



SURVEILLANCE REPORT

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

Influenza virus characterisation

Summary for Europe, December 2010

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Since the September 2010 report ([click here for September report](#)), very few samples were received from ECDC-affiliated countries until December 2010.

Table 1 shows a list of the ECDC-affiliated countries that have sent viruses or clinical samples collected since 1 September 2010. Pandemic A(H1N1) 2009 viruses were received from the UK, Italy, Spain, Portugal and Luxembourg; H3N2 viruses from the UK, Germany, Greece, Spain and Portugal. Influenza B viruses were received from the same countries with B-Victoria-lineage viruses predominating over B/Yamagata-lineage viruses.

Table 2 shows the results of haemagglutination inhibition tests carried out on A(H1N1) 2009 viruses from Europe using a panel of post-infection ferret antisera. The results show that the vast majority of viruses had similar levels and patterns of reactivity to the antisera and remained antigenically similar to the vaccine virus A/California/7/2009. One virus—A/Northern Ireland/1/2010—showed a reduced level of reactivity with the majority of sera; sequencing of this virus is ongoing.

Figure 1 shows a phylogenetic analysis of the HA1 coding region of the HA gene of H1N1 viruses. In the figure, viruses collected in ECDC-affiliated countries are highlighted in yellow and additionally colour coded by the date of collection. From the figure it can be seen that recent isolates from Europe do not cluster in the tree. A recently collected virus from Italy (A/Milano/3/2010) clustered with a genetic group that had been observed in the Southern Hemisphere in 2010*. Two viruses from England collected in November are shown in the phylogenetic tree; these viruses do not cluster together. The genetic characteristics of viruses isolated more recently in the UK have been described elsewhere†. The analysis of all A(H1N1) viruses collected in December, now received at the WHO CC from ECDC-affiliated countries, is in progress.

Fifteen H3N2 viruses have been received from ECDC-affiliated countries. These viruses have shown highly variable agglutination of red blood cells and this variable agglutination has made HI assays difficult to assess. Phylogenetic analysis of the HA1-coding region of the HA gene has been undertaken and results of representative strains are shown in Figure 2. All but one of the viruses collected recently from ECDC-affiliated countries cluster with an emerging phylogenetic group that has been recognised around the world in 2010. This phylogenetic group clusters within the A/Victoria/208/2009 genetic clade. The other virus sequence (A/England/4820166/2010) falls into the A/Perth/16/2009 phylogenetic clade and shares two additional amino acid substitutions with viruses collected previously in Greece, Argentina and the West Indies compared with the reference strains in the clade.

* Barr IG, Cui L, Komadina N, et al. A new pandemic influenza A(H1N1) genetic variant predominated in the winter 2010 influenza season in Australia, New Zealand and Singapore. *Euro Surveill.* 2010;15(42):pii=19692. [Available here.](#)

† Ellis J, Galiano M, Pebody R, et al; Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11. *EuroSurveill.* 2011;16(1): pii=19760. [Available here.](#)

Table 3 shows the results of haemagglutination inhibition tests carried out on influenza B Victoria-lineage viruses from Europe using a panel of post-infection ferret antisera. As expected, the antiserum raised against the egg-propagated vaccine virus B/Brisbane/60/2008 shows low cross-reactivity with all viruses propagated exclusively in tissue culture. Antisera raised against viruses that are phylogenetically closely related to the vaccine virus, but propagated in mammalian cells, can be used to assess the antigenic similarity of viruses within the B/Brisbane/60/2008 genetic clade. Antisera raised against B/Paris/1762/2009, B/Hong Kong/514/2009 and B/Odessa/3886/2010 show good reactivity against the majority of the viruses tested with these antisera. Two viruses showed a different pattern of reactivity; these viruses (B/England/81/2010 and B/England/121/2010) reacted well only with a sheep hyper-immune serum raised against B/Malaysia/2506/04.

Figure 3 shows the phylogenetic analysis of the HA1 coding region of the HA gene of influenza B Victoria-lineage viruses. The vast majority of the recently collected viruses from ECDC-affiliated countries fall into the Brisbane/60/2008 phylogenetic clade. However, B/England/81/2010 clustered differently with an influenza B virus collected in England earlier in the year; the altered antigenic profile of this virus is consistent with the altered phylogenetic clustering.

A haemagglutination inhibition test using a panel of post-infection ferret antisera was carried out on the two influenza B Yamagata-lineage viruses, collected from Europe, that were propagated (Table 3). Both viruses reacted well with antisera raised against B/Bangladesh/3333/2008 and against recent viruses from the B/Bangladesh/3333/2008 clade (B/Algeria/G-846/2008 and B/Wisconsin/1/2010). However, it was striking that B/Niedersachsen/1/2010 showed a good reactivity with antiserum raised against the vaccine virus (B/Florida/4/2006) recommended for use in vaccines for the 2008/2009 Northern Hemisphere influenza season. Phylogenetic analysis of viruses of the influenza B Yamagata-lineage is ongoing.

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

Collection Month	Viruses received							
	Pandemic A(H1N1)		H3		B-Victoria lineage		B-Yamagata lineage	
Country	Number received	Number grown	Number received	Number grown	Number received	Number grown	Number received	Number grown
SEPTEMBER								
United Kingdom			2	0				
OCTOBER								
France					4	4		
Germany			2	2*			1	1
Greece			1	1*				
Portugal					3	in progress		
Spain			1	1*				
United Kingdom	1	1	1	1*	2	2		
NOVEMBER								
Italy	3	3			3	in progress		
Portugal			1	1*	15	in progress		
Spain	5	3			1	in progress		
United Kingdom	6	6	2	2*	8	8	1	1
DECEMBER								
Italy	1	in progress			1	1		
Luxembourg	5	in progress			1	in progress		
Portugal	1	in progress			14	in progress		
Spain	4	in progress			1	in progress		
JANUARY								
Portugal					1	in progress		
Total = 92	26	13	10	8	54	15	2	2

* Awaiting antigenic characterisation

Table 2: Antigenic analysis of A(H1N1) viruses

Viruses	Collection date	Passage History	Haemagglutination inhibition titre ¹									
			Post infection ferret sera									
			A/Cal 4/09 C4/F14/09	A/Cal 7/09 F16/09	A/Eng 195/09 F06/10	A/Auck 3/09 C4/17/09	A/Bayern 69/09 C4/33/09	A/Lviv N6/2009 C4/34/09	A/HK 2212/2010 F18/10	A/HK 2212/2010 F21/10 Egg	A/HK 2200/2010 F22/10 Egg	A/CC 16/2010 F30/10
REFERENCE VIRUSES												
A/California/4/2009	2009-04-01	C1,E3	2560	1280	5120	2560	1280	2560	2560	2560	2560	1280
A/California/7/2009	2009-04-09	E2/E6	1280	640	640	640	640	1280	640	640	1280	1280
A/England/195/2009	2009-04-28	MDCK1/MDCK4	1280	1280	2560	2560	1280	1280	1280	1280	2560	2560
A/Auckland/3/2009	2009-04-25	Ex/E3	2560	2560	2560	2560	1280	2560	2560	1280	2560	2560
A/Bayern/69/2009	2009-07-01	MDCK4/SIAT1	40	80	80	80	320	320	80	160	160	160
A/Lviv/N6/2009	2009-10-27	MDCK4/SIAT2	320	160	160	160	640	1280	160	640	320	320
A/Hong Kong/2212/2010	2010-07-16	MDCK2/MDCK4	1280	1280	1280	1280	640	640	1280	1280	1280	2560
A/Hong Kong/2212/2010	2010-07-16	E3	2560	2560	5120	5120	1280	2560	5120	5120	5120	5120
A/Hong Kong/2200/2010	2010-07-14	E3	1280	2560	2560	2560	1280	2560	2560	2560	5120	2560
TEST VIRUSES												
A/Lyon/CHU/43.28/2010	2010-10-23	MDCK2/MDCK1	640	1280	1280	1280	640	640	640	ND	ND	ND
A/Northern Ireland/1/2010	2010-10-29	M2/MDCK1	80	80	80	80	320	1280	160	160	160	ND
A/Navarra/RR6915/10	2010-11-04	SIAT1/MDCK1	640	640	640	1280	640	640	640	ND	ND	ND
A/Navarra/RR6914/10	2010-11-11	SIAT1/MDCK1	640	1280	640	1280	640	1280	1280	1280	1280	5120
A/Madrid/SO8034/10	2010-11-18	SIAT2/MDCK1	1280	1280	1280	1280	1280	1280	1280	2560	1280	5120
A/England/118/2010	2010-11-19	S1/MDCK1	1280	1280	1280	1280	2560	2560	2560	1280	2560	1280
A/England/127/2010	2010-11-22	SIAT1/MDCK2	640	1280	1280	1280	640	640	1280	640	1280	640
A/England/143/2010	2010-11-23	SIAT1/MDCK3	640	640	1280	640	1280	2560	640	1280	640	640
A/England/151/2010	2010-11-23	SIAT1/MDCK2	640	1280	1280	1280	640	1280	1280	1280	1280	640
A/Milano/3/2010	2010-11-24	MDCK1/MDCK1	1280	1280	1280	1280	1280	1280	2560	2560	1280	5120
A/Perugia/1/2010	2010-11-24	MDCK3/MDCK1	2560	2560	5120	2560	1280	2560	5120	2560	2560	2560
A/England/119/2010	2010-11-25	SIAT1/MDCK1	1280	2560	2560	2560	640	1280	1280	2560	2560	1280
A/England/142/2010	2010-11-26	SIAT1/MDCK4	640	640	640	640	640	1280	640	1280	640	640
A/Milano/4/2010	2010-11-29	MDCK2/MDCK1	640	1280	1280	1280	1280	1280	2560	2560	1280	5120
A/Pais Vasco/RR6909/10	2010-12-01	SIAT1/MDCK1	1280	1280	1280	1280	640	1280	1280	1280	1280	1280

1 = <40, ND = not done

Vaccine virus

Table 3: Antigenic analysis of influenza B Victoria-lineage viruses

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/England 393/08 F31/08	B/Bris 60/08 F2/09	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/E5	2560	640	640	320	80	160	<	<	<
B/Victoria/304/2006	2006-06-06	E2/E4	640	640	160	320	80	160	<	<	<
B/England/393/2008	2008-08-29	E1/E6	640	640	160	160	160	640	40	20	40
B/Brisbane/60/2008	2008-08-04	E4/E4	640	640	160	160	160	640	40	20	40
B/Paris/1762/2008		C2/MDCK2	<	1280	20	10	40	40	80	160	160
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK2	<	1280	20	10	40	80	160	160	160
B/Odessa/3886/2010	2010-03-19	MDCK2/MDCK1	<	1280	20	10	80	80	80	160	160
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/England/78/2010	2010-10-12	SIAT1/MDCK1	<	640	10	10	20	10	80	80	80
B/Lyon/809/2010	2010-10-22	MDCK2/MDCK1	<	1280	20	<	40	20	80	320	160
B/Marseille/889/2020	2010-10-26	MDCK2/MDCK1	<	2560	40	<	40	20	80	320	160
B/Marseille/890/2020	2010-10-26	MDCK2/MDCK1	<	1280	20	<	40	20	40	160	80
B/Marseille/891/2020	2010-10-28	MDCK2/MDCK1	<	640	20	<	40	20	40	160	160
B/England/96/2010	2010-11-02	SIAT1/MDCK1	<	640	20	10	40	40	80	160	80
B/England/123/2010	2010-11-13	SIAT1/MDCK1	<	1280	20	10	40	40	80	160	160
B/England/105/2010	2010-11-15	SIAT1/MDCK1	<	640	20	10	40	80	160	160	160
B/England/128/2010	2010-11-23	SIAT1/MDCK1	<	1280	20	10	40	40	80	160	160
B/Roma/2/2010	2010-11-26	MDCK1/MDCK1	<	1280	20	<	40	40	80	80	ND
B/Roma/1/2010	2010-11-27	MDCK1/MDCK1	<	1280	20	<	40	40	80	160	ND
B/England/137/2010	2010-11-29	SIAT1/MDCK1	<	1280	20	10	40	40	80	160	160
B/England/149/2010	2010-11-30	SIAT1/MDCK1	<	1280	20	10	40	40	160	160	160
B/England/150/2010	2010-11-30	SIAT1/MDCK1	<	1280	20	10	80	80	160	160	160
B/Genova/2/2010	2010-12-01	MDCK1/MDCK1	<	1280	20	<	40	40	80	80	ND
B/Ghana/FS/10-47/16/2010	2010-12-02	MDCK3	<	2560	20	<	40	80	80	160	ND

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

Vaccine strain

Table 4: Antigenic analysis of influenza B Yamagata-lineage viruses

Viruses	Collection date	Passage History	Haemagglutination inhibition titre							
			Post infection ferret sera							
			B/FI ³ 4/06 SH479	B/Eg ¹ 144/05 F3/04	B/FI ¹ 4/06 F1/10	B/Bris ¹ 3/07 F24/07	B/Eng ² 145/08 F9/08	B/Bang ² 3333/08 F25/08	B/AIg ² G-486/10 F15/10	B/Wis ² 1/10 F23/10
REFERENCE VIRUSES										
B/Egypt/144/2005	2005-05-01	E9	5120	80	640	640	40	160	20	80
B/Florida/4/2006	2006-12-15	E7	5120	320	1280	1280	160	320	80	320
B/Brisbane/3/2007	2007-09-03	E3	5120	160	640	640	80	160	20	80
B/England/145/2008		Ex/E4	320	<	80	40	80	10	10	10
B/Bang/3333/2008	2007-08-07	E7	2560	80	320	160	40	160	40	80
B/AIg/G-486/2010	2010-06-06	SIAT0/MDCK3	5120	160	320	160	320	160	1280	160
B/Wisconsin/1/2010	2010-02-20	E5	1280	40	160	160	20	80	20	80
TEST VIRUSES										
B/Niedersachsen/1/2010	2010-10-18	SIAT2/MDCK1	2560	160	2560	2560	640	640	1280	160
B/England/110/2010	2010-11-11	SIAT1/MDCK1	160	320	160	160	5120	160	1280	160

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

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

Vaccine strain

Reference strains

Collection date

Jul - Aug 2010

Sep - Oct 2010

Nov - Dec 2010

■ Oseltamivir resistant

D222 G/N/E substitutions are shown

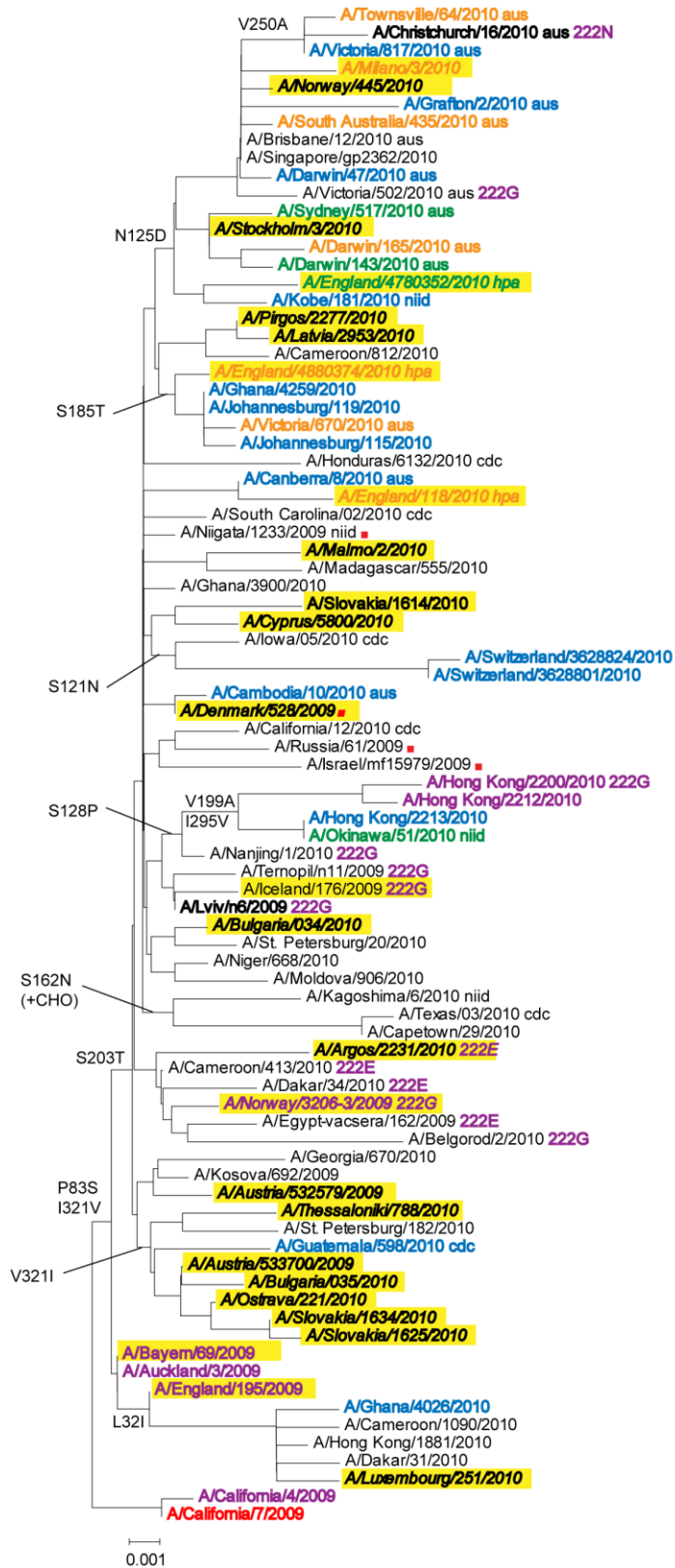


Figure 2: Phylogenetic comparison of influenza A(H3N2) HA genes

Vaccine strain

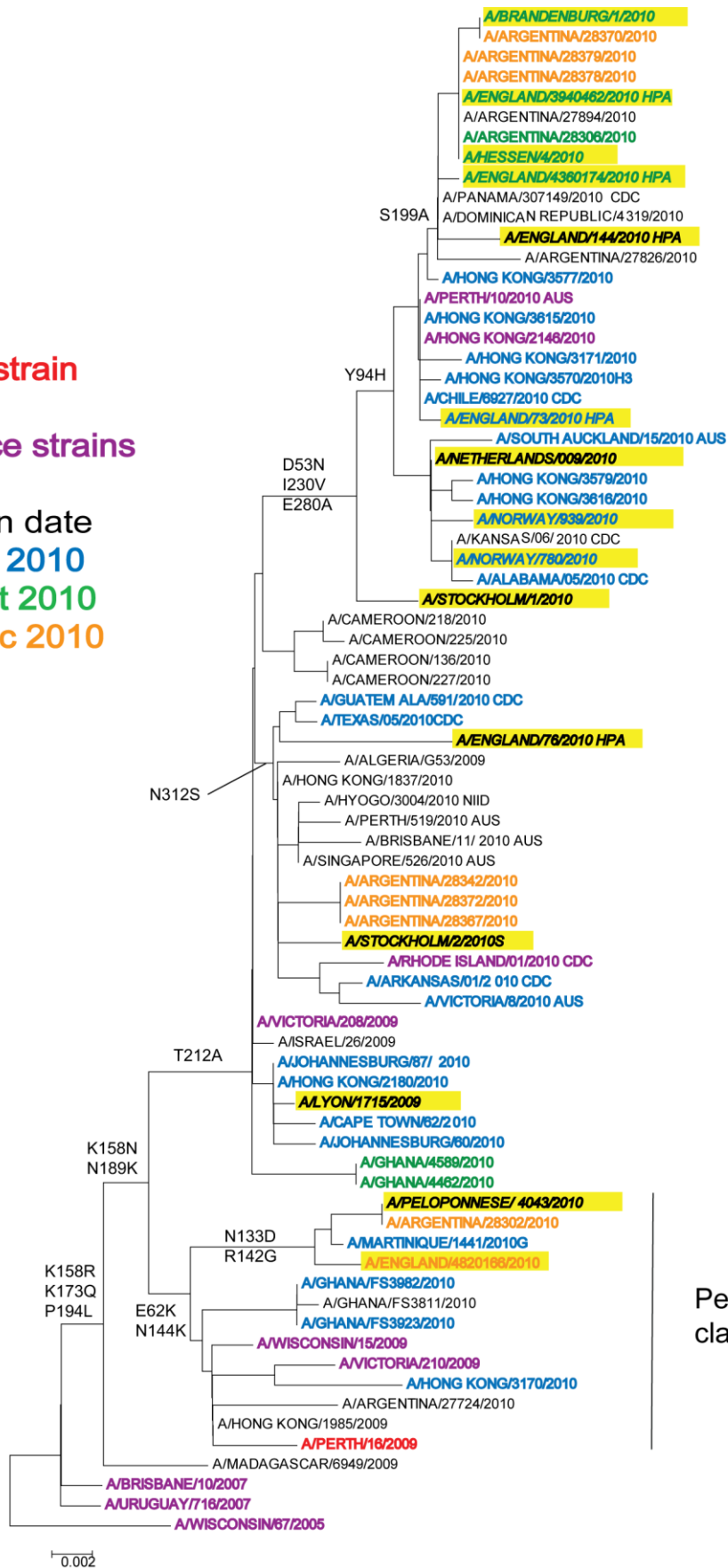
Reference strains

Collection date

Jul - Aug 2010

Sep - Oct 2010

Nov - Dec 2010



Victoria/208 clade

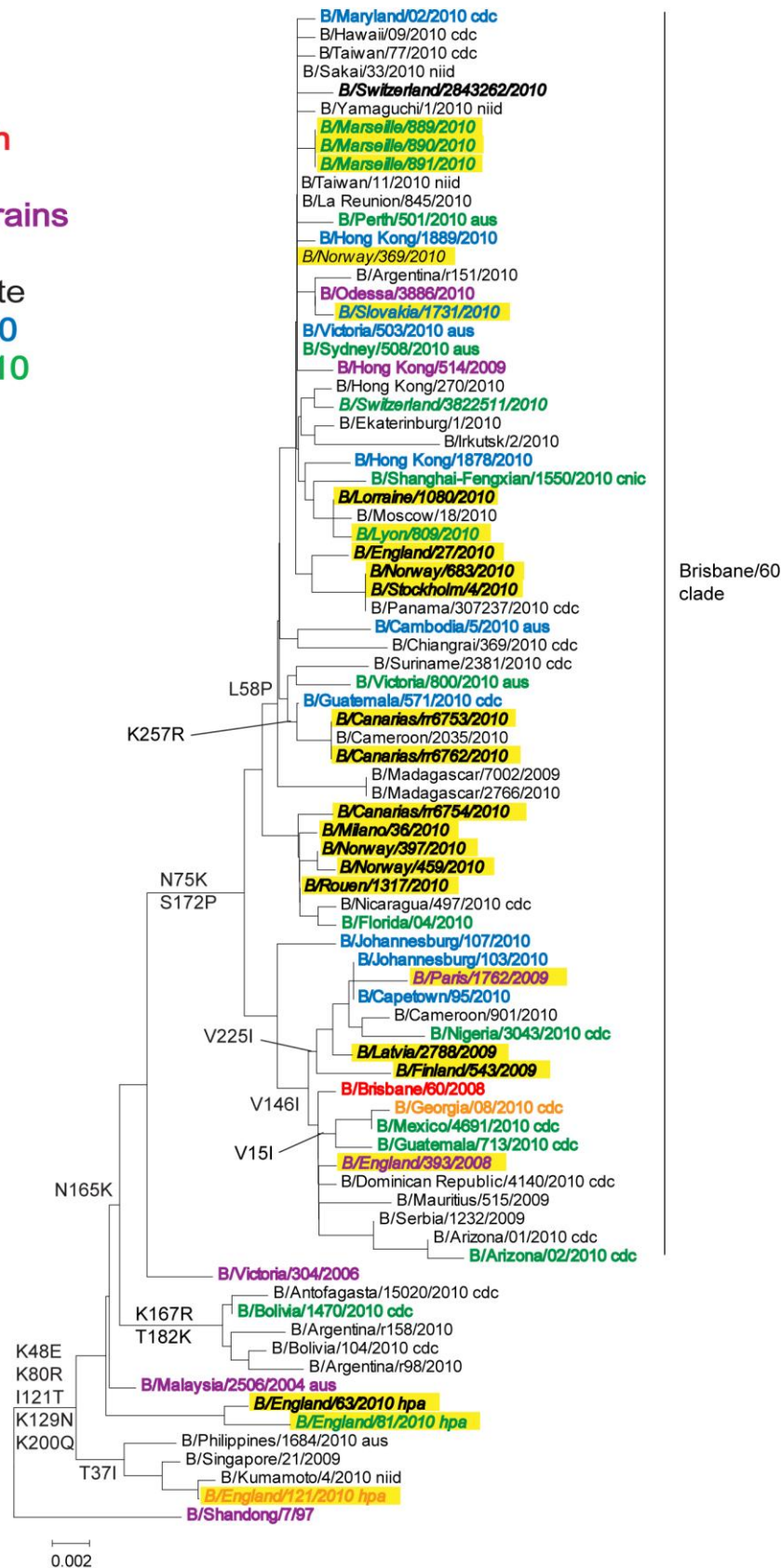
Perth/16 clade

Figure 3: Phylogenetic comparison of influenza B HA1 genes (Victoria-lineage)

Vaccine strain

Reference strains

Collection date
 Jul - Aug 2010
 Sep - Oct 2010
 Nov 2010



Note to the figures:

The phylogenetic trees were constructed using maximum parsimony in PAUP (Sinauer Associates). The bars indicate the proportion of nucleotide changes in the sequence. Reference strains are viruses to which post-infection ferret antisera have been developed. The colours indicate the date of sample collection. Isolates from ECDC countries are in italics and highlighted in yellow. Sequences for some of the viruses from non-European countries were recovered from GISAID and we acknowledge all laboratories submitting sequences.