



SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, November 2012

Summary

During the 2011/2012 season, A(H1N1)pdm09, A(H3N2) and B/Victoria and B/Yamagata lineage influenza viruses, with collection dates between 1 January and 30 September 2012 (weeks 1–39), have been detected in ECDC-affiliated countries.

- Type A viruses predominated over type B.
- A(H3N2) viruses predominated over A(H1N1)pdm09 viruses.
- A(H1N1)pdm09 viruses continued to show genetic drift from the vaccine virus, A/California/07/2009, but the vast majority remained antigenically similar to it.
- Antigenic drift of A(H3N2) viruses compared to the A/Perth/16/2009 vaccine virus resulted in a recommendation to change to an A/Victoria/361/2011-like component for the 2012/2013 influenza season.
- B/Victoria lineage viruses fell within the B/Brisbane/60/2008 genetic clade and were antigenically similar to reference cell-propagated viruses of the B/Brisbane/60/2008 genetic clade.
- Recent B/Yamagata-lineage viruses fell into two genetic clades in approximately equal proportions: clade 3 represented by the recommended vaccine component for the 2012/2013 influenza season, B/Wisconsin/1/2010, and clade 2 represented by B/Estonia/55669/2012. Viruses in each clade are antigenically distinguishable.

A summary table of viruses received from EU and EEA countries by the MRC National Institute for Medical Research WHO Collaborating Centre for Reference and Research on Influenza and collected between 1 January and 30 September 2012 is shown in Table 1.

Viruses and/or clinical samples have been received from 21 EU/EEA countries. The majority (68%) of viruses received have been influenza A(H3N2) viruses. Among the influenza B viruses received, those of the B/Yamagata and B/Victoria lineages were received in approximately equal proportions. Influenza A(H1N1)pdm09 viruses were received in low numbers and from only eight countries, with Norway providing nearly 50% (18/39). Table 1 is an update of the one in the previous [report](#) (October 2012). The two new A(H1N1)pdm09 viruses in the table both came from the Netherlands and were collected in August.

This report was prepared by Rod Daniels, Vicki Gregory and John McCauley on behalf of the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL), under contract to the European Centre for Disease Prevention and Control (ECDC).

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Table 1 Summary of clinical samples and isolates received from ECDC-affiliated countries, collection dates since 1 January 2012

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
Country		Not subtyped	Number received	Number propagated ¹	Number received	Number propagated ²	Lineage unknown	Number received	Number propagated ¹	Number received	Number propagated ¹
UNKNOWN											
Czech Republic ³	26				10	10		14	10	2	2
JANUARY											
Austria	6				4	4		1	1	1	1
Bulgaria	4				4	3					
Denmark	2				2	1					
Estonia	1				1	0					
Finland	3				3	2					
France	4				4	4					
Germany	15				14	13				1	1
Greece	18				8	7	5	1	1	4	4
Iceland	9				9	7					
Ireland	9				7	2		1	1	1	1
Italy	14				13	12				1	1
Latvia	7				6	5		1	1		
Netherlands	2				2	2					
Norway	16		5	2	11	11					
Portugal	7				7	4					
Romania	3				3	3					
Slovenia	3				3	3					
Spain	21		1	0	18	10		2	2		
Sweden	11		2	2	8	7		1	1		
United Kingdom	4				3	3		1	1		
FEBRUARY											
Bulgaria	8				8	8					
Denmark	11				6	4		2	2	3	3
Estonia	18				18	2					
Finland	5				4	2		1	1		
France	5		2	2	3	3					
Greece	24				14	13	4	2	2	4	4
Iceland	11				11	11					
Ireland	3		1	1	1	1		1	1		
Italy	12				6	5		1	1	5	5
Norway	29		10	6	15	15		1	1	3	3
Portugal	3				1	1				2	1
Slovenia	12		1	1	9	7		1	0	1	0
Sweden	5		3	3				1	1	1	1
United Kingdom	7				5	4		1	1	1	1
MARCH											
Denmark	13		3	0	8	7		1	0	1	0
Estonia	11				11	7					
Finland	1				1	1					
France	19		2	2	11	10		4	4	2	2
Iceland	3				3	2					
Ireland	4				4	4					
Italy	11				4	1		1	0	6	6
Norway	14		3	0	6	6		2	1	3	3
Portugal	10				6	4		1	1	3	2
Slovenia	16		1	0	9	7		4	1	2	0
Sweden	2				1	1				1	1
United Kingdom	10		1	1	7	4		1	1	1	1
APRIL											
Denmark	6				4	3		1	1	1	0
Estonia	8				7	3				1	1
France	6							3	3	3	3
Iceland	1				1	1					
Ireland	10				10	2					
Norway	12				9	9		1	1	2	2
Slovenia	2				1	1	1				
Sweden	5		1	1	3	2				1	1
United Kingdom	10				7	6		2	2	1	1
MAY											
Finland	1							1	1		
Norway	2				2	2					
United Kingdom	3				2	2		1	1		
JUNE											
Norway	3				1	1		1	1	1	1
United Kingdom	1									1	1
AUGUST											
Ireland	1		1	0							
Netherlands	2		2	2							
United Kingdom	2				2	2					
SEPTEMBER											
Norway	2									2	2
	529	0	39	23	361	277	10	57	46	62	55
				7.4%		68.2%			10.0%		11.7%

1. Propagated to sufficient titre to perform HI assay

2. Propagated to sufficient titre to perform HI assay in presence of 20nM oseltamivir

3. Package received 2012-05-10

Influenza A(H1N1)pdm09 virus analyses

The results of an HI assay carried out to examine the two oseltamivir-resistant A(H1N1)pdm09 viruses received from the [Netherlands](#) since the [October report](#) are shown in Table 2. A/Netherlands/492/2012 showed good reactivity with post-infection ferret antisera raised against the panel of reference viruses, including antiserum raised against the vaccine virus. A/California/7/2009. A/Netherlands/507/2012 showed at least four-fold reduced reactivity with eight of nine antisera in the panel, including that raised against the vaccine strain. Sequence analysis of the HA gene of A/Netherlands/507/2012 revealed an amino acid polymorphism at position N156D>N in the glycoprotein, which has previously been associated with low reactivity in HI assays and commonly results from propagation of viruses in certain tissue culture cells. Phylogenetic analysis of the HA gene of representative viruses (Figure 1) shows that the most recently detected H1N1 viruses from EU/EEA countries cluster within Groups 6 and 7, as [previously described](#). Both Netherlands viruses fall within Group 6.

Table 2 Antigenic analysis of A(H1N1)pdm09 viruses by HI

Haemagglutination inhibition titre ¹											
Viruses	Collection date	Passage History	Post infection ferret sera								
			A/Cal 7/09 F29/11	A/Bayern 69/09 F11/11	A/Lviv N6/2009 C4/34/09	A/C'church 16/2010 F30/10	A/HK 3934/2011 F21/11	A/Astrak 1/2011 F22/11	A/St. P'burg 27/11 F23/11	A/St. P'burg 100/11 F24/11	A/HK 5659/2012 F30/12
REFERENCE VIRUSES											
A/California/7/2009	2009-04-09	E1/E4	2560	2560	2560	1280	2560	2560	1280	5120	5120
A/Bayern/69/2009	2009-07-01	MDCK4/MDCK1	80	320	160	80	40	80	80	40	80
A/Lviv/N6/2009	2009-10-27	M4/S1/M2	640	2560	1280	320	160	160	320	160	640
A/Christchurch/16/2010	2010-07-12	E2/E2	5120	2560	2560	5120	2560	2560	2560	5120	5120
A/Hong Kong/3934/2011	2011-03-29	M2/M2	640	160	320	640	640	640	640	1280	2560
A/Astrakhan/1/2011	2011-02-28	M1/M5	1280	640	640	1280	1280	1280	2560	2560	2560
A/St. Petersburg/27/2011	2011-02-14	E1/E2	2560	1280	2560	1280	2560	2560	2560	5120	5120
A/St. Petersburg/100/2011	2011-03-14	E1/E2	2560	640	1280	1280	2560	2560	2560	5120	5120
A/Hong Kong/5659/2012	2012-05-21	MDCK4	1280	320	640	640	1280	1280	1280	2560	2560
TEST VIRUSES											
A/Netherlands/492/2012	2012-08-14	x/MDCK2/MDCK1	2560	640	1280	1280	2560	2560	1280	5120	5120
A/Netherlands/507/2012	2012-08-17	x/MDCK2/MDCK1	640	320	320	160	160	160	160	320	320

1. < = <40

Vaccine virus

Influenza A(H3N2) virus analyses

No new HI assays on influenza A(H3N2) viruses have been carried out since the [previous report](#) which gave results for A/Norway/1422/2012 and A/Norway/1443/2012.

Phylogenetic analysis of the HA gene sequences of representative viruses has been carried out (Figure 2). Viruses from the EU/EEA collected since January have HA genes that fall into the A/Victoria/208 clade, genetic groups 5 and 6, and subgroups 3A, 3B and 3C.

The HA1 amino acid substitutions that are associated with each of these groupings are:

- Subgroup 3A: V223I, N144D (resulting in the loss of a glycosylation site) and N145S, e.g. A/Stockholm/18/2011;
- Subgroup 3B: V223I, N145S, A198S & N312S, e.g. A/Athens/GR112/2012;
- Subgroup 3C: V223I, S45N, T48I, A198S & N312S, e.g. the prototype vaccine virus A/Victoria/361/2011, with some viruses also carrying the substitutions Q33R and N278K (e.g. A/Berlin/93/2011);
- Group 5: D53N, Y94H, I230V and E280A, e.g. A/Alabama/5/2010 and A/Norway/1443/2012;
- Group 6: D53N, Y94H, S199A, I230V and E280A, e.g. A/Iowa/19/2010 and A/Norway/1422/2012.

Influenza B virus analyses

B/Victoria-lineage virus

No new HI assays on influenza B (Victoria-lineage) viruses have been carried out since the [previous report](#) which gave results for B/Norway/1477/2012.

Phylogenetic analysis of the HA genes of representative B/Victoria lineage viruses is shown in Figure 3. All recently collected viruses received from EU and EEA laboratories carried HA genes that fell into clade 1, the B/Brisbane/60 clade, inclusive of B/Norway/1477/2012.

B/Yamagata-lineage viruses

No new HI assays on influenza B (Yamagata-lineage) viruses have been carried out since the [previous report](#) which gave results for B/Norway/1494/2012, B/Norway/1836/2012 and B/Norway/1864/2012.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata lineage viruses. The HA genes of these viruses fall into two genetic clades, one defined as clade 3, represented by the vaccine virus B/Wisconsin/1/2010 and reference viruses B/Bangladesh/3333/2007 and B/Stockholm/12/2011, with the other defined as clade 2 and represented by the reference viruses B/Brisbane/3/2007 and B/Estonia/55669/2011. Viruses falling within these two clades are antigenically distinguishable.

The two clades are differentiated by substitutions at HA1 residues 48, 108, 150, 165, 181 and 229. The HA genes of viruses in clade 2 encode K48, A108, S150, N165, A181 and G229. The HA genes of viruses in clade 3 encode R48, P108, I150, Y165, T181 and D229.

Clade 2 appears to be genetically homogenous and contains viruses B/Norway/1494/2012 and B/Norway/1836/2012, but clade 3 can be sub-divided into four genetic groups:

- a group defined by the amino acid substitution N202S similar to B/Wisconsin/1/2010;
- a group defined by the substitution T181K (e.g. B/Ireland/M1522/2012);
- a group defined by the substitution M251V with the substitutions T181A and K253R (e.g. B/Serbia/1894/2011);
- a group defined by the substitution M251V with the substitutions V29A and L172Q (e.g. B/Stockholm/12/2011), often with N123T (e.g. B/Norway/1864/2012).

Influenza A(H3N2)v virus

As noted [previously](#), on 3 August 2012 the United States Centers for Disease Control issued a [Health Advisory](#) describing an increase in the number of influenza A(H3N2)v infections in three US States and CDC have prepared further [background information](#) and have provided [updated](#) case counts. As of 1 December 2012 there were 308 confirmed cases. Antigenic and genetic characterisation of H3N2v viruses has been described by [Lindstrom et al., 2012](#). The virus was characterised as being antigenically distinct from currently circulating human seasonal influenza viruses and to be a reassortant virus with seven genes from swine influenza 'triple reassortant' H3N2 viruses and the M-gene from an influenza A(H1N1)pdm09 virus.

Risk assessments for these A(H3N2)v viruses, as a risk to public health, have been posted by the [United States CDC](#) and [ECDC](#).

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza, based at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva on 20–22 February 2012 and Beijing, China on 17–19 September 2012 can be found at:

<http://www.nimr.mrc.ac.uk/documents/about/interim-report-feb-2012.pdf> and
http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_September_2012_2.pdf

Note on the figures

The phylogenetic trees were constructed using RAXML and drawn using FigTree. 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 month of sample collection. Isolates from WHO National Influenza Centres (NICs) in ECDC-affiliated countries are highlighted in boxes. Sequences for some of the viruses from non-EU/EEA countries were recovered from GISAID (Global Initiative on Sharing All Influenza Data) and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.

Figure 1 Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes**Vaccine virus****Reference viruses**

Collection date

Apr 2012

May - Jun 2012

Jul 2012

Aug 2012

ECDC-affiliated countries

HA2 numbering

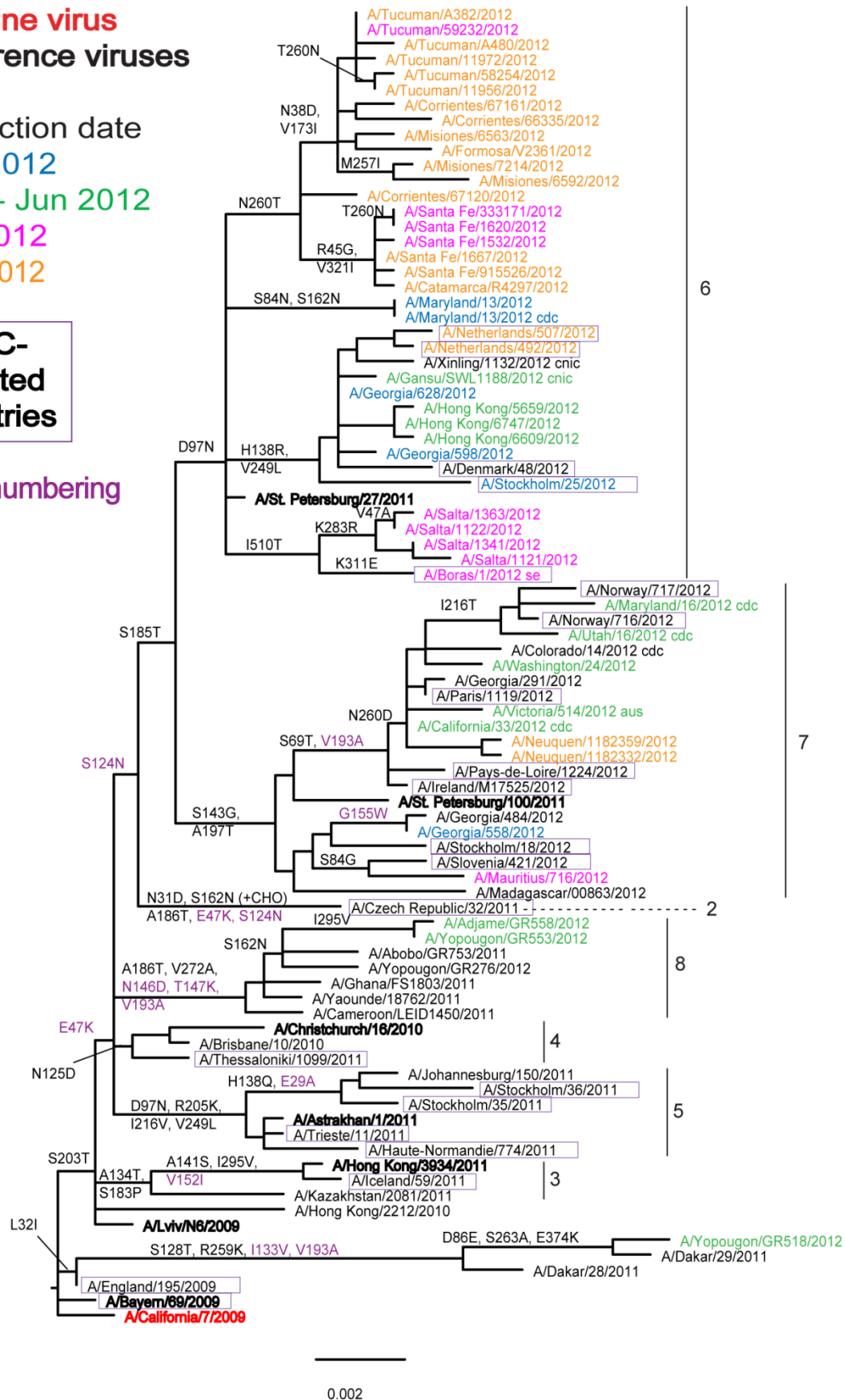


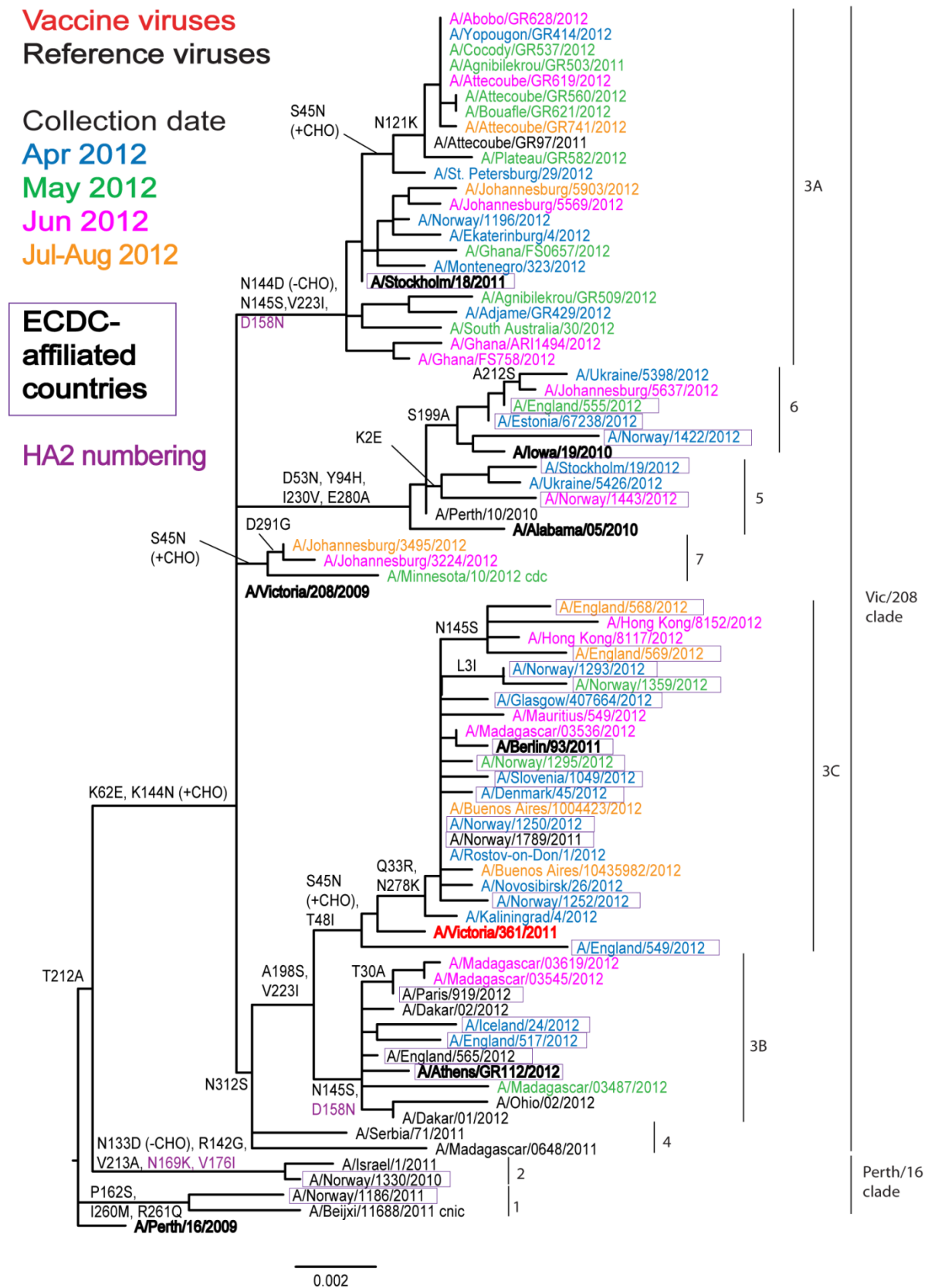
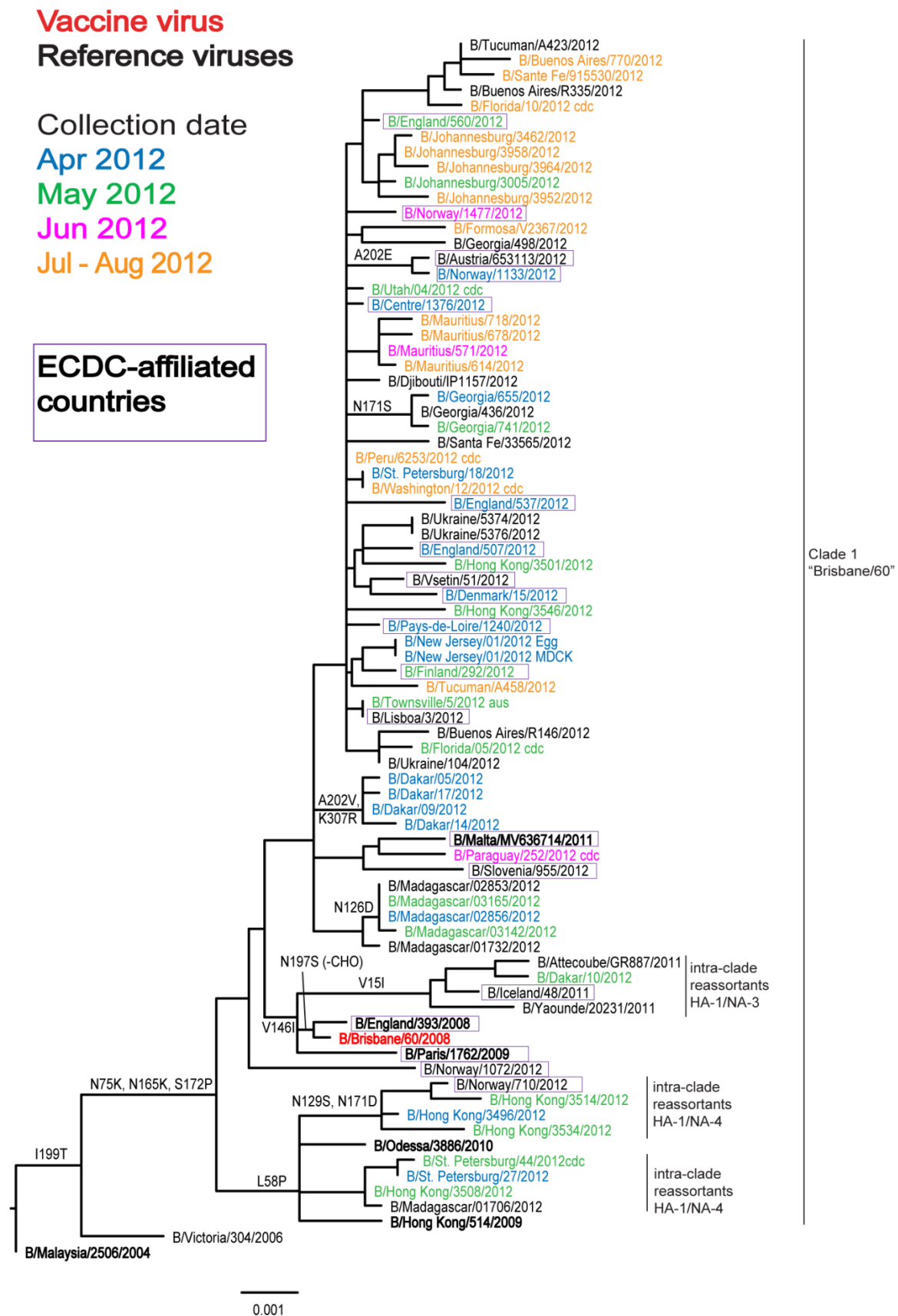
Figure 2 Phylogenetic comparison of influenza A(H3N2) HA genes

Figure 3 Phylogenetic comparison of influenza B/Victoria-lineage HA genes

Vaccine virus
Reference viruses

Apr 2012

May 2012

Jun 2012

Jul-Sep 2012

ECDC-affiliated countries



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