SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, July 2013

Summary

In the course of the 2012–13 season, A(H1N1)pdm09, A(H3N2) and B/Victoria- and B/Yamagata-lineage influenza viruses have co-circulated in ECDC-affiliated countries over what was an extended influenza season. The relative prevalences of each virus type/subtype has varied between countries.

- Type A and type B viruses have been detected in similar proportions but with type A peaking and declining slightly before type B.
- A(H1N1)pdm09 viruses have been detected at approximately twice the level of A(H3N2) viruses.
- The vast majority of A(H1N1)pdm09 viruses have remained antigenically similar to the vaccine virus, A/California/07/2009, but continued to show genetic drift with an increasing prevalence of genetic group 6 viruses.
- The vast majority of A(H3N2) viruses have been antigenically and genetically similar to cell-propagated A/Victoria/361/2011, a genetic group 3C virus and the prototype vaccine virus for the 2012–13 influenza season; group 3C viruses have circulated exclusively in recent months and the recommended vaccine virus for the 2013–14 season, A/Texas/50/2012, is in this genetic group.
- Viruses of the B/Yamagata-lineage have predominated over those of the B/Victoria-lineage.
- B/Victoria-lineage viruses have remained antigenically similar to cell-propagated reference viruses of the B/Brisbane/60/2008 genetic clade.
- B/Yamagata-lineage viruses formed two antigenically distinguishable genetic clades: clade 3 represented by B/Wisconsin/1/2010 (the recommended vaccine component for the 2012–13 influenza season) and, in increasing numbers, clade 2 represented by B/Massachusetts/2/2012 (the recommended vaccine component for the 2013–14 influenza season).

Viruses from specimens collected between 1 January 2013 and 31 May 2013, spanning the peak of the 2012–13 season, were received from 25 countries in the EU/EEA region at the MRC National Institute for Medical Research, WHO Collaborating Centre for Reference and Research on Influenza. A summary of specimens received is shown in Table 1.

The overall proportions of influenza type A (58%) and type B (42%) viruses received have become increasingly similar, reflecting the decreasing proportion of influenza A towards the end of the season at the same time as the numbers of influenza virus detections were also falling. For type A, H1N1pdm09 viruses were received in greater numbers than H3N2 viruses (ratio 2:1). Among influenza B receipts, viruses of the B/Yamagata and B/Victoria lineages were received at a ratio of 5:1.

This report was prepared by Rod Daniels, Vicki Gregory and John McCauley on behalf of the European Reference Laboratory Network for Human Influenza (ERLI-Net), under contract to the European Centre for Disease Prevention and Control (ECDC).

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, with collection dates since 1 January 2013

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]
JANUARY											
Belgium	22	2	8	5	1	1				11	8
Czech Republic	11		9	9	2	2					
Denmark	3		1	1	2	2					
Estonia	21	2	9	5	2	1	5			3	3
Finland	7		3	3	3	3		1	1		
France	1		1	1							
Germany	9		2	2	2	2		1	1	4	4
Greece	7	1	2	1	3	1				1	1
Ireland	6				2	2		1	1	3	3
Italy	32		17	17	5	5		3	3	7	7
Latvia	7		3	3	3	3				1	1
Luxembourg	13		8	7						5	4
Malta	24		18	2	1	1				5	5
Netherlands	2		1	1						1	1
Norway	4		4	3							
Portugal	24		11	9	4	4		2	2	7	7
Romania	8		5	5				1	1	2	2
Slovenia	18		4	1	5	3	1	5	4	3	3
Spain	22		10	10	6	6				6	6
Sweden	8				3	3				5	5
United Kingdom	6				5	5		1	1		
FEBRUARY											
Belgium	12		2	2	2	2				8	8
Bulgaria	22		8	7	2	2				12	12
Czech Republic	4									4	4
Greece	2		2	2							
Hungary	12		6	4				3	3	3	2
Iceland	1									1	1
Italy	22		11	11	1	1		1	1	9	9
Luxembourg	5		1	0	4	0					
Norway	1		1	in process							
Portugal	10		4	4				4	4	2	2
Romania	12		7	7						5	5
Slovakia	11		2	2	3	3				6	6
Slovenia	15		6	6	4	4		1	1	4	3
Spain	10		9	7	1	1					
Sweden	8				4	4				4	4
United Kingdom	8		3	3	2	2		1	1	2	2
MARCH											
Belgium	16		4	3	3	3				9	9
Bulgaria	2		1	1						1	1
Czech Republic	1									1	1
Estonia	21	14			2	2	5				
Hungary	11		1	1				1	1	9	3
Iceland	3				1	1				2	2
Italy	2		1	1						1	1
Luxembourg	1									1	1
Norway	22		6	in process	9	9		1	1	6	in process
Portugal	13		5	5				3	3	5	5
Romania	5		1	1	1	1				3	3
Slovakia	13		4	4	3	3		2	2	4	4
Slovenia	3							2	1	1	1
Spain	9		3	2	4	4				2	1
Sweden	1		1	1							
APRIL											
Belgium	3				2	2				1	1
Estonia	14	5					9				
Hungary	4	1						2	2	1	1
Iceland	3		1	1	1	1				1	1
Norway	16		3	in process	4	4		1	1	8	in process
Portugal	2		1	1						1	1
Romania	2		1	1						1	1
Slovakia	1									1	1
Slovenia	2		1	1						1	1
Spain	3		1	0	1	1				1	1
MAY											
Iceland	3		1	1						2	2
Norway	4		1	in process	2	in process				1	in process
	590	25	215	164	105	94	20	37	35	188	160
			36.4%		17.8%			6.3%		31.9%	
25 Countries			58%				42%				

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 HI assays carried out on influenza A(H1N1)pdm09 viruses since the June report [1] are shown in Tables 2 and 3. All 56 test viruses showed good reactivity with post-infection ferret antiserum raised against the vaccine virus, A/California/7/2009, with all titres being within fourfold of its recognition of the homologous virus. However, seven of the test viruses (A/Belgium/G1061/2013, A/Castelo Branco PT/85/2013, A/Iceland/28/2012, A/Lisboa PT/137/2013, A/Portalegre PT/142/2013, A/Stockholm/4/2013 and A/Trieste/22/2013) reacted poorly with antisera raised against A/Hong Kong/3934/2011, A/Astrakhan/1/2011, A St Petersburg/27/2011, A/St Petersburg/100/2011 and A/Hong Kong/5659/2012. HA gene sequencing of six of these seven test viruses was complete at the time of preparation of this report, and all six carried amino acid substitution or polymorphism at HA1 positions 155 and/or 156 (Tables 2 and 3). Amino acid substitution or polymorphism in the 153–157 region of HA1 can affect the antigenicity of the virus and commonly emerges during propagation of viruses in cell culture. The seven 'low-reacting' viruses show similar HI reactivity profiles to the reference virus A/Bayern/69/2009 which carries G155E substitution in HA1. The effect of G155E substitution is less pronounced in the A/Lviv/N6/2009 reference virus, which contains G155E>G polymorphism and an additional D222G HA1 substitution that is known to alter the receptor-binding properties of HA.

As described previously [2], antiserum raised against A/Christchurch/16/2010, a virus from a genetic group not seemingly in circulation at present (group 4), reacted less well than the other antisera with the test viruses: this ferret antiserum reacted with 45 of the 56 test viruses, with titres reduced eightfold or greater compared with the titre of the antiserum with the homologous virus.

Phylogenetic analysis of the HA genes of representative viruses (Figure 1) shows that the H1N1 viruses from EU/EEA countries collected during the 2012–13 season cluster within genetic groups 6 and 7, with viruses belonging to group 6 predominating, notably so for those with collection dates since 1 March 2013. HA gene sequencing was performed on 25 test viruses, and their genetic grouping is shown in Tables 2 and 3; 22 were in genetic group 6, and 3 in genetic group 7.

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

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1]									
				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	A/HK 3934/11 F21/11	A/Astrak 1/11 F22/11	A/St. P 27/11 F23/11	A/St. P 100/11 F24/11	A/HK 5659/12 F30/12	
							Group 4	Group 3	Group 5	Group 6	Group 7	Group 6	
REFERENCE VIRUSES													
A/California/7/2009		2009-04-09	EP1/E2	640	640	640	160	160	160	160	320	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	160	80	40	40	40	40	40	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	1280	640	160	80	160	320	160	320	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	2560	5120	1280	1280	1280	5120	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK4	320	160	320	320	1280	640	640	1280	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	1280	640	2560	1280	2560	5120	2560	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	640	1280	640	1280	2560	2560	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	320	640	640	640	640	1280	2560	1280	
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	640	320	640	640	1280	1280	1280	2560	2560	
TEST VIRUSES													
A/Santarem PT/1/2013		2013-01-10	SIAT1/MDCK1	640	1280	640	160	160	320	640	320	1280	
A/Coimbra PT/10/2013		2013-01-10	SIAT1/MDCK1	1280	640	2560	640	2560	2560	5120	5120	5120	
A/Leiria PT/14/2013		2013-01-21	SIAT1/MDCK1	1280	1280	2560	2560	2560	2560	2560	5120	5120	
A/Castelo Branco PT/15/2013		2013-01-23	SIAT1/MDCK1	1280	640	1280	1280	1280	1280	2560	5120	2560	
A/Braga PT/19/2013		2013-01-23	SIAT1/MDCK1	2560	1280	1280	1280	2560	2560	2560	5120	2560	
A/Castelo Branco PT/36/2013		2013-01-26	SIAT1/MDCK1	1280	640	1280	1280	2560	1280	2560	5120	2560	
A/Belgium/S0299/2013	6	2013-02-04	MDCK2	1280	640	1280	1280	2560	1280	2560	5120	2560	
A/Lisboa PT/43/2013		2013-02-05	SIAT1/MDCK3	320	320	640	320	640	640	640	1280	1280	
A/Belgium/G0534/2013	6	2013-02-05	MDCK2	1280	640	1280	1280	2560	1280	1280	5120	2560	
A/Aveiro PT/61/2013		2013-02-06	SIAT3/MDCK1	1280	640	1280	1280	1280	1280	2560	5120	2560	
A/Lisboa PT/79/2013		2013-02-08	SIAT2/MDCK1	1280	640	1280	1280	2560	1280	2560	5120	2560	
A/Castelo Branco PT/85/2013		2013-02-22	SIAT2/MDCK1	160	640	320	80	80	160	160	80	160	
A/Lisboa PT/137/2013	6	2013-03-01	SIAT4/MDCK1	160	640	320	80	80	80	160	80	160	G155E
A/Castelo Branco PT/130/2013	6	2013-03-03	SIAT1/MDCK1	1280	640	1280	1280	2560	1280	2560	5120	2560	
A/Coimbra PT/102/2013	6	2013-03-04	SIAT1/MDCK1	640	320	640	640	1280	1280	2560	2560	2560	
A/Norway/1675/2013		2013-03-06	MDCK2/MDCK1	320	320	320	320	640	640	640	1280	640	
A/Belgium/G1042/2013		2013-03-18	MDCK3/MDCK1	320	160	320	160	640	640	640	1280	640	
A/Belgium/G1041/2013	6	2013-03-18	MDCK2	640	640	640	640	1280	640	1280	1280	1280	
A/Portalegre PT/142/2013	6	2013-03-22	SIAT1/MDCK1	160	640	320	160	80	160	160	160	320	G155E>G
A/Belgium/G1061/2013	6	2013-03-22	MDCK2	640	320	320	320	160	320	640	320	320	G155G>E, N156N>R
A/Norway/1954/2013		2013-03-25	MDCK2/MDCK1	640	320	640	320	1280	640	1280	2560	1280	
A/Norway/1960/2013		2013-03-27	MDCK1/MDCK1	320	160	320	320	640	640	640	1280	1280	
A/Madeira PT/135/2013	6	2013-03-28	SIAT3/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	
A/Acores PT/139/2013	6	2013-04-01	SIAT1/MDCK1	320	320	640	640	1280	640	1280	2560	1280	

Sequences in phylogenetic tree (Figure 1)

Vaccine

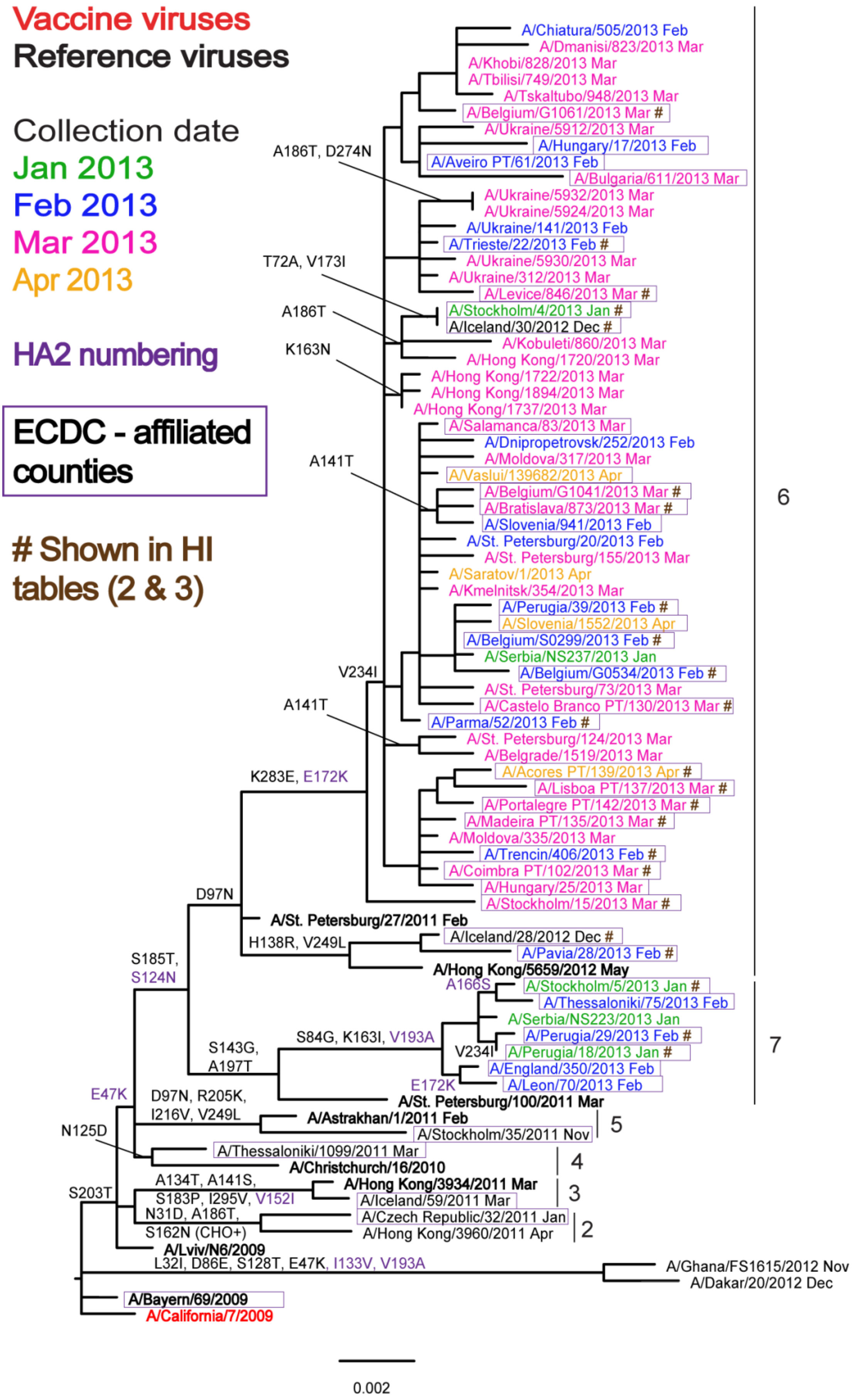
Table 3. Antigenic analysis of A(H1N1)pdm09 viruses by HI

				Haemagglutination inhibition titre[1]									
				Post infection ferret antisera									
Viruses	Genetic group	Collection date	Passage History	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 Group 4	A/HK 3934/11 F21/11 Group 3	A/Astrak 1/11 F22/11 Group 5	A/St. P 27/11 F23/11 Group 6	A/St. P 100/11 F24/11 Group 7	A/HK 5659/12 F30/12 Group 6	
REFERENCE VIRUSES													
A/California/7/2009		2009-04-09	EP1/E2	640	640	640	320	320	320	320	320	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	160	320	160	80	80	80	80	80	80	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	640	320	160	160	320	160	320	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	1280	5120	1280	1280	1280	2560	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK4	640	320	640	640	2560	1280	1280	2560	2560	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	640	640	640	1280	1280	1280	2560	2560	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	2560	1280	2560	1280	2560	2560	2560	5120	5120	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	320	640	640	1280	640	640	2560	1280	
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	
TEST VIRUSES													
A/Iceland/28/2012	6	2012-12-14	MDCK3	160	640	320	160	160	160	160	160	320	G155E>G
A/Iceland/30/2012	6	2012-12-26	MDCK1/MDCK1	1280	640	1280	640	1280	1280	1280	2560	2560	
A/Firenze/12/2013		unknown	MDCK2/MDCK1	640	320	640	320	640	640	640	1280	1280	
A/Iceland/03/2013		2013-01-04	MDCK1/MDCK1	320	160	640	320	640	640	640	1280	1280	
A/Iceland/13/2013		2013-01-10	MDCKx/MDCK1	640	160	640	320	1280	640	640	1280	1280	
A/Stockholm/4/2013	6	2013-01-11	MDCK2/MDCK1	640	640	640	320	160	320	320	160	640	G155E>G
A/Parma/13/2013		2013-01-17	MDCK1/MDCK1	640	320	640	640	1280	1280	1280	2560	2560	
A/Stockholm/5/2013	7	2013-01-19	MDCK2/MDCK1	320	640	320	320	640	640	640	1280	1280	
A/Perugia/7/2013		2013-01-21	MDCK2/MDCK1	640	320	640	640	1280	640	640	1280	1280	
A/Trieste/07/2013		2013-01-21	Cx/MDCK1	320	160	320	320	640	640	640	1280	1280	
A/Perugia/18/2013	7	2013-01-26	MDCK1/MDCK1	640	640	1280	640	1280	640	1280	1280	1280	
A/Parma/28/2013		2013-02-02	MDCK1/MDCK1	640	320	640	640	1280	1280	1280	2560	1280	
A/Pavia/28/2013	6	2013-02-02	Cx/MDCK2	1280	640	1280	320	640	640	640	1280	1280	
A/Firenze/9/2013		2013-02-04	MDCK2/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	
A/Perugia/29/2013	7	2013-02-04	MDCK1/MDCK1	320	320	320	320	640	640	640	1280	640	
A/Roma/15/2013		2013-02-08	Cx/MDCK1	640	320	640	640	1280	640	640	2560	1280	
A/Trencin/406/2013	6	2013-02-08	MDCK1/MDCK1	640	160	640	320	1280	640	1280	2560	1280	
A/Trieste/22/2013	6	2013-02-12	Cx/MDCK1	640	320	320	160	160	320	160	160	160	N156D>N
A/Pavia/47/2013		2013-02-12	Cx/MDCK1	640	320	640	320	1280	640	640	2560	1280	
A/Komarno/457/2013	6	2013-02-12	MDCK1/MDCK1	640	320	640	320	1280	640	640	1280	640	
A/Parma/52/2013	6	2013-02-18	MDCK1/MDCK1	640	640	640	640	1280	1280	640	2560	1280	
A/Perugia/39/2013	6	2013-02-18	MDCK2/MDCK1	640	320	640	320	1280	640	640	1280	640	
A/Trieste/27/2013		2013-02-22	Cx/MDCK1	640	640	1280	640	2560	1280	1280	2560	2560	
A/Trieste/30/2013		2013-02-26	Cx/MDCK1	1280	640	640	640	640	640	640	1280	640	
A/Bratislava/732/2013		2013-03-01	MDCK2/MDCK1	640	160	320	320	640	640	640	1280	1280	
A/Perugia/44/2013		2013-03-06	MDCK1/MDCK1	640	320	640	320	1280	640	640	1280	640	
A/Levice/846/2013	6	2013-03-12	MDCK1/MDCK1	1280	640	1280	640	1280	1280	1280	2560	2560	
A/Bratislava/872/2013		2013-03-13	MDCK1/MDCK1	320	320	640	320	640	640	640	1280	640	
A/Bratislava/873/2013	6	2013-03-13	MDCK1/MDCK1	320	320	320	320	640	640	640	1280	640	
A/Stockholm/15/2013	6	2013-03-26	MDCK0/MDCK1	640	640	640	1280	1280	1280	1280	2560	2560	
A/Iceland/55/2013		2013-04-22	MDCK1/MDCK1	640	640	1280	2560	1280	1280	1280	2560	2560	
A/Iceland/58/2013		2013-05-07	MDCK1/MDCK1	640	320	1280	640	2560	2560	1280	2560	2560	

🟩 Sequences in phylogenetic tree (Figure 1)

🟨 Vaccine

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



Influenza A(H3N2) virus analyses

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 as described before [3]. The change in agglutination of red blood cells is associated with a reduced avidity of H3N2 viruses for the sialic acid receptor on the surface of the cell (Lin et al. 2012) [4]. Antigenic analyses of viruses conducted since the June report [1] are shown in Table 4. HI assays were carried out using guinea pig red blood cells in the presence of 20nM oseltamivir, added to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010) [5]. The test viruses reacted poorly with post-infection ferret antiserum raised against the egg-propagated vaccine virus for 2012–13, A/Victoria/361/2011, compared with the titre against the homologous virus.

Generally, the test viruses also reacted poorly with antisera raised against other reference viruses and previous vaccine viruses propagated in eggs (A/Perth/16/2009, A/Victoria/208/2009, A/Iowa/19/2010 and A/Hawaii/22/2012). However, overall the panel of test viruses showed better reactivity with antiserum raised against egg-propagated A/Texas/50/2012 (the H3N2 vaccine virus recommendation for the northern hemisphere 2013–14) [6], compared with the titre of the antiserum with the homologous virus, than they did against other egg-propagated viruses. In Table 4, antiserum raised against A/Texas/50/2012 recognised 11 out of 38 test viruses at titres within fourfold of the titre to the homologous virus.

The test viruses reacted well with antisera raised against reference viruses exclusively propagated in MDCK cells, and/or the derivative MDCK-SIAT-1 cells, when compared to the titres with the homologous viruses. These antisera were raised against cell-propagated virus isolates of A/Victoria/361/2011, A/Alabama/5/2010, A/Stockholm/18/2011, A/Berlin/93/2011 and A/Athens/112/2012.

Phylogenetic analysis of the HA gene sequences of representative viruses is shown in Figure 2. Viruses from EU/EEA countries collected since 1 January 2013 have HA genes that fall predominantly into genetic group 3C, as is the case for all 15 test viruses sequenced during the preparation of this report. Viruses carrying HA genes falling into groups 3A and 3B (described in previous reports), 5 (e.g. A/Plzen/22/2013) and 6 (e.g. A/Lisboa/SU91/2012) have also been isolated earlier in the EU/EEA 2012–13 influenza season.

The amino acid substitutions in **HA1/HA2** associated with these groupings of recently collected viruses are:

- Group 3 viruses: **N145S** and **V223I**, with viruses in groups 3B and 3C also carrying **A198S**, **N312S** and in
- Group 3C: **S45N** (resulting in gain of a potential glycosylation site) and **T48I**, e.g. the prototype vaccine virus A/Victoria/361/2011; the great majority of viruses also carry the substitutions **Q33R** and **N278K** (e.g. A/Berlin/93/2011); an emerging subgroup also carries the substitutions **T128A** (resulting in the loss of a potential glycosylation site) and **R142G**;
- Group 3B: **D158N**;
- Group 3A: **N144D** (resulting in the loss of a potential glycosylation site), **D158N**;
- Group 5 viruses: **D53N**, **Y94H**, **I230V** and **E280A** (e.g. A/Alabama/05/2010), often in combination with **K2E** and **N8D** (resulting in the loss of a potential glycosylation site);
- Group 6 viruses: **D53N**, **Y94H**, **S199A, I230V** and **E280A** (e.g. A/Iowa/19/2010).

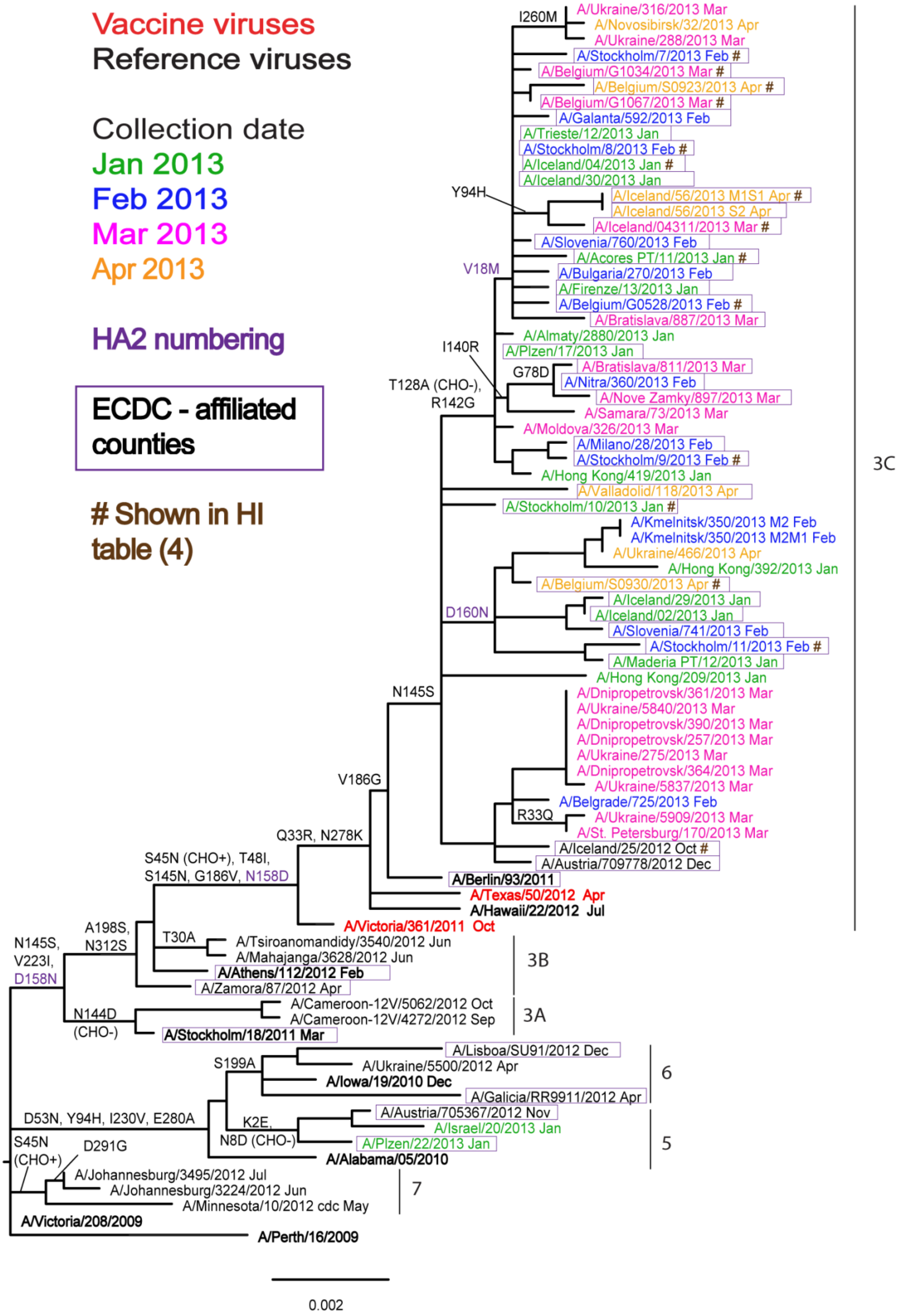
There is no evidence for antigenic change associated with any of the genetic groups or emerging subgroups, including the emerging subgroup in group 3C that carries substitutions in HA1 at amino acid residues 128 and 142.

Table 4. 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 208/09	A/Ala 5/10	A/Stock 18/11	A/Iowa 19/10	A/Vic 361/11	A/Berlin 93/11	A/Vic 361/11	A/Athens 112/12	A/Texas 50/12	A/Hawaii 22/12
				F35/11	F7/10	F27/10	F28/11	F15/11	Egg F35/12	T/C F17/12	T/C F34/12	F16/12	Egg F36/12	F37/12
	Genetic group					group 5	group 3A	group 6	group 3C	group 3C	group 3C	group 3B	group 3C	group 3C
REFERENCE VIRUSES														
A/Perth/16/2009		2009-07-04	E3/E2	640	40	80	80	160	160	320	160	320	320	80
A/Victoria/208/2009		2009-06-02	E3/E2	640	5120	1280	1280	2560	2560	5120	1280	2560	5120	5120
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	40	<	80	80	80	80	320	160	320	320	80
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	40	80	40	160	80	80	320	160	320	640	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	1280	640	1280	2560	1280	2560	1280	1280	5120	1280
A/Victoria/361/2011	3C	2011-10-24	E3/E2	320	1280	320	320	640	2560	1280	640	320	5120	1280
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT6	320	160	80	640	160	320	640	1280	640	1280	320
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT2	160	160	160	640	320	320	1280	640	640	1280	640
A/Athens/112/2012	3B	2012-02-01	SIAT6	80	160	80	320	160	160	640	320	640	640	320
A/Texas/50/2012	3C	2012-04-15	E5/E1	640	1280	320	1280	1280	1280	2560	640	1280	5120	1280
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	640	1280	320	640	1280	1280	2560	640	1280	5120	5120
TEST VIRUSES														
A/Iceland/25/2012	3C	2012-10-17	SIAT2	160	160	80	320	80	160	1280	640	640	1280	320
A/Belgium/S0932/2013		2012-12-20	SIAT2	40	80	80	160	160	160	320	320	320	640	320
A/Iceland/16879/2012		2012-12-31	SIAT3	80	80	160	320	80	160	640	640	640	640	320
A/Iceland/04/2013	3C	2013-01-04	SIAT2	160	160	160	320	160	160	1280	1280	640	1280	640
A/Madeira PT/12/2013		2013-01-07	SIAT4/SIAT1	80	160	160	320	160	160	640	320	640	1280	320
A/Stockholm/1/2013		2013-01-13	MDCK1/SIAT1	40	80	80	320	160	160	640	320	640	1280	320
A/Acores PT/11/2013	3C	2013-01-19	SIAT1/SIAT1	40	80	80	160	160	160	640	320	640	640	320
A/Stockholm/12/2013		2013-01-21	MDCK0/SIAT1	80	80	160	160	320	160	640	320	640	640	320
A/Stockholm/10/2013	3C	2013-01-30	MDCK1/SIAT1	40	80	80	160	80	160	640	320	640	640	320
A/Belgium/G0508/2013		2013-02-04	SIAT2	<	40	40	160	80	80	320	160	320	320	160
A/Belgium/G0528/2013	3C	2013-02-04	SIAT2	<	40	40	160	80	80	320	160	320	320	160
A/Stockholm/11/2013	3C	2013-02-09	MDCK1/SIAT1	40	80	80	160	80	160	640	320	640	640	320
A/Stockholm/9/2013	3C	2013-02-17	MDCK1/SIAT1	80	80	80	320	160	160	640	320	640	1280	320
A/Stockholm/8/2013	3C	2013-02-22	MDCK0/SIAT1	<	80	80	160	80	160	640	320	320	640	320
A/Stockholm/7/2013	3C	2013-02-23	MDCK1/SIAT1	<	40	40	160	80	160	320	320	320	320	160
A/Norway/1693/2013		2013-03-06	MDCK2/SIAT1	<	80	80	160	80	160	640	320	320	640	320
A/Norway/1665/2013		2013-03-07	MDCK2/SIAT1	40	80	40	160	80	80	640	160	320	640	320
A/Norway/1683/2013		2013-03-07	MDCK2/SIAT1	40	80	80	160	80	160	640	320	320	640	320
A/Norway/1736/2013		2013-03-07	MDCK2/SIAT1	80	80	80	160	80	160	320	320	320	640	320
A/Norway/1780/2013		2013-03-09	MDCK1/SIAT1	80	160	160	320	160	160	1280	320	640	1280	320
A/Estonia/76614/2013		2013-03-11	MDCK1/SIAT1	160	160	320	640	320	320	1280	640	1280	2560	640
A/Estonia/76663/2013		2313-03-13	MDCK2/SIAT1	<	40	80	160	80	160	320	320	320	320	160
A/Estonia/76676/2013		2313-03-13	MDCK2/SIAT1	160	160	320	640	640	320	1280	640	1280	2560	640
A/Iceland/4311/2013	3C	2013-03-13	SIAT2	320	160	160	320	160	320	1280	1280	1280	2560	640
A/Norway/1894/2013		2013-03-17	MDCK1/SIAT1	40	80	80	320	80	80	640	320	320	640	320
A/Norway/1940/2013		2013-03-18	MDCK2/SIAT1	40	80	40	160	80	160	320	160	320	320	160
A/Belgium/G1034/2013	3C	2013-03-18	SIAT2	<	80	80	160	160	160	320	320	320	640	320
A/Norway/1861/2013		2013-03-19	MDCK1/SIAT1	40	80	80	160	80	80	320	320	320	640	320
A/Norway/1905/2013		2013-03-24	MDCK2/MDCK1	<	<	<	160	<	80	320	160	160	320	160
A/Belgium/G1067/2013	3C	2013-03-25	SIAT2	40	80	80	160	160	160	640	320	320	640	320
A/Belgium/G1071/2013		2013-03-26	SIAT2	<	80	40	160	80	80	320	160	320	320	160
A/Norway/2010/2013		2013-04-07	MDCK2/SIAT1	40	80	80	160	80	160	320	320	320	640	160
A/Norway/2160/2013		2013-04-07	MDCK1/SIAT1	40	160	80	320	80	160	640	320	640	640	320
A/Norway/2200/2013		2013-04-10	MDCK1/SIAT1	80	160	160	640	160	320	1280	640	640	1280	640
A/Belgium/S0923/2013	3C	2013-04-10	SIAT2	<	80	40	160	80	80	320	160	320	320	160
A/Belgium/S0930/2013	3C	2013-04-11	SIAT2	<	80	40	160	80	80	320	160	320	320	160
A/Norway/2255/2013		2013-04-23	MDCK1/SIAT1	80	160	160	320	320	320	1280	640	640	1280	320
A/Iceland/56/2013	3C	2013-04-26	SIAT2	80	80	80	160	80	80	640	640	320	640	320
Sequences in phylogenetic tree (Figure 2)									Vaccine 2012-2013				Vaccine 2013-2014	

. < = <40

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



Influenza B virus analyses

B/Victoria-lineage viruses

Table 5 shows the results of antigenic analyses for viruses of the B/Victoria-lineage performed since the June report [1]. All test viruses were isolated and propagated in MDCK cells or a derivative thereof (SIAT-1) and, compared with the titre against the homologous virus in HI assays, all but two test viruses (B/Firenze/3/2013 and B/Lisboa PT/13/2013) showed poor reactivity with post-infection ferret antiserum raised against the egg-propagated virus B/Brisbane/60/2008, a component of trivalent vaccines for the 2010–11 season and a recommended component of quadrivalent vaccines [6] for the 2013–14 northern hemisphere influenza season.

Generally, the test viruses also showed similarly reduced reactivities with antisera raised against other reference viruses propagated in hens' eggs: B/England/393/2008, B/Malta/636714/2011 and B/Johannesburg/3964/2012. These observations probably relate to the loss of an N-linked carbohydrate site at position 197 of HA1 which is commonly associated with growth of B/Victoria-lineage viruses in hens’ eggs, resulting in the exposure of a dominant antigenic site. The HAs of eight of the nine test viruses sequenced retained the glycosylation site (NET) while B/Firenze/3/2013 showed polymorphism (NEX with X = T/I). All test viruses showed a consistent reactivity pattern, with titres close to the homologous titres, for antisera raised against reference viruses genetically closely related to B/Brisbane/60/2008 but propagated in cells; these post-infection ferret antisera were raised against B/Paris/1762/2008, B/Hong Kong/514/2009, B/Odessa/3886/2010 and B/Formosa/V2367/2012.

Phylogenetic analysis of the HA genes of representative B/Victoria-lineage viruses is shown in Figure 3. All the viruses received with collection dates in 2013 from EU/EEA laboratories carried HA genes that fell into genetic clade 1A. The amino acid substitution associated with the separation of clade 1 into clades 1A and 1B, L58P, has no apparent effect on antigenicity. The HAs of recent viruses show few amino acid substitutions compared with B/Brisbane/60/2008.

Table 5. Antigenic analysis of influenza B viruses (Victoria-lineage) by HI

				Haemagglutination inhibition titre										
					Post infection ferret antisera									
Viruses		Collection date	Passage History	B/Bris[2] 60/08 Sh 522	B/Mal[1] 2506/05 F37/11	B/Eng[1] 393/08 F05/11	B/Bris[1] 60/08 F22/12	B/Paris[1] 1762/08 F07/11	B/HK[1] 514/09 F13/10	B/Odessa[1] 3886/10 F19/11	B/Malta[1] 636714/11 F33/11	B/Jhb[1] 3964/12 F01/13	B/For[1] V2367/12 F04/13	
	Genetic group					1A	1A	1A	1B	1B	1A	1A	1A	
REFERENCE VIRUSES														
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	320	40	80	<	<	<	80	80	80	
B/England/393/2008	1A	2008-08-29	E1/E2	2560	40	320	320	40	40	80	320	320	320	
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	2560	80	320	320	80	40	80	320	320	320	
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	10	20	40	40	40	80	40	40	80	
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	20	10	20	80	80	160	20	20	80	
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	2560	20	80	80	40	40	80	80	160	160	
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	320	320	40	40	80	320	320	320	
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	160	320	640	80	80	80	320	640	640	
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	2560	10	40	40	40	40	80	40	80	160	
TEST VIRUSES														
B/Firenze/1/2013		2013-01-16	MDCK2/MDCK1	2560	<	20	40	80	40	40	10	<	80	
B/Firenze/3/2013	1A	2013-01-21	MDCK2/MDCK2	2560	80	80	160	40	20	20	80	160	160	197-199 NEX
B/Madeira PT/109/2013		2013-01-28	SIAT1/MDCK2	2560	20	10	20	80	80	80	20	<	40	
B/Lisboa PT/13/2013	1A	2013-01-28	SIAT1/MDCK1	5120	<	40	80	160	80	80	20	40	80	
B/Lisboa PT/50/2013		2013-02-13	SIAT1/MDCK2	2560	20	10	20	80	80	80	20	<	40	
B/Lisboa PT/74/2013	1A	2013-02-19	SIAT1/MDCK1	2560	10	20	<	80	40	40	10	20	40	
B/Milano/101/2013	1A	2013-02-19	MDCK1/MDCK1	2560	<	20	40	80	40	40	20	40	80	
B/Portalegre PT/101/2013	1A	2013-02-24	SIAT1/MDCK1	2560	10	20	<	80	40	40	20	40	80	
B/Castelo Branco PT/176/2013		2013-02-28	SIAT1/MDCK1	2560	20	20	40	80	80	80	40	<	80	
B/Castelo Branco PT/177/2013	1A	2013-03-04	SIAT1/MDCK1	5120	20	20	40	80	80	160	40	40	40	
B/Trnava/783/2013	1A	2013-03-05	MDCK1/MDCK1	5120	<	20	40	160	80	80	20	40	80	
B/Lisboa PT/232/2013		2013-03-17	SIAT1/MDCK1	2560	<	20	40	80	80	80	40	40	40	
B/Portalegre PT/233/2013	1A	2013-03-18	SIAT1/MDCK1	2560	20	10	20	80	80	80	20	<	40	
B/Bratislava/911/2013	1A	2013-03-20	MDCK1/MDCK1	2560	<	20	40	80	40	40	20	40	80	
B/Norway/1958/2013		2013-03-25	MDCK1/MDCK1	1280	<	<	10	40	40	80	10	<	40	
B/Norway/2090/2013		2013-04-14	MDCK1/MDCK1	1280	10	<	10	80	40	80	10	<	40	

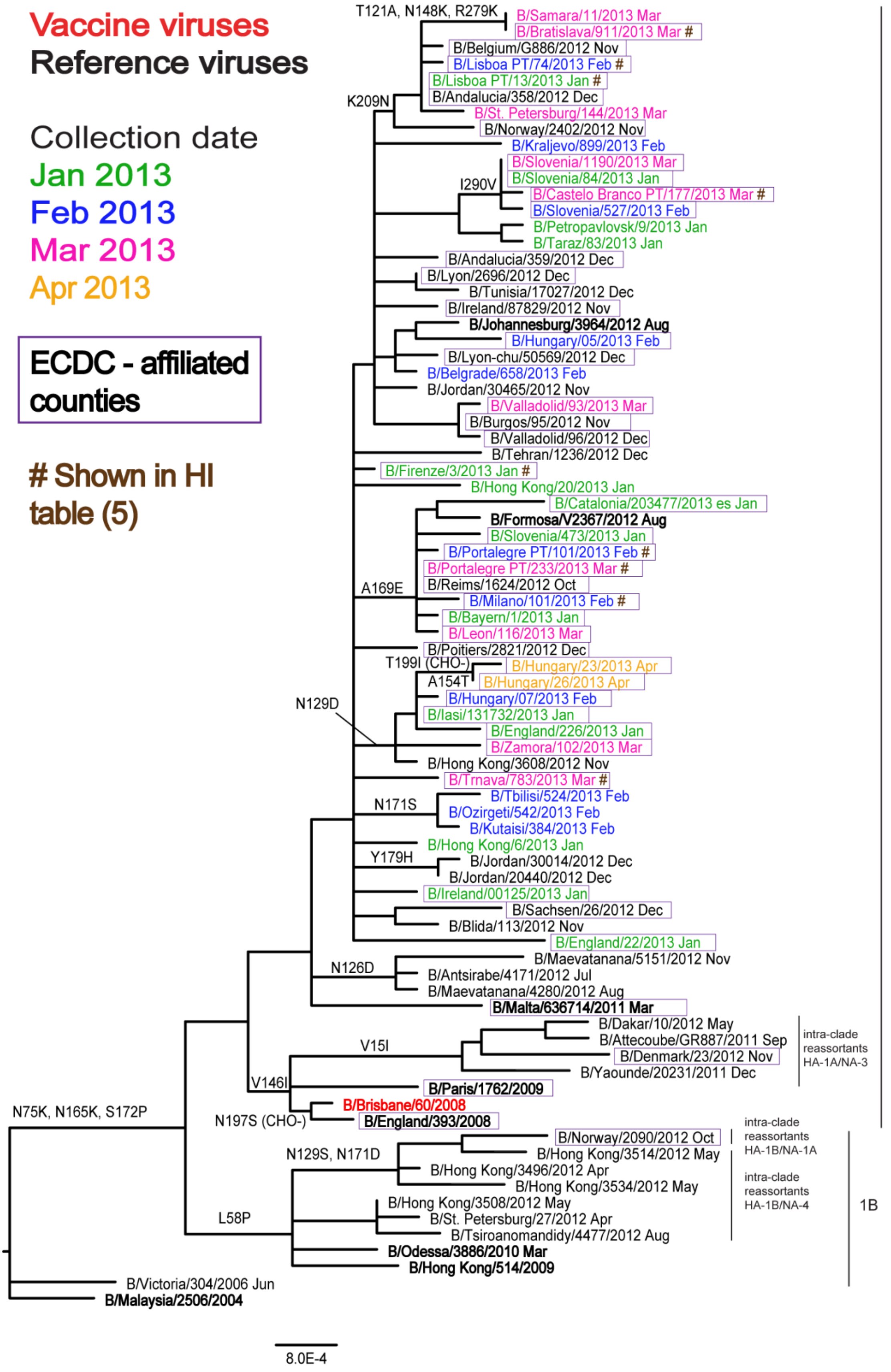
1. < = <10; 2. hyperimmune sheep serum

Vaccine*

Sequences in phylogenetic tree (Figure 3)

* Recommended B-Victoria lineage component for quadravalent vaccine 2012-13 and 2013-14

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



B/Yamagata-lineage viruses

Tables 6 and 7 show the results of HI analyses of B/Yamagata lineage viruses tested since the June report [1]. The genetic clade into which sequenced HA genes of test viruses fall is indicated.

All 87 test viruses showed good reactivity (within fourfold of the homologous titre) with antiserum raised against the egg-propagated vaccine virus recommended for the northern hemisphere winter 2013–14 influenza season [6], B/Massachusetts/02/2012. Antiserum raised against egg-propagated B/Wisconsin/1/2010, the virus used in the vaccine for 2012–13, also showed reactivity within fourfold of the titre against the homologous virus for 66 of the test viruses. Antisera raised against cell-propagated viruses, whether of clade 2 or clade 3, showed good reactivity (within fourfold of the homologous titre) against the majority of test viruses. Twenty-eight of the test viruses had been genetically characterised at the time of preparation of this report, with 23 falling into genetic group 2 and five into genetic group 3.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. The analysis shows that the HA genes of recent viruses continue to fall into two genetic clades: clade 3 (represented by the vaccine virus B/Wisconsin/1/2010 and reference viruses B/Stockholm/12/2011 and B/Novosibirsk/1/2012) and clade 2 (represented by the reference viruses B/Brisbane/3/2007, B/Estonia/55669/2011, B/Hong Kong/3577/2012 and the 2013–14 vaccine virus B/Massachusetts/02/2012). The two clades are differentiated by substitutions at HA1 residues 48, 108, 150, 165, 181 and 229. The HA genes of viruses of clade 2 encode **K48**, **A108**, **S150**, **N165**, **A181** and **G229**; the HA genes of viruses in clade 3 encode **R48**, **P108**, **I150**, **Y165**, **T181** and **D229**. The proportion of viruses received with HA genes that fall into clade 2 has continued to increase over the number with HA genes falling into clade 3.

Table 6. Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglution Inhibition Titre									
					Post infection ferret antisera								
				B/Fl[3] 4/06 SH479	B/Fl[1] 4/06 F1/10 Group 1	B/Bris[2] 3/07 F21/12 Group 2	B/Wis[2] 1/10 F24/12 Group 3	B/Stock[2] 12/11 F12/12 Group 3	B/Estonia[2] 55669/11 F26/11 Group 2	B/Novo[2] 1/12 F31/12 Group 3	B/HK[2] 3577/12 F33/12 Group 2	B/Mass[2] 2/12 Egg F2/13 Group 2	B/Mass[2] 2/12 T/C F3/13 Group 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E7	5120	1280	1280	640	1280	320	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	1280	1280	320	640	160	40	640	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	640	640	320	1280	40	80	80	640	40
B/Stockholm/12/2011	3	2007-08-07	E4/E1	2560	320	160	160	320	20	40	40	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK2	1280	160	80	40	40	640	80	640	160	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	640	160	80	80	160	40	80	160	160	40
B/Hong Kong/3577/2012	2	2012-06-13	MDCK4	1280	160	80	80	80	640	80	640	160	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	2560	640	640	160	320	160	20	320	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK4	5120	640	320	160	320	640	160	640	640	640
TEST VIRUSES													
B/Belgium/G0525/2013		2013-01-28	MDCK3	1280	160	80	40	80	640	80	320	160	160
B/Belgium/G0537/2013		2013-01-29	MDCK2	1280	160	80	80	80	640	80	640	160	160
B/Belgium/G0506/2013		2013-02-04	MDCK2	1280	160	80	40	80	320	40	320	160	80
B/Belgium/S0283/2013		2013-02-02	MDCK2	1280	160	80	40	80	320	40	320	160	160
B/Belgium/G0509/2013	2	2013-02-04	MDCK2	1280	80	80	40	80	320	80	320	160	80
B/Belgium/G0532/2013		2013-02-04	MDCK2	2560	160	160	80	160	640	80	640	320	160
B/Belgium/G0535/2013		2013-02-04	MDCK2	2560	160	160	80	160	640	80	640	320	160
B/Belgium/G0536/2013		2013-02-04	MDCK2	1280	160	80	80	80	640	80	640	320	160
B/Belgium/G0538/2013	2	2013-02-05	MDCK2	1280	80	80	40	40	320	40	320	160	80
B/Belgium/S0327/2013		2013-02-06	MDCK2	2560	160	160	80	160	640	40	640	320	160
B/Lisboa PT/138/2013		2013-03-04	SIAT1/MDCK1	2560	160	160	80	320	640	160	640	160	640
B/Norway/1677/2013		2013-03-08	MDCK1/MDCK1	1280	160	80	40	80	640	80	640	160	320
B/Norway/1654/2013		2013-03-11	MDCK1/MDCK1	2560	320	160	160	320	1280	160	640	320	640
B/Castelo Branco PT/178/2013		2013-03-14	SIAT1/MDCK1	2560	160	160	160	320	160	160	160	160	160
B/Lisboa PT/210/2013		2013-03-14	SIAT1/MDCK1	1280	80	80	40	40	320	40	320	160	160
B/Belgium/G1044/2013	2	2013-03-19	MDCK2	640	80	80	40	80	320	40	320	160	80
B/Belgium/G1062/2013		2013-03-21	MDCK2	2560	160	160	80	80	640	40	640	320	160
B/CasteloBranco PT/179/2013	3	2013-03-21	SIAT1/MDCK1	2560	160	160	160	160	160	320	320	160	320
B/Norway/1887/2013		2013-03-22	MDCK1/MDCK1	2560	160	160	160	320	640	160	640	160	640
B/Norway/2020/2013		2013-03-22	MDCK1/MDCK1	1280	160	80	80	80	640	80	320	160	320
B/Belgium/G1076/2013		2013-03-23	MDCK2	1280	160	160	40	80	320	40	320	160	160
B/Belgium/G1064/2013	2	2013-03-25	MDCK2	1280	160	160	80	80	640	40	640	320	160
B/Belgium/G1068/2013		2013-03-25	MDCK2	1280	160	80	40	80	320	40	320	160	160
B/Belgium/G1082/2013		2013-03-25	MDCK2	1280	160	80	40	80	320	40	320	160	160
B/Belgium/G1086/2013	3	2013-03-25	MDCK2	1280	160	80	80	160	80	160	80	160	80
B/Norway/1924/2013		2013-03-26	MDCK1/MDCK1	2560	160	160	80	160	640	160	640	320	640
B/Belgium/G1077/2013		2013-03-26	MDCK2	1280	160	80	40	80	320	20	320	160	160
B/Portalegre PT/229/2013	2	2013-04-01	SIAT1/MDCK1	2560	160	80	80	80	640	80	640	160	160
B/Belgium/S0929/2013	2	2013-04-05	MDCK2	2560	160	160	80	160	320	80	320	320	160
B/Norway/2057/2013		2013-04-07	MDCK2/MDCK1	2560	160	80	80	80	640	80	640	160	320
B/Norway/2103/2013		2013-04-07	MDCK1/MDCK1	2560	160	80	80	160	320	160	640	160	320
B/Norway/2161/2013		2013-04-08	MDCK1/MDCK1	2560	160	80	80	160	640	80	320	160	320
							Vaccine 2012-2013					Vaccine 2013-2014	

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

🟩 Sequences in phylogenetic tree (Figure 4)

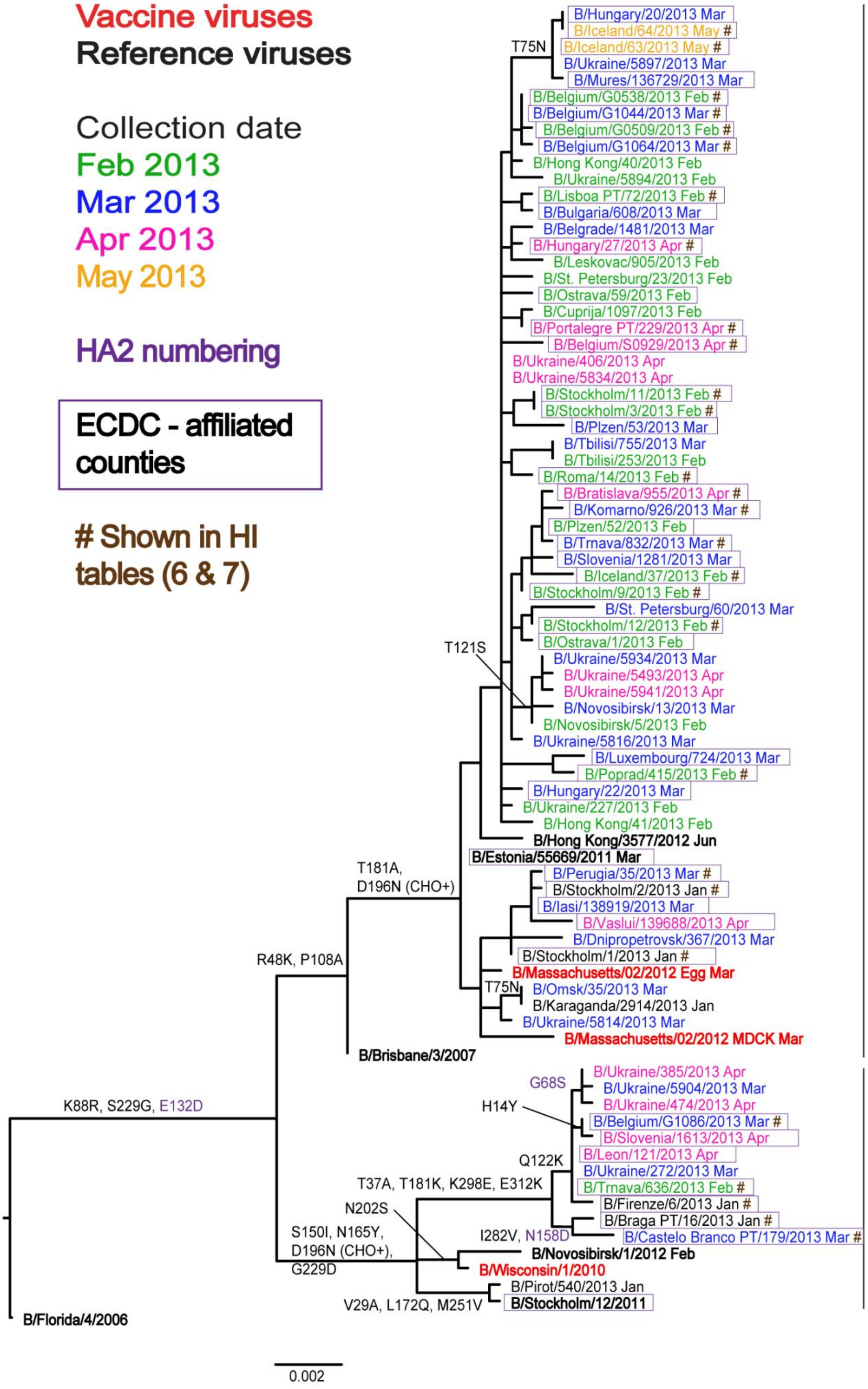
Table 7. Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

					Haemagglution Inhibition Titre									
					Post infection ferret antisera									
Viruses	Genetic group	Collection date	Passage History	B/Fl[3] 4/06 SH479	B/Fl[1] 4/06 F1/10 Group 1	B/Bris[2] 3/07 F21/12 Group 2	B/Wis[2] 1/10 F24/12 Group 3	B/Stock[2] 12/11 F12/12 Group 3	B/Estonia[2] 55669/11 F26/11 Group 2	B/Novo[2] 1/12 F31/12 Group 3	B/HK[2] 3577/12 F33/12 Group 2	B/Mass[2] 2/12 Egg F2/13 Group 2	B/Mass[2] 2/12 T/C F3/13 Group 2
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	5120	640	640	160	640	160	40	320	640	80
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	1280	1280	320	640	160	40	320	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	640	320	320	640	20	80	80	640	40
B/Stockholm/12/2011	3	2007-08-07	E4/E1	1280	160	160	160	320	<	40	40	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	2560	160	160	80	160	640	160	640	320	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK3	2560	160	160	160	320	160	320	320	320	320
B/Hong Kong/3577/2012	2	2012-06-13	MDCK4	2560	160	160	80	160	640	160	640	320	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	2560	640	640	320	640	160	40	320	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK4	5120	640	320	160	320	640	160	640	640	640
TEST VIRUSES														
B/Iceland/26/2012		2012-10-17	MDCK1/MDCK1	5120	160	160	160	320	160	320	320	160	320
B/Iceland/29/2012		2012-12-25	MDCK1/MDCK1	2560	320	160	160	320	640	80	640	320	320
B/Firenze/11/2013		unknown	MDCK2/MDCK1	2560	320	160	160	320	640	80	640	320	160
B/Stockholm/6/2013		2013-01-01	MDCK1/MDCK1	1280	160	80	40	80	640	40	320	160	160
B/Stockholm/1/2013	2	2013-01-02	MDCK0/MDCK1	1280	160	80	40	80	640	40	320	160	160
B/Stockholm/5/2013		2013-01-03	MDCK1/MDCK1	2560	320	160	80	160	640	40	640	320	160
B/Stockholm/2/2013	2	2013-01-06	MDCK0/MDCK1	1280	160	80	40	80	640	40	320	160	80
B/Stockholm/7/2013		2013-01-07	MDCK0/MDCK1	2560	160	160	80	160	640	40	640	320	160
B/Pavia/7/2013		2013-01-15	Cx/MDCK1	2560	320	160	160	320	640	80	640	320	320
B/Lisboa PT/8/2013		2013-01-18	SIAT3/MDCK1	1280	160	80	40	80	640	40	320	160	160
B/Porto PT/12/2013		2013-01-21	SIAT1/MDCK1	2560	160	160	160	160	80	320	160	160	160
B/Braga PT/17/2013		2013-01-22	SIAT1/MDCK1	1280	160	160	80	160	640	40	640	320	160
B/Vila Real PT/15/2013		2013-01-25	SIAT1/MDCK1	2560	160	160	80	160	640	40	640	320	160
B/Braga PT/16/2013	3	2013-01-26	SIAT1/MDCK1	1280	160	80	80	160	40	80	80	160	40
B/Milano/72/2013		2013-01-28	MDCK1/MDCK1	1280	160	160	80	160	640	40	640	320	320
B/Parma/50/2013		2013-01-29	MDCK2/MDCK2	2560	320	160	160	320	1280	160	1280	320	640
B/Perugia/6/2013		2013-01-30	MDCK1/MDCK1	2560	320	160	160	160	320	40	640	320	160
B/Firenze/6/2013	3	2013-01-31	MDCK2/MDCK1	2560	320	160	320	320	160	320	320	320	320
B/Stockholm/3/2013	2	2013-02-04	MDCK0/MDCK1	1280	160	80	40	80	320	40	320	160	80
B/Trieste/06/2013		2013-02-04	Cx/MDCK1	2560	320	320	160	320	640	80	1280	320	320
B/Nitra/359/2013		2013-02-05	MDCK1/MDCK1	2560	160	160	80	160	640	40	640	320	160
B/Poprad/415/2013	2	2013-02-08	MDCK1/MDCK1	1280	160	80	80	80	640	40	640	320	160
B/Bratislava/451/2013		2013-02-11	MDCK1/MDCK1	2560	160	160	80	160	640	40	640	320	160
B/Trnava/486/2013		2013-02-13	MDCK1/MDCK1	1280	160	160	80	80	640	40	640	320	160
B/Lisboa PT/72/2013	2	2013-02-13	SIAT1/MDCK1	1280	160	80	80	80	640	80	320	160	320
B/Pavia/52/2013		2013-02-13	Cx/MDCK1	2560	320	160	160	160	1280	160	1280	320	640
B/Bratislava/501/2013		2013-02-14	MDCK1/MDCK1	1280	160	80	80	80	640	40	640	320	160
B/Stockholm/12/2013	2	2013-02-16	MDCK1/MDCK1	640	80	80	40	40	320	40	320	160	160
B/Trieste/11/2013		2013-02-17	Cx/MDCK1	2560	160	160	80	160	640	80	640	320	320
B/Stockholm/11/2013	2	2013-02-18	MDCK0/MDCK1	1280	80	80	40	80	320	40	320	160	80
B/Trieste/15/2013		2013-02-19	Cx/MDCK1	2560	320	160	160	320	640	80	640	320	320
B/Perugia/19/2013		2013-02-20	MDCK1/MDCK1	2560	320	160	320	320	320	160	640	320	160
B/Roma/14/2013	2	2013-02-21	MDCK2/MDCK1	1280	160	160	80	160	640	80	640	160	320
B/Stockholm/9/2013	2	2013-02-21	MDCK1/MDCK1	1280	160	160	80	80	640	40	320	320	160
B/Pavia/76/2013		2013-02-21	Cx/MDCK1	1280	160	160	80	160	640	160	640	320	640
B/Parma/129/2013		2013-02-22	MDCK1/MDCK1	2560	320	160	320	320	320	320	640	160	640
B/Trnava/636/2013	3	2013-02-25	MDCK1/MDCK1	1280	80	80	80	80	20	80	80	160	40
B/Iceland/37/2013	2	2013-02-25	MDCK1/MDCK1	1280	160	160	40	80	80	20	160	320	80
B/Hungary/08/2013		2013-02-27	MDCK1/E3/MDCK3	5120	1280	640	320	640	320	160	640	640	640
B/Perugia/25/2013		2013-02-27	MDCK1/MDCK1	1280	160	160	160	160	320	40	640	320	320
B/Lisboa PT/135/2013		2013-02-28	SIAT1/MDCK1	1280	160	80	80	80	640	80	640	160	320
B/Iceland/39/2013		2013-03-04	MDCK1/MDCK1	2560	160	160	160	320	640	80	640	320	320
B/Lisboa PT/136/2013		2013-03-06	SIAT1/MDCK1	1280	160	80	80	80	320	80	320	160	160
B/Perugia/35/2013	2	2013-03-06	MDCK1/MDCK1	2560	160	160	160	160	640	80	640	320	320
B/Poprad/801/2013		2013-03-07	MDCK1/MDCK1	1280	160	80	80	80	320	40	320	320	160
B/Trnava/832/2013	2	2013-03-08	MDCK1/MDCK1	1280	160	80	80	80	640	40	640	320	160
B/Hungary/09/2013		2013-03-11	MDCK1/E2/MDCK3	5120	320	320	320	640	1280	640	1280	640	1280
B/Iceland/44/2013		2013-03-20	MDCK1/MDCK1	2560	160	160	160	160	640	40	640	320	160
B/Komarno/926/2013	2	2013-03-22	MDCK1/MDCK1	2560	160	160	80	80	640	40	640	320	160
B/Bratislava/939/2013		2013-03-28	MDCK1/MDCK1	1280	160	80	80	80	640	80	640	160	320
B/Hungary/27/2013	2	2013-04-03	MDCK1/E1/MDCK1	1280	160	160	40	80	80	20	160	320	80
B/Bratislava/955/2013	2	2013-04-05	MDCK1/MDCK1	2560	160	160	80	80	640	40	640	320	160
B/Iceland/52/2013		2013-04-10	MDCK1/MDCK1	2560	160	160	160	160	640	40	640	320	160
B/Iceland/63/2013	2	2013-05-16	MDCK1/MDCK1	1280	160	80	80	160	320	40	320	160	160
B/Iceland/64/2013	2	2013-05-17	MDCK1/MDCK1	2560	160	160	160	320	640	80	640	320	320
							Vaccine 2012-2013					Vaccine 2013-2014	

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

Sequences in phylogenetic tree (Figure 4)

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



Influenza A(H7N9) virus

On 1 April 2013, the WHO Global Alert and Response [7] reported that the China Health and Family Planning Commission notified the World Health Organization (WHO) of three cases of human infection with influenza A(H7N9). The cases were confirmed by laboratory testing on 29 March by the Chinese CDC. A description of the characteristics of H7N9 viruses can be found on the WHO website [8]. WHO is updating information on the outbreak regularly [8], and ECDC is posting epidemiological updates [9]. A Rapid Risk Assessment [10] for these A(H7N9) viruses has been carried out and posted by ECDC on 3 April 2013, and an updated risk assessment has been posted by WHO [11]. As of 20 July 2013, WHO reported [12] 134 laboratory-confirmed cases and 43 associated fatalities.

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 in Beijing, China, on 17–19 September 2012 and at WHO Geneva on 18–20 February 2013, can be found at:
http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_September_2012_2.pdf [13]

http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February 2013.pdf [14]

Note on the figures

The phylogenetic trees were constructed using RAxML and drawn using FigTree. 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 some of the viruses from non-EU/EEA countries were recovered from GISAID. We acknowledge all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

References

1 European Centre for Disease Prevention and Control. Influenza virus characterisation – Summary Europe, June 2013. Stockholm: ECDC; 2013 [cited 2013 Jun 29].

2 European Centre for Disease Prevention and Control. Influenza virus characterisation – Summary Europe, April 2013. Stockholm: ECDC; 2013 [cited 2013 Jun 1].

3 European Centre for Disease Prevention and Control. Influenza virus characterisation – Summary Europe, April 2011. Stockholm: ECDC; 2011 [cited 2013 Jun 1].

4 Lin YP, Xiong X, Wharton SA, Martin SR, Coombs PJ, Vachieri SG, et al. Evolution of the receptor binding properties of the influenza A(H3N2) hemagglutinin. Proc Natl Acad Sci USA. 2012 Dec 26;109(52):21474-9.

5 Lin YP, Gregory V, Collins P, Kloess J, Wharton S, Cattle N, et al. Neuraminidase receptor binding variants of human influenza A(H3N2) viruses resulting from substitution of aspartic acid 151 in the catalytic site: a role in virus attachment? J Virol. 2010 Jul;84(13):6769-81.

6 World Health Organization. Recommended composition of influenza virus vaccines for use in the 2013–2014 northern hemisphere influenza season. Wkly Epidemiol Rec. 2013 Mar 8;88(10):101-14.

7 World Health Organization. Human infection with influenza A(H7N9) virus in China. Global Alert and Response (GAR) [serial on the internet]. Apr 1 2013 [cited 2013 June 1]. Available from: http://www.who.int/csr/don/2013_04_01/en/index.html

8 World Health Organization. Avian influenza A(H7N9) virus. [homepage on the internet]. 2013 [cited 2013 June 20]. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html

9 European Centre for Disease Prevention and Control. Epidemiological updates. [homepage on the internet]. 2013 [cited 2013 Jun 12]. Available from: http://ecdc.europa.eu/en/press/epidemiological_updates/Pages/epidemiological_updates.aspx

10 European Centre for Disease Prevention and Control. Rapid risk assessment – Severe respiratory disease associated with a novel influenza A virus, A(H7N9) – China, 3 April 2013. Stockholm: ECDC.

11 World Health Organization. WHO risk assessment: Human infections with avian influenza A(H7N9) virus, 7 June 2013. Geneva: WHO; 2013 [cited 2013 Jun 12]. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/RiskAssessment_H7N9_07Jun13.pdf

12 World Health Organization. Human infection with avian influenza A(H7N9) virus – update. Global Alert and Response (GAR) [serial on the internet]. Jul 20 2013 [cited 2013 July 21]. Available from: http://www.who.int/csr/don/2013_07_20/en/index.html

13 National Institute for Medical Research, WHO Influenza Centre London. Report prepared for the WHO annual consultation on the composition of influenza vaccine for the southern hemisphere 2013, 17th–19th September 2012. London: WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research; 2012.

14 National Institute for Medical Research, WHO Influenza Centre London. Report prepared for the WHO annual consultation on the composition of influenza vaccine for the northern hemisphere 2013/14, 18th–20th February 2013. London: WHO Collaborating Centre for Reference and Research on Influenza, National Institute for Medical Research; 2013.