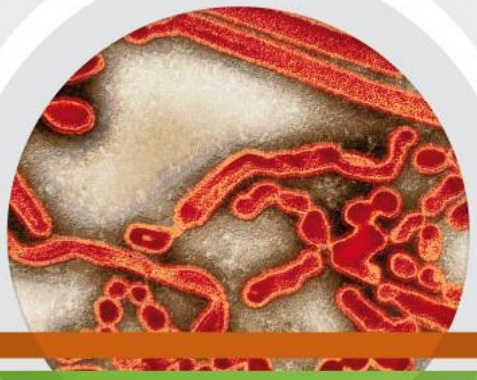

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, July 2014

Summary

During the 2013–14 season, A(H1N1)pdm09, A(H3N2), B/Victoria- and B/Yamagata-lineage influenza viruses have continued to co-circulate in EU/EEA Member States. The relative prevalence has varied between countries. Viruses with collection dates after 31 January 2014 from 21 countries have been received by the WHO Collaborating Centre in London.

Type A and type B viruses have been received at a ratio of approximately 10:1. A(H3N2) outnumbered A(H1N1)pdm09 viruses at a ratio of 1.4:1.

Recently circulating A(H1N1)pdm09 viruses in Europe, and worldwide, belonged to genetic subgroup 6B. Viruses in this subgroup are antigenically similar to the vaccine virus, A/California/07/2009.

Recently circulating A(H3N2) viruses have fallen within genetic group 3C represented by the recommended vaccine virus for the 2013–14 and 2014–15 seasons, A/Texas/50/2012. Viruses of genetic subgroup 3C.3 predominated. In recent months a cluster of viruses have been detected within subgroup 3C.3, designated 3C.3a, with HA gene sequences encoding several amino acid substitutions compared to other viruses in the 3C.3 subgroup. Antigenic analysis using antisera raised against cell-propagated H3N2 viruses indicates that the majority of circulating viruses are antigenically similar to those in circulation in the 2012–13 and 2013–14 influenza seasons but those in the newly emerging cluster, 3C.3a, are poorly recognised by some antisera.

Two genetic clades of B/Yamagata-lineage viruses continue to circulate: clade 3 represented by B/Wisconsin/1/2010 and clade 2 represented by B/Massachusetts/02/2012 (the recommended vaccine component for the 2013–14 and 2014–15 influenza seasons). Viruses in clade 3 predominate in those samples collected since 31 January 2014.

Antigenic characterisation of a small number of viruses of the B/Victoria lineage was performed in July. A single virus was recognised well by the antiserum raised against the egg-propagated reference virus, A/Brisbane/60/2008, recommended as a component of quadrivalent influenza vaccines for 2013–14 and 2014–15 influenza seasons. The test viruses were not recognised well by antisera raised against other reference viruses propagated in eggs. The majority of the test viruses were better recognised by antisera raised against reference viruses propagated exclusively in cells. Phylogenetic analysis revealed that all B/Victoria-lineage viruses received in 2014 were in genetic clade 1A, the B/Brisbane/60/2008 genetic clade.

Influenza-positive samples, viruses or clinical specimens, with collection dates after 31 January 2014 (with week 40, the start of weekly monitoring of influenza activity for the 2013–14 influenza season, commencing on 30 September 2013) have been received at the MRC National Institute for Medical Research, WHO Collaborating Centre for Reference and Research on

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Influenza (WHO CC), from 21 countries in the EU/EEA. The large majority (91%) were type A viruses, and A(H3N2) outnumbered A(H1N1)pdm09 at a ratio of 1.4:1 (Table 1). Of the small number of type B viruses received (~9% of the specimens), viruses of the B/Yamagata-lineage outnumbered those of the B/Victoria-lineages at a ratio of ~2.5:1. Some samples have yet to be fully processed (in process: Table 1).

Table 1. Summary of clinical samples and virus isolates received from EU/EEA Member States, with collection dates after 31 January 2014

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
Country			Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]
FEBRUARY											
Austria	3				3	3					
Belgium	6		3	1	3	2					
Bulgaria	27		21	21	6	5					
Cyprus	12	1	11	11							
Finland	8		3	3	5	4					
Germany	11		4	4	3	3		2	2	2	2
Iceland	6		3	3	3	3					
Ireland	4		4	4							
Italy	28		12	11	14	14				2	2
Latvia	1		1	1							
Lithuania	4	1	1	1	2	2					
Norway	13		5	in process	8	7					
Poland	9		2	1	7	5					
Portugal	6		4	4	1	1				1	1
Slovakia	5		3	3	2	2					
Slovenia	20		6	6	14	11					
Spain	1				1	0					
Sweden	7		1	1	4	4				2	2
United Kingdom	3				2	1		1	1		
MARCH											
Austria	10		2	2	5	5				3	3
Belgium	7		4	2	3	1					
Bulgaria	1		1	1							
Finland	3		1	1	2	2					
France	16		10	in process	5	in process				1	in process
Iceland	7		5	5				2	2		
Ireland	2		2	2							
Italy	3		2	2	1	1					
Latvia	11		7	5	3	3				1	1
Lithuania	10				10	10					
Norway	7				6	4		1	in process		
Poland	26	1	2	0	23	9					
Portugal	2		1	1	1	1					
Romania	13		3	3	9	8				1	1
Slovakia	3		1	1	1	1				1	1
Slovenia	3		2	2	1	1					
Spain	2		1	1						1	1
United Kingdom	8		4	4	2	2		1	1	1	1
APRIL											
Austria	3		1	1	2	2					
Belgium	10		2	1	7	4		1	1		
Finland	3				3	3					
France	8		3	in process	5	in process					
Iceland	3		2	2				1	1		
Ireland	7		1	1	6	6					
Latvia	2				2	2					
Lithuania	8	1			7	7					
Norway	1				1	in process					
Poland	3		2	0	1	1					
Portugal	1				1	1					
Slovakia	3		1	1	2	2					
Slovenia	2		1	1	1	1					
Spain	7				7	7					
MAY											
France	1									1	in process
Iceland	3				2	2				1	1
Lithuania	1				1	1					
Norway	5		2	in process	1	1				2	in process
Portugal	1									1	1
United Kingdom	1				1	1					
JUNE											
France	1		1	in process							
Iceland	1				1	1					
Norway	4				3	in process				1	in process
Spain	1				1	1					
United Kingdom	1				1	1					
JULY											
Spain	4				1	1	1			2	2
	393	4	148	114	207	160	1	9	8	24	19
			37.7%		52.7%			2.3%		6.1%	
21 Countries		91.3%					8.7%				

1. Propagated to sufficient titre to perform HI assay (the totalled number does not include any from batches that are in process)
2. Propagated to sufficient titre to perform HI assay in presence of 20nM oseltamivir (the totalled number does not include any from batches that are in process)

Influenza A(H1N1)pdm09 virus analyses

The results of haemagglutination inhibition (HI) analyses of viruses performed since the June 2014 report[1] are shown in Table 2. The test viruses were antigenically similar to the vaccine virus, A/California/7/2009; all but one of the test viruses analysed since the June 2014 report were recognised by an antiserum raised against A/California/7/2009 at titres within twofold of that obtained with the homologous virus. For viruses with known HA gene sequences, the genetic group to which the virus belongs is indicated.

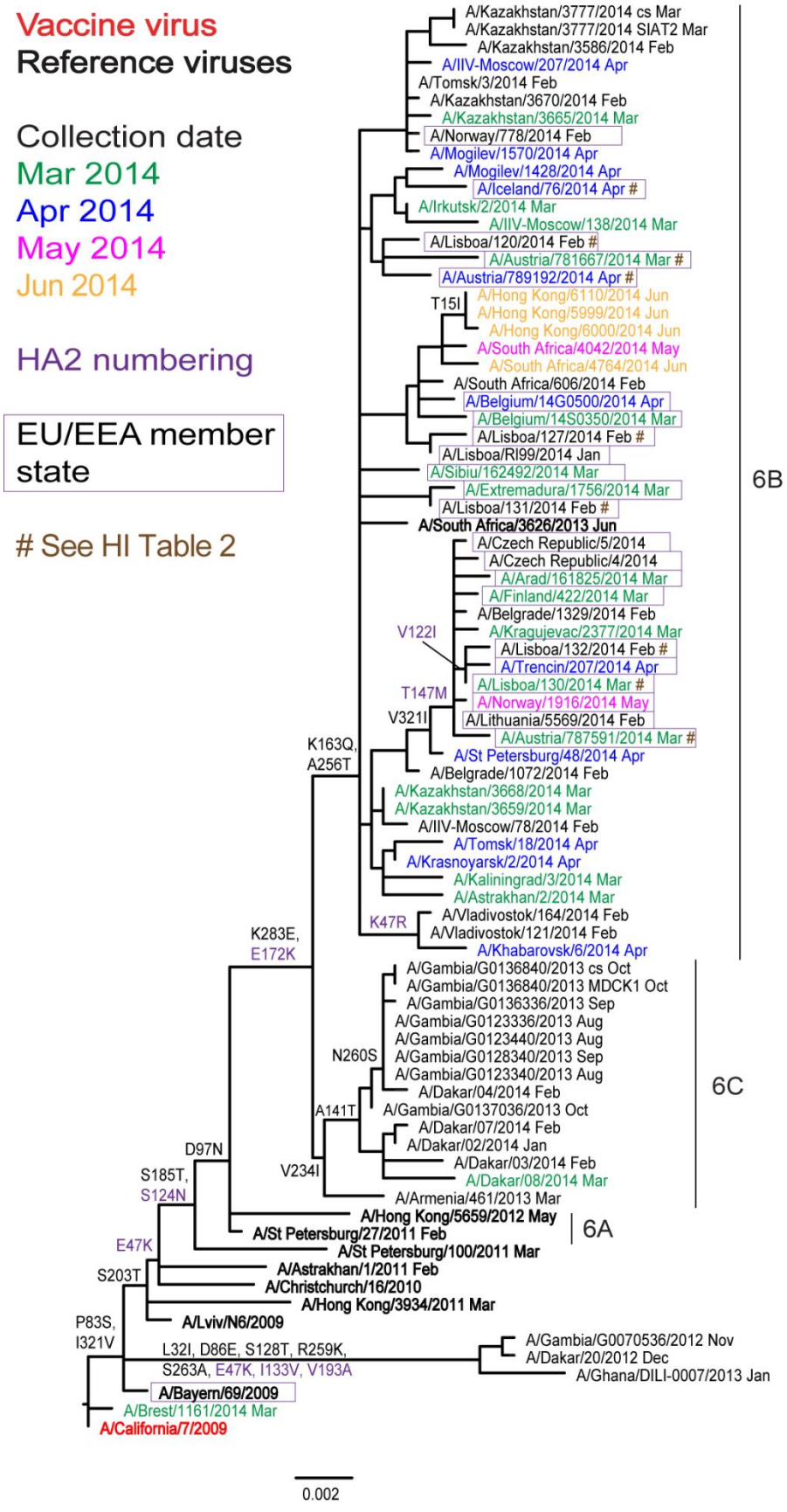
Figure 1 shows a phylogenetic tree for the HA genes of representative H1N1 viruses. Since 2009 the HA genes have evolved, and eight genetic groups have been designated, with A/California/7/2009 representing group 1. However, over the last season viruses in genetic group 6 have predominated, and all but one virus characterised in the June 2014 report and here, A/Austria/774096/2014 (Table 1, subgroup 6C), carried HA genes in genetic subgroup 6B. This subgroup carries the substitutions **D97N**, **K163Q, S185T, S203T, A256T** and **K283E** in **HA1** and **E47K, S124N and E172K** in **HA2** compared with A/California/7/2009.

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

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre: Post infection ferret antisera A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 F30/10 4	A/HK 3934/11 F21/11 3	A/Astrak 1/11 F22/13 5	A/St. P 27/11 F23/11 6	A/St. P 100/11 F24/11 7	A/HK 5659/12 F30/12 6A	A/Sth Afr 3626/13 F3/14 6B	
REFERENCE VIRUSES														
A/California/7/2009		2009-04-09	E1/E2	640	640	640	160	160	160	160	160	160	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK2	160	320	160	40	40	40	80	40	40	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	640	640	160	80	80	160	160	320	160	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	640	1280	1280	2560	1280	1280	1280	2560	1280	640	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	320	160	320	160	1280	640	640	1280	640	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK4/MDCK1	640	320	640	320	640	1280	640	2560	1280	640	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	640	640	1280	640	1280	1280	1280	2560	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	640	1280	640	1280	1280	1280	2560	1280	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	160	80	320	160	320	640	640	1280	1280	320	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	320	320	320	160	320	320	320	640	640	640	
TEST VIRUSES														
A/Austria/774096/2014	6C	unknown	SIAT0/MDCK1	1280	1280	1280	1280	1280	1280	2560	5120	2560	1280	
A/Iceland/6/2014	6B	2014-01-22	MDCK1/MDCK1	320	160	320	320	640	640	640	1280	1280	640	
A/Iceland/10/2014		2014-01-27	MDCK1/MDCK1	640	640	1280	640	1280	1280	1280	5120	2560	1280	
A/Iceland/22/2014		2014-02-04	MDCK1/MDCK1	640	320	640	640	1280	1280	1280	2560	1280	1280	
A/Lisboa/132/2014	6B	2014-02-05	SIAT1/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	2560	
A/Iceland/27/2014		2014-02-11	MDCK0/MDCK1	640	320	640	640	1280	1280	1280	2560	1280	1280	
A/Lisboa/120/2014	6B	2014-02-12	SIAT1/MDCK1	1280	640	1280	640	1280	2560	2560	5120	2560	1280	
A/Iceland/32/2014		2014-02-16	MDCK0/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	1280	
A/Lisboa/127/2014	6B	2014-02-24	SIAT1/MDCK1	1280	640	1280	1280	1280	2560	2560	5120	2560	1280	
A/Lisboa/131/2014	6B	2014-02-26	SIAT1/MDCK1	1280	1280	2560	1280	2560	2560	2560	5120	5120	2560	
A/Iceland/48/2014	6B	2014-03-01	MDCK1/MDCK1	320	160	320	160	320	320	640	640	640	640	
A/Austria/781667/2014	6B	2014-03-03	SIAT1/MDCK1	640	640	1280	640	1280	1280	1280	5120	2560	1280	
A/Lisboa/130/2014	6B	2014-03-05	SIAT1/MDCK1	1280	1280	2560	1280	2560	2560	2560	5120	5120	2560	
A/Iceland/61/2014	6B	2014-03-13	MDCK1/MDCK1	160	160	160	160	640	320	320	640	640	320	
A/Iceland/64/2014		2014-03-16	MDCK0/MDCK1	640	320	640	640	1280	1280	1280	2560	1280	1280	
A/Iceland/72/2014		2014-03-23	MDCK0/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	1280	
A/Austria/787591/2014	6B	2014-03-31	SIAT2/MDCK1	1280	1280	2560	1280	2560	2560	2560	5120	2560	2560	
A/Iceland/75/2014	6B	2014-04-03	MDCK0/MDCK1	640	320	640	640	1280	1280	1280	2560	1280	1280	
A/Austria/789192/2014	6B	2014-04-05	SIAT1/MDCK1	1280	1280	1280	1280	2560	2560	2560	5120	5120	2560	
A/Iceland/76/2014	6B	2014-04-08	MDCK0/MDCK1	640	320	640	640	640	1280	1280	2560	1280	1280	
Sequences in phylogenetic tree				Vaccine										

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, June 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/influenza-characterisation-report-june-2014.pdf

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



Influenza A(H3N2) virus analyses

As described in many previous reports[2], influenza A(H3N2) viruses continue to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pigs, turkeys and humans. All but four of the viruses examined since the June 2014 report had sufficient HA titre in assays conducted using guinea pig red blood cells in the presence of 20nM oseltamivir (added to circumvent any NA-mediated binding of H3N2 viruses to red blood cells) to be analysed by HI assay.

HI results are shown in Tables 3-1 and 3-2. The genetic group of the HA gene is indicated for viruses for which gene sequences have been determined.

All but four of the 56 test viruses analysed since the June 2014 report reacted poorly in HI assays (≥ eightfold decrease) with post-infection ferret antiserum raised against the egg-propagated vaccine virus, A/Texas/50/2012, compared with the titre of the antiserum with the homologous virus. Similar results were seen with an antiserum raised against the egg-propagated reference virus A/Hong Kong/146/2013. The test viruses were recognised better when examined with antisera raised against three other egg-propagated reference viruses, A/Serbia/NS-210/2013, A/South Africa/4655/2013 and A/Stockholm/1/2013. Notably the antiserum raised against A/South Africa/4655/2013 recognised >90% of test viruses at titres within fourfold of the titres of the antiserum with the homologous virus.

Ferret antisera raised against reference viruses exclusively propagated in tissue culture cells – A/Samara/73/2013, A/Stockholm/6/2014, Norway466/2014, and the exclusively cell-propagated cultivar of A/Victoria/361/2011 – recognised the test viruses more effectively. The reference viruses A/Stockholm/6/2014 and A/Norway/466/2014 are representative of an emerging genetic cluster of viruses, 3C.3a. Test viruses with HA genes falling into the 3C.3a genetic cluster were recognised poorly by antisera raised against the cell-propagated cultivar of A/Victoria/361/2011, and were recognised less well by the antiserum raised against A/Samara/73/2013 than those with HA genes in genetic subgroup 3C.3: ~80% of viruses in genetic subgroup 3C.3 were recognised by the antiserum at titres within fourfold of the titre for the homologous virus while only 40% of viruses in the 3C.3a cluster were recognised at titres within fourfold of the titre for the homologous virus. Antisera raised against cell-propagated reference viruses A/Stockholm/6/2014 and A/Norway/466/2014 recognised the majority of viruses from each genetic grouping at titres within twofold of the titres of the antisera with their homologous viruses.

Since 2009, seven genetic groups based on the HA gene have been defined for H3N2 viruses. Phylogenetic analysis of the HA genes of representative, recently circulating H3N2 viruses is shown in Figure 2. The vaccine virus A/Texas/50/2012 belongs to genetic subgroup 3C.1. Viruses characterised genetically since the June 2014 report fall into subgroups 3C.2, 3C.3 and 3C.3a, with viruses in 3C.3 predominating (Figure 2). Amino acid substitutions that define subgroups 3C.2, 3C.3 and 3C.3a are:

- 3C.2 **N145S** in **HA1**, and **D160N** in **HA2**, e.g. A/Hong Kong/146/2013;
- 3C.3 **T128A** (resulting in the loss of a potential glycosylation site), **R142G,** and **N145S** in **HA1**, e.g. A/Samara/73/2013;
- 3C.3a Those in 3C.3 plus **A138S**, **F159S** and **N225D** in **HA1**, many with **K326R**, e.g. A/Switzerland/9715293/2013.

Another emerging genetic cluster of viruses based on the HA gene, designated 3C.2a, has been observed. Viruses in this cluster carry the amino acid substitutions **N144S, F159Y, K160T, 225D** and **Q311H** in **HA1**. The substitutions at residues 144 and 160 result in the loss and the gain, respectively, of potential N-linked glycosylation sites at residues 144 and 158 of **HA1**.

[2] For example, the September 2013 report: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2013. Stockholm: ECDC; 2013. Available from http://www.ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-sep-2013.pdf

Table 3-1. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1]										
			Post infection ferret antisera										
			A/Perth 16/09	A/Vic 361/11	A/Texas 50/12	A/Samara 73/13	A/Serbia NS-210/13	A/HK 146/13	A/Sth Afr 4655/13	A/Stock 1/13	A/Stock 6/14	A/Nor 466/14
			F18/11	T/C F09/12	Egg F42/13	F24/13	F39/13	F40/13	F10/14	F12/14	F14/14	F13/14
	Genetic group			3C.1	3C.1	3C.3	3C.3	3C.2	3C.3 cl 101-60	3C.2 cl 36-18	3C.3a	3C.3a
REFERENCE VIRUSES												
A/Perth/16/2009	2009-07-04	E3/E3	640	160	160	160	160	160	40	80	40	40
A/Victoria/361/2011	3C.1	MDCK2/SIAT4	160	640	320	640	640	640	160	320	640	320
A/Texas/50/2012	3C.1	E5/E2	640	1280	1280	640	1280	640	80	160	160	40
A/Samara/73/2013	3C.3	C1/SIAT2	320	640	640	1280	640	1280	160	320	640	640
A/Serbia/NS-210/2013	3C.3	E5/E1	640	1280	1280	1280	1280	1280	80	160	160	160
A/Hong Kong/146/2013	3C.2	E5/E1	640	640	640	1280	640	2560	80	160	160	160
A/South Africa/4655/2013	3C.3	E7 clone 101-60	80	80	160	320	160	320	640	320	160	160
A/Stockholm/1/2013	3C.2	E7 clone 36-18	40	80	160	320	160	320	320	320	80	80
A/Stockholm/6/2014	3C.3a	SIAT2/SIAT2	40	160	80	320	320	320	80	80	320	320
A/Norway/466/2014	3C.3a	SIAT2/SIAT2	<	80	80	160	160	160	40	160	640	320
TEST VIRUSES												
A/Austria/770588/2014	2013-12-11	SIAT1/SIAT2	80	160	160	320	320	320	80	160	640	160
A/Czech Republic/10/2014	unknown	MDCK3/SIAT2	<	160	80	320	160	160	80	160	640	320
A/Stockholm/2/2014	2014-01-02	MDCK2/SIAT1	80	160	80	320	160	160	40	80	320	160
A/Austria/772655/2014	2014-01-10	SIAT2/SIAT2	80	160	160	320	160	160	80	160	640	160
A/Austria/773236/2014	2014-01-15	SIAT1/SIAT2	80	320	160	640	320	320	80	160	640	160
A/Slovenia/142/2014	2014-01-16	MDCK0/SIAT1	80	160	160	320	320	320	80	160	320	160
A/Austria/776877/2014	2014-02-04	SIAT2/SIAT2	80	320	160	640	320	320	80	160	640	160
A/Iceland/23/2014	2014-02-06	MDCK1/SIAT1	160	160	160	640	640	320	160	160	640	320
A/Austria/777909/2014	2014-02-08	SIAT1/SIAT1	40	160	40	320	160	160	40	80	160	160
A/Stockholm/14/2014	2014-02-12	MDCK1/SIAT1	80	320	160	640	320	320	80	160	320	320
A/Finland/405/2014	2014-02-13	MDCK1/SIAT1	40	160	80	320	160	160	40	80	320	160
A/Iceland/34/2014	2014-02-19	MDCK1/SIAT1	80	160	80	320	160	160	80	80	320	160
A/Stockholm/13/2014	2014-02-21	MDCK0/SIAT1	<	80	80	320	80	160	40	80	160	160
A/Stockholm/12/2014	2014-02-24	MDCK1/SIAT1	40	160	160	320	160	160	80	160	320	320
A/Finland/410/2014	2014-02-26	SIAT1/SIAT3	160	160	160	320	160	160	80	160	320	160
A/Austria/781450/2014	2014-02-27	SIAT2/SIAT2	40	160	80	320	160	160	80	80	320	160
A/Iceland/45/2014	2014-02-27	MDCK1/SIAT1	80	160	160	320	160	80	80	80	320	160
A/Finland/411/2014	2014-03-06	MDCK1/SIAT1	<	160	80	160	160	160	40	80	160	160
A/England/399/2014	2014-03-10	SIAT1/SIAT3	80	160	160	320	320	320	80	80	320	160
A/Iceland/62/2014	2014-03-13	MDCK1/SIAT3	80	160	80	320	160	160	80	80	320	160
A/Austria/784503/2014	2014-03-15	SIAT1/SIAT1	80	160	80	160	160	80	40	80	160	160
A/Austria/785558/2014	2014-03-19	SIAT1/SIAT2	80	160	160	320	160	320	80	160	640	160
A/Vrancea/162893/2014	2014-03-20	SIAT1/SIAT2	<	80	40	160	80	80	40	40	160	80
A/Botosani/162689/2014	2014-03-23	SIAT1/SIAT1	40	160	80	320	160	160	40	80	320	160
A/Botosani/162690/2014	2014-03-23	SIAT1/SIAT1	80	160	80	320	160	160	40	80	320	160
A/Iasi/162712/2014	2014-03-24	SIAT1/SIAT1	160	320	320	1280	640	640	160	320	640	320
A/Calarasi/162804/2014	2014-03-24	SIAT1/SIAT1	40	160	80	320	160	160	80	80	320	160
A/Olt/162753/2014	2014-03-24	SIAT1/SIAT3	40	160	80	320	160	160	40	80	320	160
A/Bucuresti/162834/2014	2014-03-24	SIAT1/SIAT3	80	160	160	320	160	320	80	160	640	160
A/Austria/786355/2014	2014-03-25	SIAT1/SIAT2	40	80	80	160	160	160	40	80	320	80
A/Austria/786648/2014	2014-03-26	SIAT1/SIAT1	80	80	40	160	80	160	40	40	160	160
A/Mures/163025/2014	2014-03-26	SIAT1/SIAT1	40	80	80	320	160	160	40	80	160	160
A/Austria/787217/2014	2014-03-27	SIAT2/SIAT1	40	160	80	320	160	160	40	80	160	160
A/Austria/788090/2014	2014-04-01	SIAT2/SIAT1	40	160	80	320	160	160	40	80	320	160
A/Latvia/04-003950/2014	2014-04-02	MDCK2/SIAT1	80	320	320	640	320	320	80	160	320	320
A/Latvia/04-011765/2014	2014-04-04	MDCK1/SIAT1	160	320	320	640	320	320	80	160	320	160
A/Austria/790710/2014	2014-04-12	SIAT1/SIAT1	<	80	40	160	80	80	<	40	80	80

Vaccine

1. < = <40

Sequences in phylogenetic tree

Table 3-2. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

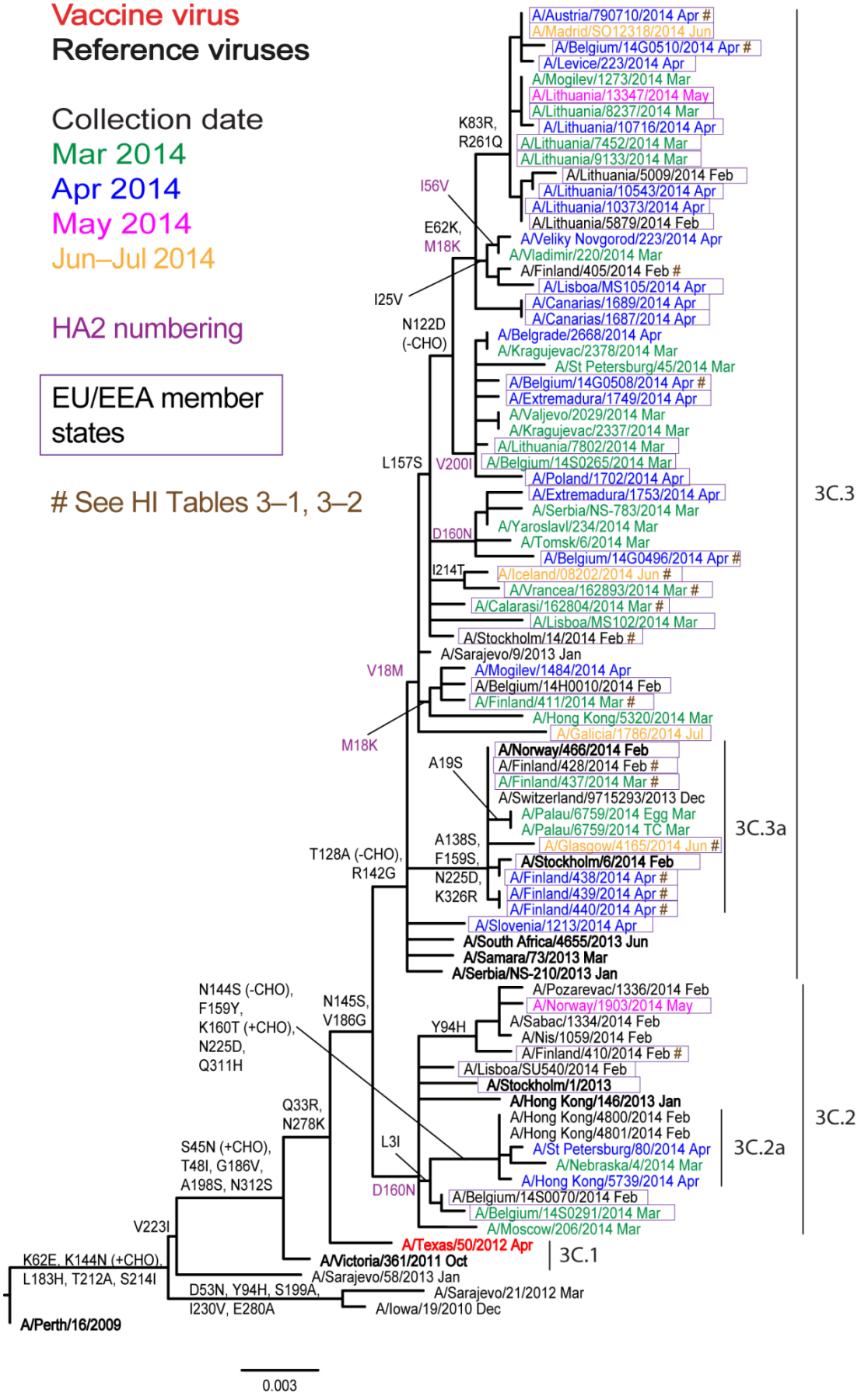
Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1]										
			Post infection ferret antisera										
			A/Perth 16/09 F18/11	A/Vic 361/11 T/C F09/12 3C.1	A/Texas 50/12 Egg F42/13 3C.1	A/Samara 73/13 F24/13 3C.3	A/Serbia NS-210/13 F39/13 3C.3	A/HK 146/13 F40/13 3C.2	A/Sth Afr 4655/13 F10/14 3C.3 cl 101-60	A/Stock 1/13 F12/14 3C.2 cl 36-18	A/Stock 6/14 F14/14 3C.3a	A/Nor 466/14 F13/14 3C.3a
Genetic group												
REFERENCE VIRUSES												
A/Perth/16/2009	2009-07-04	E3/E3	640	80	80	80	160	160	40	80	40	40
A/Victoria/361/2011	2011-10-24	MDCK2/SIAT4	320	640	640	1280	640	320	160	320	640	320
A/Texas/50/2012	2012-04-15	E5/E2	640	1280	1280	1280	1280	640	80	160	320	40
A/Samara/73/2013	2013-03-12	C1/SIAT2	320	640	640	1280	640	640	640	320	640	320
A/Serbia/NS-210/2013	2013-01-18	E5/E1	640	1280	1280	1280	1280	1280	80	160	320	160
A/Hong Kong/146/2013	2013-01-11	E5/E1	640	640	640	1280	640	1280	80	160	160	80
A/South Africa/4655/2013	2013-06-25	E7 clone 101-60	80	80	160	640	160	320	320	320	160	80
A/Stockholm/1/2013	2013-01-13	E7 clone 36-18	80	80	160	320	160	160	320	320	160	80
A/Stockholm/6/2014	2014-02-06	SIAT2/SIAT2	<	80	80	320	320	160	80	80	640	160
A/Norway/466/2014	2014-02-03	SIAT2/SIAT2	<	40	40	160	80	80	40	80	640	160
TEST VIRUSES												
A/Finland/428/2014	2014-02-17	MDCK1	<	80	80	320	160	160	80	160	640	320
A/Finland/428/2014	2014-02-17	SIAT1	<	40	40	160	160	160	40	80	640	320
A/Finland/437/2014	2014-03-24	MDCK1	<	40	40	160	160	160	40	80	640	160
A/Finland/437/2014	2014-03-24	SIAT1	<	80	80	320	160	160	80	160	640	320
A/Finland/438/2014	2014-04-03	MDCK1	<	40	40	160	160	160	40	80	640	320
A/Finland/438/2014	2014-04-03	SIAT1	<	40	40	160	80	160	40	80	640	320
A/Belgium/14G0496/2014	2014-04-03	SIAT2	160	320	320	640	320	320	80	160	640	160
A/Belgium/14H0034/2014	2014-04-10	SIAT1	80	320	160	640	320	320	160	160	640	320
A/Belgium/14G0508/2014	2014-04-16	SIAT1	80	320	160	640	320	320	80	160	640	320
A/Belgium/14G0510/2014	2014-04-16	SIAT1	80	160	160	320	320	160	40	80	320	160
A/Finland/439/2014	2014-04-23	MDCK1	<	80	40	160	160	160	40	80	640	320
A/Finland/439/2014	2014-04-23	SIAT1	<	40	40	160	160	160	40	80	640	320
A/Finland/440/2014	2014-04-28	MDCK1	<	80	80	320	160	160	80	160	640	320
A/Finland/440/2014	2014-04-28	SIAT1	<	80	80	320	160	160	80	160	640	320
A/Iceland/07150/2014	2014-05-20	SIAT1	80	160	160	640	320	320	80	160	640	160
A/Iceland/07151/2014	2014-05-20	SIAT1	80	160	160	640	320	320	80	160	640	160
A/Glasgow/4144/2014	2014-05-24	SIAT2	<	40	40	320	160	80	40	80	640	160
A/Glasgow/4165/2014	2014-06-08	SIAT1	<	40	40	80	80	80	40	80	640	160
A/Iceland/08202/2014	2014-06-10	SIAT1	80	320	160	640	320	320	80	160	640	320

Vaccine

1. < = <40

Sequences in phylogenetic tree

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



Influenza B virus analyses

The results of HI analyses for propagated viruses of the B/Victoria- and B/Yamagata-lineages from EU/EEA countries, performed since the June 2014 report[1], are shown in Tables 4 and 5, respectively. The genetic clades into which the HA genes fall are shown.

Influenza B – Victoria lineage

Six viruses of the B/Victoria lineage were received from Germany and Iceland. Post-infection ferret antiserum raised against B/Brisbane/60/2008, an exclusively egg-propagated virus previously recommended as a component of the trivalent influenza vaccine and currently recommended as a component of the quadrivalent influenza vaccine, recognised five of the six test viruses at titres fourfold or eightfold reduced compared to the titre with the homologous virus (Table 4). Antisera raised against other viruses exclusively propagated in eggs, B/Malaysia/2506/2006, B/Malta/636714/2011, B/Johannesburg/3964/2012 and B/South Australia/81/2012, recognised the cell-propagated test viruses poorly. Ferret antiserum raised against viruses propagated exclusively in cells, B/Paris/1762/2009, B/Hong Kong/514/2009, B/Odessa/3886/2010 and B/Formosa/V2367/2012, showed varying recognition of the test viruses. Although the titres of the antisera for the homologous cell-propagated viruses were low, ranging from 40 to 160, the antisera recognised the majority of test viruses at titres within fourfold of the titres with the homologous viruses.

Figure 3 shows a phylogenetic analysis of the HA genes of representative B/Victoria-lineage viruses. The HA genes of viruses collected since 1 January 2014 fall into the B/Brisbane/60/2008 genetic clade (Clade 1A).

Table 4. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

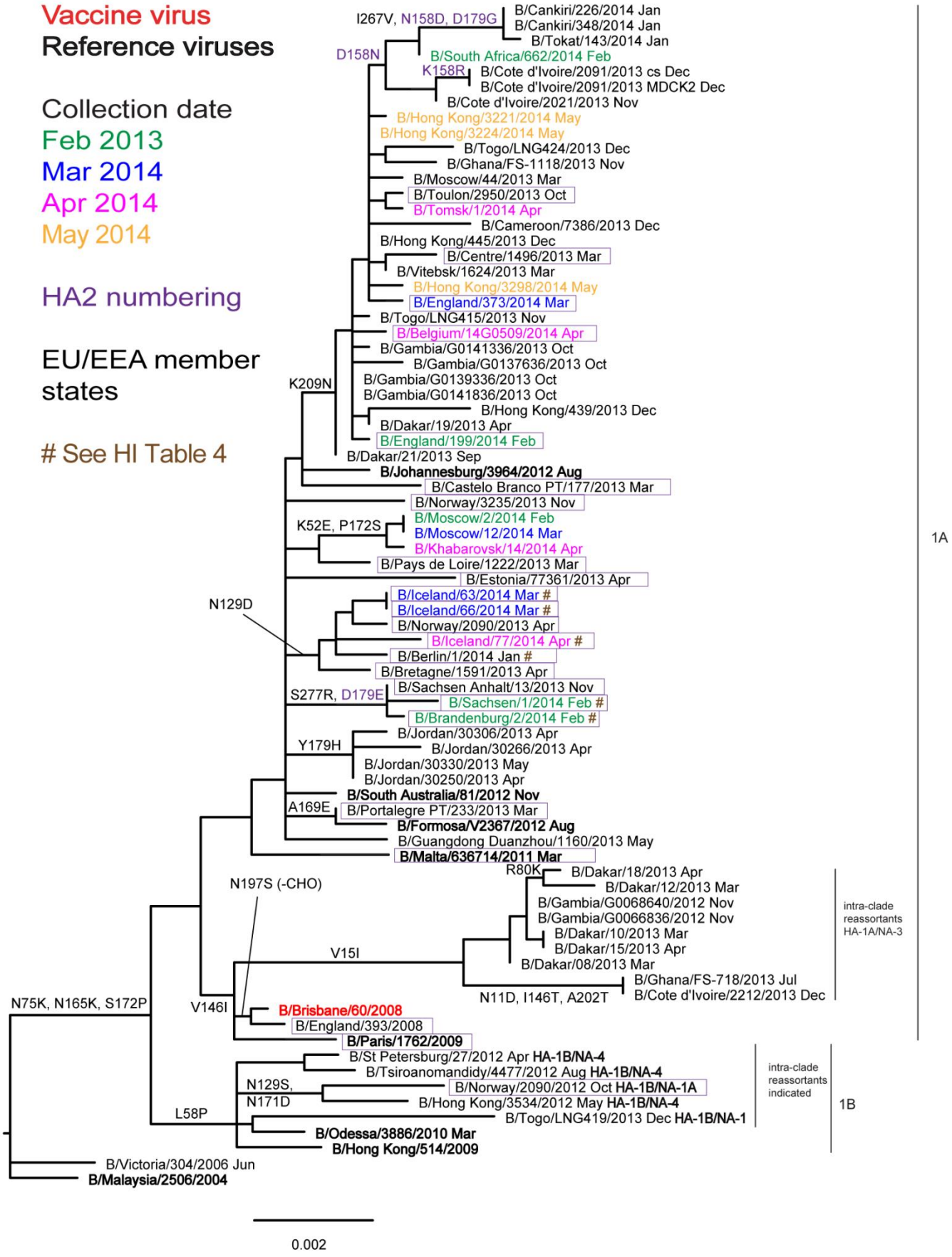
				Haemagglutination inhibition titre										
				Post infection ferret antisera										
Viruses		Collection date	Passage History	B/Bris[1,4] 60/08 Sh 522	B/Mal[2] 2506/04 F37/11	B/Bris[2] 60/08 F26/13	B/Paris[2] 1762/09 F07/11	B/HK[2] 514/09 F9/13	B/Odessa[2] 3886/10 F19/11	B/Malta[4] 636714/11 F29/13	B/Jhb[2] 3964/12 F01/13	B/For[3] V2367/12 F04/13	B/Sth Aus[2] 81/12 F41/13
	Clade			1A		1A	1A	1B	1B	1A	1A	1A	1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	320	80	<	10	<	80	160	80	320
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	80	320	40	40	40	320	320	160	1280
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK1	2560	10	80	40	80	80	40	20	40	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	2560	20	160	80	160	160	160	80	80	320
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	2560	40	320	40	80	80	160	160	160	640
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	320	40	40	40	320	160	160	640
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	320	1280	20	160	80	640	1280	640	1280
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	2560	20	160	80	80	40	160	80	160	640
B/South Australia/81/2012	1A	2012-11-28	E4/E1	5120	160	1280	80	160	<	320	640	320	1280
TEST VIRUSES													
B/Berlin/1/2014	1A	2014-01-15	C2/MDCK1	1280	10	80	20	20	10	10	20	40	40
B/Sachsen/1/2014	1A	2014-02-03	C2/MDCK2	1280	10	20	<	40	<	10	10	20	40
B/Brandenburg/2/2014	1A	2014-02-10	C2/MDCK2	5120	10	160	40	80	80	20	20	80	80
B/Iceland/63/2014	1A	2014-03-15	MDCK1/MDCK1	1280	10	40	20	40	20	10	20	40	40
B/Iceland/66/2014	1A	2014-03-18	MDCK1/MDCK1	1280	10	40	40	40	40	20	40	40	80
B/Iceland/77/2014	1A	2014-04-29	MDCK1/MDCK1	1280	10	80	40	40	40	20	40	40	80

1. < = <40; 2. < = <10; 3. < = <20; 4. hyperimmune sheep serum. Vaccine*

Sequences in phylogenetic tree

* B/Victoria-lineage virus recommended for use in quadravalent vaccines

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



Influenza B – Yamagata lineage

Post-infection ferret antiserum raised against the current, egg-propagated, vaccine virus B/Massachusetts/02/2012 recognised five of the six test viruses at titres twofold or fourfold reduced compared to the titre with the homologous virus. A ferret antiserum raised against a cell-propagated cultivar of B/Massachusetts/02/2012 recognised four of the test viruses at titres within fourfold of its titre with the homologous virus. The antisera raised against cell-cultivars of B/Estonia/55669/2011 and B/Hong Kong/3577/2012, viruses belonging to the B/Massachusetts/02/2012 clade (clade 2), recognised five and four of the test viruses, respectively, at titres within fourfold of the titres of the antisera with their homologous viruses. All six test viruses were recognised well by antisera raised against the previous vaccine virus, B/Wisconsin/1/2010, or the reference virus B/Stockholm/12/2011, another virus in the B/Wisconsin/1/2010 clade (clade 3). Five of the six viruses were recognised at titres within fourfold of the homologous titre by antiserum raised against cell-propagated B/Novosibirsk/1/2012, another virus belonging to clade 3.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. The HA genes of viruses collected since 1 January 2014 fall into the B/Massachusetts/02/2012 clade (clade 2) and the B/Wisconsin/1/2010 clade (clade 3), with those in clade 3 being in the majority in recent months.

Table 5. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

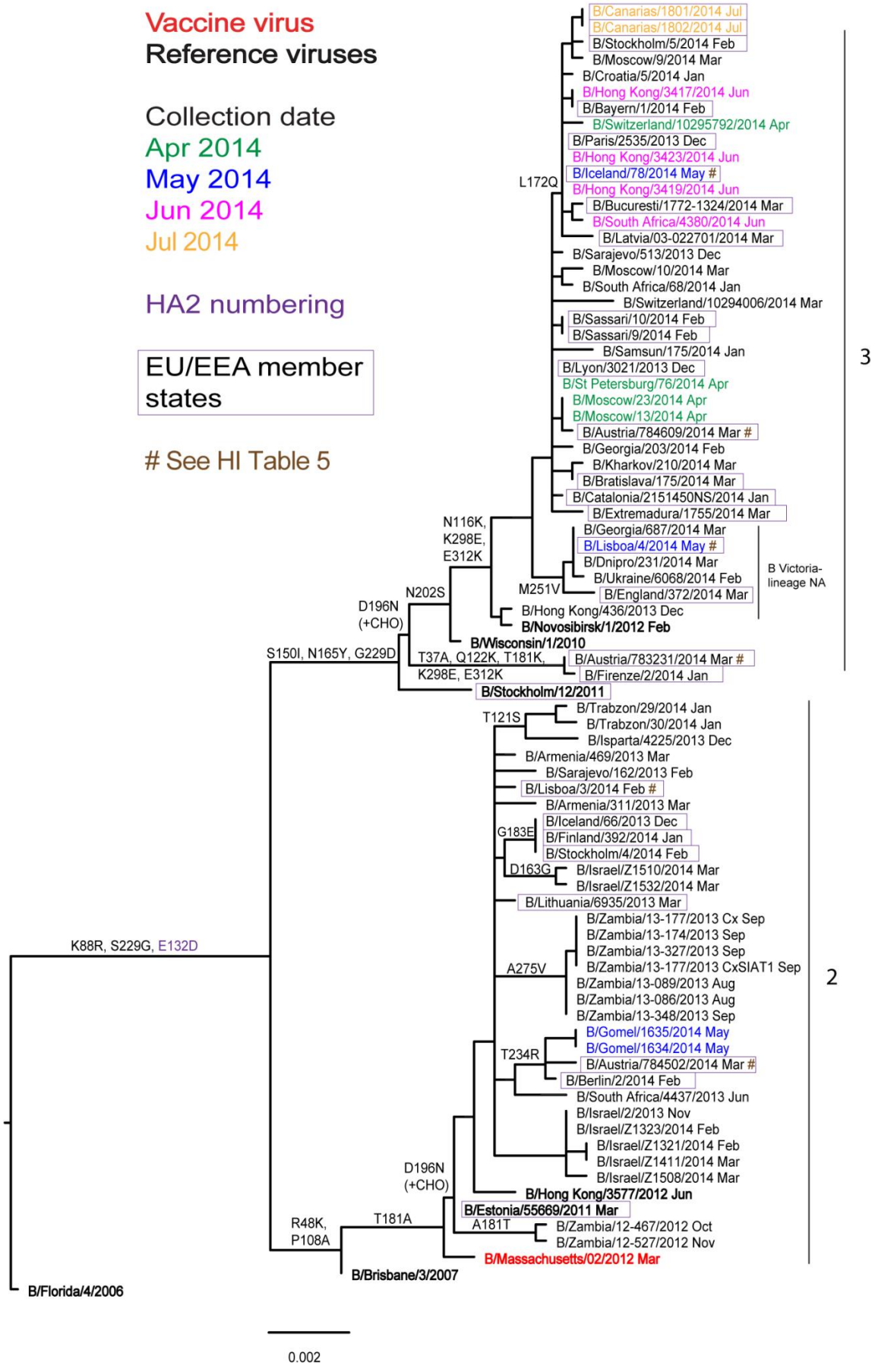
Viruses	Clade	Collection date	Passage History	Haemagglution Inhibition Titre: Post infection ferret antisera									
				B/Fl[1,3] 4/06 SH479 1	B/Fl[1] 4/06 F1/10 1	B/Bris[2] 3/07 F21/12 2	B/Wis[2] 1/10 F10/13 3	B/Stock[2] 12/11 F12/12 3	B/Estonia[2] 55669/11 F26/11 2	B/Novo[2] 1/12 F31/12 3	B/HK[2] 3577/12 F33/12 2	B/Mass[2] 02/12 Egg F2/13 2	B/Mass[2] 02/12 T/C F15/13 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E7/E1	2560	640	640	160	640	80	40	640	640	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	1280	1280	320	1280	160	40	1280	1280	320
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	640	320	160	160	640	<	40	160	320	40
B/Stockholm/12/2011	3	2007-08-07	E4/E1	1280	160	80	80	320	<	40	160	320	40
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	640	160	80	40	40	640	40	2560	160	640
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK3	1280	320	160	320	640	160	320	1280	320	320
B/Hong Kong/3577/2012	2	2012-06-13	MDCK4	640	80	40	40	80	320	40	2560	160	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E4	2560	640	640	160	640	80	20	640	640	160
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	5120	640	640	320	640	640	80	1280	640	640
TEST VIRUSES													
B/Lisboa/3/2014	2	2014-02-27	MDCK1	1280	160	160	160	320	640	160	640	320	640
B/Austria/783231/2014	3	2014-03-10	SIAT1/MDCK1	2560	320	160	320	640	160	320	320	160	320
B/Austria/784502/2014	2	2014-03-15	SIAT1/MDCK1	5120	640	320	160	640	1280	160	1280	320	1280
B/Austria/784609/2014	3	2014-03-17	SIAT1/MDCK1	2560	320	320	640	640	320	640	640	320	640
B/Lisboa/4/2014	3	2014-05-19	MDCK1	320	80	40	160	160	20	80	80	160	80
B/Iceland/78/2014	3	2014-05-28	MDCK0/MDCK1	320	40	40	160	160	20	40	40	80	40

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

Vaccine

Sequences in phylogenetic tree

Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes



Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [1] reported that the China Health and Family Planning Commission notified the WHO of three cases of human infection with influenza A(H7N9). The cases were confirmed by laboratory testing on 29 March 2013 by the Chinese CDC. A description of the characteristics of H7N9 viruses can be found on the WHO website [2]. Increased numbers of cases have been reported over the course of the 2013–14 season, continuing into June 2014. A revised Rapid Risk Assessment [3] for these A(H7N9) viruses was carried out by ECDC and posted on 27 January 2014, and an updated summary of human infection was posted by WHO on 31 January 2014 [4] followed by an updated risk assessment on 27 June 2014 [5]. The most recent update of the epidemiological situation published by WHO was posted on 27 June 2014. WHO summarised the numbers of cases of human infection and their geographic location on 14 July 2014 [6].

WHO CC reports

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva on 23–25 September 2013 and 17–19 February 2014, can be found at:

http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Sep2013final.pdf

http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Feb2014-web.pdf

Note on the figures

The phylogenetic trees were constructed using RAxML, drawn using FigTree and annotated using Adobe Illustrator. The bars indicate the proportion of nucleotide changes between sequences. Reference strains are viruses to which post-infection ferret antisera have been raised. The colours indicate the month of sample collection. Isolates from WHO NICs in ECDC countries are highlighted within boxes. Sequences for many viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu database which were downloaded for use in the preparation of this report (all submitters of data may be contacted directly via the GISAID website), along with all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

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