SURVEILLANCE REPORT

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

Influenza virus characterisation

Summary Europe, March 2011

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Over 660 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 is incomplete are shown as in progress.

Influenza A(H1N1) virus analysis

Table 2 shows representative HI results for viruses received since the previous report. The majority of viruses react well with the panel of post-infection ferret antisera including the serum raised against the vaccine virus A/California/7/2009. Close to 20% of viruses analysed by HI assay (11 of 54) showed titres of eightfold or lower with an individual antiserum or multiple antisera of the panel when compared with the titre given for the homologous reference virus. The viruses that showed reduced HI activity were derived from specimens collected in Greece, the Netherlands, Romania, Spain, Slovenia and Northern Ireland.

The nucleotide sequence of the HA1 coding region of the HA gene of the majority (8/11) of the samples with low reactivity were analysed. Of the eight viruses, the HA gene of six showed changes that encoded substitutions between residues 153 and 157, either present as a mixture or as a single species (either K153X, G155E or G155X); the two viruses that did not show this pattern were from Slovenia and no explanation for low reactivity with the antiserum panel is evident. Substitution between residues 153 and 157 are not infrequent and are often observed in viruses that have been passed in MDCK-SIAT1 cells – for example, the reference viruses A/Bayern/69/2009 and A/Lviv/N6/2009.

A phylogenetic analysis of the HA gene of recently collected H1N1 viruses is shown in Figure 1. The HA genes of these viruses continue to cluster generally into four genetic groups as described in the previous report (*ibid*). These groups are defined by amino acid substitutions:

i) A134T and S183P;
ii) N125D;
iii) S185T with many viruses carrying the additional substitution D97N in this group or an alternative additional substitution of A197T; and
iv) D97N, R205K, I216V and V249L.

Viruses from each of the four genetic groups have been seen in Europe and elsewhere. None of the genetic groups are considered antigenically distinct.

Influenza A(H3N2) virus analysis

Influenza A(H3N2) viruses have been successfully isolated on MDCK-SIAT1 cells but it is important to note that many of the viruses do not agglutinate red blood cells and the isolation of the majority of H3N2 viruses was monitored at the WHO CC in London by virus-induced cytopathology in tissue culture cells and the detection of virus by a sialidase assay for virus neuraminidase. As a consequence of the unusual red blood cell agglutinating properties of H3N2 viruses, antigenic analysis of H3N2 viruses has become increasingly difficult over recent influenza seasons. HI assays are impossible to carry out for viruses that do not agglutinate red blood cells at all; those H3N2 viruses that agglutinate red blood cells through their neuraminidase, agglutinating the red blood cells in an oseltamivir sensitive manner, will give information about the antigenic properties of the neuraminidase rather than of the virus haemagglutinin (the main target for neutralizing antibody). Recent antigenic analyses have been carried out at the WHO CC in London using virus neutralization tests, shown in the previous report.

Genetic analysis of the HA gene has been carried out and a phylogenetic tree of the HA1 coding region of the HA gene is shown in Figure 2. Recent viruses from Europe have fallen into both genetic clades of H3N2 viruses that have been observed since the new antigenic variants of H3N2 viruses emerged in 2009, although in Europe and elsewhere viruses of the A/Victoria/208 clade have been seen most frequently. There are five main genetic subgroups of the HA gene 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 and V213A; and, within the A/Victoria/208 clade, iii) N145S and V223I, iv) N312S, and v) D53N, Y94H, I230V, E280A and S312N. Viruses with HA genes from each group have been received from EU and affiliated countries but some recently collected H3N2 viruses from EU and affiliated countries belong to less well-defined genetic groups.

Influenza B virus analyses

As in our previous report, influenza B viruses of the B/Victoria/2/87 lineage have predominated (90%) over those of the B/Yamagata/16/88 lineage (10%).

B/Victoria lineage viruses

Representative results of antigenic analysis of influenza B/Victoria viruses from EU and affiliated countries as assessed by HI assay using turkey red blood cells are shown in Table 3. Many 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 3 the reference viruses B/Paris/1762/2008, B/Odessa/3886/2010 and B/Hong Kong/514/2009, used to raise post-infection ferret antisera, are cell-propagated and genetically closely related to the vaccine virus B/Brisbane/60/2008. All the test viruses analysed in Table 3 showed good reactivity with antisera raised against these three viruses and so are considered to be antigenically similar to the vaccine virus.

Phylogenetic analysis of representative influenza B/Victoria lineage viruses has been carried out on the HA1 coding region of the HA gene (Figure 3). For viruses from the EU and affiliated countries, the HA genes clustered into the B/Brisbane/60/2008 genetic clade and showed only few amino acid substitutions compared with the vaccine virus. The main substitutions I146V and L58P compared with the vaccine virus do not define antigenically distinct groups.

B/Yamagata lineage viruses

Table 4 shows the results of antigenic analysis of representative 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 generally showed better reactivity with antisera raised against the reference viruses B/Bangladesh/3333/2007 and B/Wicsonsin/1/2010, than they did with antisera raised against the most recently used vaccine virus of the B/Yamagata lineage, B/Florida/4/2006.

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 two viruses (A/Finland/33/2010 and A/Finland/39/2010) fell into the B/Bangaldesh/3333/2007 genetic clade. Minor genetic subgroups can be discerned within this clade, notably a subgroup defined by the amino acid substitution M251V, with some viruses carrying HA genes that encode additional substitutions G183R and T121A. The previous report showed results of HI assay of the two genetically distinct viruses from Finland; these viruses showed a similar pattern of reactivity to members of the B/Bangladesh/3333/2007 clade of B/Yamagata lineage viruses.

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 March 2011

MONTH	A	H1N1pdm		H3N2		B	B Yamagata lineage		B Victoria lineage	
Country		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	1	10	8			1			2	2
Italy		8	6	1	in process				9	9
Latvia		5	5						2	2
Luxembourg		5	3						1	1
Malta		6	4							
Netherlands				1	1					
Norway		1	1	1	1				7	6
Portugal	1	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		14	13	1	1		3	3	8	8
JANUARY										
Belgium	1	1	1						2	1
Czech Republic		12	in process						1	in process
Estonia		9	in process							
Germany									2	2
France							1	1	3	3
Greece	1	30	16	2	1	3				
Ireland				2	0					
Italy		50	48	3	in process	3	2	2	23	23
Latvia	1	6	in process							
Malta		2	2							
Netherlands		5	5	1	1		2	2		
Portugal	2	1	0	2	0				8	6
Romania		2	2	1	0					
Slovenia		14	13						3	3
Spain		30	21			1			5	5
Sweden				1	1					
United Kingdom		1	1							
FEBRUARY										
Greece	1	5	in process							
Spain		5	4	2	in process				5	5
Estonia		31	in process			2				
Czech		1	1						3	in process
Latvia		1	in process				1	in process	3	in process
Italy		1	1							
Malta		2	1							
MARCH										
Estonia		6	in process			6				
Greece	4	1	0			8				
Romania	1									
Spain									1	1
Latvia									2	in process
Total Received = 663	15	368	246	47	22	34	21	20	178	142

* 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 Egg	A/C'church 16/2010 F30/10
REFERENCE VIRUSES									
A/California/7/2009	2009-04-09	E2/E4	2560	2560	5120	1280	2560	2560	2560
A/England/195/2009	2009-04-28	MDCK1/MDCK2	2560	2560	2560	1280	2560	2560	2560
A/Auckland/3/2009	2009-04-25	Ex/E3	2560	2560	5120	1280	2560	2560	2560
A/Bayern/69/2009	2009-07-01	MDCK4/MDCK1	320	160	80	640	320	160	160
A/Lviv/N6/2009	2009-10-27	MDCK5	1280	160	160	2560	2560	640	640
A/Hong Kong/2212/2010	2010-07-16	E3	2560	5120	5120	2560	5120	5120	5120
A/Christchurch/16/2010	2010-07-12	E2/E1	2560	5120	5120	2560	5120	5120	5120
TEST VIRUSES									
A/Czech Republic/32/2011		MDCK4/MDCK1	1280	2560	2560	640	2560	2560	1280
A/Czech Republic/4/2011		MDCK4/MDCK1	1280	320	320	1280	1280	1280	640
A/Castilla La Mancha/RR6924/2010	2010-12-15	SIAT1/MDCK2	2560	2560	5120	1280	2560	2560	2560
A/Croatia/18009-2/2010	2010-12-15	E3/E4	1280	2560	1280	1280	2560	1280	1280
A/Croatia/18275-1/2010	2010-12-20	E3/E3	2560	2560	2560	1280	2560	2560	1280
A/La Rioja/RR6966/2010	2010-12-23	SIAT1/MDCK1	640	640	1280	640	1280	1280	2560
A/Madrid/SO8171/2010	2010-12-23	MDCK2/MDCK1	1280	1280	1280	640	1280	2560	1280
A/Lisboa/MS43+/2010	2010-12-28	MDCK3	2560	2560	2560	1280	2560	2560	2560
A/Madrid/SO8189/2010	2010-12-28	MDCK1/MDCK1	1280	1280	1280	640	1280	2560	1280
A/Lisboa/6/2010	2010-12-29	SIAT1/MDCK1	640	1280	1280	640	1280	1280	5120
A/Netherlands/2/2011	2011-01-03	xMDCK2/MDCK1	320	160	160	640	640	640	320
A/Malta/5/2011	2011-01-10	MDCK2	2560	2560	2560	640	2560	2560	1280
A/Milano/17/2011	2011-01-10	MDCK1/MDCK1	2560	2560	2560	1280	2560	5120	5120
A/Soria/9/2011	2011-01-10	MDCK1/MDCK2	640	640	640	320	640	640	320
A/Leon/12/2011	2011-01-11	MDCK1/MDCK4	160	640	640	160	320	640	320
A/Netherlands/151/2011	2011-01-12	xMDCK2/MDCK1	1280	1280	2560	1280	1280	2560	640
A/Trieste/12/2011	2011-01-13	MDCK1/MDCK1	320	160	160	640	640	320	160
A/Ceuta/RR7174/2011	2011-01-14	MDCK1/MDCK1	1280	1280	2560	640	1280	1280	1280
A/Netherlands/139/2011	2011-01-14	xMDCK2/MDCK1	80	160	40	160	160	320	160
A/Parma/38/2011	2011-01-14	MDCK1/MDCK1	2560	2560	2560	1280	2560	2560	2560
A/Kalamata/GR215/2011	2011-01-17	1st/MDCK1	640	640	640	1280	2560	640	640
A/Crete /GR185/2011	2011-01-18	1st/MDCK2	1280	1280	2560	1280	2560	1280	1280
A/Firenze/6/2011	2011-01-18	MDCK1/MDCK2	640	1280	1280	640	640	1280	1280
A/Athens/224/2011	2011-01-18	1st/MDCK2	2560	2560	2560	2560	2560	2560	2560
A/Athens/238/2011	2011-01-19	P1/MDCK3	2560	2560	5120	1280	2560	5120	2560
A/Milano/62/2011	2011-01-19	MDCK1/MDCK1	5120	5120	5120	2560	2560	5120	5120
A/Palencia/20/2011	2011-01-19	MDCK1/MDCK2	5120	2560	2560	1280	2560	2560	1280
A/Perugia/02/2011	2011-01-19	MDCK1/MDCK1	1280	1280	2560	640	1280	2560	1280
A/Parma/38/2011	2011-01-20	MDCK1/MDCK1	2560	1280	2560	640	1280	2560	1280
A/Firenze/5/2011	2011-01-21	MDCK2/MDCK2	640	1280	1280	640	1280	1280	1280
A/Leon/28/2011	2011-01-21	MDCK1/MDCK2	5120	2560	5120	2560	5120	2560	2560
A/Perugia/03/2011	2011-01-24	MDCK1/MDCK1	2560	2560	2560	1280	2560	5120	2560
A/Valladolid/29/2011	2011-01-24	MDCK1/MDCK1	2560	2560	5120	1280	2560	2560	2560
A/Parma/92/2011	2011-01-25	MDCK1/MDCK1	5120	2560	2560	1280	2560	5120	2560
A/Burgos/30/2011	2011-01-26	MDCK1/MDCK3	1280	2560	2560	640	640	2560	1280
A/Malta/7/2011	2011-01-26	MDCK2	1280	1280	1280	1280	2560	2560	1280
A/Valladolid/32/2011	2011-01-31	MDCK1/MDCK2	1280	2560	2560	640	1280	2560	1280
A/Soria/33/2011	2011-02-01	MDCK1/MDCK2	1280	1280	1280	640	640	1280	640
A/Trieste/31/2011	2011-02-02	MDCK1/MDCK1	2560	2560	2560	640	2560	2560	1280
A/Burgos/36/2011	2011-02-07	MDCK1/MDCK2	1280	1280	1280	640	1280	1280	640
A/Palencia/37/2011	2011-02-10	MDCK1/MDCK2	1280	1280	1280	640	1280	1280	640
A/Segovia/41/2011	2011-02-17	MDCK1/MDCK1	1280	1280	1280	640	1280	1280	640
A/Iasi/47707/2011	2011-01-03	MDCK1/MDCK2	320	320	320	640	1280	640	320
A/Slovenia/158/2011	2011-01-10	MDCKx/MDCK1	1280	2560	640	640	1280	1280	1280
A/Northern Ireland/2/2011	2011-01-10	MDCK1/MDCK1	160	160	160	2560	2560	640	320
A/Slovenia/167/2011	2011-01-10	MDCKx/MDCK1	320	640	640	160	640	640	320
A/Slovenia/146/2011	2011-01-11	MDCKx/MDCK1	160	320	160	40	160	320	160
A/Soria/16/2011	2011-01-13	MDCK1/MDCK1	640	640	1280	640	640	1280	2560
A/Slovenia/234/2011	2011-01-13	MDCKx/MDCK1	80	80	80	40	80	160	80
A/Larissa/GR190/2011	2011-01-17	1st/MDCK2	1280	2560	2560	1280	2560	2560	1280
A/Trikala/GR199/2011	2011-01-18	1st/MDCK2	160	160	160	1280	1280	320	320
A/Athens/230/2011	2011-01-18	1st/MDCK1	160	160	160	1280	640	320	320
A/Athens/351/2011	2011-01-20	MDCK3	160	80	80	640	1280	160	160
A/Larissa/GR332/2011	2011-01-20	MDCK3	160	160	160	320	640	160	160

Sequences included in HA phylogeny

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

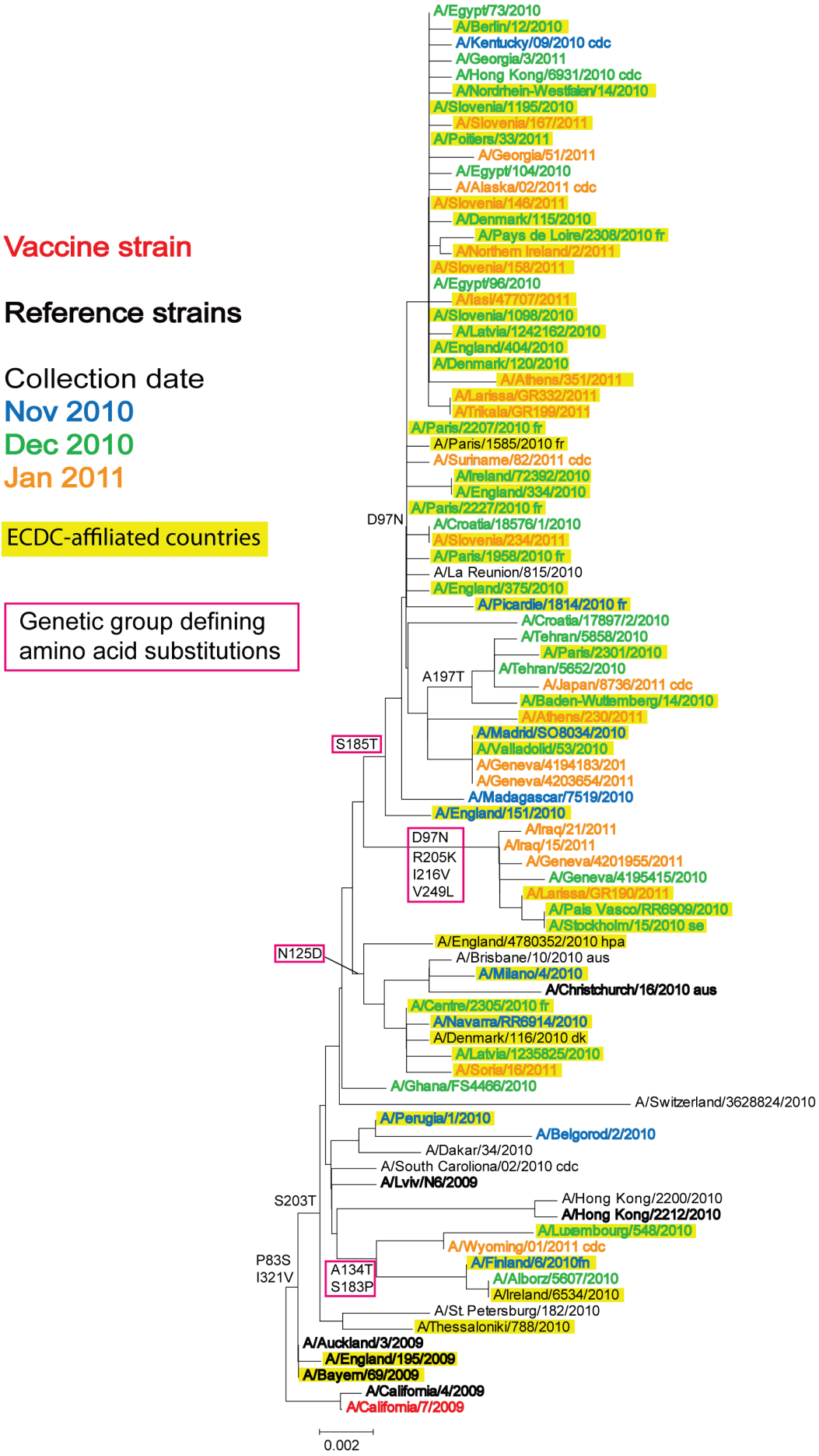

Figure 2 Phylogenetic comparison of influenza A(H3N2) HA genes (HA1 region)

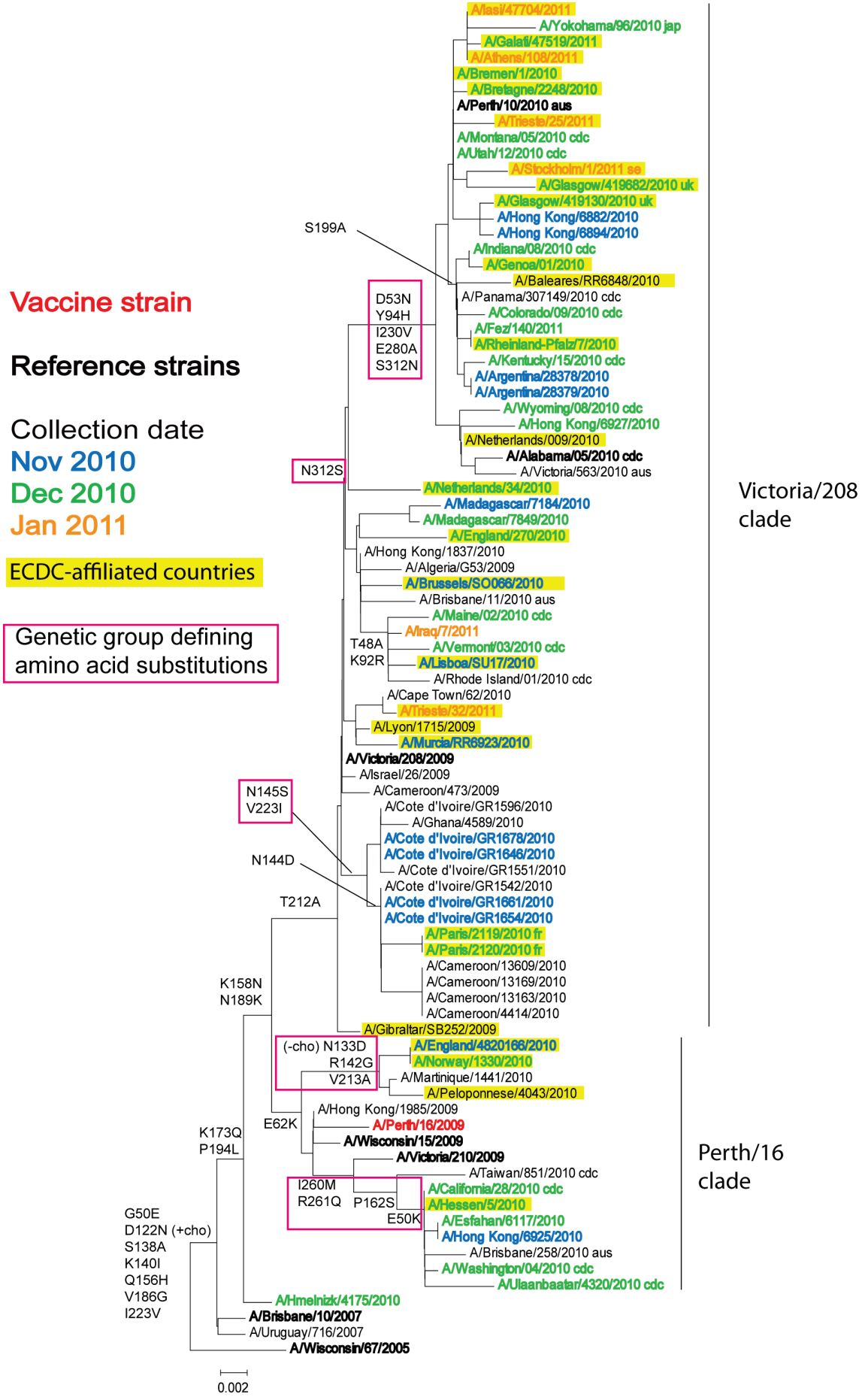

Table 3 Antigenic analysis of influenza B/Victoria lineage viruses by HI (turkey RBCs)

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 F13/10	B/Odessa 3886/10 F17/10
REFERENCE VIRUSES									
B/Malaysia/2506/2004	2004-12-06	E3/E3	640	640	160	80	<	<	<
B/England/393/2008	2008-08-29	E1/E6	640	160	320	320	40	40	40
B/Brisbane/60/2008	2008-08-04	E4/E4	640	160	160	320	40	20	40
B/Paris/1762/2008	2009-02-09	C2/MDCK4	640	10	<	20	80	160	80
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK1	640	<	<	20	80	160	80
B/Odessa/3886/2010	2010-03-19	C2/MDCK3	640	10	<	40	80	320	160
TEST VIRUSES									
B/Czech Republic/22/2011		MDCK2/MDCK1	640	10	<	40	80	160	160
B/Leon/34/2011	2011-02-02	MDCK1/MDCK2	1280	10	<	80	160	160	160
B/Brussels/S0160/2010	2010-12-13	MDCK3	1280	40	40	160	160	320	160
B/La Rioja/RR6969/2010	2010-12-14	SIAT1/MDCK1	1280	160	1280	1280	160	320	320
B/Valladolid/65/2010	2010-12-15	MDCK1/MDCK1	1280	40	80	160	160	320	320
B/Genoa/04/2010	2010-12-20	MDCK2/MDCK1	1280	40	40	160	160	320	320
B/Ceuta/RR7185/2010	2010-12-21	MDCK1/MDCK1	2560	<	160	80	320	320	320
B/Madrid/SO8183/2010	2010-12-27	MDCK1/MDCK1	640	<	40	40	160	320	160
B/Perugia/2/2010	2010-12-29	MDCK1/MDCK1	1280	20	40	160	160	320	160
B/Genoa/08/2010	2010-12-30	MDCK2/MDCK1	1280	40	40	160	160	320	320
B/Baleares/RR7035/2011	2011-01-01	SIAT1/MDCK1	640	<	40	40	160	320	160
B/Centre/2413/2010	2011-01-03	C1/MDCK1	2560	40	80	320	320	320	160
B/Ceuta/RR7186/2011	2011-01-04	MDCK1/MDCK1	640	<	20	20	80	160	80
B/Parma/02/2011	2011-01-04	MDCK1/MDCK1	1280	20	160	160	80	320	160
B/Rheinland-Pfalz/1/2011	2011-01-04	SIAT2/MDCK1	1280	20	40	160	160	320	160
B/Lisboa/1/2011	2011-01-05	SIAT1/MDCK1	2560	<	<	80	160	640	320
B/Lisboa/2/2011	2011-01-05	SIAT1/MDCK1	1280	<	<	80	160	640	320
B/Parma/3/2011	2011-01-07	MDCK1/MDCK1	1280	40	80	320	160	320	160
B/Lisboa/4/2011	2011-01-08	SIAT2/MDCK2	1280	<	<	80	160	640	320
B/Milano/03/2011	2011-01-10	MDCK1/MDCK1	1280	<	40	20	160	320	160
B/Valladolid/10/2011	2011-01-10	MDCK1/MDCK3	320	10	20	20	80	80	160
B/Milano/05/2011	2011-01-14	MDCK1/MDCK1	320	<	40	20	160	160	160
B/Milano/08/2011	2011-01-17	MDCK1/MDCK1	640	<	40	20	160	160	160
B/Perugia/02/2011	2011-01-17	MDCK2/MDCK1	640	<	40	20	160	160	80
B/Perugia/03/2011	2011-01-17	MDCK1/MDCK1	1280	<	40	40	160	320	160
B/Palencia/23/2011	2011-01-18	MDCK1/MDCK1	640	<	40	40	160	320	160
B/Leon/21/2011	2011-01-19	MDCK1/MDCK2	640	<	40	40	160	320	160
B/Trieste/02/2011	2011-01-19	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Parma/18/2011	2011-01-21	MDCK1/MDCK1	640	<	40	40	80	160	80
B/Parma/21/2011	2011-01-24	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Parma/24/2011	2011-01-24	MDCK1/MDCK1	640	<	<	40	160	640	160
B/Trieste/07/2011	2011-01-25	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Trieste/08/2011	2011-01-25	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Trieste/11/2011	2011-01-26	MDCK1/MDCK1	640	<	40	80	160	320	160
B/Salamanca/35/2011	2011-02-04	MDCK1/MDCK1	640	<	40	80	160	640	320
B/Segovia/42/2011	2011-02-18	MDCK1/MDCK1	640	<	<	80	160	640	320
B/Segovia/43/2011	2011-02-18	MDCK1/MDCK1	640	<	<	80	160	640	320
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/Leon/46/2011	2011-03-01	MDCK1/MDCK2	640	<	<	20	160	160	160
B/Zamora/52/2010	2010-12-01	MDCK1/MDCK2	320	10	10	20	40	80	40
B/Genova/2/2010	2010-12-01	MDCK1/MDCK1	640	20	20	40	80	80	80
B/Lisboa/8/2010	2010-12-02	MDCK2/MDCK1	640	10	10	20	40	80	160
B/Ireland/72067/2010	2010-12-06	MDCK1/MDCK1	2560	40	80	160	160	320	320
B/Lisboa/14/2010	2010-12-07	MDCK1/MDCK2	1280	20	40	40	80	320	160
B/Lisboa/20/2010	2010-12-07	MDCK1/MDCK2	640	20	40	40	80	320	160
B/Lisboa/13/2010	2010-12-07	MDCK1/MDCK1	640	10	10	40	40	80	160
B/Lisboa/21/2010	2010-12-08	MDCK1/MDCK2	1280	20	40	40	80	320	160
B/Stockholm/10/2010	2010-12-10	C0/MDCK1	640	20	40	40	80	160	640
B/Latvia/12-41046/2010	2010-12-23	MDCK1/MDCK1	640	20	40	40	160	320	160
B/Latvia/12-43110/2010	2010-12-27	MDCKx/MDCK2	640	10	20	20	40	160	80
B/Luxembourg/558/2010	2010-12-29	C1/MDCK1	320	10	10	20	40	80	80
B/Ireland/00132/2010	2010-12-31	MDCK1/MDCK1	2560	640	640	1280	160	160	160
B/Genoa/02/2011	2011-01-03	MDCK2/MDCK1	640	20	20	80	160	160	160

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

Sequences included in HA phylogeny

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

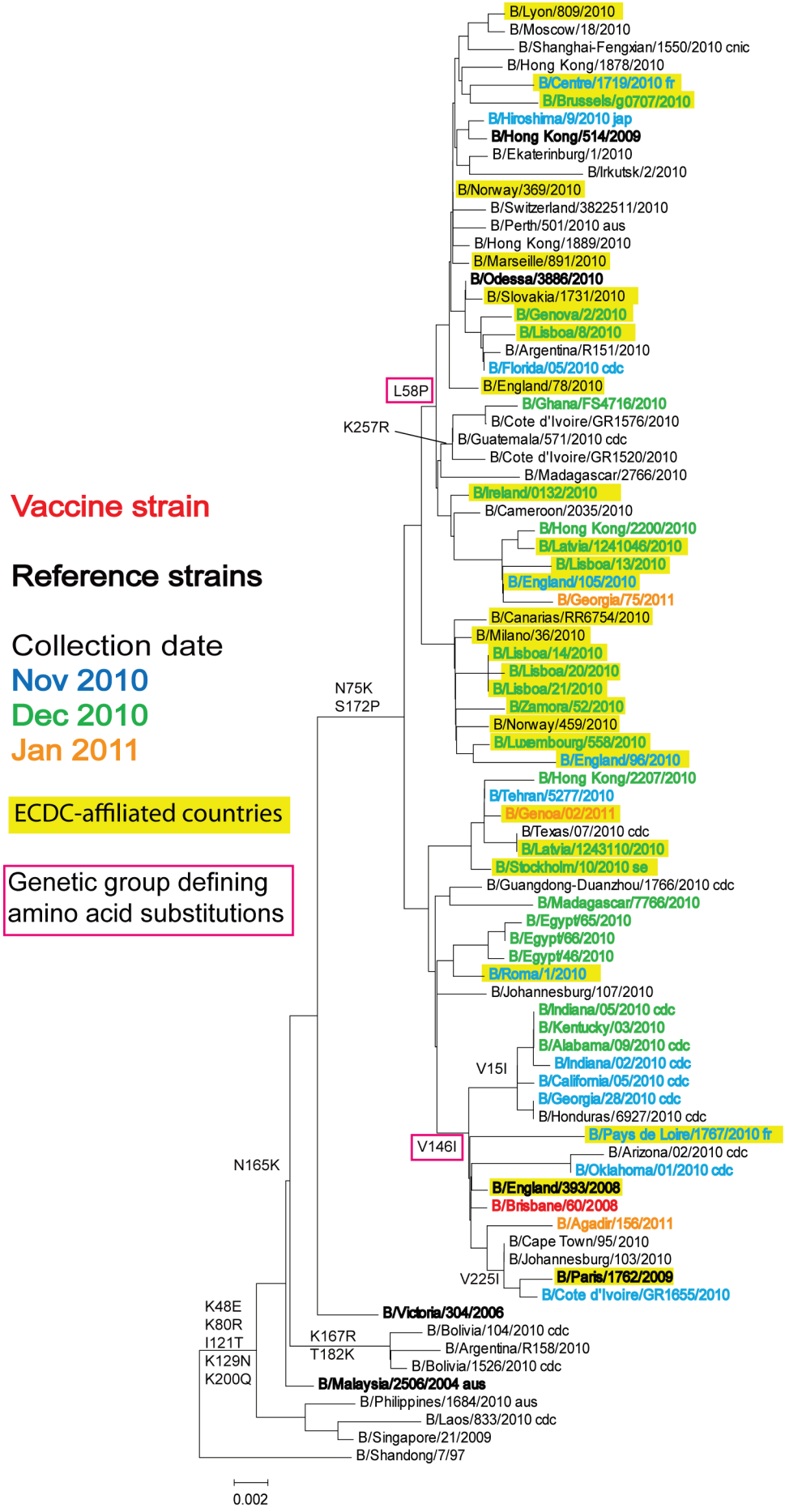

Table 4 Antigenic analysis of influenza B/Yamagata lineage viruses by HI (turkey RBCs)

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 F10/07	B/Bris[1] 3/07 F24/07	B/Eng[2] 145/08 F9/08	B/Bang[2] 3333/07 F24/10	B/Wis[2] 1/10 F23/10
REFERENCE VIRUSES									
B/Egypt/144/2005	2005-05-01	E3/E6	2560	640	640	640	40	80	160
B/Florida/4/2006	2006-12-15	E3/E4	5120	640	2560	640	160	160	320
B/Brisbane/3/2007	2007-09-03	E2/E1	5120	640	1280	640	40	80	160
B/England/145/2008		Ex/E4	320	40	80	<	80	10	20
B/Bangladesh/3333/2007	2007-08-07	E3/E4	2560	320	320	160	40	160	320
B/Wisconsin/1/2010	2010-02-20	E3/E2	1280	160	320	80	40	80	320
TEST VIRUSES									
B/England/170/2010	2010-12-01	SIAT1/MDCK1	5120	320	640	80	320	320	320
B/England/499/2010	2010-12-14	SIAT1/MDCK1	5120	320	640	320	320	320	320
B/Brussels/G0724/2010	2010-12-15	MDCK3	5120	160	320	80	160	160	160
B/Brussels/G0786/2010	2010-12-18	MDCK2/MDCK1	5120	320	640	160	320	160	160
B/England/512/2010	2010-12-20	SIAT1/MDCK1	5120	640	1280	320	320	320	320
B/Poitiers/128/2011	2010-12-29	MDCKx+1/MDCK1	5120	320	1280	320	320	320	320
B/Lyon/68/2011	2011-01-05	MDCK2/MDCK1	5120	320	640	160	320	320	320
B/Netherlands/234/2011	2011-01-16	xMDCK2/MDCK1	2560	640	320	160	640	640	640
B/Milano/10/2011	2011-01-17	MDCK1/MDCK1	1280	320	160	40	320	640	320
B/Trieste/05/2011	2011-01-19	MDCK1/MDCK1	1280	320	160	80	80	80	160
B/Brussels/G0710/2010	2010-12-13	MDCK2	5120	80	160	80	160	80	320
B/Niedersachsen/2/2010	2010-12-16	C2/MDCK1	1280	40	80	40	80	80	160
B/Stockholm/11/2010	2010-12-20	C1/MDCK2	2560	320	320	160	320	320	320
B/Nordrhein-Westfalen/1/2010	2010-12-22	C1/MDCK2	5120	160	320	640	320	320	1280
B/Berlin/1/2010	2010-12-27	C2/MDCK1	5120	80	160	160	160	160	640
B/Bretagne/2278/2010	2010-12-27	C1/MDCK1	1280	80	160	80	160	80	320
B/Berlin/2/2010	2010-12-28	C2/MDCK1	5120	160	160	160	320	160	640

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

Sequences included in HA phylogeny

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