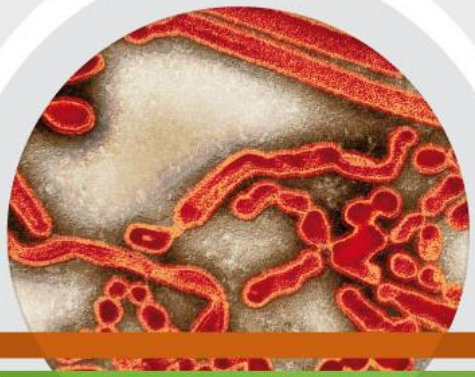


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, April 2013

Summary

During the 2012–13 season, A(H1N1)pdm09, A(H3N2) and B/Victoria- and B/Yamagata-lineage influenza viruses have been detected in ECDC-affiliated countries. The relative prevalence varied between countries.

- Type A and type B viruses have continued to co-circulate in similar proportions.
- A(H1N1)pdm09 viruses have been detected at comparable levels to A(H3N2) viruses.
- A(H1N1)pdm09 viruses continued to show genetic drift from the vaccine virus, A/California/07/2009, but the vast majority remained antigenically similar to it.
- The vast majority of A(H3N2) viruses have been antigenically and genetically similar to cell-propagated A/Victoria/361/2011, the prototype vaccine virus for the 2012–13 influenza season.
- Viruses of the B/Yamagata lineage predominated over those of the B/Victoria lineage.
- B/Victoria lineage viruses were antigenically similar to cell-propagated reference viruses of the B/Brisbane/60/2008 genetic clade.
- Recent B/Yamagata-lineage viruses fell into two antigenically distinguishable genetic clades: clade 2, represented by B/Estonia/55669/2012, and clade 3, represented by B/Wisconsin/1/2010 (the recommended vaccine component for the 2012–13 influenza season).

Viruses collected between 1 December 2012 and 15 April 2013, spanning the prolonged 2012–13 season, have been received from 22 countries in the EU/EEA region by 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 proportions of influenza type A (62%) and type B (38%) viruses received were similar. For type A, H1N1pdm09 viruses have been received in slightly 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 approximately 4:1.

This report was prepared by Rod Daniels, Vicki Gregory and John McCauley on behalf of the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL), 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 December 2012

MONTH / Country	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
			Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]
DECEMBER											
Austria	8		2	0	2	2		2	0	2	0
Belgium	41	1	13	11	4	3				23	12
Czech Republic	2		2	in process							
Denmark	2				1	1				1	1
France	33		7	7	15	15		6	6	5	5
Greece	1				1	0					
Ireland	12		1	1	3	1	2			6	4
Italy	15				3	3				12	12
Latvia	2		1	1						1	1
Luxembourg	3		1	1						2	1
Netherlands	3		1	1	2	2					
Norway	37		34	18	1	1				2	2
Portugal	9		1	0	1	1		1	1	6	6
Slovenia	5		3	3				1	0	1	0
Spain	14						1	7	7	6	6
United Kingdom	19		2	2	12	9		1	1	4	4
JANUARY											
Belgium	19	2	7	5	1	1				9	6
Czech Republic	11		9	in process	2	in process					
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	17		13	13	1	1		1	1	2	2
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	9		5	3	2	2				2	2
Romania	7		4	4				1	1	2	2
Slovenia	18		4	in process	5	in process	1	5	in process	3	in process
Spain	22		10	in process	6	6				6	6
United Kingdom	6				5	5		1	1		
FEBRUARY											
Bulgaria	22	1	7	7	2	2				12	12
Czech Republic	4									4	4
Greece	2		1	1	1	1					
Luxembourg	5		1	0	4	0					
Slovenia	16	3	5	in process	3	in process		1	in process	4	in process
Spain	10		9	in process	1	in process					
United Kingdom	8		3	3	2	2		1	1	2	2
MARCH											
Bulgaria	2		1	1						1	1
Czech Republic	1									1	in process
Luxembourg	1									1	1
Slovenia	3							2	in process	1	in process
Spain	12		3	in process	4	in process		3	in process	2	in process
APRIL											
Slovenia	2		1	in process						1	in process
Spain	3		1	in process	1	in process				1	in process
	510	10	204	111	103	73	9	36	22	148	114
				40.0%		20.2%			7.1%		29.0%
22 Countries			317 (62%)					193 (38%)			

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

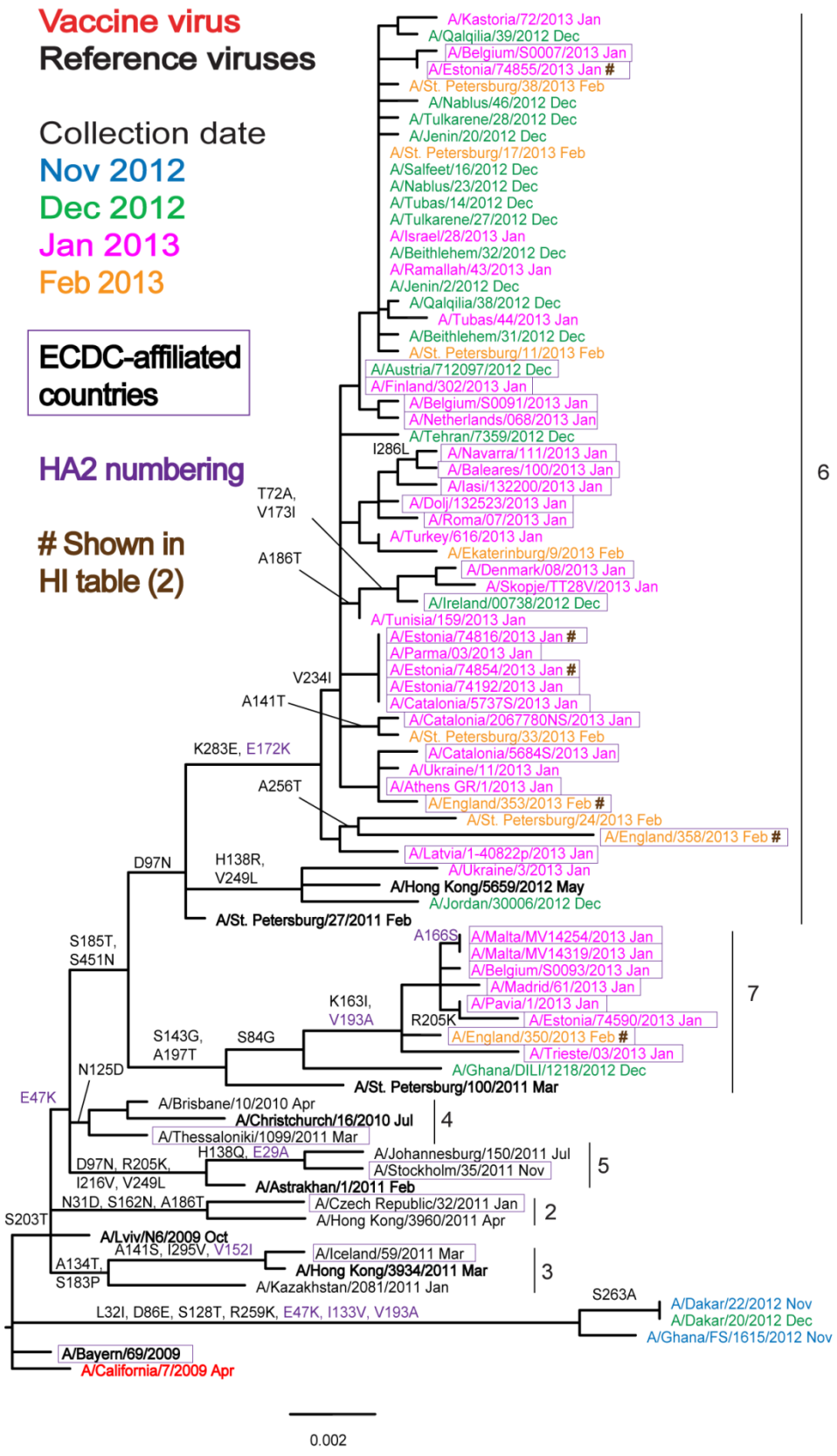
The results of HI assays carried out on influenza A(H1N1)pdm09 viruses since the March report are shown in Table 2. The test viruses showed good reactivity with post-infection ferret antisera raised against the panel of reference viruses, including antiserum raised against the vaccine virus, A/California/7/2009. 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 – approximately 40% (10/24) of the titres with test viruses were reduced eightfold or more compared to the titre with the homologous virus. A single virus, A/Florina/60/2013, showed a 16-fold reduction in titre compared to the homologous titre with antiserum raised against A/Lviv/N6/2009; HA and NA gene sequencing of this virus is pending.

Phylogenetic analysis of the HA gene 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. Where known, the genetic group of a virus is indicated in Table 2.

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

				Haemagglutination inhibition titre								
				Post infection ferret sera								
Viruses	Genetic group	Collection date	Passage History	A/Cal 7/09 F29/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/34/09	A/Chch 16/10 F30/10 4	A/HK 3934/11 F21/11 3	A/Astrak 1/11 F22/11 5	A/St. P 27/11 F23/11 6	A/St. P 100/11 F24/11 7	A/HK 5659/12 F30/12 6
REFERENCE VIRUSES												
A/California/7/2009		2009-04-09	E1/E2	640	640	640	320	320	320	640	320	320
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	320	160	80	40	80	80	80	80
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	640	320	160	160	320	160	320
A/Christchurch/16/2010	4	2010-07-12	E2/E2	2560	1280	2560	5120	2560	2560	2560	5120	5120
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	160	640	320	1280	640	640	1280	1280
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	320	640	640	1280	1280	1280	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	2560	1280	640	1280	2560	2560	2560	5120	5120
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	2560	640	2560	1280	2560	2560	2560	5120	5120
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	640	160	320	640	1280	640	640	1280	1280
TEST VIRUSES												
A/Luxembourg/628/2012		2012-12-13	MDCK1/SIAT2	640	160	640	640	1280	640	1280	2560	1280
A/Luxembourg/13/2013		2013-01-04	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	2560	2560
A/Luxembourg/18/2013		2013-01-07	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	5120	2560
A/Luxembourg/34/2013		2013-01-08	MDCK1/MDCK1	1280	640	640	1280	2560	1280	1280	5120	2560
A/Luxembourg/62/2013		2013-01-11	MDCK1/MDCK2	320	160	320	320	640	640	640	640	640
A/Luxembourg/80/2013		2013-01-16	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	5120	2560
A/Luxembourg/119/2013		2013-01-21	MDCK1/MDCK2	640	640	640	640	1280	1280	1280	2560	2560
A/Estonia/74816/2013	6	2013-01-25	MDCK3	5120	2560	5120	2560	5120	5120	5120	5120	5120
A/Luxembourg/186/2013		2013-01-25	MDCK1/SIAT2	320	320	640	320	640	640	640	1280	1280
A/Estonia/74854/2013	6	2013-01-28	MDCK2	1280	640	1280	1280	2560	1280	2560	5120	2560
A/Estonia/74855/2013	6	2013-01-28	MDCK2	2560	640	1280	1280	2560	2560	2560	5120	5120
A/England/350/2013	7	2013-02-06	SIAT1/MDCK1	640	640	640	640	640	640	640	1280	1280
A/Bulgaria/260/2013		2013-02-08	C1/MDCK1	640	320	640	1280	1280	1280	1280	2560	2560
A/Bulgaria/277/2013		2013-02-08	C1/MDCK1	640	640	640	1280	1280	1280	640	2560	1280
A/England/353/2013	6	2013-02-11	MDCK1/MDCK2	640	320	1280	640	1280	1280	1280	2560	2560
A/Florina/60/2013		2013-02-13	E2/E1	640	640	40	640	640	640	640	1280	1280
A/Bulgaria/364/2013		2013-02-14	C1/MDCK1	1280	320	1280	1280	2560	2560	1280	2560	5120
A/Bulgaria/421/2013		2013-02-14	C1/MDCK1	1280	640	1280	1280	2560	2560	2560	2560	5120
A/Thessaloniki/75/2013		2013-02-18	E2/E1	640	640	640	640	1280	1280	1280	2560	1280
A/England/358/2013	6	2013-02-18	SIAT1/MDCK1	1280	640	1280	1280	2560	2560	1280	5120	5120
A/Bulgaria/477/2013		2013-02-23	C2/MDCK1	640	320	640	640	640	1280	640	2560	2560
A/Bulgaria/492/2013		2013-02-23	C2/MDCK1	1280	640	1280	1280	2560	2560	1280	2560	2560
A/Bulgaria/494/2013		2013-02-23	C2/MDCK1	1280	320	640	640	1280	1280	1280	2560	2560
A/Bulgaria/611/2013		2013-03-04	C2/MDCK1	1280	640	1280	1280	2560	1280	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 have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells from guinea pigs, turkeys and humans as described before. Antigenic analyses of recently collected viruses, conducted since the March report, are shown in Table 3. HI assays are 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). The test viruses reacted poorly with post-infection ferret antiserum raised against the egg-propagated vaccine virus, 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/previous vaccine viruses propagated in eggs (A/Perth/16/2009, A/Victoria/208/2009, A/Iowa/19/2010 and A/Hawaii/22/2012). However, over the season many viruses reacted better with antiserum raised against egg-propagated A/Texas/50/2012 (vaccine recommendation for the northern hemisphere 2013–14), compared with the titre of the antiserum with the homologous virus. A/Texas/50/2012 is like cell-propagated A/Victoria/361/2011 but egg propagation of A/Texas/50/2012 did not select for an HA1 H156Q/R substitution, which can alter antigenic characteristics, as occurred in egg-propagated A/Victoria/361/2011.

The test viruses reacted well with antisera raised against reference viruses exclusively propagated in cells when compared to the titres with the homologous viruses. These antisera were raised against virus isolates propagated exclusively in MDCK cells, or a derivative thereof (SIAT-1), for 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 December 2012 have HA genes that fall predominantly into genetic groups 3C, with some in group 5. Occasionally, viruses falling into group 3A (e.g. A/Stockholm/39/2012) and 3B (e.g. A/Belgium/G0044/2013) have been isolated; see February and March reports.

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**, **D158N**.
- Group 5 viruses: **D53N**, **Y94H**, **I230V** and **E280A** (e.g. A/Alabama/05/2010), often in combination with **K2E**, **N8D** (resulting in the loss of a potential glycosylation site) and **S124N**.

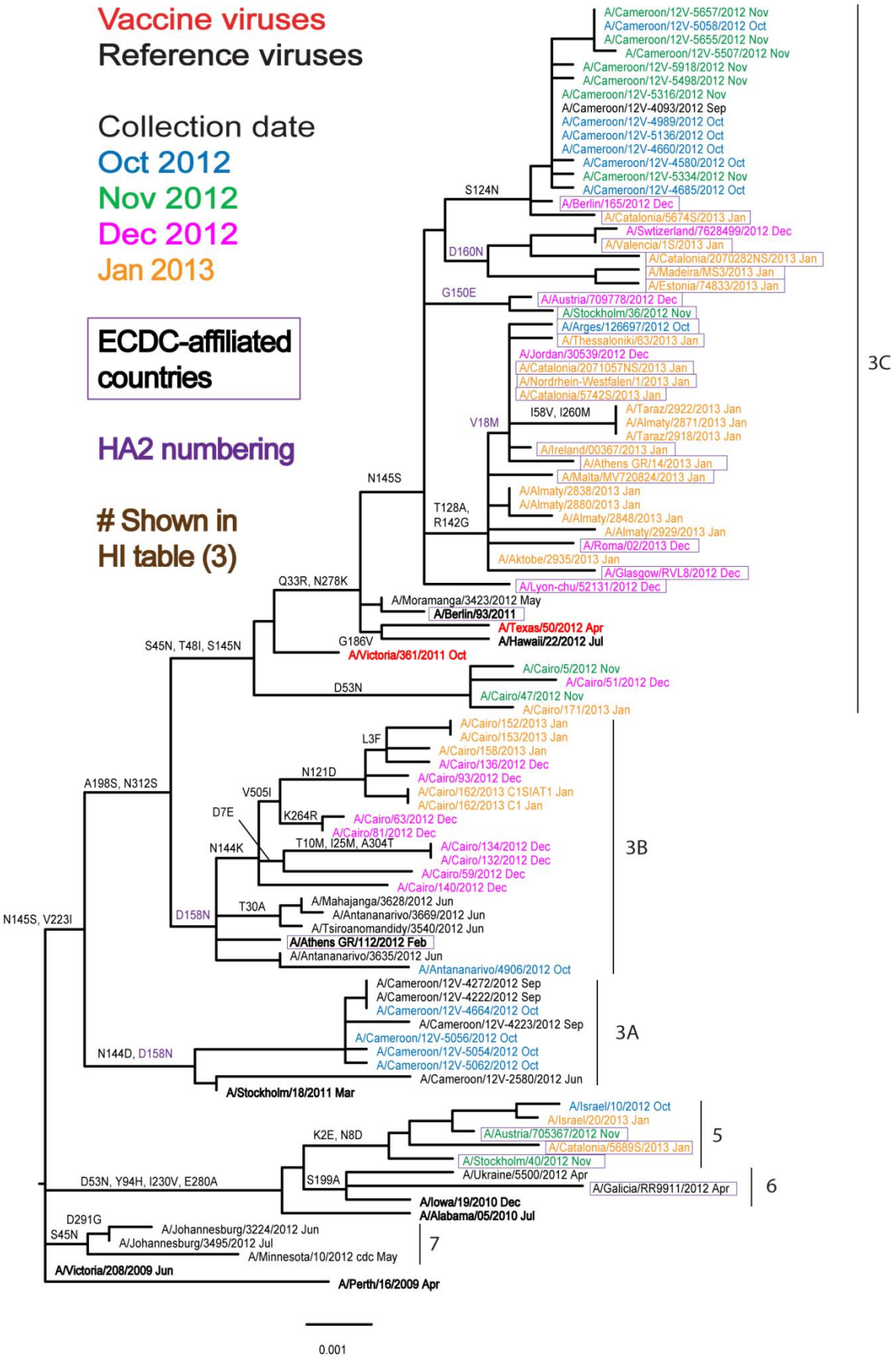
There is no evidence for antigenic change associated with any of the genetic groups or emerging subgroups, including the emerging subgroup in group 3C that carries substitutions in HA1 at amino acid residues 128 and 142.

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

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1] — Post infection ferret sera										
				A/Perth 16/09	A/Vic 208/09	A/Ala 5/10	A/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 F11/12	T/C F34/12	F16/12	Egg F36/12	F37/12
	Genetic group					5	3A	6	3C	3C	3C	3B	3C	3C
REFERENCE VIRUSES														
A/Perth/16/2009		2009-07-04	E3/E2	640	40	160	160	160	160	320	320	320	320	160
A/Victoria/208/2009		2009-06-02	E3/E2	1280	2560	640	1280	2560	2560	2560	1280	2560	5120	2560
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	40	40	80	160	80	160	160	160	320	320	80
A/Stockholm/18/2011	3A	2011-03-28	SIAT4	80	160	80	320	160	320	640	320	640	640	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	640	320	1280	2560	640	2560	640	1280	2560	640
A/Victoria/361/2011	3C	2011-10-24	E3/E2	160	1280	160	160	640	1280	640	320	160	2560	640
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT6	80	80	80	160	160	160	640	320	640	1280	320
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT3	160	160	80	320	160	160	640	320	640	640	320
A/Athens/112/2012	3B	2012-02-01	SIAT8	80	160	160	320	160	160	640	320	640	640	320
A/Texas/50/2012	3C	2012-04-15	E5/E2	640	1280	320	1280	1280	640	2560	640	1280	5120	2560
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	320	640	320	640	1280	640	1280	640	1280	5120	2560
TEST VIRUSES														
A/England/119/2013		2013-01-09	MDCK2/SIAT1	40	80	80	160	80	160	640	160	160	640	320
A/England/104/2013		2013-01-09	MDCK2/SIAT1	40	40	80	160	40	160	640	640	640	640	320
A/Valencia/1S/2013	3C	2013-01-10	SIAT2	<	40	40	160	80	80	320	160	320	640	160
A/Catalonia/2070282NS/2013	3C	2013-01-22	SIAT2	<	80	40	160	80	80	320	320	320	640	160
A/Catalonia/5742S/2013	3C	2013-01-23	SIAT2	<	40	40	160	80	80	320	320	320	640	160
A/Catalonia/2071057NS/2013	3C	2013-01-24	SIAT2	<	40	40	160	80	80	320	160	320	640	160
A/England/188/2013		2013-01-29	SIAT1/SIAT1	40	40	40	320	40	160	640	640	640	640	640
A/England/256/2013		2013-01-30	MDCK1/SIAT1	40	40	40	160	40	160	1280	640	640	640	320
A/England/280/2013		2013-01-31	MDCK1/SIAT1	40	80	80	320	160	640	1280	640	640	1280	640
A/Bulgaria/270/2013		2013-02-04	C2/SIAT1	320	160	160	640	160	320	1280	640	1280	2560	640
A/Bulgaria/253/2013		2013-02-08	C2/SIAT1	40	80	80	320	80	160	640	320	640	640	320
A/England/279/2013		2013-02-09	SIAT1/SIAT1	40	40	40	160	40	160	640	640	640	640	320
A/England/308/2013		2013-02-15	SIAT1/SIAT1	<	40	40	160	40	80	640	320	320	320	320
Sequences in phylogenetic tree (Figure 2)									Vaccine 2012-13				Vaccine 2013-14	

< = <40

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



Influenza B virus analyses

B/Victoria-lineage virus

Table 4 shows the results of antigenic analyses of viruses performed since the March report for the B/Victoria lineage. Both test viruses showed low reactivity, compared with the titre against the homologous virus in HI assays, with post-infection 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 for the 2012–13 and 2013–14 northern hemisphere influenza seasons. The test viruses showed a similar reduction in reactivity with antisera raised against other egg-propagated reference viruses: B/England/393/2008, B/Malta/636714/2011 and B/Johannesburg/3964/2012. The test viruses reacted better with 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 and 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 discernible effect on antigenicity. The HAs of recent viruses show only a small number of amino acid substitutions compared with that of B/Brisbane/60/2008.

Table 4. Antigenic analysis of influenza B viruses (Victoria lineage) by HI

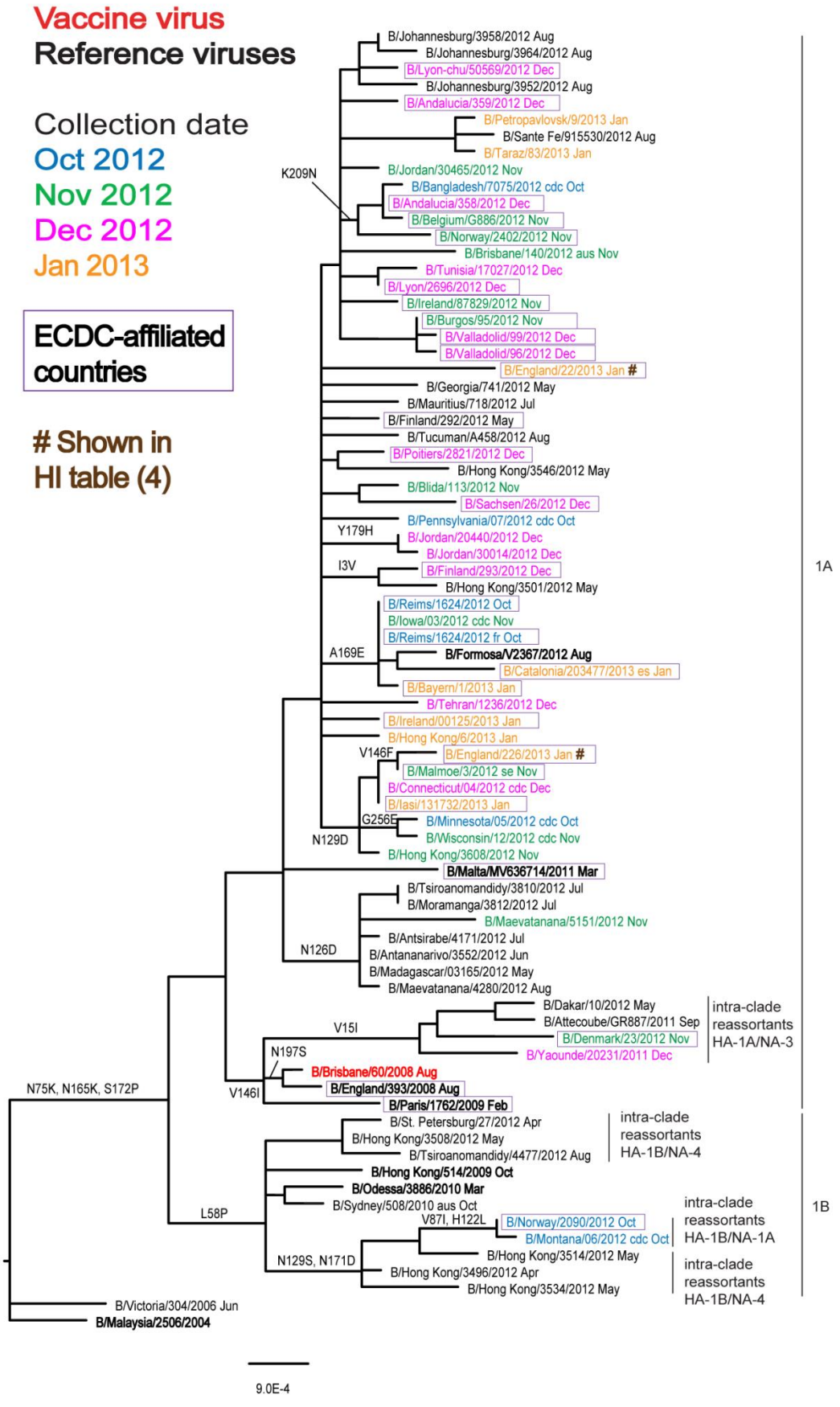
				Haemagglutination inhibition titre									
				Post infection ferret sera									
Viruses	Genetic group	Collection date	Passage History	B/Bris[2] 60/08 Sh 523	B/Mal[1] 2506/05 F37/11	B/Eng[1] 393/08 F05/11 1A	B/Bris[1] 60/08 F22/12 1A	B/Paris[1] 1762/08 F11/09 1A	B/HK[1] 514/09 F13/10 1B	B/Odessa[1] 3886/10 F19/11 1B	B/Malta[1] 636714/11 F33/11 1A	B/Jhb[1] 3964/12 F01/13 1A	B/For[1] V2367/12 F04/13 1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	320	40	80	<	<	<	80	160	80
B/England/393/2008	1A	2008-08-29	E1/E2	2560	80	320	320	40	20	40	160	320	160
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	80	320	320	40	20	40	160	320	160
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	20	20	20	40	20	40	20	40	40
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	<	10	20	40	40	80	10	40	40
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	2560	20	160	80	20	20	40	80	160	160
B/Malta/636714/2011	1A	2011-03-07	E4/E1	2560	80	640	320	40	40	40	320	320	320
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E1	2560	80	320	320	40	20	40	160	640	320
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	2560	20	80	160	20	20	20	80	160	160
TEST VIRUSES													
B/England/22/2013	1A	2013-01-02	MDCK1/MDCK1	5120	<	20	20	40	40	80	20	40	80
B/England/226/2013	1A	2013-02-01	MDCK1/MDCK1	5120	<	40	40	80	80	80	40	80	80

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

Sequences in phylogenetic tree (Figure 3) Vaccine*

* 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

Table 5 shows the results of HI analyses of B/Yamagata lineage viruses tested since the March report. The genetic clade into which sequenced HA genes of test viruses fall is indicated.

All test viruses (23) showed good reactivity (within fourfold of the homologous titre) with antisera raised against cell-propagated clade 2 viruses B/Estonia/55669/2011, B/Hong Kong/3577/2012 and B/Massachusetts/02/2012. Only three of the test viruses had been genetically characterised at the time of preparation of this report, with one falling into genetic group 3 and two into group 2. One of the test viruses, B/England/213/2013, showed low reactivity with most of the antisera, notably those raised against egg-propagated viruses; sequence analysis suggests this could be associated with a polymorphism of P240L/P in HA1. A similar reactivity profile was observed with four viruses from Luxembourg (isolates 83, 92, 101 and 177).

Antiserum raised against egg-propagated B/Wisconsin/1/2010 showed low reactivity to the majority of test viruses. In contrast, antisera raised against egg-grown B/Massachusetts/02/2012 reacted within fourfold of the titre against the homologous virus with all but the B/England/213/2013 and four Luxembourg viruses indicated above, hence the recommendation to use egg-grown B/Massachusetts/02/2012 in the vaccine for the northern hemisphere 2013–14 influenza season.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata lineage viruses. The phylogeny shows that the HA genes of recent viruses 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 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**.

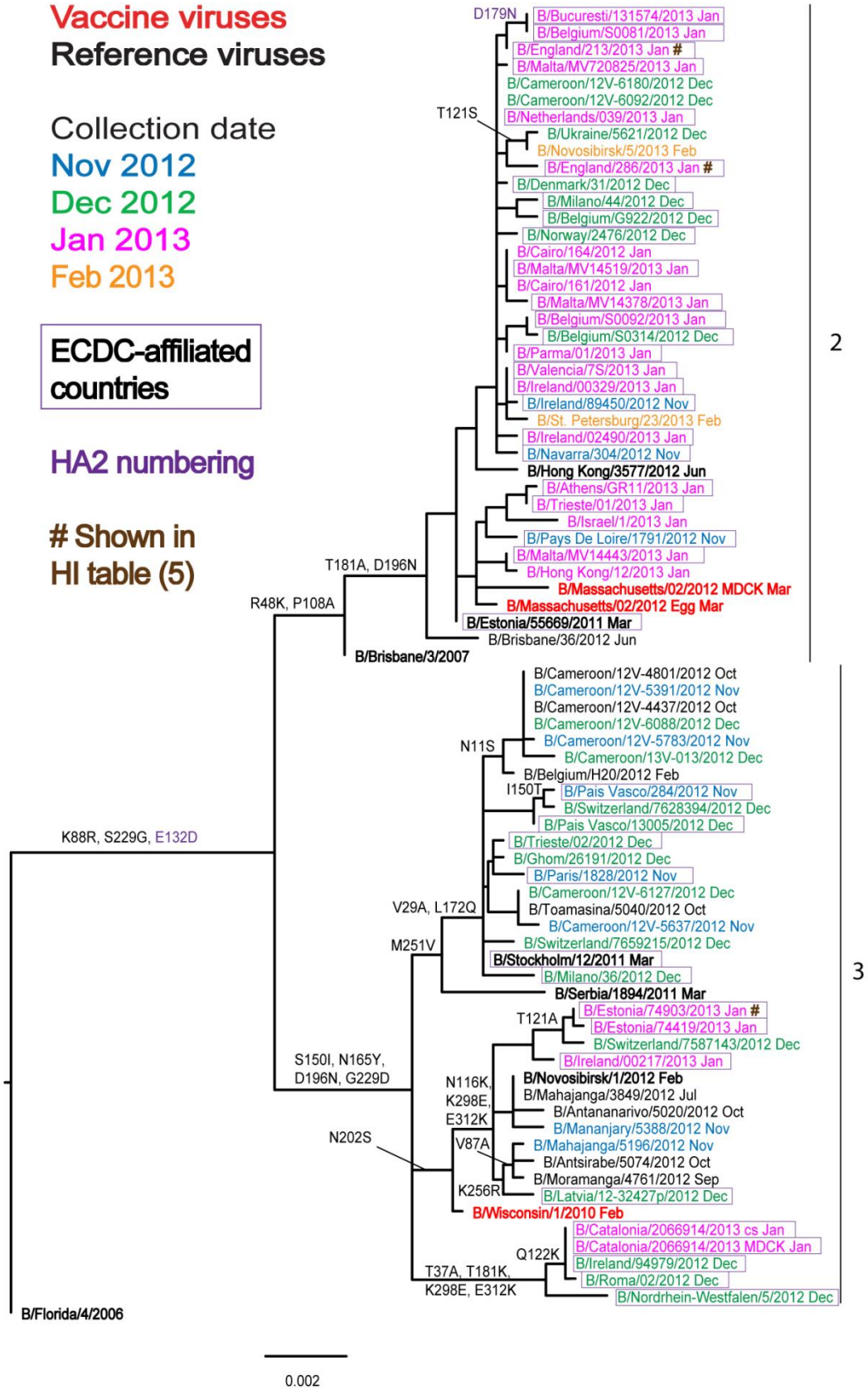
The proportion of viruses received with HA genes that fall into clade 2 has continued to increase over the number with HA genes falling into clade 3.

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

Viruses	Genetic group	Collection date	Passage History	Haemagglution Inhibition Titre									
				Post infection ferret sera									
				B/Fi[3] 4/06 SH479	B/Fi[1] 4/06 F1/10 1	B/Bris[2] 3/07 F21/12 2	B/Wis[2] 1/10 F26/10 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] 2/12 Egg F02/13 2	B/Mass[2] 2/12 T/C F03/13 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E3/E3	5120	1280	1280	320	640	320	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	1280	640	320	640	160	40	320	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	640	320	640	640	20	80	80	1280	80
B/Stockholm/12/2011	3	2007-08-07	E4/E2	1280	160	160	160	320	<	40	20	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	160	80	20	80	640	80	640	320	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	2560	160	80	80	160	160	320	320	320	320
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK1	2560	160	80	40	80	640	80	640	320	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E2	2560	640	640	160	640	80	40	320	1280	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK2	1280	160	80	40	80	320	20	320	320	160
TEST VIRUSES													
B/Luxembourg/650/2012		2012-12-18	MDCK2	5120	320	640	320	1280	2560	80	640	320	640
B/Luxembourg/83/2013		2013-01-16	MDCK1/MDCK1	1280	80	40	20	80	640	40	320	160	160
B/Luxembourg/92/2013		2013-01-17	MDCK1/MDCK1	1280	80	80	20	40	640	20	320	160	160
B/Luxembourg/101/2013		2013-01-17	MDCK1/MDCK1	1280	80	80	40	80	320	40	320	160	160
B/Luxembourg/177/2013		2013-01-24	MDCK1/MDCK1	640	80	80	20	40	320	20	320	160	160
B/Estonia/74786/2013		2013-01-24	MDCK3	2560	160	160	160	320	160	320	320	320	160
B/Estonia/74903/2013	3	2013-01-28	MDCK2	5120	320	160	160	320	160	320	320	320	160
B/England/286/2013	2	2013-02-03	MDCK1/MDCK1	5120	640	160	160	320	640	320	640	320	640
B/England/213/2013	2	2013-02-04	SIAT1/MDCK1	5120	160	80	20	80	640	40	640	160	160
B/Bulgaria/173/2013		2013-02-04	C1/MDCK1	2560	320	320	80	320	1280	80	1280	320	320
B/Bulgaria/178/2013		2013-02-04	C1/MDCK1	1280	320	320	80	320	1280	80	1280	640	640
B/Bulgaria/340/2013		2013-02-08	C1/MDCK1	2560	320	160	80	320	1280	160	1280	320	640
B/Bulgaria/327/2013		2013-02-11	C1/MDCK1	2560	320	160	160	320	160	320	320	320	160
B/Bulgaria/297/2013		2013-02-12	C1/MDCK1	1280	320	160	40	160	1280	40	1280	320	320
B/Bulgaria/298/2013		2013-02-12	C1/MDCK1	2560	320	160	40	160	1280	40	640	320	320
B/Bulgaria/351/2013		2013-02-12	C1/MDCK1	2560	320	320	80	320	1280	80	1280	320	640
B/Bulgaria/348/2013		2013-02-13	C1/MDCK1	2560	320	320	80	320	1280	80	1280	640	320
B/Bulgaria/337/2013		2013-02-14	C1/MDCK1	2560	640	320	160	640	1280	160	1280	640	1280
B/Bulgaria/365/2013		2013-02-14	C1/MDCK1	1280	160	160	80	160	1280	80	1280	320	640
B/Bulgaria/402/2013		2013-02-20	C1/MDCK1	1280	160	160	40	160	640	40	640	320	320
B/Bulgaria/505/2013		2013-02-23	C2/MDCK1	2560	320	160	80	160	1280	160	1280	320	640
B/Bulgaria/608/2013		2013-03-04	C1/MDCK1	2560	320	160	80	320	1280	160	1280	320	640
B/Luxembourg/724/2013		2013-03-07	MDCK2	2560	320	1280	320	1280	2560	320	640	320	1280
Sequences in phylogenetic tree (Figure 4)							Vaccine 2012-13					Vaccine 2013-14	

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

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 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. WHO is updating information on the outbreak regularly and ECDC is posting epidemiological updates. A Rapid Risk Assessment for these A(H7N9) viruses has been carried out and posted by ECDC on 3 April 2013, and a description of their characteristics can be found on the WHO Regional Office for Europe website. As of 08 May 2013, WHO reported 131 laboratory-confirmed cases and 32 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
http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February_2013.pdf

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.