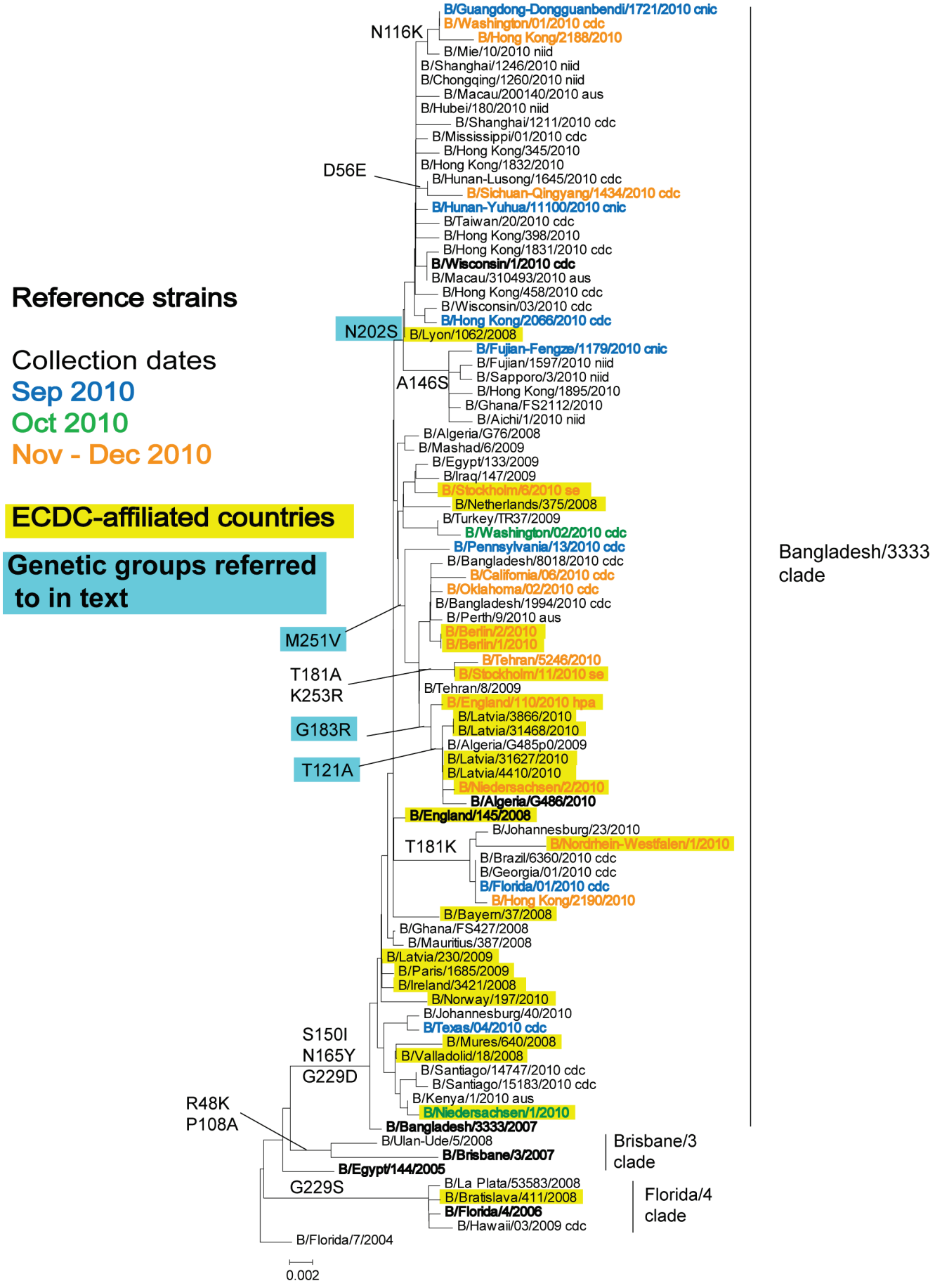


Figure 6 Phylogenetic comparison of influenza B HA1 genes (Yamagata-lineage)



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 WHOCC.