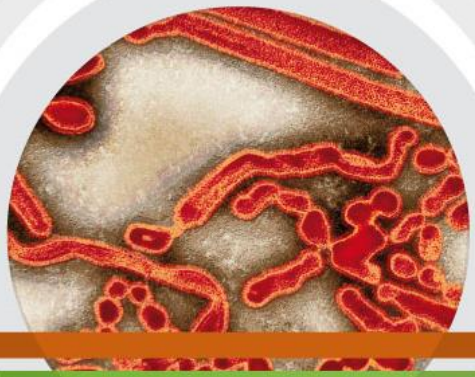

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, September 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/lineage has varied between countries.

- Type A (~60%) and type B (~40%) 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, predominant in the 6C subgroup.
- The vast majority of A(H3N2) viruses have been antigenically and genetically similar to cell-propagated A/Victoria/361/2011, a genetic subgroup 3C virus and the prototype vaccine virus for the 2012–13 influenza season; subgroup 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 (75% of B/Yamagata detections recently) 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 28 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 1.5:1). 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).

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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											
Austria	3		2	2	1	1					
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
Iceland	8		2	2	6	5					
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
Lithuania	1									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							
Poland	13	1	8	2	3	0				1	1
Portugal	24		11	9	4	4		2	2	7	7
Romania	9		6	6				1	1	2	2
Slovenia	18		4	1	5	3	1	5	4	3	3
Spain	22		10	10	6	6				6	6
Sweden	10		2	2	3	3				5	5
United Kingdom	6				5	5		1	1		
FEBRUARY											
Austria	6		4	4				1	1	1	1
Belgium	12		2	2	2	2				8	8
Bulgaria	22		8	7	2	2				12	12
Czech Republic	4									4	4
France	12		2	2	1	1		5	5	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
Lithuania	45	18	7	3	11	7	6			3	3
Luxembourg	5		1	0	4	0					
Norway	1		1	1							
Poland	4									4	4
Portugal	10		4	4				4	4	2	2
Romania	13		8	8						5	5
Slovakia	11		2	2	3	3				6	6
Slovenia	15		5	5	4	4		1	1	5	4
Spain	10		9	6	1	1					
Sweden	8				4	4				4	4
United Kingdom	8		3	3	2	2		1	1	2	2
MARCH											
Austria	7				1	1		1	1	5	5
Belgium	15		4	3	3	3				8	8
Bulgaria	2		1	1						1	1
Czech Republic	1									1	1
Estonia	21	4	7	7	6	6				4	4
France	13		2	2	3	3		4	4	4	4
Hungary	11		1	1				1	1	9	3
Iceland	3				1	1				2	2
Ireland	11				11	4					
Italy	2		1	1						1	1
Lithuania	38	1	2	0	8	5	19			8	8
Luxembourg	1									1	1
Norway	22		6	6	9	9		1	1	6	6
Poland	7						1			6	6
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	12		3	2	4	4		3	3	2	1
Sweden	1		1	1							
APRIL											
Belgium	3				2	2				1	1
Estonia	13	2	1	1	1	1		1	1	8	8
France	6				1	1		1	1	4	4
Hungary	4	1						2	2	1	1
Iceland	3		1	1	1	1				1	1
Ireland	5				5	3					
Lithuania	4	3								1	1
Norway	16		3	3	4	4		1	1	8	8
Poland	1						1				
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
United Kingdom	6				6	0					
MAY											
Austria	1									1	1
Iceland	3		1	1						2	2
Norway	4		1	1	2	2				1	1
	786	35	255	202	167	132	33	53	51	243	230
			32.4%		21.2%			6.7%		30.9%	
28 Countries			58.1%					41.9%			

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 July report [1] are shown in Table 2. All 27 test viruses showed good reactivity with antiserum raised against the vaccine virus, A/California/7/2009, with all titres being within twofold of its recognition of the homologous virus. 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: the titre was reduced eightfold or greater compared with the titre of the antiserum with the homologous virus for 25 of the 27 test viruses.

Figure 1 shows a phylogenetic tree for the HA genes of representative H1N1 viruses. Over the course of the last four years the HA genes have been observed to fall into eight designated genetic groups, with a ninth 'outlier' group largely restricted to countries of west Africa. However, viruses collected after 31 January 2013 fell into genetic groups 6 and 7, with group 6 viruses clustering in three subgroups, 6A–C (Figure 1). Both groups carry the substitutions **S185T** and **S203T** in **HA1** and **E47K** and **S124N** in **HA2** compared to A/California/7/2009.

The two groups and three subgroups are further defined by the following amino acid substitutions in **HA1/HA2**.

Group 6 viruses:

D97N, with the subgroups defined by the substitutions:

- 6A: **H138R** and **V249L**, e.g. A/Hong Kong/5659/2012.
- 6B: **K163Q**, **A256T**, **K283E** and **E172K**, e.g. A/Norway/2417/2013.
- 6C: **V234I**, **K283E** and **E172K** e.g. A/Estonia/76677/2013.

Group 7 viruses:

- S143G and A197T, often with S84G, K163I and V193A, e.g. A/Norway/1675/2013.

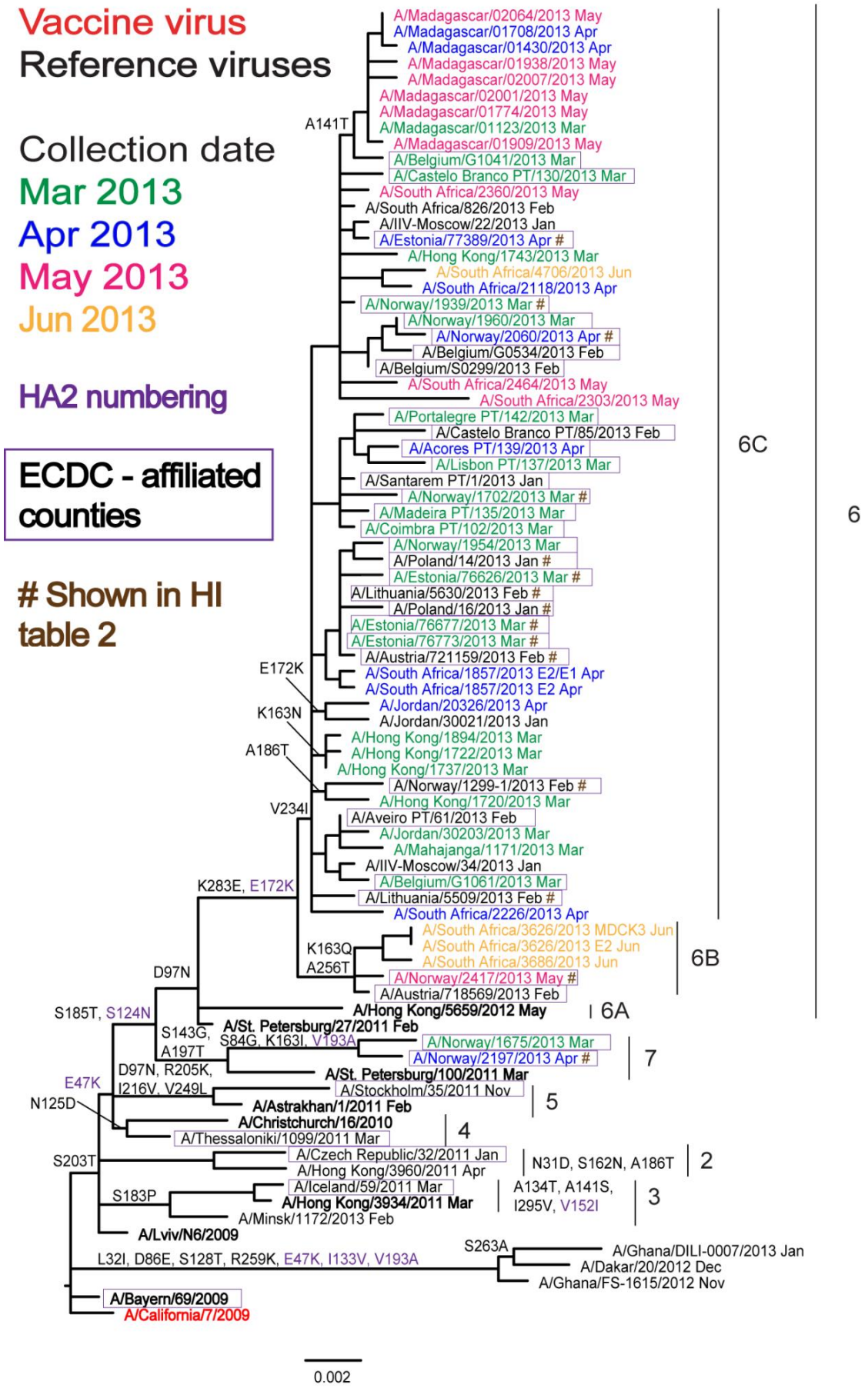
The majority of H1N1 viruses from EU/EEA countries collected during the 2012–13 season cluster within genetic groups 6 and 7, with viruses belonging to subgroup 6C predominating, notably so for those with collection dates since 1 March 2013. HA gene sequencing was performed on 16 of the 27 test viruses, and their genetic grouping is shown in Table 2; 14 were in genetic subgroup 6C, one in subgroup 6B, and one in group 7.

Table 2. 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 6A	
REFERENCE VIRUSES													
A/California/7/2009		2009-04-09	E1/E2	640	640	640	320	320	320	320	320	320	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	160	320	160	40	40	80	80	40	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	1280	320	160	320	320	160	320	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	2560	5120	1280	2560	1280	5120	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK4	640	320	640	640	1280	1280	1280	2560	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	320	320	640	320	640	640	1280	1280	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	1280	2560	1280	2560	2560	2560	5120	2560	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	640	640	640	1280	640	640	2560	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK1	320	160	320	320	640	640	640	1280	1280	
TEST VIRUSES													
A/Poland/14/2013	6C	2013-01-03	MDCKx/MDCK2	640	640	640	320	1280	1280	1280	1280	1280	
A/Austria/713625/2013		2013-01-08	SIAT1/MDCK1	640	320	640	320	640	640	640	1280	1280	
A/Poland/16/2013	6C	2013-01-09	Ex/E1	1280	640	640	320	160	320	320	320	320	
A/Austria/717240/2013		2013-01-28	SIAT1/MDCK2	640	320	320	320	640	640	640	1280	2560	
A/Austria/717999/2013		2013-02-01	SIAT1/MDCK2	640	320	640	640	1280	1280	1280	2560	5120	
A/Austria/718287/2013		2013-02-05	SIAT1/MDCK1	640	320	640	320	1280	640	1280	1280	1280	
A/Austria/718985/2013	6C	2013-02-08	SIAT2/MDCK2	640	320	640	320	1280	640	640	2560	2560	
A/Norway/1299-1/2013	6C	2013-02-14	MDCK1/MDCK2	640	160	640	320	320	320	320	320	320	
A/Lithuania/5509/2013	6C	2013-02-18	MDCK3	1280	640	640	640	1280	1280	1280	2560	2560	
A/Lithuania/5630/2013	6C	2013-02-20	MDCK3	640	320	320	160	320	320	320	320	320	
A/Austria/721159/2013	6C	2013-02-21	SIAT1/MDCK1	640	320	640	1280	1280	1280	1280	2560	2560	
A/Lithuania/6526/2013		2013-02-26	MDCK3	1280	640	1280	640	1280	1280	1280	5120	2560	
A/Norway/1702/2013	6C	2013-03-02	MDCK1/MDCK1	320	160	640	320	640	640	640	1280	1280	
A/Norway/1774/2013		2013-03-04	MDCK1/MDCK2	640	320	640	640	1280	640	1280	2560	1280	
A/Estonia/76520/2013		2013-03-08	MDCK1/MDCK1	640	640	1280	640	2560	1280	1280	2560	2560	
A/Estonia/76534/2013		2013-03-11	MDCK1/SIAT2	640	320	640	320	1280	640	1280	2560	1280	
A/Estonia/76626/2013	6C	2013-03-12	MDCK2/MDCK1	320	640	320	160	160	320	320	160	320	
A/Estonia/76677/2013	6C	2013-03-13	MDCK2/MDCK1	1280	320	1280	640	1280	1280	1280	5120	2560	
A/Estonia/76745/2013		2013-03-14	MDCK2/MDCK1	640	320	640	320	1280	1280	1280	2560	1280	
A/Estonia/76773/2013	6C	2013-03-15	MDCK2/MDCK2	640	320	640	320	640	640	640	1280	1280	
A/Norway/1939/2013	6C	2013-03-19	MDCK2/MDCK2	320	320	640	320	1280	1280	1280	2560	1280	
A/Estonia/76993/2013		2013-03-21	MDCK2/SIAT2	640	320	640	320	1280	640	640	1280	640	
A/Norway/1983/2013		2013-04-01	MDCK1/MDCK2	640	640	640	640	1280	1280	1280	2560	2560	
A/Norway/2060/2013	6C	2013-04-05	MDCK2/MDCK2	640	320	640	640	1280	1280	1280	2560	1280	
A/Estonia/77389/2013	6C	2013-04-08	MDCK2/MDCK1	1280	640	1280	1280	2560	2560	1280	5120	2560	
A/Norway/2197/2013	7	2013-04-12	MDCK2/MDCK1	640	320	640	640	1280	640	1280	2560	1280	
A/Norway/2417/2013	6B	2013-05-03	MDCK2	1280	640	1280	640	1280	1280	1280	2560	2560	

Sequences in phylogenetic tree (Figure 1) 1. < = <40 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 July report [1] are shown in Table 3. HI assays were carried out using guinea pig red blood cells in the presence of 20 nM oseltamivir, added to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010) [5].

All 27 test viruses were propagated in MDCK-SIAT1 cells and reacted poorly in HI assays (≥ eightfold titre decrease), with post-infection ferret antiserum raised against the previous egg-propagated vaccine virus, A/Victoria/361/2011, compared to the titre of the antiserum with the homologous virus. 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/Iowa/19/2010 and A/Hawaii/22/2012). Somewhat better reactivity was seen with antiserum raised against egg-propagated A/Texas/50/2012 (the H3N2 vaccine virus recommendation for the northern hemisphere 2013–14 and southern hemisphere 2014) [6]. Although this virus induced an antiserum with a high homologous titre (2560), 21/27 (78%) of test viruses reacted within fourfold of the titre with 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 the 19 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 the subgroups defined by the substitutions:
- 3A: **N144D** (resulting in the loss of a potential glycosylation site) and **D158N**, e.g. A/Stockholm/18/2011;
- 3B: **A198S**, **N312S** and **D158N**, e.g. A/Athens/112/2012;
- 3C: **S45N** (resulting in gain of a potential glycosylation site), **T48I**, **A198S** and **N312S**, e.g. the prototype vaccine virus A/Victoria/361/2011.

The 3C subgroup can be subdivided into three subsets:

- 3C.1 **Q33R**, **S145N** and **N278K**, e.g. A/Berlin/93/2011, A/Texas/50/2012 and A/Hawaii/22/2012;
- 3C.2 As 3C.1 plus **N145S**, e.g. A/Ireland/M28390/2013;
- 3C.3 As 3C.2 plus **T128A** (resulting in the loss of a potential glycosylation site) and **R142G**, e.g. A/Samara/73/2013

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, e.g. A/Plzen/22/2013);

Group 6 viruses:

- As group 5 plus **S199A** (e.g. A/Iowa/19/2010).

Note: In Figure 2, the G186V substitutions in HA1 occur during adaptation of H3N2 viruses to passage in hens' eggs and are not group-specific.

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

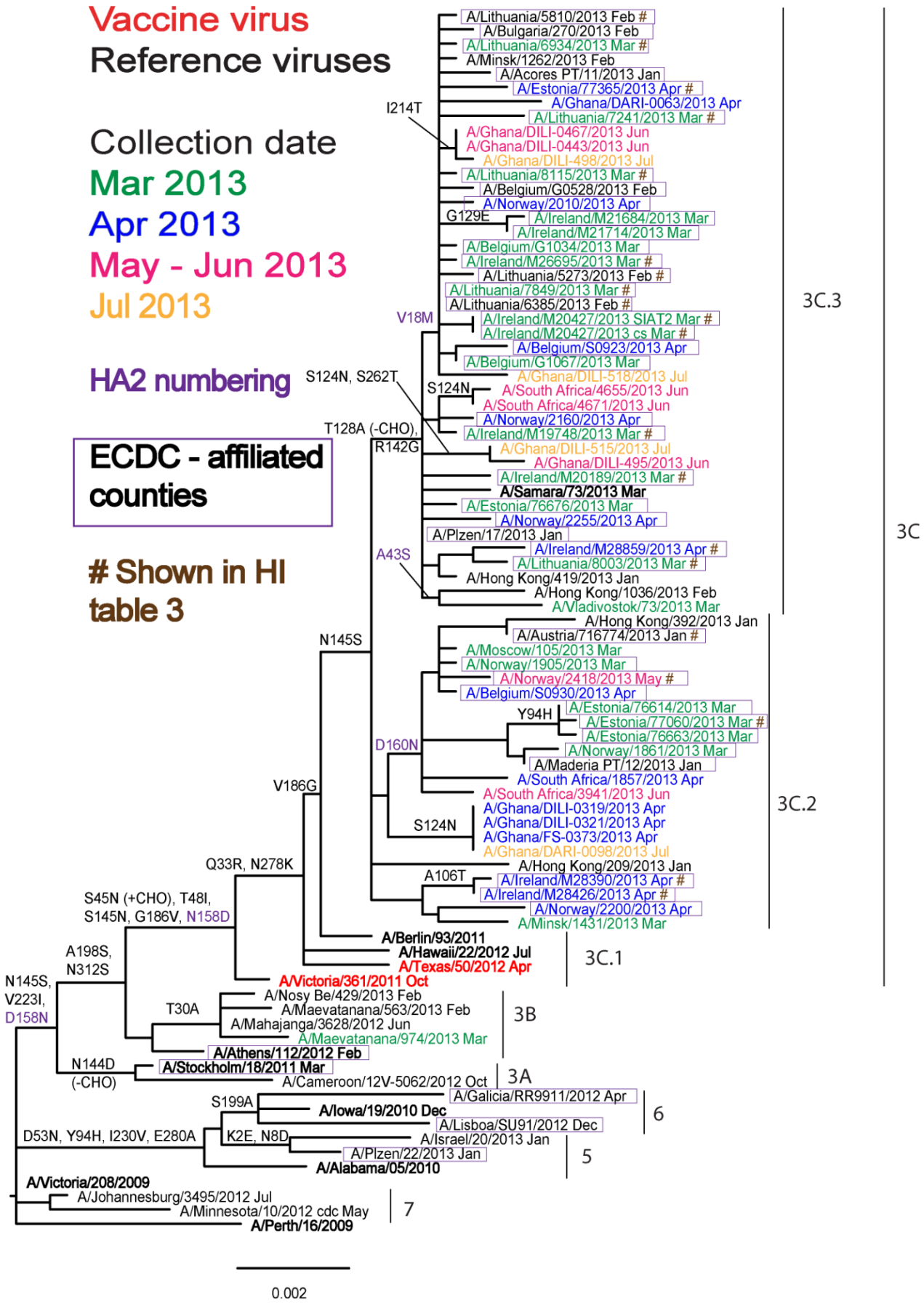
Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20 nM oseltamivir)

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1]									
				Post infection ferret antisera									
				A/Perth 16/09 F35/11	A/Ala 5/10 F27/10 group 5	A/Stock 18/11 F28/11 group 3A	A/Iowa 19/10 F15/11 group 6	A/Vic 361/11 Egg F35/12 group 3C.1	A/Berlin 93/11 T/C F17/12 group 3C.1	A/Vic 361/11 T/C F34/12 group 3C.1	A/Athens 112/12 F16/12 group 3B	A/Texas 50/12 Egg F36/12 group 3C.1	A/Hawaii 22/12 F37/12 group 3C.1
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E2	640	160	160	160	160	320	320	320	320	80
A/Victoria/208/2009		2009-06-02	E3/E2	640	1280	1280	2560	2560	2560	1280	2560	5120	2560
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	40	80	160	160	80	320	160	320	160	80
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	40	80	160	80	80	320	160	320	320	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	640	1280	1280	640	1280	1280	1280	2560	1280
A/Victoria/361/2011	3C.1	2011-10-24	E3/E2	320	640	320	640	2560	1280	640	320	5120	1280
A/Berlin/93/2011	3C.1	2011-12-07	NVD3/SIAT6	160	160	320	320	160	640	640	640	1280	320
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT4	80	160	320	160	160	640	640	640	640	320
A/Athens/112/2012	3B	2012-02-01	SIAT6	80	80	160	80	160	640	320	640	640	320
A/Texas/50/2012	3C.1	2012-04-15	E5/E1	320	640	1280	1280	1280	1280	640	1280	2560	1280
A/Hawaii/22/2012	3C.1	2012-07-09	E4/E1	320	640	640	1280	640	1280	640	1280	5120	5120
TEST VIRUSES													
A/Austria/716774/2013	3C.2	2013-01-25	SIAT1/SIAT1	40	80	160	80	80	640	320	640	640	640
A/Lithuania/5271/2013		2013-02-05	SIAT2	40	160	320	320	160	640	640	640	1280	640
A/Lithuania/5273/2013	3C.3	2013-02-05	SIAT2	<	40	160	160	80	320	320	320	640	320
A/Lithuania/5274/2013		2013-02-05	SIAT2	40	160	640	320	160	1280	1280	1280	1280	1280
A/Lithuania/5810/2013	3C.3	2013-02-19	SIAT2	40	160	320	160	160	640	640	640	1280	640
A/Lithuania/6105/2013		2013-02-24	SIAT2	40	160	320	160	160	1280	1280	1280	1280	640
A/Lithuania/6376/2013		2013-02-26	SIAT2	80	160	320	160	320	1280	1280	1280	1280	640
A/Lithuania/6385/2013	3C.3	2013-02-27	SIAT2	40	160	320	160	160	640	640	640	1280	640
A/Ireland/M19748/2013	3C.3	2013-03-04	SIAT2	40	160	320	160	160	640	320	1280	640	640
A/Lithuania/6934/2013	3C.3	2013-03-04	SIAT3	40	80	320	80	160	640	320	640	640	640
A/Ireland/M20427/2013	3C.3	2013-03-05	SIAT3	<	80	160	80	80	640	320	640	640	640
A/Ireland/M20189/2013	3C.3	2013-03-05	SIAT3	40	80	160	80	80	640	320	640	640	640
A/Lithuania/7241/2013	3C.3	2013-03-06	SIAT2	40	80	320	160	160	640	640	640	640	640
A/Lithuania/7849/2013	3C.3	2013-03-08	SIAT2	40	80	320	160	160	640	640	640	640	640
A/Lithuania/8003/2013	3C.3	2013-03-12	SIAT3	<	40	160	80	80	320	320	320	320	640
A/Lithuania/8115/2013	3C.3	2013-03-16	SIAT2	80	160	320	160	320	640	640	640	1280	1280
A/Estonia/76844/2013		2013-03-18	MDCK2/SIAT1	80	160	320	160	160	1280	640	640	1280	640
A/Austria/726749/2013		2013-03-21	SIAT1/SIAT1	40	80	160	80	80	640	320	640	640	640
A/Estonia/77020/2013		2013-03-25	MDCK2/SIAT1	40	80	320	80	160	640	320	640	640	320
A/Estonia/77060/2013	3C.2	2013-03-25	MDCK2/SIAT1	80	160	320	160	160	640	320	1280	640	320
A/Ireland/M26695/2013	3C.3	2013-03-26	SIAT3	40	40	160	80	40	320	320	320	320	320
A/Ireland/M28426/2013	3C.2	2013-04-02	SIAT3	<	40	80	40	40	320	160	320	320	320
A/Ireland/M28390/2013	3C.2	2013-04-02	SIAT3	<	40	80	40	40	320	160	320	320	320
A/Ireland/M28859/2013	3C.3	2013-04-03	SIAT2	40	160	320	160	160	640	320	640	640	640
A/Estonia/77365/2013	3C.3	2013-04-05	MDCK2/SIAT1	<	80	160	80	80	320	320	320	320	320
A/Norway/2418/2013	3C.2	2013-05-03	SIAT2	<	40	160	80	80	320	80	320	320	320
A/Norway/2514/2013		2013-05-03	SIAT2	40	80	160	160	160	320	320	320	640	320
								Vaccine 2012-13				Vaccine 2013-14	

1. < = <40

Sequences in phylogenetic tree (Figure 2)

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



Influenza B virus analyses

B/Victoria-lineage viruses

Table 4 shows the results of antigenic analyses for viruses of the B/Victoria-lineage performed since the July report [1]. The three test viruses were isolated and propagated in MDCK cells or a derivative thereof (SIAT-1) and gave ≥ eightfold reductions in HI titres, compared to the titres with the homologous viruses, 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 and southern hemisphere 2014 influenza seasons. 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 the test viruses sequenced retained the glycosylation site (NET). All test viruses gave titres close to the homologous titres, with antisera raised against cell-grown reference viruses B/Paris/1762/2008, B/Hong Kong/514/2009 and B/Odessa/3886/2010, which are genetically closely related to egg-propagataed B/Brisbane/60/2008. These three reference viruses are considered as surrogate cell-propagated antigens representing the egg-derived B/Brisbane/60/2008 prototype strain. Reactivity was somewhat reduced, with antiserum raised against cell-grown 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 4. Antigenic analysis of influenza B viruses (Victoria-lineage) by HI

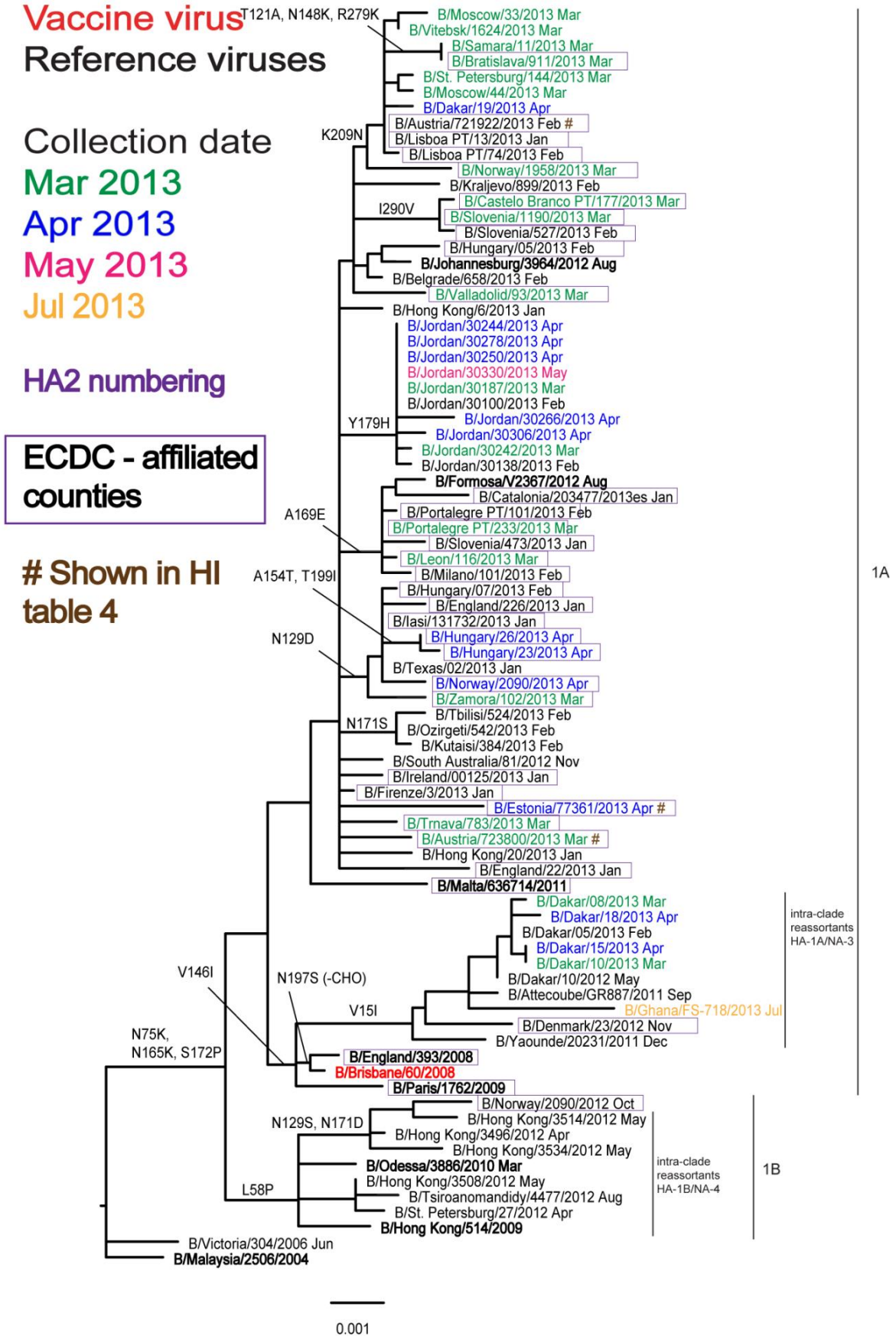
				Haemagglutination inhibition titre									
				Post infection ferret sera[1]									
Viruses		Collection date	Passage History	B/Bris[2] 60/08 Sh 522	B/Mal 2506/05 F37/11	B/Eng 393/08 F05/11	B/Bris 60/08 F22/12	B/Paris 1762/08 F07/11	B/HK 514/09 F13/10	B/Odessa 3886/10 F19/11	B/Malta 636714/11 F33/11	B/Jhb 3964/12 F01/13	B/For 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	1280	40	320	320	40	40	80	320	320	320
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	80	320	320	40	40	80	320	320	320
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	<	<	40	40	40	80	40	<	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	<	<	40	80	80	160	20	<	40
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	1280	20	80	80	40	40	80	80	80	160
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	160	320	40	40	80	320	320	160
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	2560	160	320	640	80	80	80	640	1280	640
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	1280	40	80	160	40	40	40	160	160	320
TEST VIRUSES													
B/Austria/721922/2013	1A	2013-02-26	SIAT1/MDCK1	2560	20	<	10	40	40	40	<	<	20
B/Austria/723800/2013	1A	2013-03-07	SIAT1/MDCK1	2560	20	<	20	40	40	80	10	<	20
B/Estonia/77361/2013	1A	2013-04-05	MDCK2/MDCK1	5120	40	40	40	80	80	160	40	80	80
							Vaccine*						

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

Sequences in phylogenetic tree (Figure 3)

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

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



B/Yamagata-lineage viruses

Tables 5 and 6 show the results of HI analyses of B/Yamagata lineage viruses tested since the July report [1]. The genetic clade into which sequenced HA genes of test viruses fall is indicated.

Approximately 90% (43/47) of the 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 2013–2014 and southern hemisphere 2014 influenza seasons [6], B/Massachusetts/02/2012 (clade 2). Antiserum raised against egg-propagated B/Wisconsin/1/2010, the clade 3 virus used in the vaccine for 2012-2013, showed reactivity within fourfold of the titre against the homologous virus for only ~30% (14/47) of these viruses. Many, but not all, viruses carrying clade 2 HA genes, represented by B/Massachusetts/02/2012, could be differentiated antigenically from those carrying clade 3 HA genes, represented by B/Wisconsin/1/2010, by certain post-infection ferret antisera. However, not all antisera were able to antigenically differentiate viruses falling in the alternate clades.

Twenty of the test viruses had been genetically characterised at the time of preparation of this report, with 15 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 of HA genes falling into clade 3.

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

				Haemagglution Inhibition Titre										
				Post infection ferret sera										
Viruses		Collection date	Passage History	B/Fl[3] 4/06 SH479	B/Fl[1] 4/06 F1/10	B/Bris[2] 3/07 F21/12	B/Wis[2] 1/10 F24/12	B/Stock[2] 12/11 F34/11	B/Estonia[2] 55669/11 F26/11	B/Novo[2] 1/12 F31/12	B/HK[2] 3577/12 F33/12	B/Mass[2] 2/12 Egg	B/Mass[2] 2/12 F2/13 T/C	B/Mass[2] 2/12 F3/13
	Genetic Group			1	1	2	3	3	2	3	2		2	2
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	5120	1280	1280	640	640	320	80	640	1280		160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	640	640	320	640	160	40	320	1280		160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	5120	1280	640	640	640	40	160	160	640		80
B/Stockholm/12/2011	3	2007-08-07	E4/E1	2560	320	160	160	640	10	40	40	320		20
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	160	160	80	320	640	80	640	320		320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	640	160	80	160	320	40	160	160	160		40
B/Hong Kong/3577/2012	2	2012-06-13	MDCK3	1280	160	160	80	320	640	40	640	320		160
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	2560	640	1280	320	640	160	40	320	640		80
B/Massachusetts/02/2012	2	2012-03-13	IDCK1/C2/MDCK4	5120	640	640	320	640	640	160	640	640		640
TEST VIRUSES														
B/Poland/7/2013		2013-02-04	MDCKx/MDCK1	1280	160	160	80	320	640	40	320	160		160
B/Poland/8/2013	2	2013-02-11	MDCKx/MDCK1	1280	160	80	80	160	640	80	640	80		320
B/Poland/10/2013		2013-02-14	MDCKx/MDCK1	1280	160	80	40	160	320	80	320	160		320
B/Poland/9/2013		2013-03-04	MDCKx/MDCK1	1280	80	80	40	160	640	40	320	160		160
B/Estonia/76502/2013		2013-03-08	MDCK1/MDCK1	1280	160	160	160	320	40	80	80	160		40
B/Estonia/76615/2013	3	2013-03-11	MDCK1/MDCK1	1280	160	160	160	320	40	80	80	160		40
B/Poland/6/2013	2	2013-03-12	MDCKx/MDCK1	1280	160	80	80	320	80	160	160	160		80
B/Estonia/77011/2013		2013-03-22	MDCK2/MDCK1	1280	160	80	80	160	320	40	320	160		160
B/Norway/1946/2013	2	2013-03-23	MDCK1/MDCK2	2560	160	160	80	320	640	80	640	320		320
B/Poland/12/2013		2013-03-25	MDCKx/MDCK1	1280	160	80	80	160	640	160	320	160		640
B/Estonia/77112/2013	2	2013-03-27	MDCK2/SIAT1	2560	320	320	160	640	640	80	640	320		320
B/Estonia/77128/2013		2013-03-27	MDCK2/SIAT1	1280	80	80	40	80	160	40	160	80		160
B/Estonia/77368/2013		2013-04-08	MDCK1/MDCK1	1280	160	80	80	160	320	40	320	160		160
B/Estonia/77387/2013		2013-04-08	MDCK2/MDCK1	2560	160	160	80	320	320	40	320	160		160
B/Estonia/77391/2013	2	2013-04-08	MDCK1/MDCK1	1280	160	80	80	320	320	40	320	160		160
B/Estonia/77427/2013	2	2013-04-08	MDCK2/MDCK1	5120	320	160	160	320	1280	320	640	320		640
B/Estonia/77447/2013	2	2013-04-09	MDCK1/MDCK1	1280	160	160	80	320	320	40	320	160		160
B/Norway/2162/2013		2013-04-10	MDCK1/MDCK1	1280	160	160	160	320	160	80	80	160		40
B/Estonia/77471/2013		2013-04-10	MDCK2/MDCK1	1280	160	160	80	320	320	40	320	160		160
B/Estonia/77557/2013		2013-04-12	MDCK1/MDCK1	1280	160	160	80	320	320	40	320	160		160
B/Norway/2531/2013	3	2013-04-17	MDCK1/MDCK1	1280	160	80	80	640	40	80	80	160		40
B/Estonia/77676/2013		2013-04-18	MDCK2/MDCK1	2560	160	160	80	320	320	40	320	160		160
B/Norway/2254/2013		2013-04-21	MDCK1/MDCK1	5120	320	320	320	640	320	640	640	320		640
B/Norway/2277/2013		2013-04-28	MDCK1/MDCK1	5120	320	320	160	320	640	160	640	320		640
B/Norway/2281/2013	2	2013-04-29	MDCK1/MDCK1	1280	160	160	80	320	320	40	320	160		160
							Vaccine 2012-13					Vaccine 2013-14		

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

Sequences in phylogenetic tree (Figure 4)

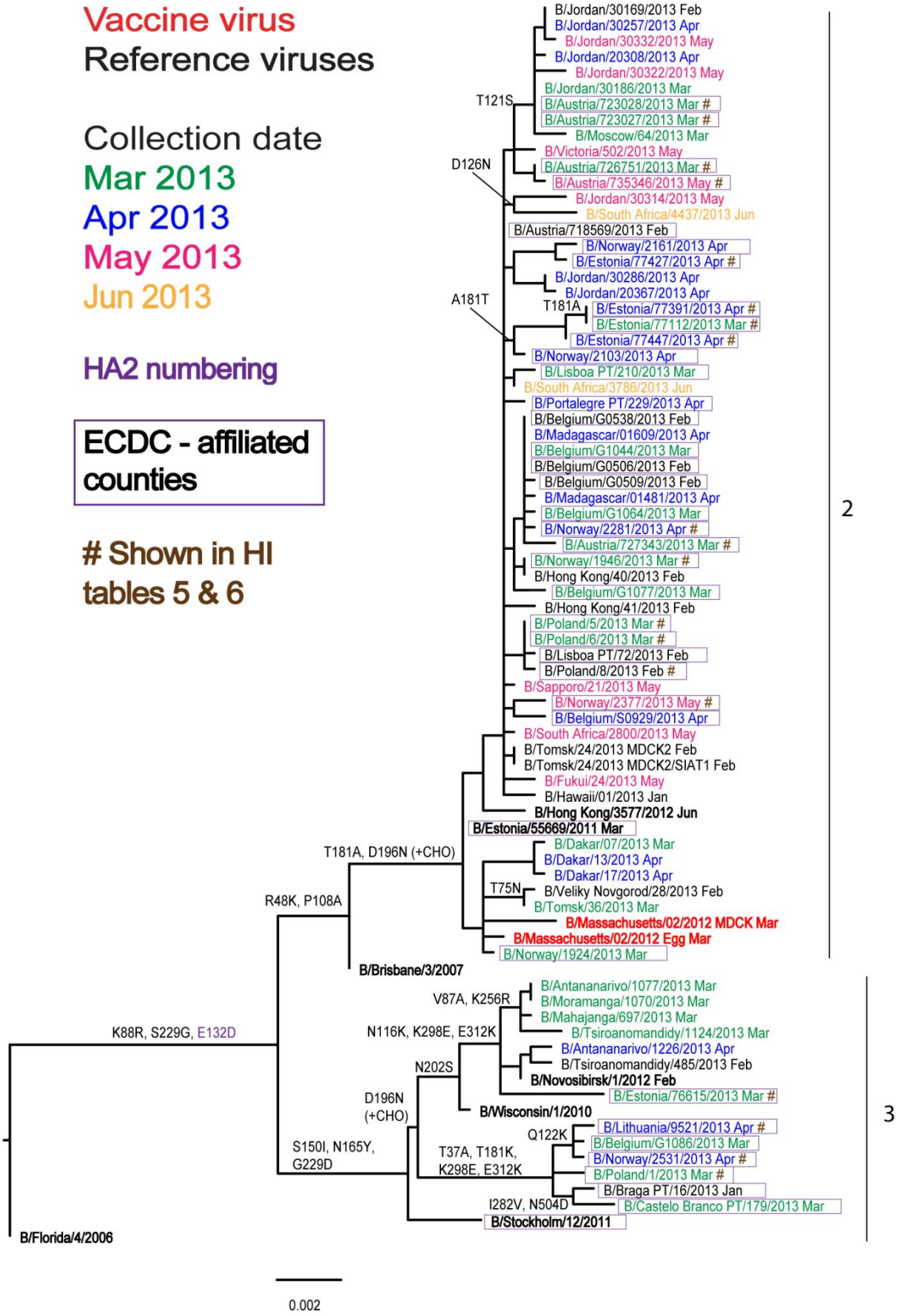
Table 6. Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

				Haemagglution Inhibition Titre									
				Post infection ferret sera									
Viruses		Collection date	Passage History	B/Fl[3] 4/06 SH479	B/Fl[1] 4/06 F1/10	B/Bris[2] 3/07 F21/12	B/Wis[2] 1/10 F24/12	B/Stock[2] 12/11 F12/12	B/Estonia[2] 55669/11 F26/11	B/Novo[2] 1/12 F31/12	B/HK[2] 3577/12 F33/12	B/Mass[2] 2/12 Egg F2/13	B/Mass[2] 2/12 T/C F3/13
	Genetic Group			1	1	2	3	3	2	3	2	2	2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E7/E1	5120	1280	1280	640	640	320	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	1280	1280	320	640	160	80	320	1280	80
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	5120	640	320	640	640	40	160	80	640	80
B/Stockholm/12/2011	3	2007-08-07	E4/E1	1280	320	160	160	320	10	40	40	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	80	80	40	160	640	40	320	160	160
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	640	80	80	160	320	80	160	80	160	40
B/Hong Kong/3577/2012	2	2012-06-13	MDCK3	1280	80	80	80	160	640	40	320	160	160
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/MDCK2	1280	320	320	80	320	320	40	320	640	160
TEST VIRUSES													
B/Poland/4/2013		2013-01-28	MDCKx/MDCK1	2560	320	80	80	320	640	40	640	320	160
B/Lithuania/3693/2013		2013-01-31	MDCK2/MDCK1	2560	160	160	80	320	640	160	640	160	320
B/Austria/717998/2013		2013-02-01	SIAT1/MDCK1	2560	160	80	80	320	320	80	320	160	160
B/Lithuania/3726/2013		2013-02-01	MDCK2/MDCK1	1280	160	80	80	320	640	80	640	160	160
B/Poland/2/2013		2013-02-20	MDCKx/MDCK1	2560	320	80	160	320	640	320	640	320	320
B/Lithuania/6530/2013		2013-02-25	MDCK3	1280	80	40	40	320	20	40	40	<	20
B/Lithuania/6453/2013		2013-02-28	MDCK2	2560	320	160	80	320	640	80	1280	160	320
B/Austria/723028/2013	2	2013-03-04	SIAT1/MDCK1	2560	80	80	80	160	320	40	320	160	160
B/Austria/723027/2013	2	2013-03-04	SIAT1/MDCK2	2560	160	80	80	320	320	40	320	160	160
B/Poland/1/2013	3	2013-03-04	MDCKx/MDCK1	2560	160	80	160	320	160	320	320	160	320
B/Poland/3/2013		2013-03-04	MDCKx/MDCK1	2560	160	80	80	320	640	80	640	160	160
B/Poland/5/2013	2	2013-03-04	MDCKx/MDCK1	1280	80	80	80	160	640	40	320	160	160
B/Lithuania/6935/2013		2013-03-04	MDCK3	1280	160	160	80	320	640	40	640	40	160
B/Lithuania/6942/2013		2013-03-04	MDCK2	5120	320	320	320	1280	160	160	160	160	80
B/Lithuania/6931/2013		2013-03-05	MDCK2	2560	320	160	160	320	320	80	640	160	160
B/Austria/726751/2013	2	2013-03-21	SIAT1/MDCK1	1280	160	160	40	320	640	40	640	320	160
B/Austria/727096/2013		2013-03-26	SIAT2/MDCK1	2560	160	160	40	160	320	160	320	160	320
B/Austria/727343/2013	2	2013-03-27	SIAT1/MDCK1	2560	320	320	160	320	320	40	320	320	320
B/Lithuania/9556/2013	3	2013-03-28	MDCK2/MDCK1	2560	160	160	160	320	320	320	640	160	640
B/Lithuania/9521/2013	3	2013-04-02	MDCK2/MDCK1	1280	160	160	160	320	320	320	320	160	320
B/Austria/735346/2013	2	2013-05-13	SIAT1/MDCK1	2560	160	160	80	320	320	80	320	160	320
B/Norway/2377/2013	2	2013-05-21	MDCK2	1280	160	80	80	160	320	40	320	160	160
							Vaccine 2012-13 2013					Vaccine 2013-14	

1. < = <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 [11] on 3 April 2013, and an updated risk assessment has been posted by WHO [12]. As of 11 August 2013, WHO reported [13] 135 laboratory-confirmed cases and 44 associated fatalities (case fatality ratio [CFR] of 33%).

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 18–20 February 2013 and 23–25 September 2013, can be found at: http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February 2013.pdf [14] and http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Sep2013final.pdf [15]

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.

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