



SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, March 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 co-circulated 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 2012/2013 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 2012/2013 influenza season).

Viruses collected between 1 September 2012 and 28 February 2013 have been received from twenty 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 (58%) and type B (42%) viruses received were similar. For type A, H1N1pdm09 viruses have been received in slightly greater numbers than H3N2 viruses (ratio 3:2). Among influenza B receipts, viruses of the B/Yamagata and B/Victoria lineages were received at a ratio of approximately 4:1.

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, with collection dates since 1 September 2012

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
SEPTEMBER											
Denmark	2				2	2					
France	2									2	2
Norway	2									2	2
Spain	1				1	0					
OCTOBER											
France	6				3	3		2	2	1	1
Germany	2				1	1				1	1
Norway	14		6	0	2	2		1	1	5	4
Romania	1				1	1					
Spain	2	1								1	0
Sweden	2		1	1	1	1					
United Kingdom	8		1	0	2	2		2	2	3	3
NOVEMBER											
Austria	1				1	1					
Belgium	3		1	1				1	1	1	1
Denmark	7		1	1	1	1		2	2	3	3
Finland	1							1	1		
France	11		5	5	1	1				5	5
Germany	8				5	2				3	3
Ireland	10							1	1	9	9
Italy	5		2	2	1	1				2	2
Netherlands	1									1	1
Norway	27		13	9	1	1		2	2	11	11
Portugal	2				1	1				1	1
Spain	10				1	1	2	1	1	6	5
Sweden	7		1	1	5	5				1	1
United Kingdom	3				2	2		1	1		
DECEMBER											
Austria	8		2	0	2	2		2	0	2	0
Belgium	41	1	13	11	4	3				23	12
Denmark	2				1	1				1	1
France	33		7	7	15	15		6	6	5	5
Germany	23		6	6	12	12		1	1	4	4
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
Netherlands	3		1	1	2	2					
Norway	37		34	18	1	1				2	2
Portugal	9		1	0	1	1		1	1	6	6
Spain	14						1	7	7	6	6
Slovenia	5		3	3				1	0	1	0
United Kingdom	19		2	2	12	9		1	1	4	4
JANUARY											
Belgium	19	2	7	5	1	1				9	6
Denmark	3		1	1	2	2					
Estonia	21	2	9	in process	2	1	7			1	1
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
Malta	31		18	2	2	2				11	11
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	7		3	0	2	0		2	0		
Spain	22		8	8	6	2	2			6	6
United Kingdom	6				5	in process		1	in process		
FEBRUARY											
Greece	2		1	in process	1	in process					
United Kingdom	8		3	in process	2	in process		1	in process	2	in process
20 Countries											
	557	7	190	120	128	100	14	42	35	176	152
			34.1%		23.0%			7.5%		31.6%	
			325 (58%)					232 (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 [February 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; many of the titres with test viruses were reduced eight-fold or more compared to the titre with the homologous virus.

Phylogenetic analysis of the HA gene of representative viruses (Figure 1) shows that the H1N1 viruses from EU/EEA countries collected in 2013 all cluster within genetic groups 6 and 7.

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

Haemagglutination inhibition titre												
Post infection ferret sera												
Viruses		Collection date	Passage History	A/Cal 7/09 F29/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/34/09	A/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
Genetic group							Group 4	Group 3	Group 5	Group 6	Group 7	Group 6
REFERENCE VIRUSES												
A/California/7/2009		2009-04-09	E1/E2	2560	1280	2560	1280	1280	2560	2560	2560	5120
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	320	320	160	320
A/Christchurch/16/2010	4	2010-07-12	E2/E2	2560	2560	5120	5120	2560	2560	5120	5120	320
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	160	640	640	1280	1280	640	1280	1280
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	1280	640	1280	1280	1280	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	2560	1280	2560	1280	2560	2560	2560	5120	5120
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	2560	1280	2560	1280	2560	2560	2560	5120	5120
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	640	320	640	640	1280	1280	640	2560	2560
TEST VIRUSES												
A/Belgium/G944/2012	7	2012-12-11	MDCK2	1280	640	1280	1280	1280	1280	2560	5120	2560
A/Belgium/S0294/2012		2012-12-19	MDCK2	2560	640	1280	1280	2560	2560	2560	5120	5120
A/Belgium/G992/2012		2012-12-20	MDCK3	1280	640	1280	640	2560	1280	1280	2560	2560
A/Belgium/G1019/2012	6	2012-12-26	MDCK2	2560	640	2560	2560	5120	2560	5120	5120	5120
A/Belgium/S0318/2012		2012-12-27	MDCK4	640	640	1280	640	1280	1280	1280	5120	2560
A/Belgium/S0334/2012		2012-12-27	MDCK3	640	640	640	640	1280	1280	1280	2560	1280
A/Belgium/S0345/2012	6	2012-12-28	MDCK3	640	320	1280	640	1280	1280	1280	5120	2560
A/Ireland/00738/2012		2012-12-31	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	5120
A/Belgium/S0007/2013		2013-01-01	MDCK2	1280	640	2560	1280	2560	2560	1280	5120	5120
A/Belgium/G0001/2013		2013-01-02	MDCK2	1280	640	640	2560	1280	1280	1280	2560	2560
A/Belgium/S0003/2013		2013-01-02	MDCK2	1280	1280	1280	2560	2560	2560	2560	5120	5120
A/Malta/MV14254/2013	7	2013-01-10	MDCK2	1280	640	1280	1280	1280	1280	1280	5120	2560
A/Catalonia/5684S/2013		2013-01-11	MDCK1	640	640	1280	320	640	640	640	640	1280
A/Malta/MV14319/2013	7	2013-01-12	MDCK2	1280	640	640	640	1280	1280	1280	2560	2560
A/Valencia/2S/2013		2013-01-12	MDCK2	640	640	1280	640	1280	1280	1280	5120	2560
A/Belgium/S0091/2013	6	2013-01-14	MDCK2	640	640	640	640	1280	1280	1280	2560	2560
A/Belgium/S0093/2013		2013-01-14	MDCK2	1280	640	2560	1280	1280	2560	2560	5120	5120
A/Catalonia/5737S/2013		2013-01-22	MDCK1	1280	640	1280	1280	2560	2560	1280	5120	5120

Sequences in phylogenetic tree (Figure 1)

Figure 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes**Vaccine virus****Reference viruses**

Collection date

Oct 2012

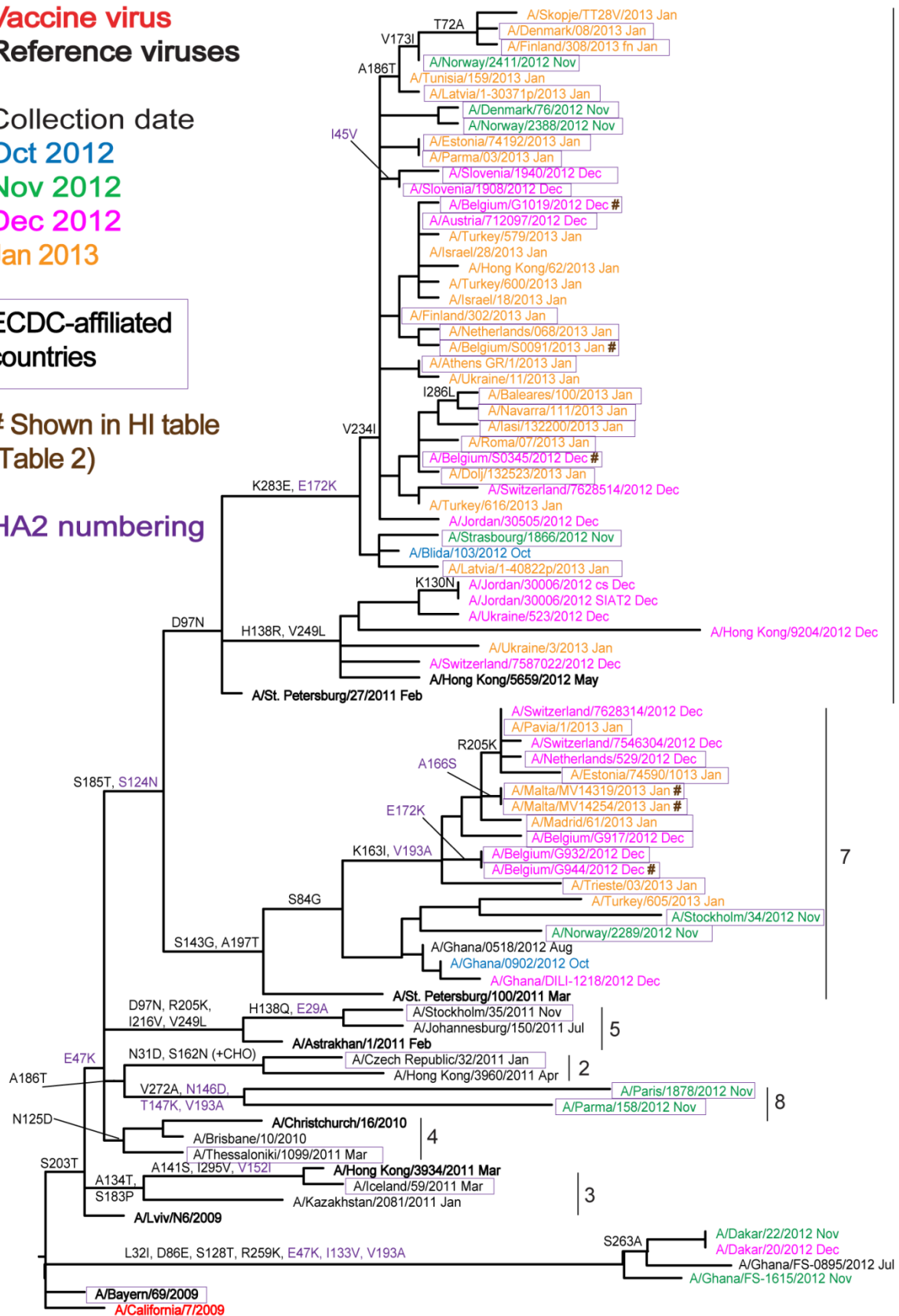
Nov 2012

Dec 2012

Jan 2013

ECDC-affiliated
countries# Shown in HI table
(Table 2)

HA2 numbering



0.002

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 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, 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 an isolate of A/Victoria/361/2011 propagated in MDCK cells in parallel with cell-propagated viruses, 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 September 2012 have HA genes that fall into genetic groups 5 and 3C, with one exception in the Figure, A/Belgium/G0044/2013, falling into group 3B.

The amino acid substitutions in the HA associated with these groupings of recently collected viruses are:

- Group 3 viruses: **N145S** & **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: **D487N**;
- 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**.

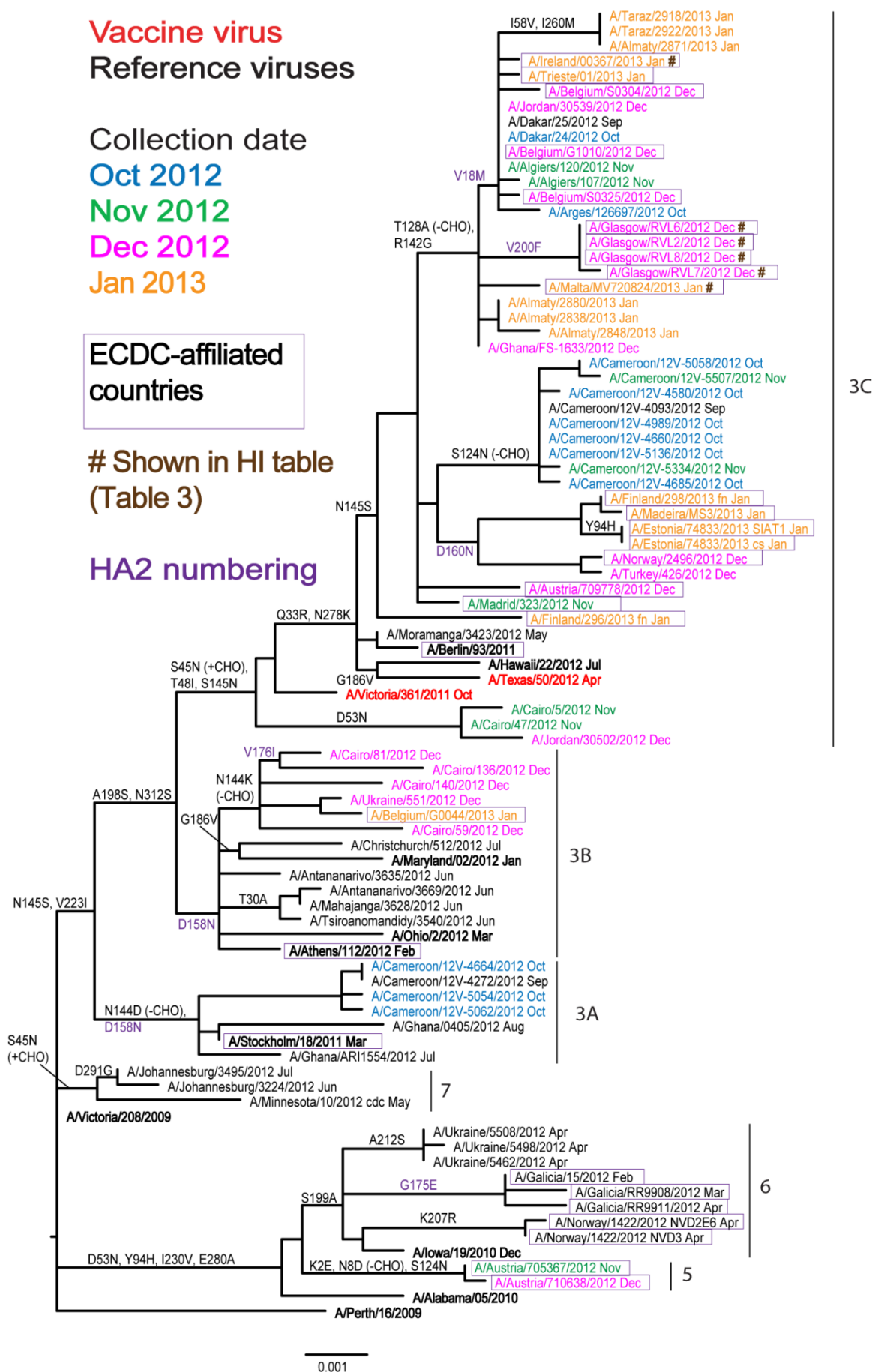
There is no evidence for antigenic change associated with any of the genetic groups or emerging subgroups, including the emerging subgroup in group 3C that carries substitutions in the HA at amino acid residues 128 and 142.

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

Viruses		Collection Date	Passage History	Haemagglutination inhibition titre											
				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	F36/12
Genetic group					group 5	group 3A	group 6	group 3C	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	80	320	160	320	320	80	
A/Victoria/208/2009		2009-06-02	E3/E2	640	2560	640	1280	2560	2560	5120	1280	2560	5120	2560	
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	<	<	80	80	160	80	160	160	320	160	40	
A/Stockholm/18/2011	3A	2011-03-28	SIAT4	80	160	160	640	320	160	1280	640	640	1280	640	
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	640	320	640	1280	640	2560	1280	1280	2560	1280	
A/Victoria/361/2011	3C	2011-10-24	E3/E2	320	640	320	80	640	2560	640	320	160	2560	1280	
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT3	160	160	160	320	320	320	640	640	640	1280	640	
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT2	160	160	320	320	320	320	1280	640	1280	1280	640	
A/Athens/112/2012	3B	2012-02-01	SIAT7	80	160	160	320	320	160	640	320	640	1280	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	320	1280	320	640	1280	1280	1280	640	1280	5120	2560	
TEST VIRUSES															
A/Glasgow/RVL2/2012	3C	2012-12-19	SIAT3	40	80	80	160	160	160	640	320	320	320	320	
A/Glasgow/RVL6/2012	3C	2012-12-21	SIAT4	40	80	80	160	160	160	640	320	640	640	320	
A/Glasgow/RVL5/2012		2012-12-21	SIAT3	<	80	40	160	160	160	640	320	640	640	320	
A/Glasgow/RVL7/2012	3C	2012-12-21	SIAT3	40	160	80	320	160	320	640	320	640	640	320	
A/Glasgow/RVL8/2012	3C	2012-12-21	SIAT4	40	160	160	320	320	320	1280	320	640	1280	320	
A/Ireland/00367/2013	3C	2013-01-02	SIAT4	<	40	40	160	160	160	320	160	320	640	160	
A/Catalonia/5674S/2013		2013-01-15	SIAT1	40	80	80	160	80	80	320	320	640	640	160	
A/Malta/MV720824/2013	3C	2013-01-15	MDCK3	40	160	160	160	320	320	1280	640	1280	1280	640	
A/Catalonia/5689S/2013		2013-01-17	SIAT1	160	160	320	640	640	320	2560	1280	1280	2560	320	

Sequences in phylogenetic tree (Figure 2)

< = <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 [February 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 2013–14 northern hemisphere influenza season. 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

Viruses	Collection date	Passage History	Haemagglutination inhibition titre									
			Post infection ferret sera									
			B/Bris ² 60/08 Sh 523	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 F11/09	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 clade					1A	1A	1A	1B	1B	1A	1A	1A
REFERENCE VIRUSES												
B/Malaysia/2506/2004	2004-12-06	E3/E6	1280	320	40	80	<	<	<	80	160	80
B/England/393/2008	2008-08-29	E1/E2	1280	80	320	320	40	40	40	320	320	320
B/Brisbane/60/2008	2008-08-04	E4/E3	2560	80	320	640	80	80	80	320	640	640
B/Paris/1762/2008	2009-02-09	C2/MDCK2	2560	<	20	40	40	40	40	20	40	80
B/Hong Kong/514/2009	2009-10-11	MDCK4	2560	<	20	20	80	80	160	20	40	80
B/Odessa/3886/2010	2010-03-19	C2/MDCK2	2560	40	160	160	40	40	40	80	160	320
B/Malta/636714/2011	2011-03-07	E4/E1	5120	80	320	640	80	40	40	320	640	320
B/Johannesburg/3964/2012	2012-08-03	E1/E1	2560	160	320	640	80	40	40	320	640	640
B/Formosa/V2367/2012	2012-08-06	MDCK1/MDCK2	2560	<	20	80	20	40	40	40	160	160
TEST VIRUSES												
B/Catalonia/5479S/2012	2012-12-04	MDCK1	2560	10	10	10	20	40	40	10	20	40
B/Ireland/87829/2012	2012-11-19	MDCK4	2560	10	20	20	40	40	40	20	80	80

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

Vaccine virus

Reference viruses

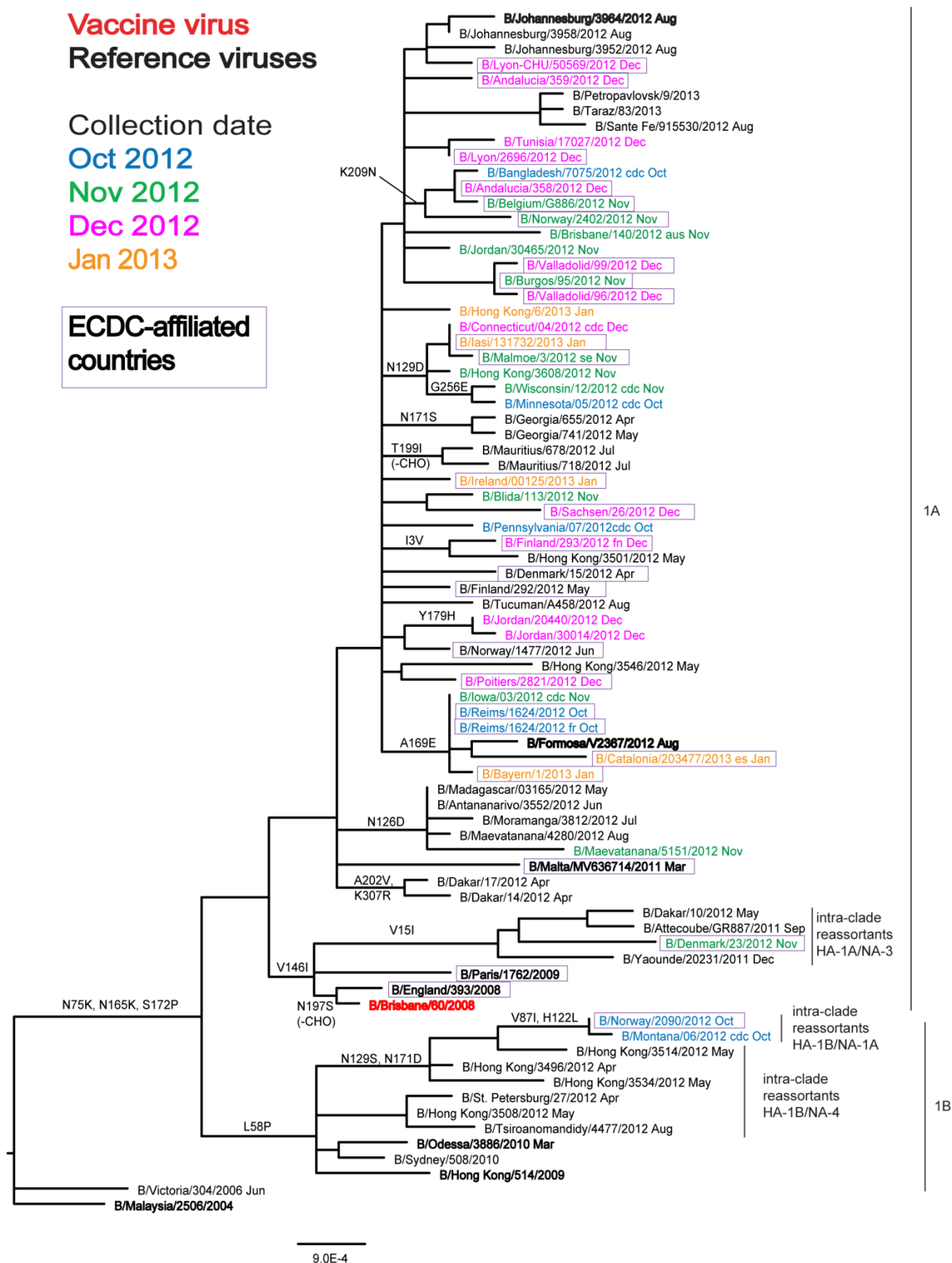
Oct 2012

Nov 2012

Dec 2012

Jan 2013

ECDC-affiliated countries



B/Yamagata-lineage viruses

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

The vast majority (39 of 43) of the viruses showed good reactivity (within four-fold 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. Of these 39, 15 were sequenced and all fell within HA clade 2. The same three antisera, raised against clade 2 viruses, showed poor reactivity (> four-fold compared to the homologous titres) with four of the 43 viruses assayed; the HA sequences of these four viruses fell within clade 3. One virus (B/Catalonia/2067780NS/2013) reacted well with antisera raised against viruses in both clades, but fell into clade 3 by HA sequence.

Antiserum raised against egg-propagated B/Wisconsin/1/2010 showed low reactivity to the majority of test viruses, regardless of the clade (2 or 3) into which their HA genes fell. In contrast, antisera raised against egg-grown B/Massachusetts/02/2012 reacted within four-fold of the titre against the homologous virus with all of the viruses tested, irrespective of the HA clade of the test virus, hence the recommendation to use this virus 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 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		Collection date	Passage History	Haemagglutination Inhibition Titre									
				B/FI ¹ 4/06 SH479	Post infection ferret sera								
					B/FI ¹ 4/06 F21/07 Clade 1	B/Bris ² 3/07 F21/12 Clade 2	B/Wis ² 1/10 F26/10 Clade 3	B/Stock ² 12/11 F12/12 Clade 3	B/Estonia ² 55669/11 F26/11 Clade 2	B/Novo 1/12 F31/12 Clade 3	B/HK 3577/12 F33/12 Clade 2	B/Egg F02/13 Clade 2	B/Mass 2/12 T/C F03/13 Clade 2
Genetic clade													
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E3/E4	5120	1280	1280	640	1280	320	80	640	1280	320
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	640	640	640	640	320	40	640	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	320	320	1280	1280	40	160	160	1280	80
B/Stockholm/12/2011	3	2007-08-07	E4/E1	1280	80	160	160	320	10	40	40	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	40	160	40	40	640	80	1280	320	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	1280	40	160	160	160	80	160	160	640	80
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK3	2560	160	160	160	160	1280	160	1280	640	640
B/Massachusetts/02/2012	2	2012-03-13	E3/E2	2560	640	640	320	320	80	40	160	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK2	2560	80	320	80	320	320	80	1280	640	320
TEST VIRUSES													
B/Belgium/G933/2012		2012-12-10	MDCK2	2560	320	320	80	160	1280	80	1280	640	320
B/Belgium/G938/2012		2012-12-10	MDCK2	2560	320	320	80	320	1280	80	1280	640	320
B/Belgium/G939/2012		2012-12-10	MDCK2	2560	160	320	40	160	640	40	640	320	320
B/Belgium/G943/2012		2012-12-10	MDCK2	2560	160	160	80	320	640	80	640	640	320
B/Belgium/G947/2012	2	2012-12-12	MDCK2	2560	80	160	40	160	640	80	640	640	320
B/Belgium/G1021/2012		2012-12-20	MDCK2	2560	80	320	40	160	1280	80	1280	640	320
B/Belgium/G1022/2012	2	2012-12-24	MDCK2	2560	160	320	40	160	640	80	1280	640	320
B/Belgium/S0310/2012		2012-12-24	MDCK2	2560	160	320	40	160	1280	80	1280	640	320
B/Belgium/S0314/2012	2	2012-12-25	MDCK2	1280	40	80	20	80	640	40	640	320	160
B/Belgium/G1023/2012		2012-12-31	MDCK2	2560	160	320	40	160	1280	80	1280	640	320
B/Malta/MV14117/2013	2	2013-01-08	MDCK2	2560	160	320	80	160	640	80	640	640	320
B/Malta/MV14378/2013	2	2013-01-14	MDCK2	5120	320	320	80	320	1280	80	1280	640	320
B/Malta/MV14443/2013	2	2013-01-15	MDCK2	2560	320	320	80	320	640	80	1280	640	320
B/Malta/MV14519/2013	2	2013-01-15	MDCK2	1280	160	160	40	160	320	40	640	320	160
B/Malta/MV720825/2013	2	2013-01-15	MDCK2	5120	320	320	160	640	1280	320	1280	640	640
B/Belgium/H20/2012	3	2012-02-06	MDCK2	1280	<	80	40	80	40	80	80	320	80

Sequences in phylogenetic tree (Figure 4)

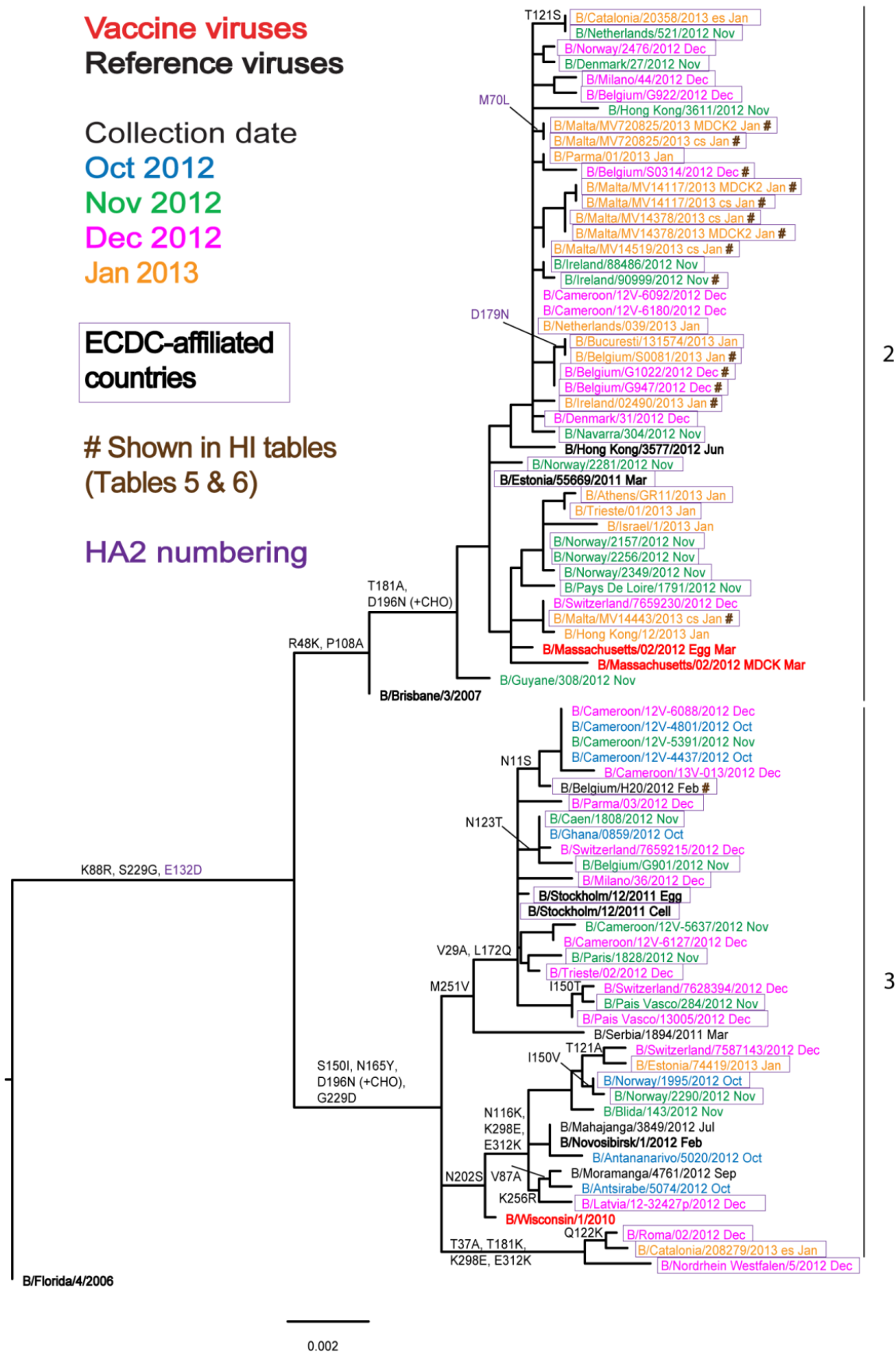
1. <= <40; 2. <= <10; 3. hyperimmune sheep serum

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

		Haemagglutination Inhibition Titre											
Viruses	Genetic clade	Collection date	Passage History	B/FI ¹ 4/06 SH479	Post infection ferret sera								
					B/FI ¹ F1/10	B/Bris ² F21/12	B/Wis ² F26/10	B/Stock ² F12/12	B/Estonia ² F26/11	B/Novo ² F31/12	B/HK ² F33/12	B/Mass ² F02/13	B/Mass ² T/C F03/13
					Clade 1	Clade 2	Clade 3	Clade 3	Clade 2	Clade 3	Clade 2	Clade 2	Clade 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E3/E3	5120	1280	1280	640	640	320	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	1280	1280	320	640	320	80	640	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	640	640	640	640	40	80	80	640	80
B/Stockholm/12/2011	3	2007-08-07	E4/E2	1280	320	160	160	320	10	40	40	320	20
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	5120	320	320	160	320	1280	320	1280	640	640
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	5120	320	320	160	320	320	640	640	640	640
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK1	5120	320	320	160	320	1280	320	1280	320	640
B/Massachusetts/02/2012	2	2012-03-13	E3/E2	2560	320	640	160	320	80	40	160	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK2	5120	640	640	160	320	640	160	1280	1280	640
TEST VIRUSES													
B/Belgium/S0039/2013		2013-01-05	MDCK2	5120	320	320	80	320	1280	80	1280	640	320
B/Belgium/S0052/2013		2013-01-08	MDCK2	5120	320	320	80	160	640	80	640	640	320
B/Belgium/S0064/2013		2013-01-10	MDCK2	5120	640	640	320	640	1280	320	1280	1280	640
B/Belgium/S0081/2013	2	2013-01-12	MDCK2	2560	160	160	40	160	320	40	640	320	160
B/Belgium/S0084/2013		2013-01-15	MDCK2	2560	160	160	40	160	640	40	640	320	160
B/Belgium/S0092/2013	2	2013-01-14	MDCK2	5120	320	320	160	320	640	160	640	320	640
B/Ireland/89450/2012	2	2012-11-22	MDCK2	2560	320	320	80	160	640	80	640	320	160
B/Ireland/90800/2012		2012-11-26	MDCK2	2560	320	320	160	320	640	160	640	320	640
B/Ireland/90999/2012	2	2012-11-25	MDCK2	2560	320	320	80	320	1280	80	640	640	320
B/Ireland/91035/2012		2012-11-28	MDCK2	2560	320	320	80	320	640	80	640	640	320
B/Ireland/94775/2012		2012-12-12	MDCK2	2560	160	160	40	160	320	40	320	320	320
B/Ireland/98426/2012		2012-12-27	MDCK3	2560	320	320	80	320	640	80	1280	640	320
B/Ireland/00329/2013	2	2013-01-02	MDCK2	2560	320	160	80	320	1280	80	640	640	320
B/Ireland/94979/2012	3	2012-12-12	MDCK2	2560	320	160	80	320	40	160	80	320	80
B/Catalonia/2061945NS/2012		2012-12-13	P0/MDCK1	5120	320	320	80	320	640	80	640	320	320
B/Catalonia/2062653NS/2012		2012-12-17	P0/MDCK1	5120	320	320	80	320	1280	80	1280	640	640
B/Catalonia/2071966NS/2013		2013-01-28	P0/MDCK1	2560	320	160	80	160	640	80	640	320	320
B/Valencia/7S/2013	2	2013-01-10	P0/MDCK1	2560	320	160	80	160	1280	160	1280	320	640
B/Catalonia/5653S/2013		2013-01-14	P0/MDCK1	2560	320	160	80	160	640	80	640	320	320
B/Catalonia/5595S/2013		2013-01-08	P0/MDCK1	2560	160	160	40	160	320	80	320	320	160
B/Catalonia/5536S/2012		2012-12-16	P0/MDCK1	5120	640	320	80	320	1280	160	1280	320	640
B/Catalonia/5485S/2012		2012-12-10	P0/MDCK1	2560	320	160	80	320	640	80	640	320	320
B/Ireland/98719/2012		2012-12-28	MDCK3	2560	160	160	40	80	320	40	320	320	160
B/Ireland/00217/2013	3	2013-01-02	MDCK3	640	160	80	80	160	10	80	40	160	20
B/Ireland/02490/2013	2	2013-01-08	MDCK3	1280	160	160	40	80	320	20	320	320	160
B/Catalonia/2066914NS/2013	3	2013-01-07	P0/MDCK1	1280	160	80	40	80	20	80	80	160	40
B/Catalonia/2067780NS/2013	3	2013-01-11	P0/MDCK1	2560	160	160	80	160	160	320	320	320	320

Sequences in phylogenetic tree (Figure 4)

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.

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.