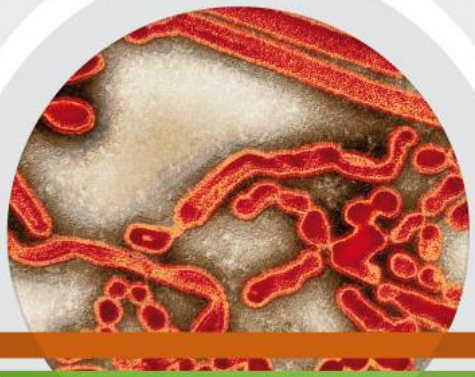


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, May 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 prevalences 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 31 May 2013, spanning the 2012–13 season, were received from 23 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 proportions of influenza type A (61%) and type B (39%) viruses received were similar. 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 4:1.

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 December 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]
DECEMBER											
Austria	8		2	0	2	2		2	0	2	0
Belgium	41	1	13	11	4	3				23	12
Czech Republic	2		2	2							
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
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	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	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	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
United Kingdom	6				5	5		1	1		
FEBRUARY											
Bulgaria	22	1	7	7	2	2				12	12
Czech Republic	4									4	4
Greece	2		2	2							
Hungary	12		6	in process				3	in process	3	in process
Luxembourg	5		1	0	4	0					
Romania	12		7	7						5	5
Slovenia	15		6	6	4	4		1	1	4	3
Spain	10		9	7	1	1					
United Kingdom	8		3	3	2	2		1	1	2	2
MARCH											
Bulgaria	2		1	1						1	1
Czech Republic	1									1	1
Hungary	11		1	in process				1	in process	9	in process
Luxembourg	1									1	1
Romania	5		1	1	1	in process				3	in process
Slovenia	3							2	1	1	1
Spain	12		3	2	4	4		3	in process	2	1
APRIL											
Romania	2		1	1						1	1
Slovenia	2		1	1						1	1
Spain	3		1	0	1	1				1	1
	575	7	229	166	116	99	9	41	29	173	135
			39.8%		20.2%			7.1%		30.1%	
23 Countries		61.2%					38.8%				

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 April report [1] are shown in Table 2. All test viruses continued to show 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, with this antiserum recognising the test viruses shown at titres within fourfold of its recognition of the homologous virus. As described previously [1], 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 approximately 30% (11/29) of the test viruses, with titres reduced eightfold or greater compared with the titre of the antiserum with the homologous virus.

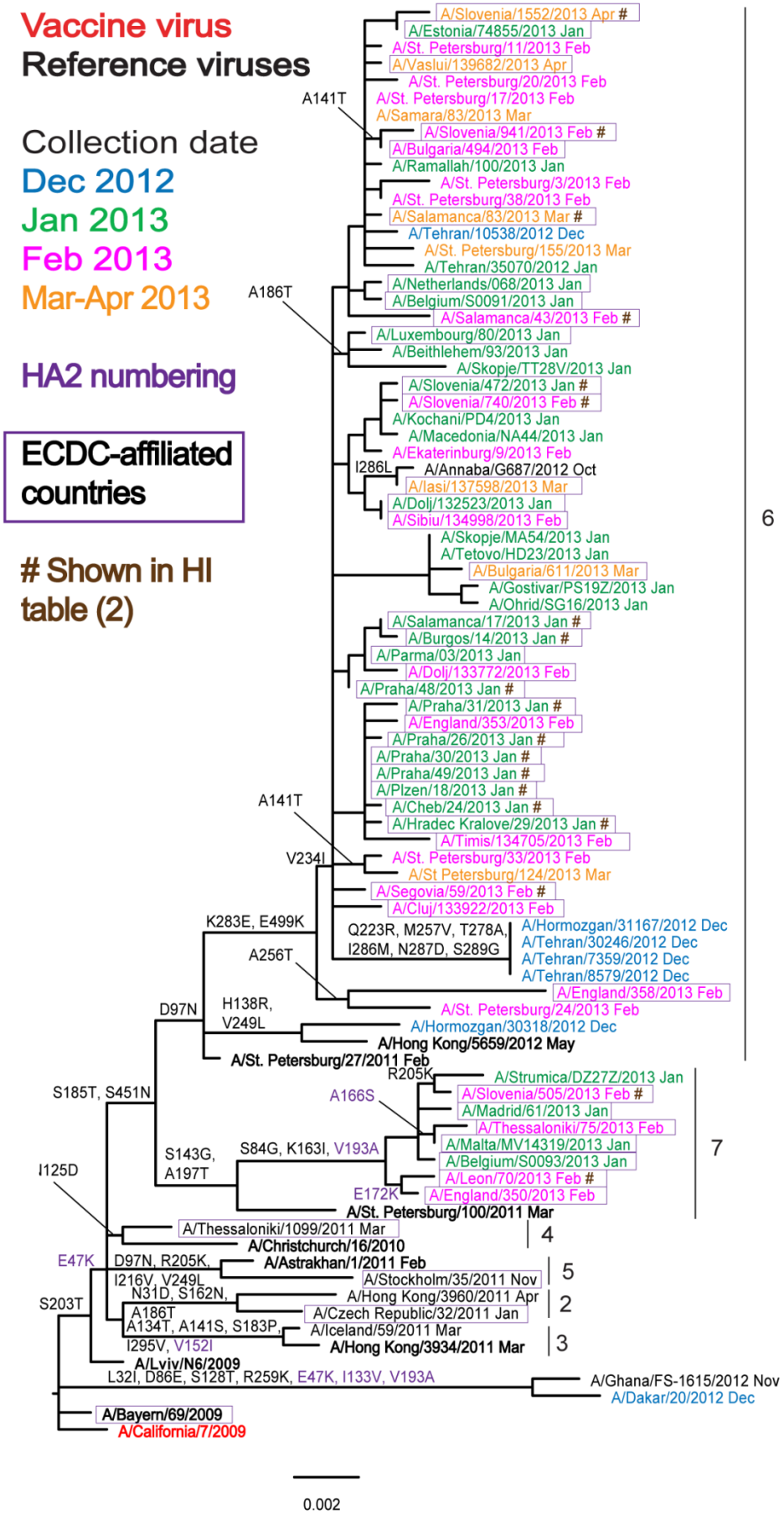
Antisera raised against several of the reference strains reacted poorly with a single virus, A/Plzen/18/2013, showing eightfold or greater reductions in titre compared to the titres of the antisera with their homologous antigens; HA gene sequencing of this virus showed that it carried a polymorphism at residue 155 (E>G) of HA1. Amino acid substitution or polymorphism in this region of HA1 can affect the antigenicity of the virus and commonly emerges during propagation of viruses in cell culture.

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, with viruses belonging to group 6 predominating. HA gene sequencing was performed on 19 test viruses and the genetic groups to which they belong are shown in Table 2; all but two were in genetic group 6.

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

				Haemagglutination inhibition titre[1]								
				Post infection ferret sera								
Viruses		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	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	EP1/E1	1280	1280	1280	640	1280	640	640	1280	1280
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	320	160	40	40	40	40	40	40
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	2560	1280	320	160	160	320	160	640
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	2560	2560	5120	1280	1280	1280	5120	2560
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK2	640	320	640	640	1280	1280	1280	2560	2560
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	1280	1280	1280	1280	2560	5120	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	1280	1280	1280	640	1280	1280	1280	5120	2560
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	640	640	1280	640	1280	1280	1280	2560	2560
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	5120
TEST VIRUSES												
A/Plzen/39/2013		2012-12-19	MDCK4/MDCK1	320	640	320	160	160	160	320	160	320
A/Plzen/18/2013	6	2012-12-24	MDCK4/MDCK1	320	320	160	80	80	160	160	160	320
A/Cheb/24/2013	6	2013-01-11	MDCK4/MDCK1	1280	640	1280	640	1280	1280	1280	2560	2560
A/Plzen/25/2013		2013-01-11	MDCK4/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560
A/Praha/26/2013	6	2013-01-11	MDCK4/MDCK1	1280	320	1280	1280	1280	1280	1280	2560	2560
A/Praha/28/2013		2013-01-11	MDCK4/MDCK1	640	320	640	640	1280	640	1280	2560	1280
A/Hradec Kralove/29/2013	6	2013-01-11	MDCK4/MDCK1	320	320	320	320	640	640	640	1280	1280
A/Praha/30/2013	6	2013-01-14	MDCK4/MDCK1	640	320	320	320	640	640	640	1280	1280
A/Praha/31/2013	6	2013-01-14	MDCK4/MDCK1	320	320	640	640	640	640	640	1280	1280
A/Plzen/48/2013	6	2013-01-23	MDCK4/MDCK1	640	640	640	640	1280	1280	1280	2560	1280
A/Plzen/49/2013	6	2013-01-28	MDCK4/MDCK1	640	320	640	640	640	1280	1280	1280	1280
A/Burgos/14/2013	6	2013-01-30	MDCK2	2560	1280	2560	1280	2560	2560	2560	5120	5120
A/Salamanca/17/2013	6	2013-01-30	MDCK2	1280	1280	2560	1280	2560	1280	2560	2560	2560
A/Slovenia/472/2013	6	2013-01-31	MDCKx/MDCK1	1280	1280	1280	2560	2560	1280	2560	5120	2560
A/Slovenia/505/2013	7	2013-02-01	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Salamanca/43/2013	6	2013-02-13	MDCK3	1280	640	1280	640	1280	640	640	2560	2560
A/Slovenia/740/2013	6	2013-02-14	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Slovenia/825/2013		2013-02-18	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Leon/70/2013	7	2013-02-18	MDCK3	320	320	640	640	640	320	640	2560	1280
A/Valladolid/54/2013		2013-02-18	MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120
A/Slovenia/870/2013		2013-02-21	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Slovenia/888/2013		2013-02-22	MDCKx/MDCK1	1280	1280	1280	1280	5120	1280	2560	5120	5120
A/Segovia/59/2013	6	2013-02-24	MDCK2	2560	1280	2560	2560	5120	1280	2560	5120	5120
A/Salamanca/61/2013		2013-02-24	MDCK3	640	640	1280	1280	2560	640	1280	2560	5120
A/Salamanca/63/2013		2013-02-24	MDCK3	1280	640	1280	1280	2560	640	1280	5120	2560
A/Slovenia/941/2013	6	2013-02-25	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Salamanca/83/2013	6	2013-03-06	MDCK2	1280	640	2560	1280	2560	640	1280	2560	2560
A/Leon/90/2013		2013-03-09	MDCK3	320	1280	5120	2560	5120	1280	2560	5120	5120
A/Slovenia/1552/2013	6	2013-04-05	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	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 [2]. 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 [3]. Antigenic analyses of recently collected viruses conducted since the April report [1] are shown in Table 3. 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 [4]. 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 [5]) compared with the titre of the antiserum with the homologous virus than they did against other egg-propagated viruses. In Table 3, antiserum raised against A/Texas/50/2012 recognised 7 out of 25 of the test viruses with titres within fourfold of the titre to the homologous virus. A/Texas/50/2012 is an A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011.

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 December 2012 have HA genes that fall predominantly into genetic group 3C. Viruses carrying HA genes falling into group 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 since December 2012.

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** and **N312S**
- 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**
- 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.

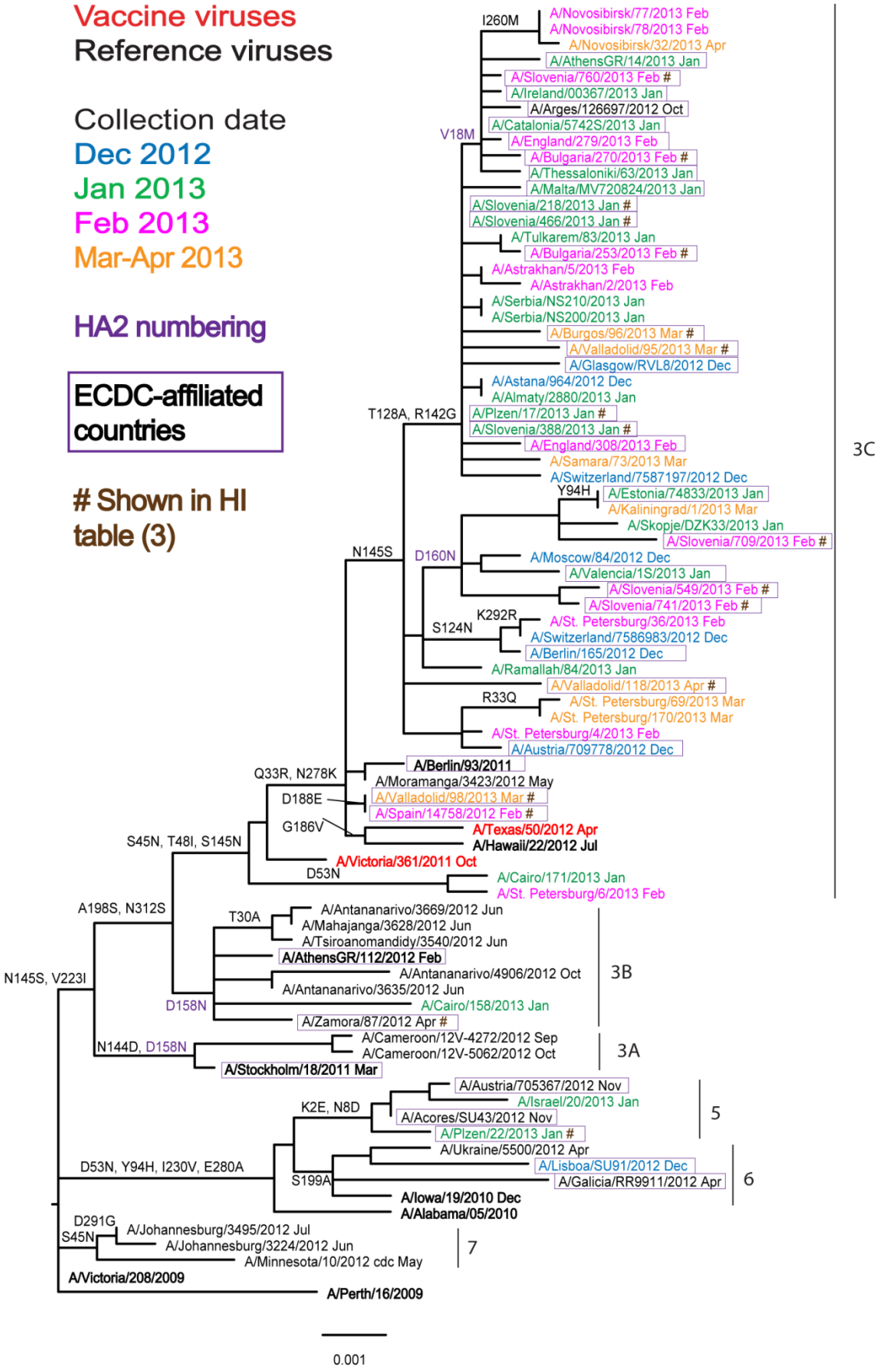
Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

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 F11/13	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	80	80	320	160	320	320	80
A/Victoria/208/2009		2009-06-02	E3/E2	640	2560	640	1280	1280	1280	2560	2560	1280	5120	2560
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	40	<	80	80	80	80	160	160	320	160	40
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	40	80	40	160	80	80	320	160	320	320	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	640	320	640	1280	640	2560	1280	1280	2560	640
A/Victoria/361/2011	3C	2011-10-24	E3/E2	640	640	160	160	320	2560	640	640	160	2560	640
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT6	160	80	80	320	160	160	640	320	640	640	320
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT3	160	80	80	320	160	160	640	320	640	640	320
A/Athens/112/2012	3B	2012-02-01	SIAT7	80	80	80	320	160	160	640	320	640	640	320
A/Texas/50/2012	3C	2012-04-15	E5/E1	640	1280	320	1280	640	1280	2560	640	2560	5120	1280
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	320	640	320	640	640	640	1280	640	1280	2560	5120
TEST VIRUSES														
A/Plzen/17/2013	3C	2013-01-03	MDCK4/SIAT1	<	40	40	160	40	80	320	160	320	320	160
A/Zamora/10/2012		2012-01-19	SIAT3	<	80	40	160	80	80	320	320	320	320	160
A/Spain/13831/2012		2012-02-16	SIAT3	80	160	160	320	160	160	640	320	640	1280	320
A/Spain/13916/2012		2012-02-17	SIAT3	80	80	80	160	80	160	320	320	320	640	160
A/Spain/14701/2012		2012-02-21	SIAT3	40	80	80	160	80	40	320	320	320	640	160
A/Spain/14758/2012	3C	2012-02-21	SIAT3	40	80	80	320	160	160	640	320	320	640	160
A/Spain/20322/2012		2012-03-10	SIAT3	40	160	160	320	160	160	640	320	640	640	320
A/Spain/23634/2012		2012-03-22	SIAT3	80	160	160	320	160	160	1280	640	640	1280	320
A/Zamora/87/2012	3B	2012-04-03	SIAT3	<	80	40	160	80	80	320	320	320	640	160
A/Plzen/22/2013	5	2013-01-10	MDCK4/SIAT1	80	160	160	320	160	160	640	320	320	640	320
A/Slovenia/218/2013	3C	2013-01-17	MDCK1/SIAT1	<	80	40	160	40	160	320	320	320	640	160
A/Slovenia/388/2013	3C	2013-01-25	MDCKx/SIAT1	40	80	80	160	80	160	320	320	640	640	160
A/Slovenia/466/2013	3C	2013-01-30	MDCKx/SIAT1	40	80	40	160	80	160	320	320	320	640	160
A/Slovenia/549/2013	3C	2013-02-04	MDCK1/SIAT1	40	80	40	160	80	160	320	160	320	640	160
A/Bulgaria/270/2013	3C	2013-02-04	C2/SIAT1	320	160	160	640	160	320	1280	640	1280	2560	640
A/Bulgaria/253/2013	3C	2013-02-08	C2/SIAT1	40	80	80	320	80	160	640	320	640	640	320
A/Slovenia/709/2013	3C	2013-02-12	MDCK1/SIAT1	160	640	320	640	160	640	1280	640	1280	2560	1280
A/Valladolid/39/2013		2013-02-14	SIAT2	320	<	160	320	160	160	1280	640	640	640	1280
A/Slovenia/741/2013	3C	2013-02-14	MDCKx/SIAT1	40	160	80	320	80	160	640	320	640	1280	640
A/Slovenia/760/2013	3C	2013-02-15	MDCKx/SIAT1	<	80	80	320	80	160	640	320	640	1280	640
A/Spain/18719/2013		2013-03-03	SIAT4	40	80	80	320	160	160	640	320	640	1280	320
A/Burgos/96/2013	3C	2013-03-12	SIAT2	160	<	80	640	160	80	640	320	640	640	2560
A/Valladolid/95/2013	3C	2013-03-14	SIAT2	160	<	80	320	160	160	640	320	640	640	320
A/Valladolid/98/2013	3C	2013-03-18	SIAT3	40	160	80	320	160	160	640	320	320	640	320
A/Valladolid/118/2013	3C	2013-04-02	SIAT3	<	80	80	160	80	80	320	320	320	320	160
									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 April report [1]. All test viruses were from Slovenia. Compared with the titre against the homologous virus in HI assays all test viruses showed low 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 [5] for the 2013–14 northern hemisphere influenza season. The test viruses showed a similarly reduced reactivity with antisera raised against other reference viruses propagated in hens' eggs: 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/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, 1B and 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

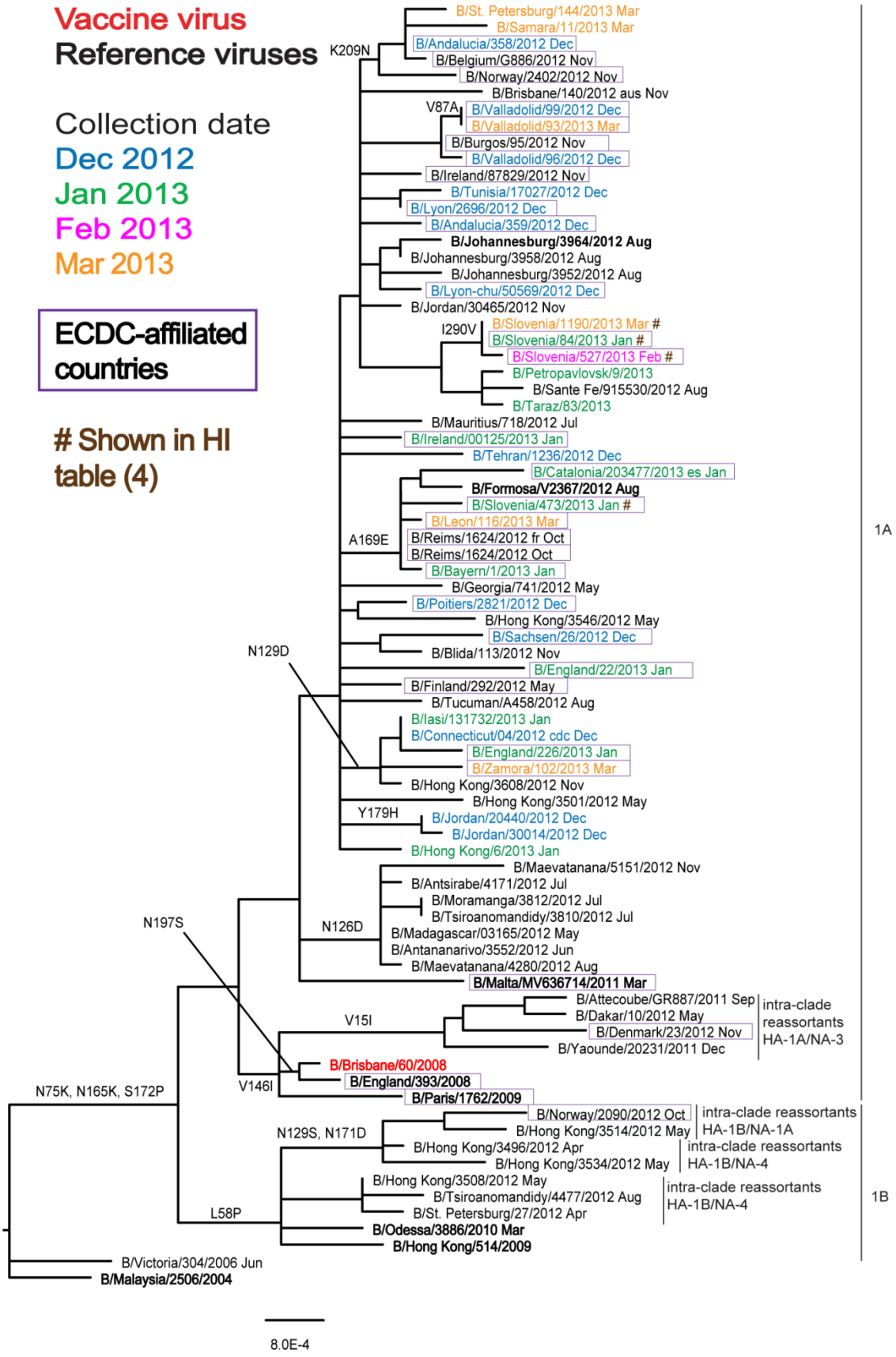
Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre: Post infection ferret sera									
				B/Bris[2] 60/08 Sh 522	B/Mal[1] 2506/05 F37/11	B/Eng[1] 393/08 F05/11 1A	B/Bris[1] 60/08 F22/12 1A	B/Paris[1] 1762/08 F07/11 1A	B/HK[1] 514/09 F13/10 1B	B/Odessa[1] 3886/10 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	640	40	80	10	<	<	80	160	80
B/England/393/2008	1A	2008-08-29	E1/E2	2560	80	320	320	80	40	40	160	320	160
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	2560	80	320	320	80	40	40	320	320	320
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	10	40	40	80	40	40	20	40	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	5120	<	20	40	160	80	80	20	80	80
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	2560	40	160	160	40	40	40	80	160	160
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	320	320	80	20	40	320	320	320
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E1	5120	160	640	640	80	40	40	320	640	640
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	2560	10	80	160	80	40	40	80	160	160
TEST VIRUSES													
B/Slovenia/84/2013	1A	2013-01-08	MDCK1/MDCK1	2560	<	20	40	80	80	80	20	80	80
B/Slovenia/176/2013		2013-01-15	MDCKx/MDCK1	2560	<	40	40	80	80	80	20	80	80
B/Slovenia/221/2013		2013-01-17	MDCKx/MDCK1	2560	<	20	40	80	40	40	20	40	40
B/Slovenia/473/2013	1A	2013-01-28	MDCKx/MDCK1	2560	<	20	40	80	80	40	20	40	80
B/Slovenia/527/2013	1A	2013-02-03	MDCKx/MDCK1	2560	<	20	40	80	40	40	20	40	40
B/Slovenia/1190/2013	1A	2013-03-11	MDCKx/MDCK1	2560	<	20	40	80	80	40	20	40	40
							Vaccine*						

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

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

Table 5 shows the results of HI analyses of B/Yamagata lineage viruses tested since the April report [1]. The genetic clade into which sequenced HA genes of test viruses fall is indicated.

All 16 test viruses showed good reactivity (within fourfold of the homologous titre) with antisera raised against the egg-propagated vaccine virus recommended for the northern hemisphere winter 2013–14 influenza season [5] 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 the majority of 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 test viruses. Nine of the test viruses had been genetically characterised at the time of preparation of this report, with six falling into genetic group 2 and three into 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.

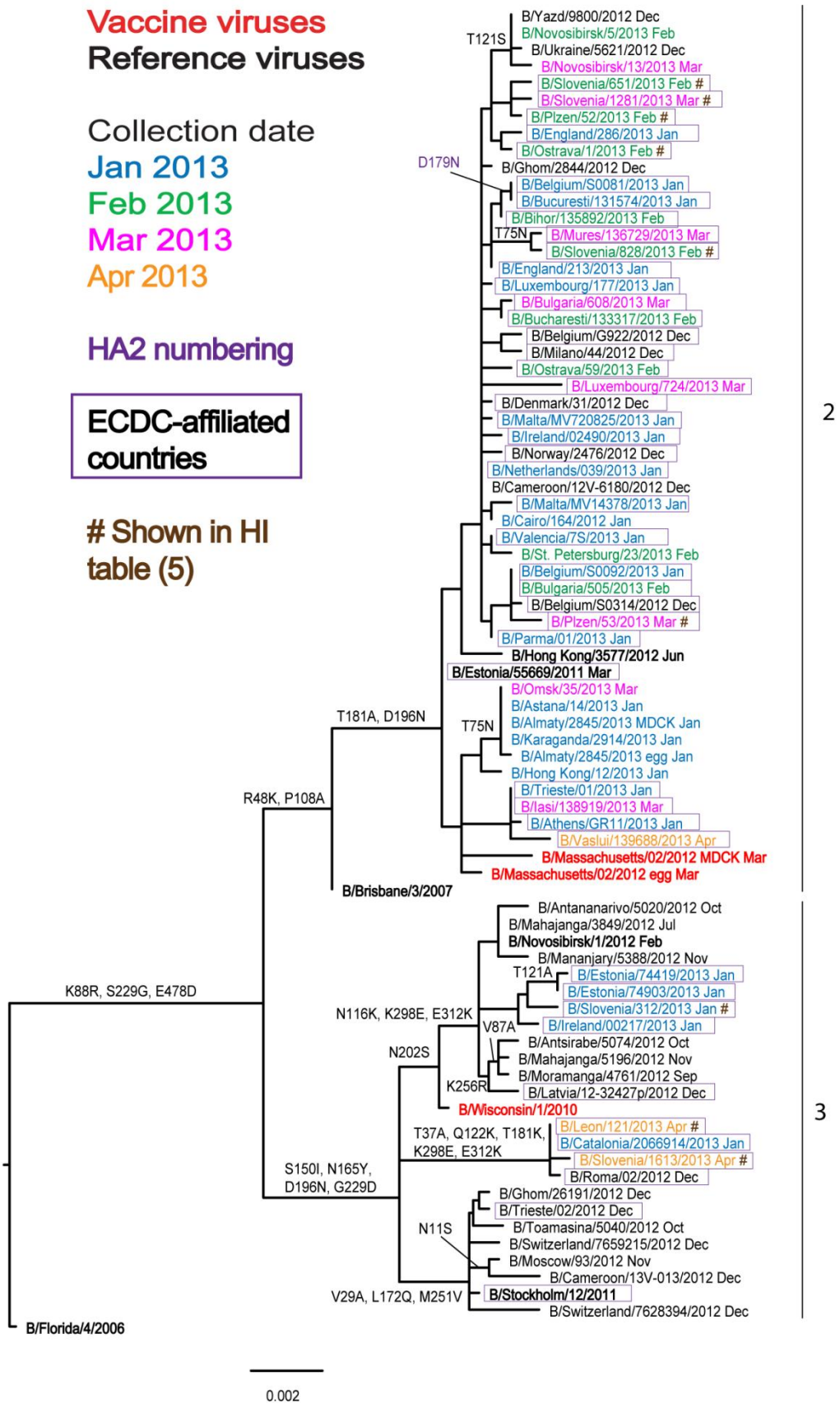
Table 5. Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

					Haemagglution Inhibition Titre								
					Post infection ferret sera								
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 F02/13 Group 2	B/Mass[2] 2/12 T/C F03/13 Group 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E7/E1	5120	640	640	320	640	320	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	1280	640	320	640	160	40	320	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	1280	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/MDCK2	1280	160	80	80	80	640	80	640	160	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK3	2560	320	160	320	320	160	320	320	320	640
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK2	2560	320	160	160	160	640	160	640	320	640
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	2560	640	640	320	320	160	40	320	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	2560	640	320	160	320	320	80	640	640	320
TEST VIRUSES													
B/Slovenia/319/2013		2013-01-21	MDCKx/MDCK1	2560	160	160	80	160	640	80	640	320	320
B/Slovenia/312/2013	3	2013-01-22	MDCKx/MDCK1	1280	160	160	160	320	80	320	160	160	160
B/Slovenia/386/2013		2013-01-25	MDCKx/MDCK1	2560	320	160	160	320	1280	160	640	320	640
B/Ostrava/1/2013	2	2013-02-08	MDCK2/MDCK1	2560	160	160	80	160	640	160	640	160	320
B/Slovenia/651/2013	2	2013-02-08	MDCKx/MDCK1	1280	160	160	80	80	640	40	640	160	160
B/Slovenia/699/2013		2013-02-12	MDCKx/MDCK1	2560	320	160	80	160	640	80	640	160	320
B/Ostrava/59/2013		2013-02-17	MDCK2/MDCK1	2560	320	320	160	320	640	80	640	320	320
B/Slovenia/828/201	2	2013-02-20	MDCKx/MDCK1	1280	160	80	80	80	1280	40	640	320	160
B/Ostrava/58/2013		2013-02-22	MDCK2/MDCK1	1280	160	160	80	160	640	80	640	320	160
B/Slovenia/942/2013		2013-02-25	MDCKx/MDCK1	2560	160	160	80	160	640	40	640	160	160
A/Plzen/52/2013	2	2013-02-28	MDCK2/MDCK1	2560	320	160	80	160	640	80	640	320	160
A/Plzen/53/2013	2	2013-03-05	MDCK2/MDCK1	2560	320	160	160	320	640	160	640	320	320
B/Slovenia/1281/2013	2	2013-03-19	MDCKx/MDCK1	2560	160	160	80	80	640	40	640	320	160
B/Leon/120/2013		2013-03-30	MDCK2	5120	320	160	40	160	80	80	80	320	80
B/Leon/121/2013	3	2013-04-02	MDCK2	2560	160	160	80	160	40	80	80	320	80
B/Slovenia/1613/2013	3	2013-04-15	MDCKx/MDCK1	2560	160	160	160	160	160	160	160	160	80
							Vaccine 2012-2013					Vaccine 2013-2014	

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 [6] 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 [7]. WHO is updating information on the outbreak regularly and ECDC is posting epidemiological updates [8]. A Rapid Risk Assessment [9] 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. As of 30 May 2013, WHO reported [10] 132 laboratory confirmed cases and 37 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 [11]
http://www.nimr.mrc.ac.uk/documents/about/Interim_Report_February_2013.pdf [12]

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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