TECHNICAL DOCUMENT

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

Influenza virus characterisation

Summary Europe, February 2012

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Summary

Influenza A(H1N1)pdm09, influenza A(H3N2), and influenza B/Victoria- and B/Yamagata-lineage viruses have been detected in ECDC-affiliated countries since 1 September 2011. This summary presents the results for viruses from EU/EEA countries collected since then by the WHO National Influenza Centres (NICs) and sent to the WHO Collaborating Centre for Reference and Research on Influenza (WHO CC) in London.

- 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. All but one of the recently analysed viruses from ECDC-affiliated countries fall within the A/Victoria/208/2009 genetic clade and there is accumulating evidence of altered antigenicity compared to the vaccine virus, A/Perth/16/2009.
- Influenza B viruses of the B/Victoria/2/87 and B/Yamagata/16/88 lineages have been detected in low numbers, with viruses of the B/Yamagata lineage being in the majority for those received at the WHO CC.
- 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.

Close to 300 viruses/clinical samples, received from WHO National Influenza Centres in the EU/EEA region with collection dates between 1 September 2011 and the end of January 2012, have been propagated and analysed at the WHO CC in London (Table 1). The majority were A(H3N2) viruses (86%); 6% of viruses were of the B/Yamagata lineage, 4% were influenza A(H1N1pdm09) viruses and 3% were of the influenza B/Victoria lineage. Viruses/clinical samples were received from 16 countries in the EU/EEA area.

Influenza A(H1N1)pdm09 virus analyses

The results of haemagglutination-inhibition (HI) analyses of A(H1N1)pdm09 influenza viruses are shown in Table 2. All the viruses analysed show good reactivity with antisera raised against the panel of viruses used, including antisera raised against the vaccine virus (A/California/7/2009), although two of the five viruses showed a four-fold reduced activity in comparison with the titre against the homologous vaccine virus.

Phylogenetic analysis of the HA gene for four of the five viruses analysed by HI along with other viruses from WHO NICs or other WHO CCs was carried out (Figure 1). Amino acid substitutions or polymorphisms between residues 153 and 157 are marked on the tree; no viruses analysed by HI assay showed amino acid substitutions or polymorphism in this region. Substitutions or polymorphism at position 223, associated with egg adaptation, are also marked.

The HA genes of H1N1 viruses cluster into eight genetic groups, previously described, defined by the following amino acid substitutions in HA1 compared with A/California/7/2009. In addition to the substitutions P83S, S203T and I321V the groups had the following substitutions:

- 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.

Recently collected viruses from the EU/EEA countries fell into a range of genetic groups, notably 5, 6 and 7, with none of the genetic groups predominating 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 previously, these viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pig, turkey and humans. Those viruses with sufficient titre in HA assays using guinea pig red blood cells in the presence of 20 nM oseltamivir, to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010), were analysed by HI assay. Approximately 75% of viruses propagated retained sufficient HA titre in the presence of oseltamivir to allow HI analysis. Virus neutralisation assays were used to complement HI.

The results of the HI assays are shown in Tables 3 to 9. The results from the plaque reduction-based virus neutralisation assays are shown in Tables 10 and 11. Of the HI assays, overall in the order of two thirds of the viruses (~67%) showed a reduction in HI titre of eight-fold or more with the post-infection ferret antisera raised against the vaccine virus, A/Perth/16/2009, compared with the homologous titres against the vaccine virus. The test viruses showed higher reactivity with antisera raised against some of the other reference viruses when compared with the homologous reactions, notably with antisera raised against A/Alabama/5/2010, A/Hong Kong/3969/2011 and A/Stockholm/18/2011- notably, all of which have been propagated only in cells.

The results of several virus neutralisation assays have been integrated in Tables 10 and 11. As in the HI analyses, antisera raised against A/Perth/16/2009 (F35/11) reacted with a titre of ≥8-fold lower, with approximately 60% of the test viruses, compared with the titre against the homologous virus. Of these assays, as for the HI assays, test viruses showed good reactivity with antisera raised against the cell propagated reference virus A/Hong Kong/3969/2011 and, and in these virus neutralisation assays, with antisera raised against the egg-propagated reference virus A/Perth/10/2010, when compared to the titres observed with the respective homologous viruses. Low reactivity was observed for the test viruses with antisera raised against A/Iowa/19/2010 compared with the homologous titre of the egg-propagated virus A/Iowa/19/2010.

Phylogenetic analysis of the HA gene sequences of representative viruses, with those for which antigenic results are available being highlighted in Tables 3 to 11, together with other viruses from WHO NICs or other WHO CCs was carried out (Figure 2). Seven genetic groups can be identified among the HA genes for recently circulating A(H3N2) influenza viruses defined by the following amino acid substitutions in HA1 compared with 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 which all carry the substitutions K62E, K144N (resulting in the gain of a glycosylation site) and T212A with respect to viruses of the Perth/16 genetic clade, there are five genetic groups, one of which can be sub-divided into three:

- 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 fell into the genetic groups 3A, 3B, 3C, 5 and 6. Both HI assays and virus neutralisation assays showed that only a minority of viruses in each of the predominating genetic groups (groups 3A, 3B, 3C, 5 and 6) retained good reactivity, i.e. no more than four-fold reduction, with post-infection ferret antisera raised against A/Perth/16/2009. However, the majority of viruses showed good reactivity in HI and virus neutralisation assays with post-infection ferret antisera raised against reference viruses with HA genes in genetic groups 3 and 5.

Influenza B virus analyses

Numbers of influenza B virus detections have been low; but nearly twice as many viruses of the B/Yamagata lineage were received from the WHO NICs in EU/EEA countries compared with 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 12. All viruses show reduced reactivity (≥8-fold) with post-infection ferret antisera raised against the egg-propagated vaccine virus B/Brisbane/60/2008 compared with the titre of the homologous virus, all viruses reacted well with antisera raised against viruses genetically closely related to the vaccine virus but propagated in cells. In Table 12, these sera raised against B/Paris/1762/2008, B/Odessa/3886/2010 and B/Hong Kong/514/2009, and these viruses are considered as surrogate cell-propagated antigens representing the egg-propagated vaccine virus. The reactivity of test viruses with antisera raised against B/Malta/MV636714/2011, another egg isolate, was low and similar to their reactivities with antisera raised against the vaccine virus.

Phylogenetic analysis of the HA1 coding region of the HA gene of representative B/Victoria lineage viruses is shown in Figure 3. The HA genes of all recently collected viruses fall into Clade 1, the B/Brisbane/60 clade.

B/Yamagata-lineage viruses

Table 13 shows the results of HI assays of influenza B/Yamagata lineage viruses received from WHO NICs in EU/EEA countries collected since 1 September 2011 and received by the WHO CC in London. Over 80% of viruses showed reduced (≥8-fold reduction compared with the homologous titre) reactivity with post-infection ferret antisera raised against the most recently chosen vaccine virus of the B/Yamagata lineage, the egg-propagated virus A/Florida/4/2006. The test viruses reacted well with sera raised against the egg-propagated prototype virus B/Bangladesh/3333/2007 with 75% of viruses showing a ≤4-fold reduction in HI titre compared with the homologous titre of the egg-propagated B/Bangladesh/3333/2007; they reacted less well with post-infection ferret antisera raised against A/Wisconsin/1/2010 with 50% of viruses showing a reduction of ≥8-fold compared with the homologous titre; the test viruses reacted well with the ferret post-infection antisera raised against the egg-propagated virus B/Stockholm/12/2011 and the cell-propagated virus B/Serbia/1984/2011. Most test viruses showed low reactivity with the post-infection ferret antisera 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 of representative B/Yamagata lineage viruses. The HA genes of all viruses collected by the WHO NICs in EU/EEA countries and received by the WHO CC in London fall into genetic Clade 3, represented by B/Bangladesh/3333/2007. For the HA gene, 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).

In the samples received at the WHO CC in London from the WHO NICs in EU/EEA countries, most of the HA gene sequences of viruses received with collection dates after 1 September 2011 fell into the latter two groups, the groups similar to B/Serbia/1894/2011 and B/Stockholm/12/2011.

From elsewhere in the world, a small number of isolates fall into the B/Brisbane/3/2007 clade (Clade 2).

Antiviral analyses

At the WHO CC, just over one hundred A(H3N2) viruses and a single influenza B virus received from WHO NICs in EU/EEA countries have been analysed for their susceptibility to the antiviral drugs oseltamivir and zanamivir. All were sensitive to both drugs and no virus displayed reduced sensitivity to either drug. Phenotypic assays for measuring susceptibility to neuraminidase inhibitors was supported by full NA gene sequencing and no amino acid substitutions related to reduced susceptibility/resistance were observed. Analysis of the M gene coding sequence of close to 50 M gene sequences of A(H3N2) viruses and six A(H1N1)pdm09 viruses received from WHO NICs in the EU/EEA region and

collected since 1 September 2011 showed that all encoded the amino acid substitution S31N that confers resistance to the adamantane class of drugs, amantadine and rimantadine.

A fuller description of these results and those obtained from other WHO NICs received by the WHO Collaborating Centre for Reference and Research based in 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 to 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 date of sample collection. Isolates from WHO NICs in ECDC countries are highlighted in yellow. Sequences for some of the viruses from non-EU/EAA countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.

Table 1. Summary of clinical samples and isolates received from ECDC-affliliated countries, collection dates since 2011-09-01

MONTH / Country	A Untyped *	H1N1pdm09		H3N2		B Untyped *	B Yamagata lineage		B Victoria lineage	
		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	in progress		1	1		
Sweden		2	1	4	4		2	2		
United Kingdom				2	2					
DECEMBER										
Belgium				6	3					
Finland				1	in progress					
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	in progress					
Slovenia				2	2					
Spain				25	in progress		3	in progress		
Sweden		2	in progress	8	8					
JANUARY										
Austria				4	4		1	in progress	1	1
Finland				3	in progress					
France				4	4					
Germany				14	13				1	1
Ireland				7	in progress		1	1	1	1
Italy				1	1					
Latvia				6	in progress					
Netherlands				2	2					
Norway				5	5					
Portugal				3	2					
Romania				3	3					
Slovenia				1	1					
Spain		1	0	10	in progress		2	in progress		
Sweden		2	in progress	5	5		1	1		
United Kingdom				3	3		1	1		
Total Received = 280	0	12	4	241	168	2	17	11	8	8
		4.3%		86.1%		0.7%	6.1%		2.8%	

Table 2. Antigenic analyses of A(H1N1)pdm09 influenza 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 N6/09 C4/34/09	A/C'church 16/10 F30/10	A/HK 3934/11 F21/11	A/Astrak 1/11 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	2560	2560	2560	640	640	640	640	640
A/Bayern/69/2009		2009-07-01	MDCK4/MDCK2	160	640	320	80	40	80	80	80
A/Lviv/N6/2009		2009-10-27	MDCK4/SIAT1/MDCK2	640	2560	1280	160	80	160	320	160
A/Christchurch/16/2010		2010-07-12	E2/E1	2560	5120	2560	5120	1280	2560	2560	5120
A/Hong Kong/3934/2011		2011-03-29	MDCK2/MDCK2	1280	320	640	640	2560	1280	1280	2560
A/Astrakhan/1/2011		2011-02-28	MDCK1/MDCK2	2560	1280	2560	1280	2560	1280	2560	5120
A/St. Petersburg/27/2011		2011-02-14	E1/E2	2560	2560	2560	1280	2560	2560	2560	5120
A/St. Petersburg/100/2011		2011-03-14	E1/E2	2560	1280	1280	640	1280	1280	1280	5120
TEST VIRUSES											
A/Stockholm/27/2011	7	2011-10-18	MDCK2/MDCK1	1280	1280	640	640	1280	640	640	2560
A/Pais Vasco/RR8716/2011		2011-10-19	E2/E3	640	640	640	320	640	640	640	2560
A/Stockholm/36/2011	5	2011-11-27	MDCK2/MDCK1	2560	640	2560	1280	5120	2560	2560	5120
A/Norway/2379/2011	7	2011-12-08	MDCK2/MDCK2	1280	640	1280	640	2560	1280	1280	2560
A/Stockholm/1/2012	6	2012-01-02	3/MDCK1	640	1280	640	160	160	160	80	160

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

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

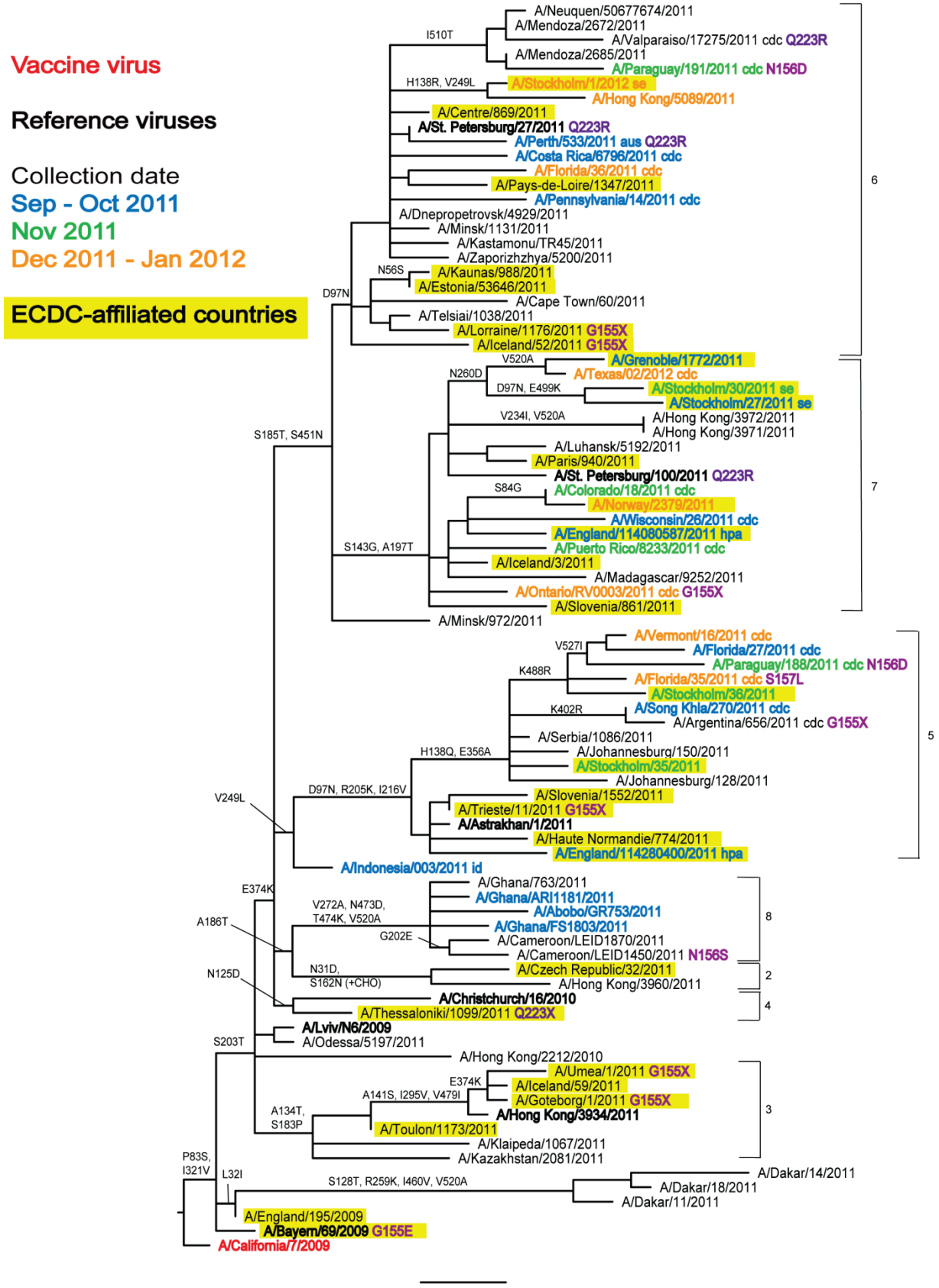


Table 3. Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir)

Viruses		Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret sera								
				A/Bris 10/07	A/Perth 16/09	A/Vic 208/09	A/Vic 210/09	A/Ala 5/10	A/Perth 10/10	A/HK 3969/11	A/Stock 18/11	A/Iowa 19/10
				F18/07	F35/11	F7/10	F11/10	F27/10	F03/11	F27/11	F28/11	F15/11
Genetic group							group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES												
A/Brisbane/10/2007		2007-02-06	E2/E1	5120	80	40	80	<	80	320	40	40
A/Perth/16/2009		2009-07-04	E3/E1	<	640	40	160	80	160	320	160	160
A/Victoria/208/2009		2009-06-02	E3/E2	1280	640	2560	2560	1280	2560	2560	1280	5120
A/Victoria/210/2009		2009-06-02	E2/E3	640	1280	1280	2560	320	1280	1280	640	1280
A/Alabama/5/2010		2010-07-13	MK2/M2/SIAT5	80	80	40	40	320	320	640	160	320
A/Perth/10/2010		2010-05-25	E2/E1	640	320	1280	2560	640	1280	2560	1280	1280
A/Hong Kong/3969/2011		2011-05-19	MDCK3	320	160	160	320	320	640	1280	320	640
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT2	160	80	40	40	80	160	640	320	160
A/Iowa/19/2010		2010-12-30	E3/E1	160	320	1280	1280	640	1280	2560	1280	5120
TEST VIRUSES												
A/England/253/2011	3C	2011-09-11	SIAT1/SIAT1	160	160	160	160	320	640	1280	640	640
A/England/257/2011	3B	2011-10-10	SIAT1/SIAT1	160	80	160	160	320	320	640	640	320
A/England/256/2011	3B	2011-10-12	SIAT1/SIAT1	160	80	160	160	160	320	640	320	320
A/England/255/2011	3B	2011-10-14	SIAT1/SIAT1	80	40	80	160	160	160	320	320	160
A/Bratislava/31/2011	3C	2011-11-03	SIAT2	160	160	320	640	640	640	1280	640	640
A/Bratislava/31/2011	3C	2011-11-03	MDCK2/SIAT1	160	160	160	160	320	320	640	320	320
A/England/258/2011	3C	2011-11-07	SIAT1/SIAT1	80	80	80	80	160	320	640	320	320
A/England/259/2011	3B	2011-11-16	SIAT1/SIAT1	80	80	80	80	160	320	640	320	160
A/Finland/190/2011	3C	2011-11-25	SIAT3/SIAT3	160	80	80	80	160	160	640	320	160

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

Table 4. 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/Bris 10/07	A/Perth 16/09	A/Vic 208/09	A/Vic 210/09	A/Ala 5/10	A/Perth 10/10	A/HK 3969/11	A/Stock 18/11	A/Iowa 19/10
				F29/09	F35/11	F7/10	F11/10	F27/10	F03/11	F27/11	F28/11	F15/11
Genetic group							group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES												
A/Brisbane/10/2007		2007-02-06	E2/E1	640	40	40	<	<	80	160	<	40
A/Perth/16/2009		2009-07-04	E3/E2	<	640	40	160	160	160	640	160	160
A/Victoria/208/2009		2009-06-02	E3/E1	320	640	2560	2560	1280	2560	2560	2560	2560
A/Victoria/210/2009		2009-06-02	E2/E3	320	2560	2560	5120	640	2560	2560	1280	1280
A/Alabama/5/2010		2010-07-13	MK1/C2/SIAT2	<	80	40	40	320	320	640	320	320
A/Perth/10/2010		2010-05-25	E2/E2	320	640	1280	2560	1280	2560	2560	1280	1280
A/Hong Kong/3969/2011		2011-05-19	MDCK2/SIAT4	80	160	80	160	320	320	1280	320	320
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT3	80	80	80	160	320	320	1280	640	320
A/Iowa/19/2010		2010-12-30	E3/E1	320	640	2560	2560	2560	2560	2560	2560	2560
TEST VIRUSES												
A/Stockholm/23/2011	3B	2011-09-05	C1/SIAT1	<	40	80	80	80	160	320	160	80
A/Stockholm/24/2011	3B	2011-09-05	C1/SIAT1	<	40	80	80	160	320	320	320	320
A/Stockholm/26/2011	3A	2011-10-05	C1/SIAT1	<	40	80	80	160	160	320	320	160
A/Norway/2047/2011	6	2011-10-10	MDCK1/SIAT1	40	160	160	160	320	640	1280	320	640
A/Norway/2125/2011	6	2011-10-26	SIAT1/SIAT1	80	320	320	320	640	1280	2560	640	1280
A/Stockholm/29/2011	3B	2011-10-31	C1/SIAT1	<	40	80	80	160	160	640	320	160
A/Norway/2146/2011	3B	2011-11-02	MDCK1/SIAT1	<	40	80	80	160	160	320	160	160
A/Bayern/87/2011	3B	2011-11-16	MDCK2/SIAT1	<	80	160	160	160	320	640	320	320
A/Stockholm/33/2011	3B	2011-11-19	C2/SIAT1	<	80	80	160	160	320	640	320	320
A/Stockholm/32/2011	3C	2011-11-27	C2/SIAT1	40	160	160	160	320	640	1280	640	640
A/Stockholm/34/2011	3B	2011-11-27	C1/SIAT1	40	40	80	80	160	160	320	160	160
A/Netherlands/702/2011	6	2011-12-02	MDCK4/SIAT1	<	80	80	80	160	320	640	160	320
A/Berlin/85/2011	3C	2011-12-07	MDCK2/SIAT1	40	160	160	160	320	640	1280	640	640
A/Berlin/86/2011	3C	2011-12-09	MDCK2/SIAT1	40	160	160	160	320	640	640	640	640
A/Berlin/87/2011	3C	2011-12-12	MDCK2/SIAT1	40	160	160	160	320	640	640	640	640

1. < = <40

Vaccine virus

Sequences in HA phylogenetic tree

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/Bris 10/07 F29/09	A/Perth 16/09 F30/09	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11	A/HK 3969/11 F27/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11
Genetic group							group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES												
A/Brisbane/10/2007		2007-02-06	E2/E1	640	<	<	<	<	80	160	40	<
A/Perth/16/2009		2009-07-04	E3/E2	<	320	40	160	160	160	640	160	160
A/Victoria/208/2009		2009-06-02	E3/E1	640	640	2560	2560	1280	2560	2560	1280	2560
A/Victoria/210/2009		2009-06-02	E2/E3	1280	1280	1280	5120	320	2560	1280	640	1280
A/Alabama/5/2010		2010-07-13	MK1/C2/SIAT2	<	80	40	40	160	320	320	160	160
A/Perth/10/2010		2010-05-25	E2/E2	640	640	1280	2560	1280	2560	2560	1280	2560
A/Hong Kong/3969/2011		2011-05-19	MDCK2/SIAT4	80	160	160	160	320	640	1280	640	320
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT3	80	160	160	160	320	320	640	1280	320
A/Iowa/19/2010		2010-12-30	E3/E1	160	1280	5120	2560	2560	5120	2560	2560	5120
TEST VIRUSES												
A/Madrid/RR8753/2011	3B	2011-11-30	SIAT1/SIAT1	40	80	160	160	160	320	640	320	320
A/Slovenia/2855/2011	5	2011-12-05	MDCKx/SIAT1	40	160	160	160	640	640	1280	320	640
A/Berlin/92/2011	3C	2011-12-07	C5/SIAT1	40	160	640	320	640	640	1280	640	640
A/Berlin/93/2011	3C	2011-12-07	C5/SIAT1	80	320	640	320	640	640	1280	640	640
A/Norway/2366/2011	3B	2011-12-07	SIAT2	160	160	160	160	320	320	640	320	320
A/Berlin/89/2011	3C	2011-12-09	C3/SIAT1	40	160	320	160	320	320	640	320	640
A/Slovenia/2970/2011	5	2011-12-12	MDCKx/SIAT1	40	320	320	320	640	640	1280	640	640
A/Berlin/88/2011	3C	2011-12-13	C1/SIAT1	40	160	320	160	320	640	1280	320	640
A/Berlin/90/2011		2011-12-21	C3/SIAT1	40	160	640	320	320	640	1280	640	640
A/Madrid/RR8856/2011	3B	2011-12-22	SIAT1/SIAT1	40	160	160	160	320	320	640	320	320
A/Berlin/91/2011	3C	2011-12-23	C2/SIAT1	40	160	640	320	640	640	1280	640	640
A/Castilla La Mancha/RR8843/2011	3C	2011-12-26	SIAT1/SIAT2	40	320	160	320	320	640	1280	640	640
A/Pais Vasco/RR8864/2011	3A	2011-12-26	SIAT1/SIAT1	<	80	160	160	160	320	320	640	320
A/Pais Vasco/RR8867/2011	3B	2011-12-27	SIAT1/SIAT2	40	80	160	160	320	320	640	320	320
A/Castilla La Mancha/RR8870/2011	3B	2011-12-27	SIAT1/SIAT1	40	160	160	160	160	320	640	320	320
A/Castilla La Mancha/RR8871/2011	6	2011-12-27	SIAT1/SIAT1	40	160	320	160	320	320	640	320	640
A/Berlin/1/2012	3B	2012-01-02	C1/SIAT1	40	160	160	160	320	320	640	320	320
A/Slovenia/9/2012	5	2012-01-03	MDCKx/SIAT1	<	80	80	80	320	320	640	320	320

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/Bris 10/07 F29/09	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Vic 210/09 F11/10	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11	A/HK 3969/11 F27/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11
Genetic group							group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES												
A/Brisbane/10/2007		2007-02-06	E2/E1	1280	80	80	160	40	160	320	80	80
A/Perth/16/2009		2009-07-04	E3/E2	<	1280	80	640	320	640	1280	320	320
A/Victoria/208/2009		2009-06-02	E3/E1	640	1280	5120	5120	1280	5120	5120	2560	5120
A/Victoria/210/2009		2009-06-02	E2/E2	640	2560	2560	5120	640	2560	1280	1280	2560
A/Alabama/5/2010		2010-07-13	MK1/M2/SIAT5	<	80	40	80	320	320	640	320	320
A/Perth/10/2010		2010-05-25	E3/E1	320	640	1280	2560	1280	2560	2560	1280	2560
A/Hong Kong/3969/2011		2011-05-19	M2/SIAT4	160	320	160	640	320	640	1280	640	640
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT3	80	160	80	160	160	320	640	640	320
A/Iowa/19/2010		2010-12-30	E3/E1	160	1280	5120	2560	2560	5120	5120	5120	5120
TEST VIRUSES												
A/Porto/EuroEva58/2011	3A	2011-11-20	SIAT1	40	160	320	80	320	640	1280	1280	320
A/Norway/2233/2011		2011-11-22	MDCK1/SIAT1	40	160	160	160	320	320	640	640	320
A/Belgium/G1063/2011	3B	2011-11-25	SIAT2	40	40	80	80	160	320	640	320	320
A/Berlin/94/2011	3C	2011-11-29	C3/SIAT1	80	320	320	320	640	1280	2560	1280	1280
A/Norway/2350/2011	3B	2011-11-30	MDCK1/SIAT1	40	80	160	160	160	320	320	320	640
A/Norway/2335/2011		2011-12-01	MDCK2/SIAT1	40	160	160	320	320	640	1280	640	640
A/Norway/2329/2011	3B	2011-12-02	MDCK1/SIAT1	40	80	160	80	160	320	320	320	320
A/Belgium/G1109/2011	3C	2011-12-05	SIAT3	40	160	320	160	640	1280	2560	640	1280
A/Norway/2334/2011		2011-12-05	MDCK2/SIAT1	40	160	160	160	160	320	1280	640	320
A/Norway/2352/2011		2011-12-05	MDKC2/SIAT1	80	160	160	160	320	320	640	640	320
A/Norway/2367/2011		2011-12-07	MDCK1/SIAT1	40	160	160	160	320	320	640	640	320
A/Belgium/G1125/2011	3B	2011-12-10	SIAT3	40	80	160	160	160	640	1280	320	320
A/Norway/2382/2011		2011-12-13	MDCK2/SIAT1	160	160	160	320	320	640	1280	640	320
A/Norway/2430/2011		2011-12-13	SIAT1/SIAT1	80	160	320	160	320	320	1280	640	640
A/Norway/2431/2011	3C	2011-12-13	SIAT1/SIAT1	80	320	320	320	640	1280	2560	1280	640
A/Norway/2432/2011		2011-12-13	SIAT1/SIAT1	40	80	160	160	160	320	640	640	320
A/Bayern/88/2011		2011-12-14	C5/SIAT1	40	160	320	320	320	640	1280	320	640
A/Norway/2400/2011	3C	2011-12-14	SIAT1/SIAT1	80	320	640	640	1280	1280	2560	1280	1280
A/Belgium/G1147/2011	3A	2011-12-16	SIAT2	80	160	320	160	320	640	1280	1280	640
A/Norway/2406/2011		2011-12-17	SIAT1/S1	80	160	320	160	320	640	1280	640	640
A/Norway/2426/2011		2011-12-20	SIAT1/S1	40	160	160	160	320	320	640	640	640
A/Norway/2418/2011		2011-12-21	SIAT1/S1	160	160	160	160	320	640	1280	640	320
A/Norway/2442/2011	3B	2011-12-23	SIAT1/S1	40	160	160	160	160	320	1280	640	320
A/Via Real/SU5/2012	6	2012-01-03	SIAT1	40	160	320	160	640	1280	1280	640	1280
A/Porto/EuroEva69/2012	6	2012-01-04	SIAT1	40	160	320	160	640	640	1280	640	1280
A/Baden-Wurttemberg/1/2012	3A	Jan 2012	C2/SIAT1	40	80	160	80	160	320	640	640	320

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

Table 7. 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/Bris 10/07 F29/09	A/Perth 16/09 F35/11	A/Vic 208/09 F7/10	A/Vic 210/09 F10/11	A/Ala 5/10 F27/10	A/Perth 10/10 F03/11	A/HK 3969/11 F27/11	A/Stock 18/11 F28/11	A/Iowa 19/10 F15/11
Genetic group							group 1	group 5	group 5	group 3C	group 3A	group 6
REFERENCE VIRUSES												
A/Brisbane/10/2007		2007-02-06	E2/E1	5120	40	40	40	40	80	160	<	40
A/Perth/16/2009		2009-07-04	E3/E2	<	1280	40	160	160	160	640	160	160
A/Victoria/208/2009		2009-06-02	E3/E1	320	1280	2560	2560	1280	5120	2560	2560	5120
A/Victoria/210/2009		2009-06-02	E2/32	320	2560	1280	2560	320	1280	1280	640	1280
A/Alabama/5/2010		2010-07-13	MK1/M2/SIAT2	<	80	40	40	160	80	640	160	160
A/Perth/10/2010		2010-05-25	E2/E2	160	640	2560	2560	1280	2560	2560	1280	2560
A/Hong Kong/3969/2011		2011-05-19	MDCK2/SIAT4	80	160	160	160	320	320	1280	640	320
A/Stockholm/18/2011		2011-03-28	MDCK2/SIAT3	<	80	80	40	80	160	320	320	80
A/Iowa/19/2010		2010-12-30	E3/E1	80	640	2560	2560	2560	5120	2560	2560	5120
TEST VIRUSES												
A/Paris/1744/2011		2011-10-05	MDCK3/SIAT1	40	160	160	160	320	160	640	640	640
A/Lyon CHU/46.334/2011		2011-11-15	MDCK3/SIAT1	40	160	160	160	320	320	640	640	320
A/Parma/171/2011	3A	2011-11-24	MDCK2/SIAT1	<	40	40	<	40	80	160	160	80
A/Firenze/1/2011	6	2011-11-25	MDCK2/SIAT1	<	40	40	80	160	160	160	320	160
A/Catalonia/S4345/2011		2011-11-28	MDCK0/SIAT1	40	160	320	320	640	640	640	640	1280
A/Milano/260/2011		2011-11-30	MDCK1/SIAT1	<	40	40	80	80	160	320	320	160
A/Trieste/58/2011		2011-11-30	MDCK2/SIAT1	<	80	160	80	160	320	320	320	160
A/Milano/256/2011	3A	2011-12-01	MDCK1/MDCK1	<	80	160	80	160	160	320	320	160
A/Paris/2013/2011		2011-12-06	MDCK1/SIAT1	40	160	320	160	320	640	1280	640	640
A/Parma/169/2011		2011-12-06	MDCK2/SIAT1	<	160	160	80	160	320	320	640	320
A/Marseille/2240/2011		2011-12-08	MDCK2/SIAT1	<	80	160	160	160	320	640	640	320
A/Lorraine/2073/2011		2011-12-08	MDCK1/SIAT1	40	320	320	160	640	640	1280	640	1280
A/Toulouse/2187/2011		2011-12-09	MDCK2/SIAT1	40	160	160	160	320	320	640	640	320
A/Rheinland-Pfalz/75/2011	3B	2011-12-09	C6/SIAT1	<	80	160	160	160	160	320	320	160
A/Berlin/2/2012		2011-12-09	C2/SIAT1	40	320	160	160	640	640	640	640	640
A/Milano/258/2011		2011-12-09	MDCK1/SIAT1	<	80	80	80	160	160	320	320	160
A/Trieste/59/2011	3B	2011-12-09	MDCK2/SIAT1	<	40	80	80	80	160	320	160	160
A/Norway/2433/2011		2011-12-12	SIAT1/SIAT2	<	80	160	160	160	160	320	320	160
A/Lorraine/2056/2011		2011-12-12	MDCK1/SIAT1	40	160	160	160	160	320	640	320	640
A/Valladolid/48/2011		2011-12-13	MDCKx/SIAT1	<	80	80	80	160	80	160	320	160
A/Milano/265/2011	3A	2011-12-13	MDCK1/SIAT1	<	80	80	80	80	160	320	320	160
A/Parma/170/2011		2011-12-13	MDCK2/SIAT1	40	160	160	160	320	320	640	640	320
A/Firenze/3/2011	6	2011-12-14	MDCK2/SIAT1	40	320	320	320	640	640	640	320	640
A/Paris/2097/2011		2011-12-15	MDCK2/SIAT1	40	160	160	160	320	320	1280	320	640
A/Milano/268/2011	3A	2011-12-15	MDCK1/SIAT2	<	80	80	80	80	160	320	320	160
A/Paris/2100/2011		2011-12-16	MDCK2/SIAT1	<	80	160	80	160	160	640	320	80
A/Valladolid/49/2011		2011-12-16	MDCK1/SIAT1	<	80	80	80	160	160	320	320	160
A/Paris/2114/2011		2011-12-19	MDCK2/SIAT1	<	160	160	320	320	320	640	320	320
A/Ireland/11M92381/2011		2011-12-19	MDCK3/SIAT1	<	80	40	<	80	80	320	320	80
A/Parma/168/2011		2011-12-19	MDCK3/SIAT1	40	160	160	160	160	320	1280	640	320
A/Paris/2116/2011		2011-12-20	MDCK2/SIAT1	<	160	160	320	320	320	640	320	640
A/Paris/2133/2011		2011-12-22	MDCK1/SIAT1	<	80	80	80	80	160	160	160	80
A/Ireland/11M92761/2011		2011-12-22	MDCK2/SIAT1	<	80	160	80	160	160	320	160	160
A/Parma/172/2011		2011-12-22	MDCK2/SIAT1	40	160	320	160	320	640	640	1280	320
A/Parma/175/2011		2011-12-22	MDCK2/SIAT1	40	320	320	320	320	640	1280	1280	640
A/Lyon/2264/2011		2011-12-23	MDCK2/SIAT1	40	320	320	160	320	640	640	640	640
A/Catalonia/S4320/2011	6	2011-12-27	C0/SIAT1	40	160	320	320	640	640	640	640	1280
A/Pays de Loire/2149/2011		2011-12-27	MDCK2/SIAT1	<	80	160	80	320	320	640	320	320
A/Trieste/62/2011		2011-12-27	MDCK2/SIAT1	<	160	160	80	160	320	320	640	320
A/Parma/177/2011		2011-12-27	MDCK1/SIAT1	40	160	320	160	320	320	640	1280	160
A/Paris/2154/2011		2011-12-28	MDCK1/SIAT1	<	80	160	80	320	320	640	320	160
A/Paris/7/2012		2011-12-28	MDCK1/SIAT1	<	80	40	80	160	320	320	320	160
A/Salamanca/50/2011		2011-12-29	MDCK1/SIAT1	<	160	160	80	160	320	640	1280	320
A/Trieste/63/2011	3A	2011-12-29	MDCK2/SIAT1	<	160	80	80	160	160	320	640	320
A/Parma/173/2011		2011-12-29	MDCK2/SIAT1	40	160	160	160	320	640	640	1280	320
A/Parma/174/2011		2011-12-30	MDCK2/SIAT1	<	80	80	80	80	160	320	320	160
A/Parma/176/2011	3A	2011-12-30	MDCK2/SIAT1	40	320	320	320	320	320	640	1280	640
A/Baden-Wurttemberg/2/2012	3C	2012-01-01	C2/SIAT1	40	320	320	320	640	640	640	640	640
A/Lyon/40/2012		2012-01-02	MDCK2/SIAT1	40	160	320	320	320	640	640	640	640
A/Via Real/SU6/2012		2012-01-03	SIAT2	<	160	160	160	640	640	640	640	640
A/Paris/27/2012		2012-01-03	MDCK2/SIAT1	<	80	160	80	160	160	320	320	160
A/Parma/01/2012	3B	2012-01-04	MDCK1/SIAT1	<	<	<	<	40	80	160	80	40
A/Lyon CHU/01.593/2011		2012-01-06	MDCK2/SIAT1	40	160	160	160	320	320	640	640	320
A/Valladolid/1/2012		2012-01-09	MDCK1/SIAT1	<	160	160	160	320	640	640	640	640
A/Segovia/2/2012		2012-01-09	MDCK1/SIAT1	<	80	80	80	160	320	320	160	320
A/Berlin/3/2012	3C	2012-01-10	C2/SIAT1	40	160	160	80	320	640	640	320	320
A/Salamanca/4/2012	6	2012-01-12	MDCK1/SIAT1	<	80	160	80	160	160	320	160	320

1. < = <40

Vaccine virus

Sequences in phylogenetic tree

Table 8. 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 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 19/10 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	640	80	160	640	160	160	160	160	320
A/Victoria/208/2009		2009-06-02	E3/E1	640	1280	1280	2560	2560	2560	2560	2560	2560
A/Alabama/5/2010		2010-07-13	MK1/C2/SIAT1	40	40	320	320	160	160	160	80	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	40	80	160	320	640	160	320	160	320
A/Iowa/19/2010		2010-12-30	E3/E1	1280	2560	1280	2560	2560	2560	2560	2560	2560
A/Finland/190/2011		2011-11-25	Cx/SIAT1	160	160	160	640	320	160	640	320	640
A/England/259/2011		2011-11-16	Cx/SIAT1	160	160	160	640	320	160	640	640	640
A/Norway/1789/2011			Cx/SIAT1	320	320	320	1280	640	640	2560	640	1280
TEST VIRUSES												
A/Denmark/87/2011		2011-09-04	SIAT1/SIAT1	80	80	160	320	160	160	640	320	640
A/Denmark/90/2011		2011-11-28	MDCK1/SIAT1	80	80	160	640	320	160	640	320	640
A/Netherlands/710/2011	6	2011-12-06	MDCK2/SIAT1	40	80	160	160	80	160	160	160	320
A/Milano/268/2011	3A	2011-12-15	MDCK1/SIAT2	40	40	40	160	160	80	160	160	160
A/Norway/99/2012		2011-12-16	LLC-MK2-MDCK1/SIAT1	320	160	320	640	640	640	1280	640	1280
A/Turkey/19/2011		2011-12-18	SIAT1/SIAT1	320	320	320	1280	1280	320	1280	640	1280
A/Ireland/11M92698/2011	3B	2011-12-20	SIAT2	40	80	80	160	320	160	320	160	320
A/Ireland/11M92761/2011		2011-12-21	SIAT2	40	80	80	160	320	80	160	160	320
A/Trieste/60/2011	3A	2011-12-21	MDCK2/SIAT2	40	40	40	160	160	80	160	160	160
A/Norway/2448/2011		2011-12-21	SIAT3	320	160	640	1280	640	1280	1280	640	1280
A/Ireland/11M92761/2011		2011-12-21	SIAT2	80	80	80	160	320	160	320	320	320
A/Ireland/11M92922/2011		2011-12-22	SIAT1/SIAT2	40	80	80	320	640	160	320	160	320
A/Norway/39/2012		2011-12-22	LLC-MK2-MDCK1/SIAT1	40	40	80	160	160	80	160	160	160
A/Norway/96/2012		2011-12-22	SIAT2	40	40	80	160	160	80	160	160	320
A/Ireland/11V9451/2011	3B	2011-12-25	MDCK1/SIAT1	80	80	160	320	320	160	640	320	640
A/Norway/38/2012		2011-12-25	SIAT2	80	80	160	320	320	160	320	320	640
A/Latvia/12-47890p/2011		2011-12-28	MDCK3/SIAT1	80	80	160	320	160	160	320	320	640
A/Netherlands/713/2011	3C	2011-12-29	C1/SIAT1	40	80	160	320	160	160	320	160	320
A/Norway/3/2012		2012-01-02	MDCK1/SIAT1	40	80	160	160	320	80	160	160	320
A/Norway/75/2012		2012-01-02	MDCK-SIAT1/SIAT1	80	80	160	320	320	160	640	320	640
A/Norway/97/2012		2012-01-02	LLC-MK2-MDCK1/SIAT1	320	160	320	1280	640	640	1280	640	1280
A/Austria/654044/2012		2012-01-03	C2/SIAT1	40	40	40	160	80	80	160	160	160
A/Ireland/12M90/2012		2012-01-03	SIAT3	80	160	160	640	320	320	640	320	640
A/Lyon/37/2012		2012-01-04	MDCK2/SIAT2	40	80	80	160	320	80	160	160	320
A/Netherlands/001/2012	6	2012-01-05	MDCK2/SIAT1	320	320	640	1280	640	640	1280	640	1280
A/Norway/73/2012		2012-01-05	SIAT1/SIAT1	320	160	640	640	640	1280	1280	640	1280
A/Berlin/6/2012		2012-01-09	C3/SIAT1	160	160	640	1280	640	640	1280	640	1280
A/Hamburg/1/2012		2012-01-09	C2/SIAT1	160	160	320	640	320	320	160	320	640
A/Austria/654591/2012		2012-01-09	C1/SIAT1	160	320	640	1280	320	640	1280	640	1280
A/Latvia/1-34462p/2012		2012-01-09	MDCK1/SIAT1	80	80	160	320	320	160	320	320	640
A/Latvia/1-34610/2012		2012-01-09	MDCK1/SIAT1	80	80	80	320	160	160	320	320	320
A/Ireland/12v397/2012		2012-01-10	SIAT3	40	80	80	160	320	80	160	160	320
A/England/12/2012		2012-01-10	SIAT1/SIAT1	160	160	320	640	320	320	1280	320	640
A/Norway/114/2012		2012-01-10	MDCK1/SIAT1	40	40	80	160	160	80	160	160	320
A/Latvia/1-35583/2012		2012-01-10	MDCKx/SIAT1	80	80	160	640	160	160	640	320	640
A/Austria/655242/2012		2012-01-11	C1/SIAT1	80	160	320	640	320	320	1280	320	640
A/Netherlands/002/2012	3B	2012-01-12	MDCK2/SIAT1	<	40	80	160	80	40	160	80	160
A/Rheinland-Pfalz/1/2012		2012-01-12	C2/SIAT1	160	160	160	640	320	320	640	320	640
A/Berlin/4/2012		2012-01-13	C2/SIAT1	160	160	160	640	320	320	640	320	640
A/Baden-Württemberg/3/2012		2012-01-13	C2/SIAT1	80	160	160	320	160	160	640	160	640
A/Latvia/1-37528/2012		2012-01-13	MDCKx/SIAT1	80	80	160	320	160	160	320	320	320
A/England/21/2012		2012-01-16	SIAT1/SIAT1	40	40	40	80	80	40	160	160	160
A/Berlin/5/2012		2012-01-16	C2/SIAT1	80	160	160	640	320	160	640	320	640
A/Berlin/7/2012		2012-01-16	C2/SIAT1	80	160	160	640	320	160	640	320	1280
A/Brandenburg/1/2012		2012-01-16	C2/SIAT1	320	160	640	1280	640	640	1280	640	1280
A/England/20/2012		2012-01-17	SIAT1/SIAT1	80	40	80	320	160	80	320	160	320
A/Berlin/8/2012		2012-01-17	C2/SIAT1	80	160	160	320	160	160	640	320	640
A/Austria/653679/2012		Jan 2012	SIAT2/SIAT1	40	80	80	160	80	80	320	160	160

1. < = <40

Vaccine virus

Sequences in phylogenetic trees

Table 9. 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 18/11	A/Iowa 19/10	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	640	80	160	640	160	160	160	160	320
A/Victoria/208/2009	2009-06-02	E3/E1	1280	2560	1280	5120	2560	5120	5120	2560	2560
A/Alabama/5/2010	2010-07-13	MK1/C2/SIAT2	40	40	160	320	80	160	80	80	160
A/Hong Kong/3969/2011	2011-05-19	MDCK2/SIAT4	160	160	320	1280	320	320	1280	640	640
A/Stockholm/18/2011	2011-03-28	MDCK2/SIAT3	40	40	80	320	320	160	160	160	320
A/Iowa/19/2010	2010-12-30	E3/E1	1280	5120	1280	5120	2560	5120	5120	1280	5120
A/Finland/190/2011	2011-11-25	Cx/SIAT1	160	160	160	1280	320	320	1280	320	640
A/England/259/2011	2011-11-16	Cx/SIAT1	160	160	320	1280	640	320	1280	640	640
A/Norway/1789/2011		Cx/SIAT1	160	160	320	1280	320	320	640	320	640
IVR-164(A/Brisbane/299/2011)		E5/E1	320	640	640	1280	640	1280	640	320	640
TEST VIRUSES											
A/Stockholm/37/2011	2011-11-27	C3/SIAT1	80	80	160	320	160	160	640	160	320
A/Stockholm/42/2011	2011-12-02	C3/SIAT1	160	160	640	640	320	640	1280	320	640
A/Stockholm/2011-21445/2011	2011-12-06	C1/SIAT1	80	80	160	320	320	160	640	320	320
A/Suceava/87402/2012	2011-12-12	MDCK3/SIAT1	160	160	320	640	320	320	640	640	640
A/Stockholm/40/2011	2011-12-13	C2/SIAT1	<	40	40	160	80	80	80	80	160
A/Stockholm/39/2011	2011-12-15	C1/SIAT1	80	80	80	320	320	160	640	160	640
A/Bacau/88448/2012	2011-12-19	MDCK3/SIAT1	160	160	320	640	320	320	640	640	640
A/Stockholm/43/2011	2011-12-26	C3/SIAT1	40	40	80	160	160	80	320	80	160
A/Stockholm/12-00978/2012	2011-12-26	C1/SIAT1	160	160	640	640	320	640	640	320	640
A/Stockholm/1200979/2012	2011-12-27	C1/SIAT1	160	160	640	640	320	640	640	640	1280
A/Galati/88977/2012	2011-12-27	MDCK2/SIAT1	160	160	160	640	320	320	640	640	640
A/Stockholm/44/2011	2011-12-28	C0/SIAT1	160	160	320	640	320	640	320	320	320
A/Bacau/89197/2012	2012-01-03	MDCK2/SIAT1	160	160	320	640	320	320	640	640	1280
A/Braila/89501/2012	2012-01-04	MDCK3/SIAT1	80	80	160	640	160	160	640	320	640
A/Iasi/89451/2012	2012-01-04	MDCK3/SIAT1	160	160	320	1280	640	320	1280	320	640
A/Finland/196/2012	2012-01-09	MDCKSIAT3/SIAT1	80	160	160	320	320	160	320	320	320
A/Stockholm/12-00694/2012	2012-01-11	C2/SIAT1	160	160	320	640	320	320	1280	320	640
A/Stockholm/12-00574/2012	2012-01-12	C2/SIAT1	160	160	320	640	320	640	640	640	640
A/Stockholm/12-00974/2012	2012-01-12	C1/SIAT1	40	40	80	320	160	80	160	160	320
A/Finland/197/2012	2012-01-12	MDCK-SIAT2/SIAT1	320	320	640	1280	640	1280	2560	640	2560
A/Stockholm/12-00695/2012	2012-01-13	C2/SIAT1	320	320	640	1280	640	1280	1280	640	1280
A/Stockholm/12-00752/2012	2012-01-16	C0/SIAT1	40	80	80	160	320	160	320	160	320
A/Latvia/1-39879p/2012	2012-01-16	MDCK/SIAT1	160	160	320	640	320	320	640	320	320

1. < = <40; ND = Not Done

Vaccine virus

Table 10. Antigenic analysis of influenza A(H3N2) viruses - Plaque Reduction Neutralisation[1] - MDCK-SIAT

Viruses		Collection Date	Passage History	Neutralisation titre				
				Post infection ferret sera				
				A/Bris 10/07	A/Per 16/09	A/Per 10/10	A/HK 3969/11	A/Iowa 19/10
				F18/07	F30/09	F03/11	F27/11	F15/11
Genetic group						group 5	group 3C	group 6
REFERENCE VIRUSES								
A/Brisbane/10/2007		2007-02-06	E2/E4	>5120	320	ND	320	320
A/Perth/16/2009		2009-07-04	E3/E1	40	1280	ND	320	80
A/Hong Kong/3969/2011		2011-05-19	MDCK3	160	80	ND	320	320
A/Iowa/19/2010		2010-12-30	E3/E1	160	640	ND	2560	5120
TEST VIRUSES								
A/Bratislava/31/2011	3C	2011-11-03	MDCK1/SIAT1	40	160	640	1280	320
A/Finland/190/2011	3C	2011-11-25	SIAT3/SIAT3	80	80	320	640	640

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

Vaccine virus

ND = Not Done

Sequences in phylogenetic trees

Table 11. Antigenic analysis of influenza A(H3N2) viruses - Plaque Reduction Neutralisation[1] - MDCK-SIAT

Viruses		Collection Date	Passage History	Neutralisation titre[2] Post infection ferret sera A/Bris 10/07	A/Per 16/09 F35/11	A/Per 10/10 F8/11	A/HK 3969/11 F27/11	A/Iowa 19/10 F15/11
Genetic group						group 5	group 3C	group 6
REFERENCE VIRUSES								
A/Brisbane/10/2007		2007-02-06	E2/E4	5120	80	80	80	80
A/Perth/16/2009		2009-07-04	E3/E1	<	640	80	160	80
A/Perth/10/2010		2010-05-25	E2/E2	40	320	640	640	640
A/Hong Kong/3969/2011		2011-05-19	MDCK3	<	80	160	160	80
A/Iowa/19/2010		2010-12-30	E3/E1	80	640	2560	2560	5120
TEST VIRUSES								
A/Stockholm/23/2011	3B	2011-09-05	C1/SIAT1	<	<	40	80	40
A/England/253/2011	3C	2011-09-11	SIAT1/SIAT1	<	160	640	1280	640
A/Valladolid/47/2011	6	2011-09-13	MDCK1/SIAT1	<	320	640	2560	640
A/Stockholm/26/2011	3A	2011-10-05	C1/SIAT1	<	80	160	640	160
A/England/257/2011	3B	2011-10-10	SIAT1/SIAT1	<	80	640	1280	320
A/Norway/2047/2011	6	2011-10-10	MDCK1/SIAT1	<	80	160	160	80
A/England/256/2011	3B	2011-10-12	SIAT1/SIAT1	<	80	320	1280	320
A/England/255/2011	3B	2011-10-14	SIAT1/SIAT1	<	80	320	640	320
A/Norway/2125/2011	6	2011-10-26	SIAT1/SIAT1	<	320	1280	1280	640
A/Bratislava/31/2011	3C	2011-11-03	SIAT2	<	320	1280	2560	1280
A/England/258/2011	3C	2011-11-07	SIAT1/SIAT1	<	160	640	1280	640
A/England/259/2011	3B	2011-11-16	SIAT1/SIAT1	<	80	320	1280	320
A/Bayern/87/2011	3B	2011-11-16	MDCK2/SIAT1	<	160	640	1280	320
A/Parma/171/2011	3A	2011-11-24	MDCKx/SIAT1	<	40	40	80	40
A/Stockholm/32/2011	3C	2011-11-27	C2/SIAT1	<	80	160	320	160
A/Stockholm/34/2011	3B	2011-11-27	C1/SIAT1	<	40	80	160	80
A/Madrid/RR8753/2011	3B	2011-11-30	SIAT1/SIAT1	<	80	320	640	160
A/Norway/2335/2011		2011-12-01	MDCK2/SIAT1	<	40	160	160	80
A/Netherlands/702/2011	6	2011-12-02	MDCK4/SIAT1	<	40	160	80	80
A/Norway/2329/2011		2011-12-02	MDCK1/SIAT1	<	80	160	320	160
A/Slovenia/2855/2011	5	2011-12-05	MDCKx/SIAT1	<	160	640	640	320
A/Berlin/85/2011	3C	2011-12-07	MDCK2/SIAT1	<	320	640	1280	640
A/Berlin/93/2011	3C	2011-12-07	C5/SIAT1	<	80	160	160	160
A/Berlin/86/2011	3C	2011-12-09	MDCK2/SIAT1	<	160	640	1280	640
A/Berlin/87/2011	3C	2011-12-12	MDCK2/SIAT1	<	320	1280	2560	640
A/Berlin/88/2011	3C	2011-12-13	C1/SIAT1	<	320	1280	1280	640
A/Norway/2431/2011	3C	2011-12-13	SIAT1/SIAT1	<	160	640	1280	640
A/Firenze/3/2011	6	2011-12-14	MDCK2/SIAT1	<	160	320	640	320
A/Belgium/G1147/2011	3A	2011-12-16	SIAT2	<	80	320	640	160
A/Castilla La Mancha/RR8843/2011	3C	2011-12-26	SIAT1/SIAT2	<	160	640	1280	320
A/Castilla La Mancha/RR8870/2011	3B	2011-12-27	SIAT1/SIAT1	<	80	160	640	160
A/Parma/01/2012	3B	2012-01-04	MDCKx/SIAT1	<	<	40	80	40
A/Berlin/3/2012	3C	2012-01-10	SIAT2/SIAT1	<	80	320	640	160

1. Based on 80% plaque reduction compared to serum negative controls 2. <=<40

Vaccine virus

Sequences in phylogenetic tree

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

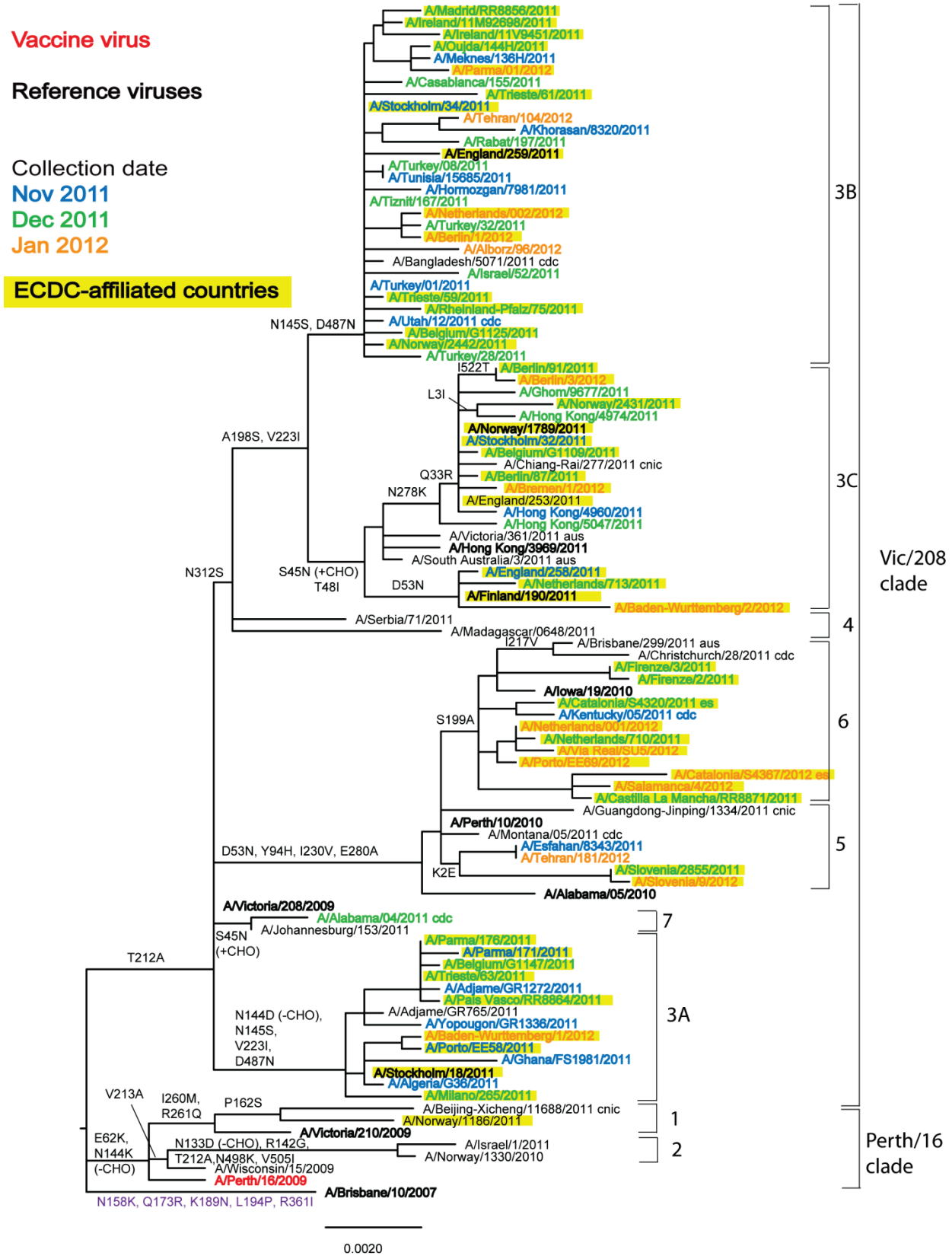


Table 12. Antigenic analyses of influenza B viruses (Victoria lineage)

Viruses	Collection date	Passage History	Haemagglutination inhibition titre[1]							
			Post infection ferret sera							
			B/Bris[2] 60/08 Sh 524	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/E5	2560	320	40	160	<	<	<	80
B/England/393/2008	2008-08-29	E1/E6	2560	160	640	1280	80	40	40	320
B/Brisbane/60/2008	2008-08-04	E8	2560	160	640	1280	40	40	40	320
B/Paris/1762/2008	2009-02-09	C2/MDCK1	2560	<	10	40	80	80	80	10
B/Hong Kong/514/2009	2009-10-11	MDCK1/MDCK1	2560	<	10	40	80	80	80	10
B/Odessa/3886/2010	2010-03-19	C2/MDCK1	2560	<	10	40	80	80	80	10
B/Malta/636714/2011	2011-03-07	E4/E1	2560	80	320	640	80	40	80	320
TEST VIRUSES										
B/Aix-en-Provence/1795/2011	2011-09-26	C2/MDCK1	5120	20	40	80	160	80	80	40
B/Norway/2075/2011	2011-10-11	MDCK2/MDCK1	1280	<	10	40	80	80	160	ND
B/Berlin/146/2011	2011-10-19	MDCK1/MDCK1	5120	40	20	80	80	80	160	ND
B/Norway/2368/2011	2011-12-05	MDCK1	5120	20	10	80	80	80	80	20
B/Norway/2429/2011	2011-12-14	MDCK1	5120	10	10	40	80	40	40	20
B/Austria/653113/2012	2012-01-01	C1/MDCK1	1280	<	<	20	80	80	40	10
B/Ireland/12M2515/2012	2012-01-09	MDCK2	2560	10	10	40	80	80	80	40
B/Sachen Anhalt/1/2012	Jan-2012	MDCK2	5120	10	20	80	80	40	80	40

1. < = <10; 2. hyperimmune sheep serum; ND = Not Determined

Vaccine virus

Sequences in phylogenetic trees

Table 13. 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 F20/07	B/Bris[1] 3/07 F24/07	B/Eng[2] 145/08 F09/08	B/Bang[2] 3333/07 F24/10	B/Wis[2] 1/10 F26/10	B/Stock[2] 12/11 F34/11	B/Estonia[2] 55669/11 F26/11	B/Serbia[2] 1894/11 F25/11
REFERENCE VIRUSES											
B/Egypt/144/2005	2005-05-01	E7	5120	160	2560	5120	80	160	160	640	80	20
B/Florida/4/2006	2006-12-15	E3/E4	5120	320	2560	5120	160	320	320	1280	160	40
B/Brisbane/3/2007	2007-09-03	E2/E3	5120	320	2560	5120	160	320	320	1280	160	20
B/England/145/2008		Ex/E5	640	40	160	160	160	20	10	160	<	10
B/Bangladesh/3333/2007	2007-08-07	E3/E4	5120	80	640	640	40	320	160	320	10	40
B/Wisconsin/1/2010	2010-02-20	E3/E2	2560	80	640	640	80	160	320	640	10	40
B/Stockholm/12/2011	2011-03-14	E4/E1	2560	80	320	320	80	80	40	320	<	20
B/Estonia/55669/2011	2011-03-14	MDCK2/MDCK2	5120	80	320	160	40	40	10	320	1280	80
B/Serbia/1894/2011	2011-03-08	MDCK1/MDCK4	5120	160	640	320	160	160	80	640	160	320
TEST VIRUSES											
B/England/254/2011	2011-10-04	SIAT1/MDCK1	1280	<	160	<	20	20	10	ND	ND	ND
B/Paris/1870/2011	2011-11-12	MDCK2/MDCK1	2560	40	160	160	40	40	40	320	160	320
B/Sweden/2/2011	2011-11-15	C1/MDCK1	2560	80	160	20	20	40	40	ND	ND	ND
B/Catalonia/S4125/2011	2011-11-16	MDCK1	2560	80	320	160	40	80	80	640	80	160
B/Paris/1900/2011	2011-11-17	MDCK2/MDCK1	2560	80	160	160	40	80	40	320	160	320
B/Stockholm/19/2011	2011-11-23	C1/MDCK1	2560	160	320	40	40	80	80	ND	ND	ND
B/Netherlands/707/2011	2011-11-29	MDCK1/MDCK1	2560	160	320	40	40	160	80	ND	ND	ND
B/Berlin/147/2011	2011-12-01	MDCK2/MDCK1	5120	80	640	80	40	160	80	ND	ND	ND
B/Catalonia/S4251/2011	2011-12-12	MDCK1	2560	80	320	320	80	80	80	640	80	160
B/England/9/2012	2012-01-05	MDCK1	5120	160	640	80	80	320	80	320	320	640
B/Stockholm/12-00971/2012	2012-01-08	C0/MDCK1	5120	40	160	20	40	80	40	320	80	160
B/Ireland/12M1522/2012	Jan 2012	MDCK1	5120	80	320	160	80	80	40	320	160	160

1. < = <40; 2. < =<10 ; 3. hyperimmune sheep serum; ND = Not Determined

Sequences in phylogenetic tree

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

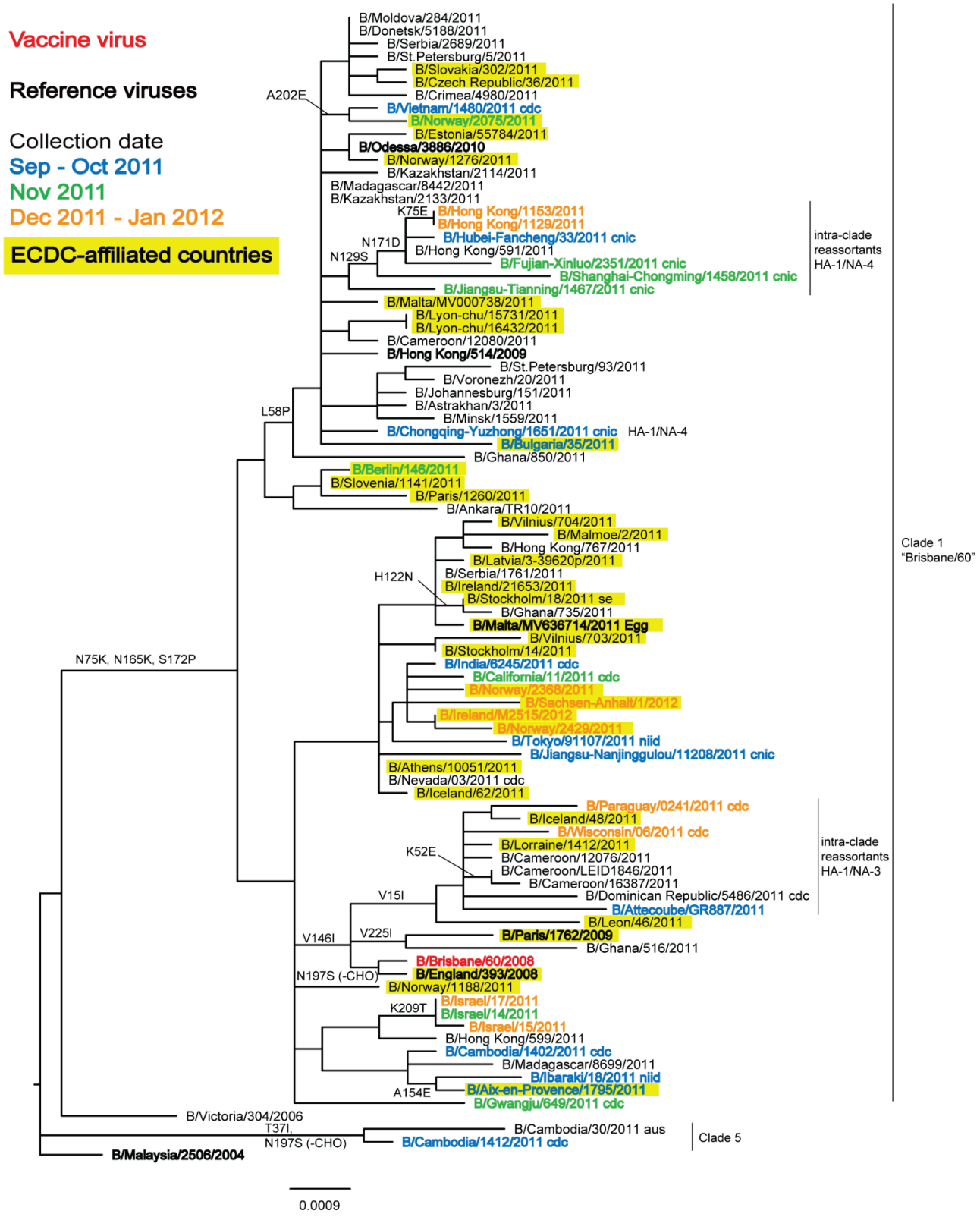
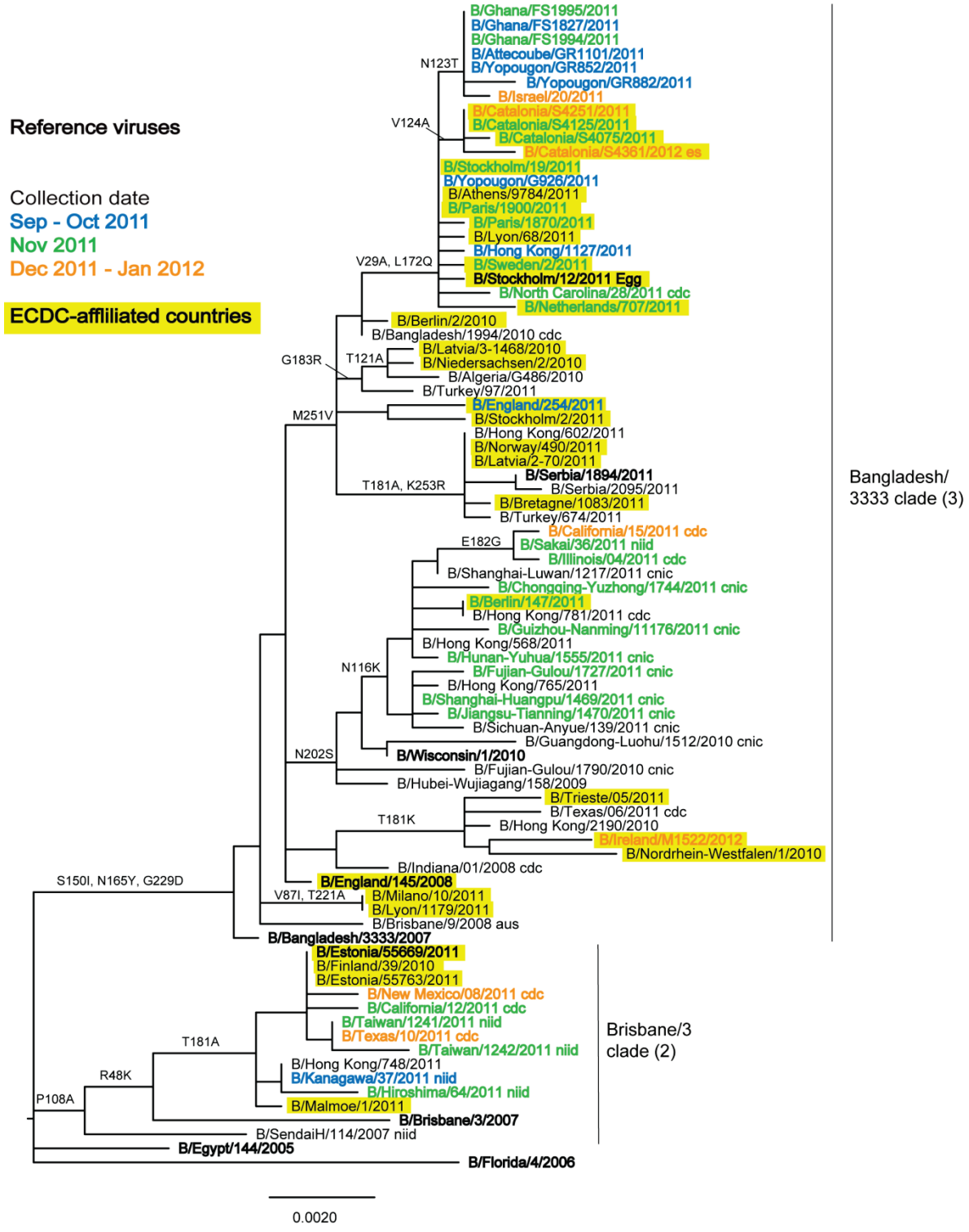


Figure 4. Phylogenetic comparison of influenza B (Yamagata-lineage) HA genes (HA1 region)



Reference

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