TECHNICAL DOCUMENT

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

Influenza virus characterisation

Summary Europe, March 2012

Summary

Since 1 September 2011, influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B/Victoria- and B/Yamagata-lineage viruses have been detected in ECDC-affiliated countries.

- Type A viruses have predominated over type B.
- A(H3N2) viruses have predominated over A(H1N1)pdm09 viruses.
- The range of influenza A(H3N2) viruses collected since 1 September 2011 fall within seven genetic groups, the majority being in group 3 which can be subdivided into three sub-groups: A, B and C.
- In haemagglutination-inhibition (HI) tests many of the recently circulating A(H3N2) viruses isolated in mammalian cells, falling within different genetic groups, yield low titres with post-infection ferret antisera raised against egg-propagated viruses, including the A/Perth/16/2009 vaccine virus. Although these observations may to a certain extent reflect virus isolation/passage history, in February 2012 WHO did recommend a change to an A/Victoria/361/2011-like virus in the composition of trivalent influenza vaccines for the Northern Hemisphere 2012–13 influenza season.
- Influenza B viruses of both the B/Victoria/2/87 and B/Yamagata/16/88 lineages have been detected in approximately equal proportions. The B/Victoria lineage has been slightly more prevalent, based on reporting to TESSy, while for specimens received at WHO Collaborating Centre in London the prevalent lineage has been B/Yamagata. This represents a considerable increase in the relative circulation of influenza B/Yamagata lineage viruses over that seen in recent seasons, a factor that influenced the WHO recommendation made in February 2012 to move from a B/Victoria lineage virus to a B/Yamagata lineage virus in trivalent vaccines for the Northern Hemisphere 2012–13 influenza season.
- The majority of influenza B viruses of the B/Yamagata-lineage fall within the B/Bangladesh/3333/2007 genetic clade and all of the B/Victoria lineage viruses fall within the B/Brisbane/60/2008 genetic clade.

A total of 355 viruses/clinical samples, received from WHO National Influenza Centres in the EU/EEA region with collection dates between 1 September 2011 and 29 February 2012 have been propagated and analysed at the WHO Collaborating Centre in London (Table 1). The majority were A(H3N2) viruses (83%), 6% of viruses were of the B/Yamagata lineage, 4% were of the influenza B/Victoria lineage and only 3% were influenza A(H1N1)pdm09 viruses. Viruses/clinical samples were received from 20 countries in the EU/EEA region.

Influenza A(H1N1)pdm09 virus analyses

Since the previous report, an additional A(H1N1)pdm09 influenza virus has been analysed by HI assay (results are shown in Table 2). The virus analysed show good reactivity with antisera raised against the panel of viruses used, including antiserum raised against the vaccine virus (A/California/7/2009).

Phylogenetic analysis of the HA gene of this virus and others from WHO National Influenza Centres or other WHO Collaborating Centres was carried out (Figure 1). The HA genes of H1N1 viruses cluster into eight genetic groups, as previously described, defined by the following amino acid substitutions in HA1 compared to A/California/7/2009. In addition to the substitutions P83S, S203T and I321V in the HA, the groups had the following substitutions in HA1:

- Group 2 N31D, S162N (resulting in the gain of a glycosylation site) & A186T**,** e.g. A/Czech Republic/32/2011;
- Group 3 A134T & S183P, e.g. A/Hong Kong/3934/2011;
- Group 4 N125D, e.g. A/Christchurch/16/2010;
- Group 5 D97N, R205K, I216V & V249L, e.g. A/Astrakhan/1/2011;
- Group 6 D97N & S185T, e.g. A/St Petersburg/27/2011;
- Group 7 S143G, S185T & A197T, e.g. A/St Petersburg/100/2011;
- Group 8 A186T & V272A, e.g. A/Ghana/763/2011.

The A/Stockholm/48/2012 virus fell into genetic group 6, with previously characterised viruses also falling into this group and groups 5 and 7, with none of the genetic groups predominant in Europe or globally.

Influenza A(H3N2) virus analyses

The majority of viruses and samples received from the WHO NICs in EU and EEA Member States were A(H3N2) viruses (Table 1).

As described in CNRL virus characterisation for August-September 2011, A(H3N2) viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pigs, turkeys and humans. Those viruses with sufficient titre in HA assays using guinea pig red blood cells in the presence of 20nM oseltamivir to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010), were analysed by means of HI assay. (Approximately 75% of viruses propagated during the period September 2011 to March 2012 retained sufficient HA titre in the presence of oseltamivir to allow HI analysis).

The results of the HI assays carried out since the last report are shown in Tables 3 to 7. When compared to the homologous reactions, the test viruses showed good reactivity with antisera raised against reference viruses propagated only in cells. These antisera were raised against A/Alabama/5/2010, A/Hong Kong/3969/2011, A/Stockholm/18/2011, A/Finland/190/2011, A/England/259/2011 and A/Norway/1789/2011, with only two of the 37 viruses analysed showing an eight-fold reduction in titre with any of the antisera, compared to the titres against the homologous viruses. In the HI assays all of the viruses showed an eight-fold or greater reduction in HI titre compared to the homologous titres for post-infection ferret antisera raised against the vaccine virus (A/Perth/16/2009) and all other reference viruses propagated in eggs (A/Victoria/208/2009 and A/Iowa/19/2010). This included three sera raised against the new prototype vaccine virus A/Victoria/361/2011 and the high growth reassortant candidate vaccine virus IVR-165 (see Tables 6 and 7). The low reactivity of test viruses with antisera raised against egg-adapted viruses, including the current vaccine virus, render these results difficult to interpret and it is possible that what might be taken as evidence of antigenic drift for many of the test viruses may, in fact, reflect isolation/passage history of the test viruses, all of which were propagated in cell culture.

The HA gene sequences of representative viruses were analysed phylogenetically (Figure 2), with those for which antigenic results are available highlighted in Tables 3 and 5. Seven genetic groups can be identified among the HA genes for recently circulating A(H3N2) influenza viruses, as defined below by the amino acid substitutions in HA1 compared to the vaccine virus A/Perth/16/2009. The seven genetic groups fall into two genetic clades, the Perth/16 clade and the Victoria/208 clade.

In the Perth/16 genetic clade there are two genetic groups:

- Group 1 P162S, I260M, R261Q, e.g. A/Victoria/210/2009
- Group 2 N133D (resulting in the loss of a glycosylation site), R142G, T212A & V213A, e.g. A/Norway/1330/2010.

In the Victoria/208 genetic clade all HA gene sequences encode the substitutions: K62E, K144N (resulting in the gain of a glycosylation site) and T212A, as regards viruses of the Perth/16 genetic clade. There are five genetic groups, one of which can be sub-divided into three sub-groups:

- Group 3A N144D (resulting in the loss of a glycosylation site), N145S & V223I, e.g. A/Stockholm/18/2011
- Group 3B N145S, A198S, V223I & N312S, e.g. A/England/259/2011
- Group 3C S45N (resulting in the gain of a glycosylation site) T48I, A198S, V223I & N312S, e.g. A/Hong Kong/3969/2011, with some viruses also carrying the substitutions D53N, or N278K, sometimes combined with Q33R, with a sub-set carrying L3I
- Group 4 N312S, e.g. A/Serbia/71/2011
- Group 5 D53N, Y94H, I230V & E280A, e.g. A/Perth/10/2010
- Group 6 D53N, Y94H, S199A, I230V & E280A, e.g. A/Iowa/19/2010
- Group 7 S45N (resulting in the gain of a glycosylation site), e.g. A/Alabama/04/2011.

Viruses collected in EU/EEA countries continue to fall predominantly into genetic groups 3A, 3B, 3C and 6.

Based on the antigenic and genetic results it is possible that the prototype virus, A/Perth/16/2009, might no longer be the most appropriate vaccine candidate for the Northern Hemisphere 2012–2013 influenza season. Hence, WHO recommended a change of the A(H3N2) component of trivalent vaccines to an A/Victoria/361/2011-like virus.

Influenza B virus analyses

The number of influenza B virus detections has been low but nearly twice as many viruses of the B/Yamagata lineage have been received from the WHO National Influenza Centres in EU/EEA countries compared to those of the B/Victoria lineage.

B/Victoria-lineage viruses

The results of HI analyses of influenza B viruses of the B/Victoria lineage can be seen in Table 8. All but one of the test viruses showed reduced reactivity (≥eight-fold reduction in titre compared to the homologous titre) with post-infection ferret antiserum raised against the egg-propagated vaccine virus B/Brisbane/60/2008. All viruses reacted well with antisera raised against viruses which were genetically closely related to the vaccine virus but propagated in cells. In Table 8, these sera were raised against B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010, viruses which are considered to be surrogate, cell-propagated antigens representing the egg-propagated vaccine virus. The reactivity of test viruses with antiserum raised against B/Malta/MV636714/2011, another egg isolate, was low and similar to their reactivity with antiserum raised against B/Brisbane/60/2008.

Phylogenetic analysis of the HA1 coding region of the HA gene for representative B/Victoria lineage viruses is shown in Figure 3. The HA genes of all recently collected viruses from EU and EEA laboratories have fallen into Clade 1, the B/Brisbane/60 clade.

B/Yamagata-lineage viruses

Table 9 shows the results of HI assays of influenza B/Yamagata lineage viruses from WHO National Influenza Centres in EU/EEA countries recently received by the WHO Collaborating Centre in London. Seventy-five per cent of viruses showed reduced reactivity (≥eight-fold reduction compared to the homologous titre) with post-infection ferret antiserum raised against the previous vaccine virus of the B/Yamagata lineage, B/Florida/4/2006. Eight of twelve test viruses also reacted poorly with the post-infection ferret antisera raised against the egg-propagated viruses B/Wisconsin/1/2010 and B/Bangladesh/3333/2007. The test viruses reacted well with the post-infection ferret antisera raised against the egg-propagated virus B/Stockholm/12/2011 and the cell-propagated virus B/Serbia/1894/2011. Most test viruses, with the exception of four viruses from Greece which have yet to be analysed genetically, showed low reactivity with the post-infection ferret antiserum raised against B/Estonia/55669/2011, a virus from a distinct genetic clade.

Figure 4 shows a phylogenetic analysis of the HA1 coding region of the HA gene for representative B/Yamagata lineage viruses. The HA genes of all viruses collected by the WHO National Influenza Centres in EU/EEA countries and received by the WHO Collaborating Centre in London fall into the HA-defined genetic Clade 3, represented by B/Bangladesh/3333/2007. 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 (marked i in Figure 4)
- a group defined by the substitution T181K (e.g. B/Ireland/M1522/2012) (marked ii)
- a group defined by the substitution M251V, with the substitutions T181A and K253R (e.g. B/Serbia/1894/2011) (marked iii)
- a group defined by the substitution M251V, with the substitutions V29A and L172Q (e.g. B/Stockholm/12/2011) (marked iv).

In the samples received at the WHO Collaborating Centre in London from the WHO National Influenza Centres in EU/EEA countries, most of the HA gene sequences of viruses received with collection dates after 1 September 2011 fell into the group similar to B/Stockholm/12/2011. From elsewhere in the world, a small number of isolates fall into the B/Brisbane/3/2007 clade (Clade 2).

Given the significant increase in B/Yamagata lineage viruses, as a proportion of the influenza B viruses that have been detected recently, and the antigenic drift that has occurred relative to the previous B/Yamagata lineage vaccine virus (B/Florida/4/2006), WHO is recommending a change in the composition of trivalent influenza vaccines for the Northern Hemisphere 2012–2013 influenza season, from a virus of the B/Victoria lineage to the B/Yamagata lineage.

A fuller description of these results and others obtained from other WHO National Influenza Centres received by the WHO Collaborating Centre for Reference and Research on Influenza, based at the MRC National Institute for Medical Research in London, can be found at: http://www.nimr.mrc.ac.uk/documents/about/interim-report-feb-2012.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 against which post-infection ferret antisera have been raised. The colours indicate the date of sample collection. Isolates from WHO National Influenza Centres in ECDC countries are highlighted in yellow. Sequences for some of the viruses from non-EU/EEA countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, collected since 1 September 2011

MONTH	A Untyped *	H1N1pdm09		H3N2		B Untyped *	B Yamagata lineage		B Victoria lineage	
Country		Number received	Number propagated	Number received	Number propagated		Number received	Number propagated	Number received	Number propagated
SEPTEMBER										
Denmark				1	1					
France		1	1						1	1
Spain				1	1					
Sweden				3	3					
United Kingdom				1	1					
OCTOBER										
Belgium						1				
France				1	1					
Germany									1	1
Norway				2	2				1	1
Sweden		1	1	2	2					
United Kingdom				3	3		1	1		
NOVEMBER										
Belgium		1	0	1	1					
Denmark				1	1					
Finland				1	1					
France				1	1		2	2		
Germany				3	2					
Ireland				2	0	1				
Italy				4	4					
Netherlands							1	1		
Norway				3	3					
Portugal				1	1					
Slovakia				2	2					
Spain				7	4		1	1		
Sweden		2	1	4	4		2	2		
United Kingdom				2	2					
DECEMBER										
Belgium				6	3					
Finland				1	1					
France				14	14					
Germany				12	12		1	1		
Ireland				6	6					
Italy				21	19					
Latvia				1	1					
Netherlands				3	3					
Norway		2	1	21	21				2	2
Romania				4	3					
Slovenia				2	2					
Spain				36	18		3	3		
Sweden		2	1	8	8					
JANUARY										
Austria				4	4		1	1	1	1
Bulgaria				4	in progress					
Finland				3	2					
France				4	4					
Germany				14	13				1	1
Greece				8	7	5	1	1	4	4
Ireland				7	2		1	1	1	1
Italy				1	1					
Latvia				6	5		1	1		
Netherlands				2	2					
Norway				5	5					
Portugal				3	2					
Romania				3	3					
Slovenia				1	1					
Spain		1	0	18	10		2	2		
Sweden		1	1	7	7		1	1		
United Kingdom				3	3		1	1		
FEBRUARY										
Bulgaria				8	in progress					
Greece				14	13	4	4	4	2	2
Total Received = 355	0	11	6	296	235	11	23	23	14	14
		3.1%		83.4%		3.1%	6.5%		3.9%	

Table 2. Antigenic analyses of A(H1N1)pdm09 viruses

				Haemagglutination inhibition titre[1]							
				Post infection ferret sera							
Viruses	Collection date	Passage History		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
Genetic group				group 1	group 1	group 1	group 4	group 3	group 5	group 6	group 7
REFERENCE VIRUSES											
A/California/7/2009	2009-04-09	E1/E2		1280	1280	1280	640	640	640	320	640
A/Bayern/69/2009	2009-07-01	MDCK5		160	640	160	80	80	160	80	80
A/Lviv/N6/2009	2009-10-27	M4/S1/M2		640	2560	1280	320	160	160	320	160
A/Christchurch/16/2010	2010-07-12	E2/E1		2560	2560	2560	5120	1280	2560	1280	5120
A/Hong Kong/3934/2011	2011-03-29	M2/M2		640	320	320	320	1280	1280	640	1280
A/Astrakhan/1/2011	2011-02-28	M1/M5		2560	1280	1280	1280	2560	2560	2560	5120
A/St. Petersburg/27/2011	2011-02-14	E1/E2		5120	2560	1280	1280	2560	2560	2560	5120
A/St. Petersburg/100/2011	2011-03-14	E1/E2/E1		1280	640	640	640	1280	1280	1280	2560
TEST VIRUSES											
A/Stockholm/48/2012	2011-12-22	1/MDCK4	6	1280	640	640	640	1280	1280	1280	2560

1. < = <40

Vaccine virus

Sequence in phylogenetic tree

Figure 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes

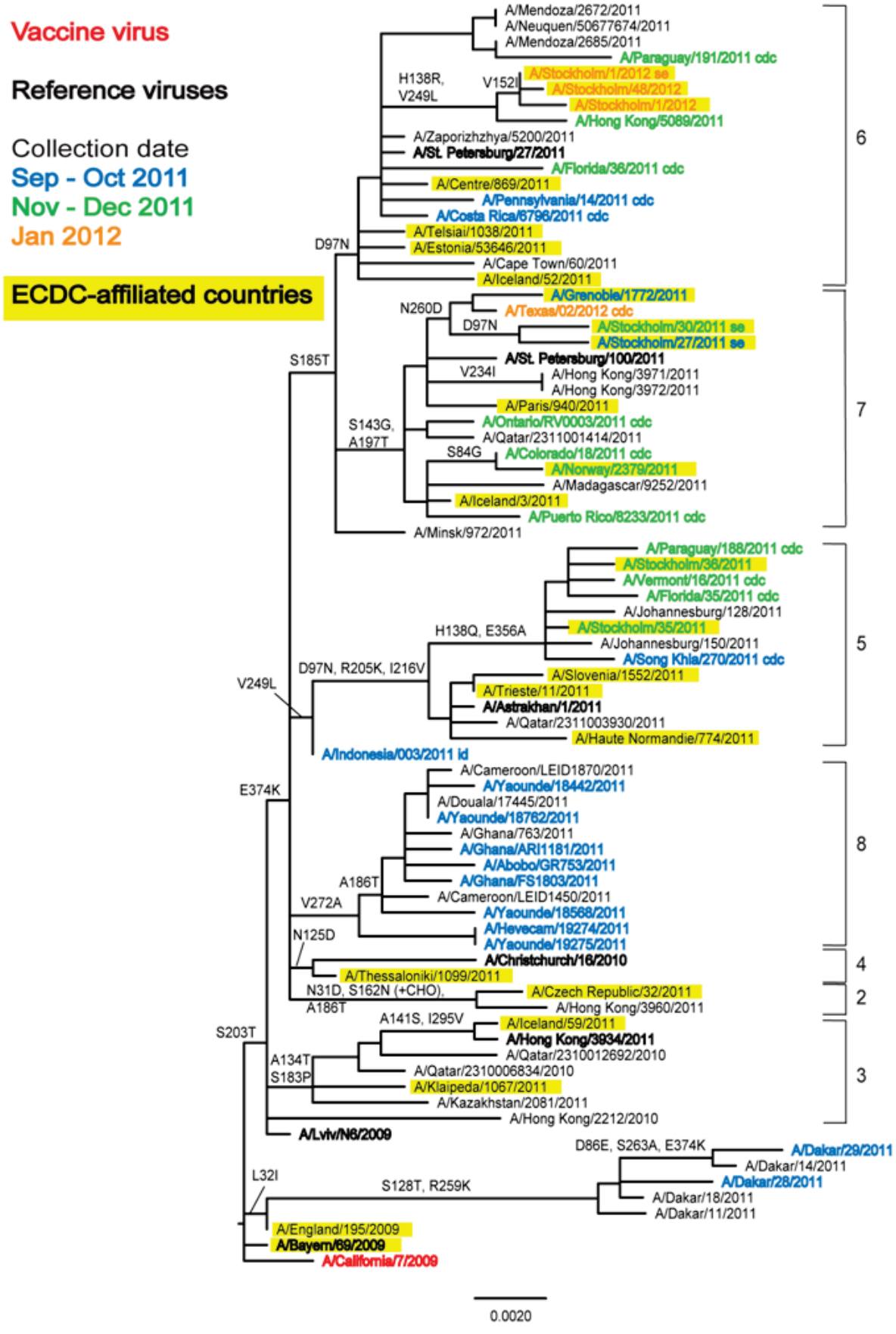


Table 3. Antigenic analyses of influenza A H3N2 viruses (Guinea Pig RBC with 20nM Oseltamivir)

				Haemagglutination inhibition titre[1]								
				Post infection ferret sera								
Viruses		Collection Date	Passage History	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Ala 5/10 F27/10	A/HK 3969/11 F27/11	A/Stock 18/2011 F28/11	A/Iowa 19/2010 F15/11	A/Fin 190/11 F01/12	A/Eng 259/11 F02/12	A/Norway 1789/11 F03/12
Genetic group						group 5	group 3C	group 3A	group 6	group 3C	group 3B	group 3C
REFERENCE VIRUSES												
A/Perth/16/2009		2009-07-04	E3/E2	1280	40	160	320	160	160	160	80	160
A/Victoria/208/2009		2009-06-02	E3/E1	2560	5120	2560	5120	5120	5120	5120	2560	2560
A/Alabama/5/2010		2010-07-13	MK1/M2/SIAT2	40	<	160	320	80	160	160	80	80
A/Hong Kong/3969/2011		2011-05-19	MDCK2/SIAT4	160	160	320	640	320	320	640	320	320
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT3	160	160	160	640	640	160	320	320	160
A/Iowa/19/2010		2010-12-30	E3/E1	1280	2560	1280	2560	2560	5120	2560	1280	2560
A/Finland/190/2011		2011-11-25	Cx/SIAT3	160	160	160	640	320	160	640	320	320
A/England/259/2011		2011-11-16	Cx/SIAT2	320	320	320	640	640	320	640	640	320
A/Norway/1789/2011		2011-08-02	Cx/SIAT3	160	160	160	640	320	160	640	320	320
TEST VIRUSES												
A/Andalucia/RR8952/2011		2011-11-17	SIAT1/SIAT2	80	160	320	320	320	320	640	160	320
A/Andalucia/RR8949/2011		2011-12-24	SIAT1/SIAT2	40	80	160	320	320	160	640	160	160
A/Andalucia/RR8942/2011		2011-12-24	SIAT1/SIAT1	160	320	320	640	640	640	1280	640	640
A/Andalucia/RR8946/2011	6	2011-12-29	SIAT1/SIAT1	80	160	320	640	320	320	640	320	320
A/Aragon/RR8907/2011		2011-12-29	SIAT1/SIAT2	80	80	320	640	320	320	640	320	320
A/LaRioja/RR8958/2011		2011-12-30	SIAT1/SIAT1	40	80	80	320	160	160	320	320	160
A/Melilla/RR8885/2012	3B	2012-01-02	SIAT1/SIAT1	80	80	80	320	320	160	640	320	160
A/Murcia/RR9012/2012		2012-01-09	SIAT1/SIAT2	80	160	160	320	320	320	640	320	160
A/Stockholm/5/2012		2012-01-12	C1/SIAT2	160	160	80	320	320	80	320	320	160
A/Galicia/RR9065/2012		2012-01-20	SIAT1/SIAT2	160	160	320	640	320	640	640	320	320
A/Aragon/RR9056/2012	6	2012-01-20	SIAT1/SIAT1	160	160	320	640	320	640	640	320	320
A/Melilla/RR9064/2012		2012-01-23	SIAT1/SIAT2	80	160	320	320	320	320	640	320	320
A/Madrid/RR9076/2012		2012-01-25	SIAT1/SIAT2	80	160	320	640	320	320	1280	160	320

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

Table 4. Antigenic analyses of influenza A H3N2 viruses (Guinea Pig RBC with 20nM Oseltamivir)

			Haemagglutination inhibition titre[1]								
			Post infection ferret sera								
Viruses	Collection Date	Passage History	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Ala 5/10 F27/10	A/HK 3969/11 F27/11	A/Stock 18/2011 F28/11	A/Iowa 19/2010 F15/11	A/Fin 190/11 F01/12	A/Eng 259/11 F02/12	A/Norway 1789/11 F03/12
Genetic group					group 5	group 3C	group 3A	group 6	group 3C	group 3B	group 3C
REFERENCE VIRUSES											
A/Perth/16/2009	2009-07-04	E3/E2	1280	80	320	640	320	320	320	320	640
A/Victoria/208/2009	2009-06-02	E3/E1	1280	5120	1280	2560	2560	5120	5120	2560	5120
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT2	40	40	160	320	160	160	160	160	320
A/Hong Kong/3969/2011	2011-05-19	MDCK2/SIAT4	160	160	320	1280	640	320	1280	640	1280
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT3	80	80	160	640	640	320	320	320	640
A/Iowa/19/2010	2010-12-30	E3/E1	2560	5120	2560	5120	5120	5120	5120	5120	5120
A/Finland/190/2011	2011-11-25	Cx/SIAT3	160	160	320	1280	640	640	1280	640	1280
A/England/259/2011	2011-11-16	Cx/SIAT2	80	80	80	320	160	160	640	320	640
A/Norway/1789/2011	2011-08-02	Cx/SIAT3	320	320	640	1280	640	640	1280	640	1280
TEST VIRUSES											
A/Andalucia/RR8953/2011	2011-11-17	SIAT1/SIAT3	160	160	320	1280	640	640	1280	320	1280
A/LaRioja/RR8961/2011	2011-12-16	SIAT1/SIAT3	160	160	160	320	640	320	320	320	640
A/Andalucia/RR8947/2011	2011-12-28	SIAT1/SIAT3	80	40	160	160	160	160	160	320	320
A/Madrid/SO8944/2012	2012-01-23	SIAT1/SIAT3	160	320	320	1280	640	640	640	640	1280

1. < = <40

Vaccine virus

Table 5. Antigenic analyses of influenza A H3N2 viruses (Guinea Pig RBC with 20nM Oseltamivir)

Viruses		Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera								
				A/Perth 16/09	A/Vic 208/09	A/Ala 5/10	A/HK 3969/11	A/Stock 18/2011	A/Iowa 19/2010	A/Fin 190/11	A/Eng 259/11	A/Norway 1789/11
				F35/11	F7/10	F27/10	F27/11	F28/11	F15/11	F01/12	F02/12	F03/12
Genetic group						group 5	group 3C	group 3A	group 6	group 3C	group 3B	group 3C
REFERENCE VIRUSES												
A/Perth/16/2009		2009-07-04	E3/E2	1280	40	160	640	160	160	160	160	320
A/Victoria/208/2009		2009-06-02	E3/E1	1280	5120	2560	5120	2560	5120	5120	2560	2560
A/Alabama/5/2010		2010-07-13	MK1/M2/SIAT2	80	<	160	320	160	160	160	160	320
A/Hong Kong/3969/2011		2011-05-19	M2/S4	160	160	320	1280	640	640	1280	640	640
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT32	80	160	160	640	640	160	640	320	640
A/Iowa/19/2010		2010-12-30	E3/E1	640	5120	1280	5120	1280	5120	5120	2560	2560
A/Finland/190/2011		2011-11-25	Cx/SIAT2	160	160	320	640	320	320	1280	640	1280
A/England/259/2011		2011-11-16	Cx/SIAT2	40	80	160	160	160	80	160	320	640
A/Norway/1789/2011		2011-08-02	Cx/SIAT2	160	160	320	640	320	320	640	320	1280
TEST VIRUSES												
A/Athens GR/59/2012	3A	2012-01-23	1st/MDCK2	80	160	160	640	640	160	320	320	640
A/Athens GR/62/2012	3B	2012-01-23	1st/MDCK2	80	80	160	320	320	160	320	320	320
A/Athens GR/73/2012		2012-01-25	1st/MDCK2	<	80	40	160	160	80	320	160	320
A/Athens GR/79/2012	3B	2012-01-26	MDCK2	80	160	80	320	320	160	320	160	320
A/Ioannina GR/81/2012		2012-01-26	MDCK2	80	160	80	320	320	160	320	160	320
A/Ioannina GR/145/2012	3A	2012-01-30	MDCK2	80	160	80	320	320	160	320	160	320
A/Ioannina GR/148/2012		2012-02-01	MDCK2	80	160	160	640	640	320	640	320	640
A/AthensGR/112/2012	3B	2012-02-01	1st/MDCK2	80	160	80	320	320	160	320	320	640
A/Athens GR/125/2012		2012-02-01	1st/MDCK2	40	80	80	320	320	160	320	160	320
A/Athens GR/131/2012	3C	2012-02-02	1st/MDCK2	80	160	160	320	320	160	640	320	1280
A/Athens GR/134/2012	3A	2012-02-02	1st/MDCK2	80	160	160	640	640	160	640	320	640
A/Athens GR/165/2012	3A	2012-02-04	MDCK2	80	160	160	640	640	320	640	320	640
A/Athens GR/166/2012		2012-02-04	MDCK2	80	160	160	640	640	320	640	320	640
A/Athens GR/173/2012	3B	2012-02-06	MDCK2	80	160	160	640	320	160	640	320	640

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

Table 6. Antigenic analyses of influenza A H3N2 viruses (Guinea Pig RBC with 20nM Oseltamivir)

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera									
			A/Perth 16/09	A/Vic 208/09	A/Ala 5/10	A/HK 3969/11	A/Stock 18/2011	A/Iowa 19/2010	A/Fin 190/11	A/Norway 1789/11	A/Vic 361/11	A/Vic 361/11
			F35/11	F7/10	F27/10	F27/11	F28/11	F15/11	F01/12	F03/12	F05/12	F06/12
Genetic group					group 5	group 3C	group 3A	group 6	group 3C	group 3C	group 3C	group 3C
REFERENCE VIRUSES												
A/Perth/16/2009	2009-07-04	E3/E1	1280	80	160	640	160	160	320	640	160	160
A/Victoria/208/2009	2009-06-02	E3/E1	1280	2560	1280	2560	2560	5120	5120	5120	2560	5120
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT1	40	40	320	320	160	160	160	320	80	160
A/Hong Kong/3969/2011	2011-05-19	MDCK3	160	320	320	640	640	640	1280	1280	640	640
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT2	80	160	160	640	640	160	320	640	640	320
A/Iowa/19/2010	2010-12-30	E3/E1	640	5120	1280	2560	2560	5120	2560	5120	2560	2560
A/Finland/190/2011	2011-11-25	Cx/SIAT3	160	320	320	640	640	640	1280	1280	640	640
A/Norway/1789/2011	2011-08-02	Cx/SIAT3	160	320	320	640	640	640	1280	1280	640	640
A/Victoria/361/2011	2011-10-24	E3/E1	320	640	640	1280	160	1280	1280	1280	5120	5120
TEST VIRUSES												
A/Athens GR/208/2012	2012-02-04	SIAT2	80	160	160	640	320	320	1280	640	160	320
A/Athens GR/237/2012	2012-02-06	SIAT3	160	320	160	1280	2560	640	1280	1280	640	640

1. < = <40

Vaccine virus

Table 7. Antigenic analyses of influenza A H3N2 viruses (Guinea Pig RBC with 20nM Oseltamivir)

			Haemagglutination inhibition titre[1]									
			Post infection ferret sera									
Viruses	Collection Date	Passage History	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Ala 5/10 F27/10	A/HK 3969/11 F27/11	A/Stock 18/2011 F28/11	A/Iowa 19/2010 F15/11	A/Fin 190/11 F01/12	A/Norway 1789/11 F03/12	A/Vic 361/11 F05/12	IVR-165 (Vic/361) NIB F11/12
Genetic group					group 5	group 3C	group 3A	group 6	group 3C	group 3C	group 3C	group 3C
REFERENCE VIRUSES												
A/Perth/16/2009	2009-07-04	E3/E1	1280	80	160	640	160	160	320	640	160	40
A/Victoria/208/2009	2009-06-02	E3/E1	1280	5120	1280	2560	2560	5120	5120	5120	2560	2560
A/Alabama/5/2010	2010-07-13	MK1/M2/SIAT1	40	40	160	320	160	160	160	320	80	<
A/Hong Kong/3969/2011	2011-05-19	MDCK3	160	320	320	1280	640	640	1280	1280	640	160
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT2	80	160	160	640	640	160	320	640	640	80
A/Iowa/19/2010	2010-12-30	E3/E1	640	5120	1280	2560	2560	5120	2560	5120	2560	1280
A/Finland/190/2011	2011-11-25	Cx/SIAT3	160	320	320	640	640	640	1280	1280	640	160
A/Norway/1789/2011	2011-08-02	Cx/SIAT3	160	320	320	640	640	640	1280	1280	640	320
A/Victoria/361/2011		E3/E1	320	640	640	1280	160	1280	1280	1280	5120	1280
IVR-165 (Vic/361/11)		E3/D6/E1	160	640	320	640	640	640	640	640	640	1280
TEST VIRUSES												
A/Ioannina GR/187/2012	2012-01-31	SIAT4	80	80	80	320	640	160	640	1280	160	40
A/Athens GR/193/2012	2012-02-07	SIAT3	40	40	80	320	160	80	320	1280	80	40
A/Athens GR/209/2012	2012-02-08	SIAT4	80	80	160	320	320	320	640	1280	160	40
A/Athens GR/248/2012	2012-02-09	SIAT3	80	80	160	320	320	160	640	640	160	40
			Vaccine virus									

1. < = <40

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

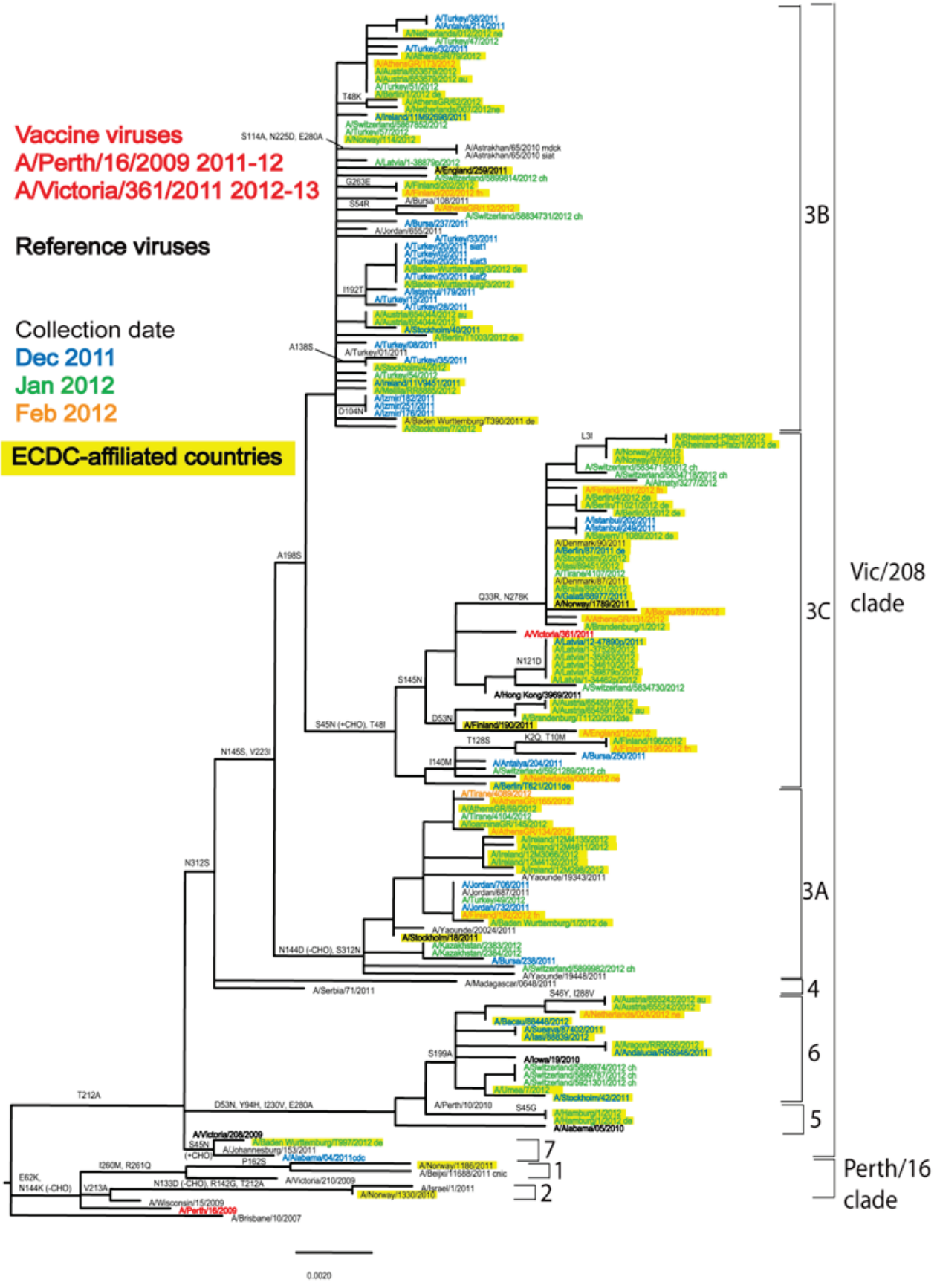


Table 8. Antigenic analyses of influenza B viruses (Victoria lineage)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]							
			Post infection ferret sera							
			B/Bris 60/08 Sh 523	B/Mal 2506/04 F37/11	B/England 393/08 F05/11	B/Bris 60/08 F06/11	B/Paris 1762/08 F07/11	B/HK 514/09 F13/10	B/Odessa 3886/10 F17/10	B/Malta 636714/11 F33/11
REFERENCE VIRUSES										
B/Malaysia/2506/2004	2004-12-06	E3/E3	1280	320	80	160	10	<	10	80
B/England/393/2008	2008-08-29	E1/E6	1280	320	320	1280	80	80	80	320
B/Brisbane/60/2008	2008-08-04	E4/E4	640	160	320	640	80	40	80	160
B/Paris/1762/2008	2009-02-09	C2/MDCK4	1280	20	80	80	80	80	80	20
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK1	5120	20	20	40	160	80	160	10
B/Odessa/3886/2010	2010-03-19	C2/MDCK3	2560	20	80	160	320	160	160	40
B/Malta/636714/2011		E4/E1	2560	40	320	640	80	40	40	320
TEST VIRUSES										
B/Lefkada GR/46/2012	2012-01-22	MDCK2	5120	10	20	40	160	80	80	40
B/Lefkada GR/58/2012	2012-01-24	1st/MDCK2	5120	10	20	40	160	80	80	40
B/Crete GR/141/2012	2012-01-26	MDCK3	5120	40	80	160	320	320	320	80
B/Athens GR/98/2012	2012-01-30	MDCK2	2560	10	20	40	40	20	40	40
B/Athens GR/147/2012	2012-02-01	MDCK2	5120	20	40	80	160	160	160	40
B/Athens GR/152/2012	2012-02-03	MDCK2	5120	20	40	80	160	160	160	40

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

Vaccine virus

Table 9. Antigenic analyses of influenza B viruses (Yamagata lineage)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre								
			Post infection ferret sera								
			B/Fl[3] 4/06 SH479	B/Eg[1] 144/05 F3/07	B/Fl[1] 4/06 F21/07	B/Bris[2] 3/07 F24/07	B/Eng[2] 145/08 F09/08	B/Bang[2] 3333/07 F21/08	B/Wis[2] 1/10 F26/10	B/Stock[2] 12/2011 F34/11	B/Estonia[2] 55669/2011 F26/11	B/Serbia[2] 1894/2011 F25/11
REFERENCE VIRUSES											
B/Egypt/144/2005	2005-05-01	E3/E5	5120	640	2560	1280	160	640	320	640	320	40
B/Florida/4/2006	2006-12-15	E3/E4	5120	640	1280	640	160	320	320	640	320	40
B/Brisbane/3/2007	2007-09-03	E2/E4	5120	160	640	320	80	160	160	320	160	10
B/England/145/2008		Ex/E3	640	40	40	20	160	10	10	320	<	<
B/Bangladesh/3333/2007	2007-08-07	E4/E1	5120	160	160	160	40	320	320	640	10	20
B/Wisconsin/1/2010	2010-02-20	E3/E1	2560	160	160	80	80	40	320	640	10	40
B/Stockholm/12/2011	2011-03-28	E4/E1	5120	160	160	160	80	160	160	640	40	80
B/Estonia/55669/2011	2011-03-14	MDCK2/MDCK2	1280	40	40	40	10	10	<	160	1280	80
B/Serbia/1894/2011	2011-03-08	MDCK1/MDCK4	2560	80	80	80	40	20	40	320	160	160
TEST VIRUSES											
B/Stockholm/12/2011	2011-03-28	Cx/MDCK3	5120	160	320	80	160	320	80	640	640	1280
B/La Rioja/RR8960/2011	2011-12-23	SIAT/MDCK2	5120	40	40	20	40	20	10	320	20	40
B/La Rioja/RR8959/2011	2011-12-27	SIAT/MDCK1	1280	<	<	<	20	20	10	320	20	40
B/Austria/653880/2012	2012-01-02	C2/MDCK2	2560	40	40	40	40	20	20	320	80	160
B/Navarra/RR9043/2012	2012-01-19	E1/E1	2560	40	80	40	20	<	40	320	<	10
B/Navarra/RR9043/2012	2012-01-19	SIAT/MDCK1	1280	40	<	80	20	20	10	320	40	80
B/Lefkada GR/64/2012	2012-01-24	MDCK2	2560	80	320	80	160	320	160	320	320	1280
B/Latvia/1-48266/2012	2012-01-27	MDCK/MDCK3	1280	80	40	40	80	40	20	640	80	160
B/Athens GR/123/2012	2012-02-01	MDCK2	2560	80	320	320	160	160	80	320	1280	1280
B/Athens GR/132/2012	2012-02-01	MDCK2	1280	40	80	40	40	40	10	160	1280	80
B/Athens GR/179/2012	2012-02-06	MDCK2	5120	40	80	80	40	40	40	320	1280	40
B/Athens GR/199/2012	2012-02-07	MDCK2	5120	160	80	80	320	160	80	640	1280	320

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

Sequences in phylogenetic tree

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

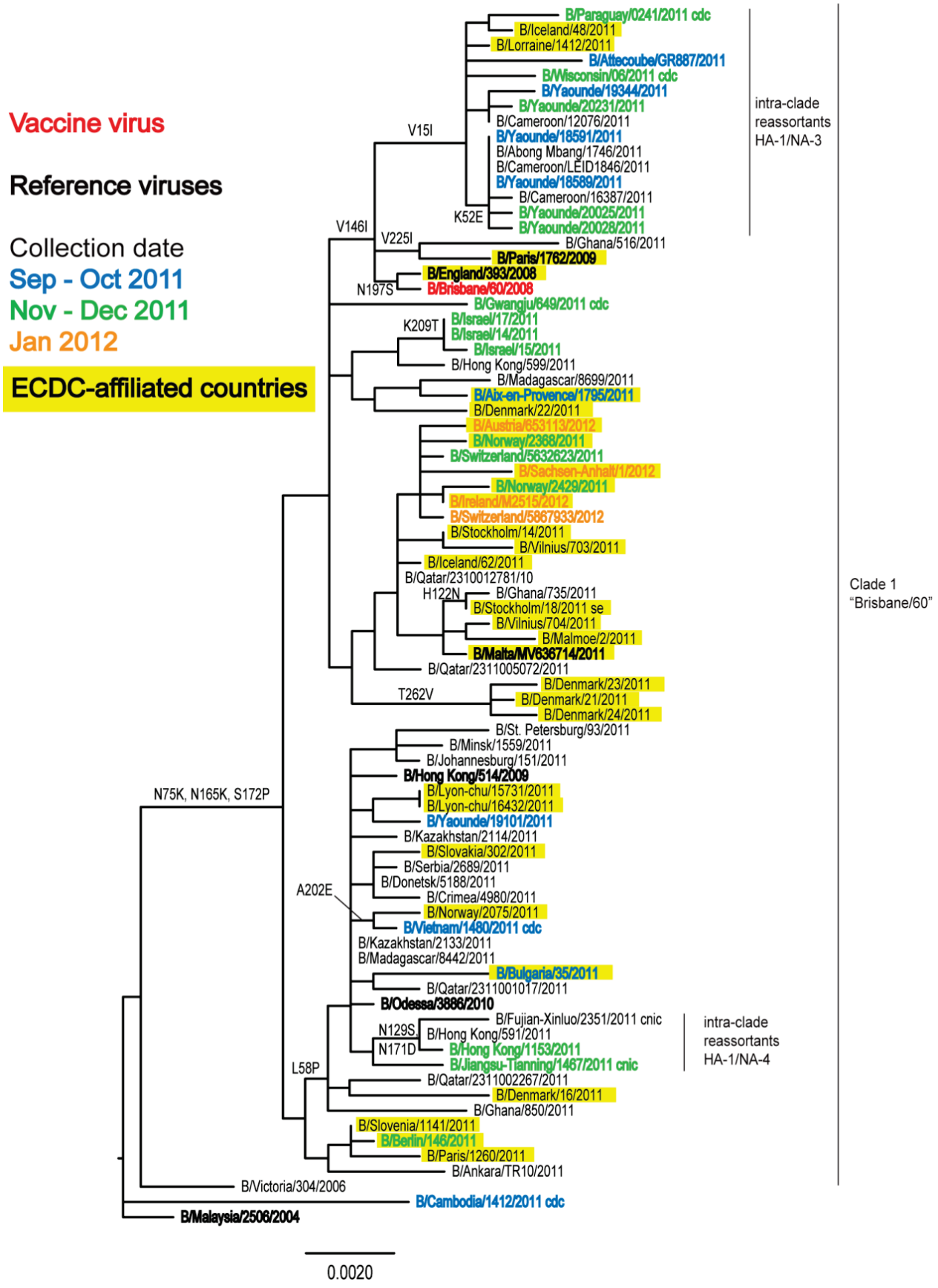
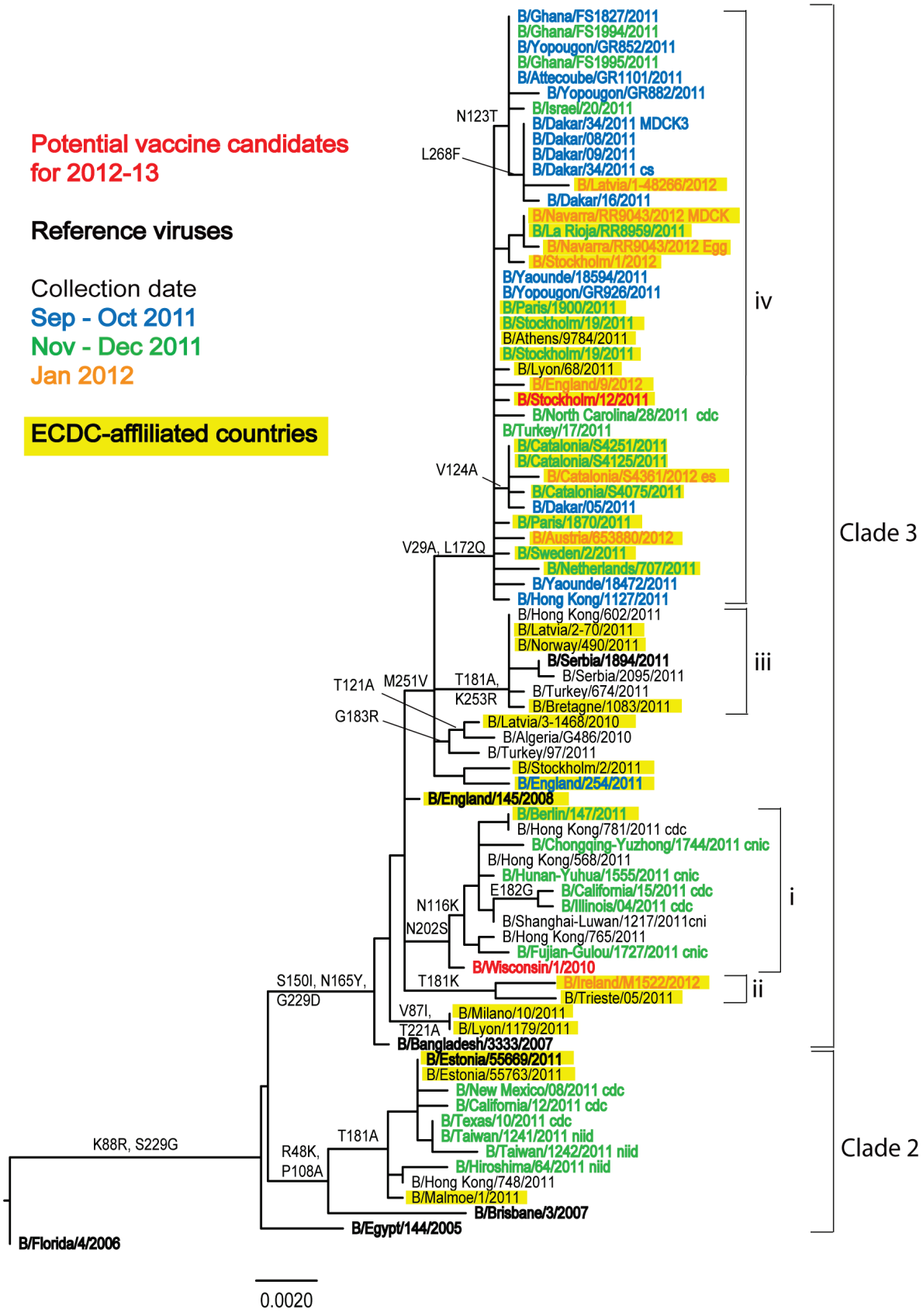


Figure 4. Phylogenetic comparison of influenza B (Yamagata-lineage) HA genes (HA1 region)



References

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