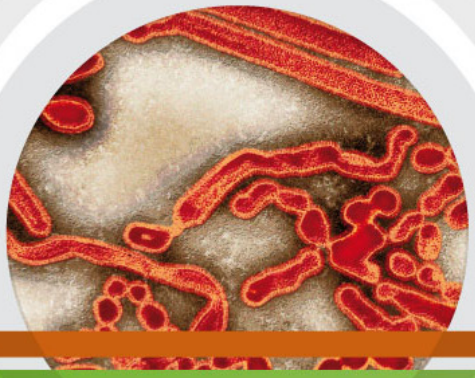


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, February 2013

Summary

During the 2012/2013 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 31 January 2013 have been received from twenty countries in the EU/EAA 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 (60%) and type B (40%) 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.

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 2012-09-01.

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	6							1	in process	5	5
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	4				
Belgium	41	1	13	in process	4	3				23	in process
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	in process	3	in process	3			5	in process
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						6	6	6	2	2
Slovenia	5		3	3				1	0	1	0
United Kingdom	18		2	2	11	in process		1	1	4	4
JANUARY											
Belgium	19	2	7	in process	1	1				9	in process
Denmark	3		1	1	2	2					
Estonia	21	2	9	in process	2	in process	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	4				1	1		1	1	2	2
Italy	17		13	13	1	1		1	1	2	2
Latvia	7		3	3	3	3				1	1
Malta	24		24	in process							
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	20		10	in process	6	in process	4				
	525	7	194	93	116	84	26	37	33	145	104
				37.0%		22.1%			7.0%		27.6%
20 Countries			317 (60%)					208 (40%)			

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 December report are shown in Tables 2 to 6. The vast majority of 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. However, one virus, A/Norway/2254/2012, showed at least four-fold reduced reactivity with seven of nine antisera in the panel , including the antiserum raised against the vaccine virus, compared with the titres of the homologous viruses. Sequence analysis of the HA gene of A/Norway/2254/2012 revealed a substitution G155E at in the HA. Other viruses that also carry substitutions or polymorphisms in this region of the HA are marked on the tables; most of the viruses carrying a substitution or polymorphism in this region show a reduction (two-fold or four-fold) in titre with antiserum raised against the panel of reference viruses. Substitutions in this region of the HA previously have been associated with low reactivity in HI assays since the early phase of the H1N1 pandemic of 2009; they commonly result from propagation of viruses in certain tissue culture cells.

Phylogenetic analysis of the HA gene of representative viruses (Figure 1) shows that the most recently detected H1N1 viruses from EU/EAA countries cluster predominantly within Groups 6 and 7, with a small number clustering in group 8.

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 F05/10	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	1280	640	1280	640	640	640	320	1280	640
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	320	80	40	<	40	40	40	40
A/Lviv/N6/2009		2009-10-27	MDCK4/SIAT1/MDCK2	640	1280	640	320	80	160	320	160	640
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	1280	5120	1280	1280	1280	2560	2560
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	320	160	320	320	640	640	640	1280	1280
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	640	640	1280	1280	1280	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	1280	640	640	640	1280	1280	1280	2560	2560
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	1280	640	1280	1280	1280	1280	1280	2560	2560
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	320	160	320	160	640	640	320	640	1280
TEST VIRUSES												
A/Slovenia/1905/2012	6	2012-12-05	MDCK1/MDCK1	1280	640	640	640	1280	1280	1280	2560	2560
A/Slovenia/1908/2012	6	2012-12-06	MDCKx/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	2560
A/Norway/2493/2012	6	2012-12-08	MDCK2	640	320	640	320	640	640	640	1280	1280
A/Norway/2531/2012	6	2012-12-11	MDCK2	640	320	640	640	1280	640	640	2560	2560
A/Norway/2552/2012	8	2012-12-17	MDCK2	1280	320	640	640	1280	640	1280	2560	2560
A/Lyon/2757/2012	7	2012-12-17	MDCK2/MDCK1	320	160	160	160	320	320	320	640	640
A/Norway/46/2013	6	2012-12-18	MDCK2	1280	320	640	640	1280	1280	1280	2560	2560
A/Norway/2667/2012	7	2012-12-19	MDCK2	640	320	320	320	640	640	640	1280	640
A/Norway/2620/2012	6	2012-12-20	MDCK2	1280	640	1280	1280	1280	1280	1280	5120	2560
A/England/658/2012	6	2012-12-20	MDCK1/MDCK1	1280	640	1280	640	1280	1280	1280	5120	2560
A/Lyon/2783/2012	6	2012-12-20	MDCK2/MDCK1	1280	640	1280	640	1280	1280	1280	2560	2560
A/Lyon-CHU/52.271/2013	7	2012-12-27	MDCK2/MDCK1	1280	640	640	1280	1280	1280	1280	5120	5120
A/Lyon/2/2013	7	2012-12-28	MDCK2/MDCK1	320	320	320	320	640	640	320	1280	1280
A/Norway/120/2013	8	2013-01-02	MDCK2	1280	640	640	1280	1280	1280	1280	5120	2560
A/Limoges/26/2013	6	2013-01-03	MDCK1/MDCK1	320	320	320	640	320	320	320	640	640

Sequences in phylogenetic trees — Vaccine

1. < = <40

Table 3 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 F05/10	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	640	640	1280	640	640	2560	640
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	320	80	40	40	40	80	40	40
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	640	160	160	160	320	160	640
A/Christchurch/16/2010	4	2010-07-12	E2/E2	2560	2560	1280	5120	2560	1280	2560	5120	320
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	320	640	640	1280	640	1280	2560	2560
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	640	1280	1280	1280	1280	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	2560	1280	1280	1280	2560	1280	2560	5120	5120
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	2560	640	1280	1280	2560	1280	2560	5120	5120
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	640	160	320	320	640	640	640	1280	1280
TEST VIRUSES												
A/Norway/2505/2012		2012-12-06	MDCK3	1280	640	1280	1280	2560	1280	2560	5120	2560
A/Norway/2607/2012		2012-12-06	MDCK2	2560	2560	1280	2560	2560	1280	2560	5120	5120
A/Norway/2494/2012		2012-12-08	MDCK2	2560	1280	1280	1280	2560	1280	2560	5120	5120
A/Lyon/2665/2012		2012-12-10	MDCK2/MDCK2	1280	640	640	640	1280	1280	1280	2560	2560
A/Norway/45/2013		2012-12-14	MDCK3	1280	640	1280	1280	2560	1280	2560	2560	2560
A/Berlin/167/2012	6	2012-12-14	C2/MDCK1	2560	640	1280	2560	2560	2560	2560	5120	5120
A/Slovenia/1940/2012	6	2012-12-14	MDCK2/SIAT2	640	320	640	640	1280	640	1280	2560	2560
A/Norway/48/2013		2012-12-15	MDCK3	1280	320	640	640	2560	2560	1280	5120	2560
A/Thüringen/37/2012		2012-12-17	C2/MDCK1	2560	640	1280	1280	2560	1280	2560	5120	2560
A/Thüringen/38/2012	6	2012-12-17	C2/MDCK1	2560	1280	2560	2560	2560	2560	2560	5120	5120
A/Thüringen/39/2012		2012-12-17	C2/MDCK1	2560	1280	2560	2560	2560	2560	2560	5120	5120
A/Baden Württemberg/173/2012	7	2012-12-17	C1/MDCK1	2560	1280	2560	2560	2560	2560	2560	5120	5120
A/Norway/62/2013		2012-12-25	MDCK3	1280	640	1280	1280	2560	1280	2560	5120	2560
A/Norway/130/2013	6	2012-12-27	MDCK3	1280	320	1280	640	1280	1280	2560	2560	2560
A/Norway/129/2013		2012-12-28	MDCK3	1280	640	1280	640	2560	2560	1280	5120	2560
A/Norway/51/2013	6	2012-12-30	MDCK3	1280	320	1280	1280	1280	1280	1280	5120	2560
A/Norway/56/2013		2012-12-30	MDCK2	2560	1280	1280	1280	2560	2560	2560	5120	1280
A/Norway/29/2013		2012-12-31	MDCK3	1280	1280	1280	2560	1280	1280	2560	5120	2560
A/Norway/35/2013	6	2013-01-02	MDCK3	1280	640	1280	1280	2560	1280	2560	2560	2560
A/Norway/108/2013	6	2013-01-03	MDCK3	1280	640	1280	1280	1280	2560	1280	2560	2560
A/Sachsen/1/2013	6	2013-01-03	C2/MDCK1	2560	1280	2560	2560	2560	2560	2560	5120	2560
Sequences in phylogenetic trees				Vaccine								

Table 4 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 F05/10	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	1280	640	1280	640	640	640	640	1280	1280
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	160	80	40	<	40	40	<	40
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	1280	640	160	80	160	160	160	320
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	1280	5120	1280	1280	1280	2560	2560
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	320	320	640	320	1280	640	640	1280	1280
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	320	640	640	1280	640	1280	2560	2560
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	1280	640	640	640	640	1280	640	2560	2560
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	1280	640	1280	640	1280	1280	1280	5120	2560
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	160	80	160	160	320	320	320	640	1280
TEST VIRUSES												
A/Paris/1667/2012		2012-11-01	MDCK2/MDCK1	640	640	640	640	1280	640	1280	2560	1280
A/Paris/1773/2012		2012-11-20	MDCK1/MDCK1	640	320	640	640	1280	1280	1280	2560	1280
A/Strasbourg/1866/2012	6	2012-11-21	MDCK2/MDCK1	1280	640	640	1280	1280	1280	1280	5120	2560
A/Paris/1836/2012		2012-11-28	MDCK2/MDCK1	640	320	320	320	640	320	640	1280	1280
A/Paris/1878/2012	8	2012-11-29	MDCK2/MDCK1	1280	640	640	640	1280	1280	1280	2560	2560
A/Berlin/166/2012		2012-12-11	C1/MDCK2	1280	1280	1280	1280	2560	1280	2560	2560	5120
A/England/661/2012		2012-12-21	SIAT1/MDCK3	1280	640	640	640	1280	1280	1280	2560	2560
A/Athens GR/1/2013	6	2013-01-02	MDCK2	640	320	640	640	1280	1280	1280	2560	1280
A/Niedersachsen/1/2013	6	2013-01-07	C1/MDCK2	1280	640	640	1280	1280	1280	1280	2560	2560
A/Lisboa/EuroEva42/2013	6	2013-01-08	MDCK2	640	320	640	320	640	640	1280	1280	1280
Sequences in phylogenetic trees				Vaccine								

1. < = < 40

Table 5 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 F05/10	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	1280	640	1280	640	640	640	640	640	640	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	160	80	40	40	40	40	40	40	
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	1280	640	160	80	160	160	160	320	
A/Christchurch/16/2010	4	2010-07-12	E2/E2	2560	1280	2560	5120	2560	1280	1280	2560	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	320	160	320	320	640	640	320	640	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	320	1280	640	1280	1280	640	2560	2560	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E2	1280	640	1280	640	1280	1280	1280	2560	2560	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	1280	640	1280	1280	1280	1280	1280	2560	2560	
A/Hong Kong/5659/2012	6	2012-05-21	MDCK4/MDCK1	320	160	320	320	640	640	320	1280	1280	
TEST VIRUSES													
A/Norway/2289/2012		2012-11-07	LLCMK2/MDCK1/MDCK1	1280	640	1280	1280	1280	1280	2560	2560	2560	
A/Belgium/G860/2012	7	2012-11-08	MDCK2	1280	640	1280	640	1280	1280	1280	2560	2560	
A/Norway/2254/2012		2012-11-09	MDCK2/MDCK2	160	640	320	80	80	80	160	80	320	G155E
A/Pavia/175/2012		2012-11-12	Cx/MDCK1	1280	640	640	640	1280	1280	1280	2560	2560	
A/Denmark/76/2012		2012-11-22	MDCK3/MDCK1	1280	640	1280	1280	2560	2560	1280	5120	2560	
A/Parma/158/2012		2012-11-23	MDCK2/MDCK1	1280	640	1280	1280	1280	1280	1280	2560	2560	
A/Belgium/G906/2012		2012-12-03	MDCK2	1280	640	1280	1280	2560	2560	2560	2560	2560	
A/Belgium/G916/2012		2012-12-07	MDCK2	2560	1280	1280	1280	2560	2560	2560	5120	5120	
A/Belgium/G917/2012	7	2012-12-07	MDCK2	640	320	640	640	1280	640	640	2560	2560	
A/Belgium/G932/2012		2012-12-10	MDCK2	1280	640	640	640	1280	1280	1280	2560	2560	
A/Norway/2597/2012		2012-12-16	MDCK4	2560	640	1280	640	2560	2560	1280	5120	2560	
A/Latvia/12-39425p/2012		2012-12-17	MDCK2/MDCK1	1280	640	1280	1280	1280	1280	1280	5120	2560	
A/Pavia/1/2013		2013-01-01	Cx/MDCK1	1280	640	1280	1280	1280	1280	2560	2560	2560	
A/Latvia/1-30371p/2013		2013-01-02	MDCK1/MDCK1	640	640	1280	640	1280	1280	1280	2560	2560	
A/Roma/01/2013		2013-01-02	Cx/MDCK1	1280	640	1280	1280	2560	1280	2560	2560	2560	
A/Navarra/19/2013		2013-01-02	E2/E1	1280	1280	1280	1280	1280	1280	1280	2560	2560	
A/Estonia/74192/2013		2013-01-02	MDCK2/MDCK1	640	320	640	640	1280	640	640	2560	2560	
A/Denmark/08/2013		2013-01-02	MDCK1/MDCK1	1280	320	640	640	2560	2560	1280	2560	2560	
A/Roma/05/2013		2013-01-03	Cx/MDCK2	640	320	640	320	1280	1280	1280	2560	2560	
A/Roma/06/2013		2013-01-04	Cx/MDCK2	1280	320	1280	1280	1280	1280	1280	2560	2560	
A/Roma/07/2013		2013-01-04	Cx/MDCK1	1280	640	640	1280	2560	2560	2560	5120	2560	
A/Acores/HAH480803/2012	6	2013-01-07	MDCK3	2560	640	1280	1280	2560	2560	2560	5120	5120	
A/Acores/HAH7518/2012	6	2013-01-07	MDCK2	640	640	640	1280	1280	1280	1280	2560	2560	
A/Latvia/1-36685/2013		2013-01-11	MDCK1/MDCK1	640	320	640	640	1280	640	640	2560	2560	
A/Navarra/111/2013		2013-01-11	SIAT1/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	
A/Madrid/61/2013		2013-01-14	SIAT1/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	2560	
A/Baleares100/2013		2013-01-14	SIAT1/MDCK1	640	320	640	640	1280	1280	640	1280	1280	
A/Navarra/108/2013		2013-01-14	SIAT1/MDCK1	1280	640	1280	640	1280	2560	1280	2560	2560	
A/Latvia/1-40822p/2013		2013-01-18	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	5120	5120	
A/Finland/30820/13	6	2013-01-21	MDCK1/MDCK1	1280	640	1280	1280	1280	1280	1280	5120	5120	
A/Finland/314/2013		2013-01-23	MDCK1/MDCK1	1280	640	1280	1280	1280	1280	1280	2560	2560	
A/Finland/302/2013	6	2013-01-24	MDCK3/MDCK1	320	320	320	320	160	320	320	320	640	G155X
Sequences in phylogenetic trees				Vaccine									

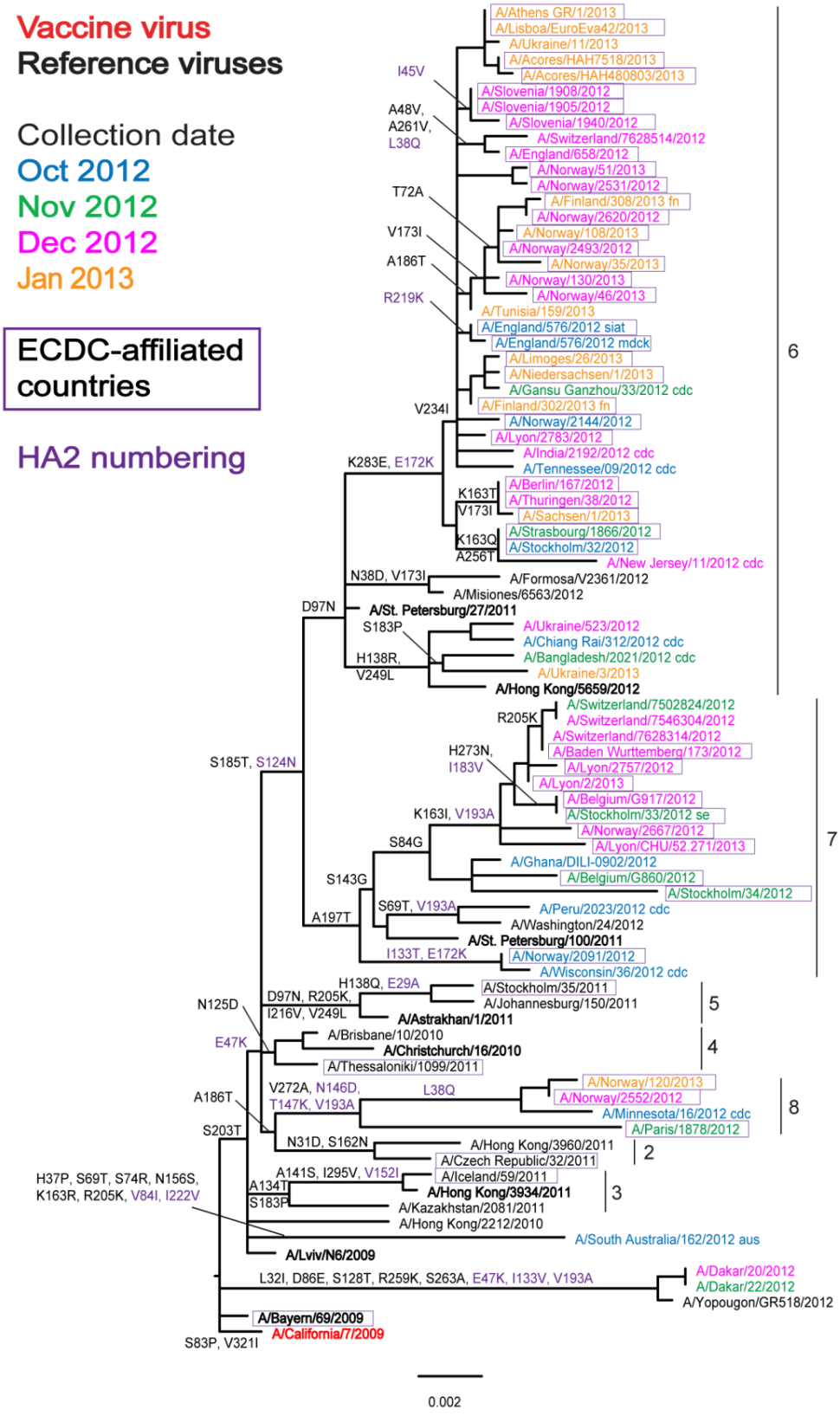
Table 6 Antigenic analysis of A(H1N1)pdm09 viruses by HI

			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 N6/2009 C4/34/09	A/Chch 16/10 F30/10 Group 4	A/HK 3934/11 F21/11 Group 3	A/Astrak 1/11 F22/11 Group 5	A/St. P 27/11 F23/11 Group 6	A/St. P 100/11 F24/11 Group 7	A/HK 5659/12 F30/12 Group 6	
REFERENCE VIRUSES												
A/California/7/2009	2009-04-09	E1/E2	1280	640	1280	640	640	640	640	640	640	
A/Bayern/69/2009	2009-07-01	MDCK5/MDCK1	80	160	80	40	40	40	40	40	40	
A/Lviv/N6/2009	2009-10-27	MDCK4/S1/MDCK3	320	1280	640	160	80	160	160	160	320	
A/Christchurch/16/2010	2010-07-12	E2/E2	2560	1280	2560	5120	2560	1280	1280	2560	2560	
A/Hong Kong/3934/2011	2011-03-29	MDCK2/MDCK3	320	160	320	320	640	640	320	640	640	
A/Astrakhan/1/2011	2011-02-28	MDCK1/MDCK5	1280	320	1280	640	1280	1280	640	2560	2560	
A/St. Petersburg/27/2011	2011-02-14	E1/E2	1280	640	1280	640	1280	1280	1280	2560	2560	
A/St. Petersburg/100/2011	2011-03-14	E1/E2	1280	640	1280	1280	1280	1280	1280	2560	2560	
A/Hong Kong/5659/2012	2012-05-21	MDCK4/MDCK1	320	160	320	320	640	640	320	1280	1280	
TEST VIRUSES												
A/Galicia/RR9910/2012	2012-03-08	SIAT1/SIAT3	2560	1280	2560	2560	5120	2560	5120	5120	5120	
A/Norway/2259/2012	2012-11-09	MDCK2/MDCK3	640	320	160	80	80	160	80	80	80	seq pending
A/Norway/2352/2012	2012-11-13	MDCK1/MDCK2	1280	1280	1280	1280	2560	2560	2560	5120	5120	
A/Norway/2362/2012	2012-11-26	MDCK1/MDCK2	1280	1280	1280	2560	2560	2560	2560	5120	5120	
A/Norway/2383/2012	2012-11-26	MDCK1/MDCK2	1280	1280	1280	1280	2560	2560	5120	5120	5120	
A/Norway/2403/2012	2012-11-28	MDCK1/MDCK2	1280	1280	1280	1280	2560	1280	5120	5120	5120	
A/Norway/2411/2012	2012-11-30	MDCK1/MDCK2	640	320	640	1280	1280	1280	1280	2560	2560	
A/Norway/2388/2012	2012-11-26	MDCK1/MDCK2	1280	640	640	320	80	320	160	160	320	N156D
A/Paris/1885/2012	2012-12-05	MDCK2/MDCK1	640	320	640	640	1280	1280	640	2560	2560	
A/Netherlands/529/2012	2012-12-16	MDCK2/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	
A/Centre/1976/2012	2012-12-17	MDCK1/MDCK1	1280	1280	1280	1280	2560	1280	2560	5120	5120	
A/Netherlands/068/13	2013-01-07	MDCK2/MDCK1	640	320	640	640	1280	1280	1280	2560	2560	
A/Parma/01/2013	2013-01-07	MDCK2/MDCK1	640	640	640	640	1280	640	1280	2560	1280	
A/Parma/06/2013	2013-01-07	MDCK2/MDCK1	640	640	640	1280	1280	1280	1280	2560	2560	
A/Parma/02/2013	2013-01-08	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	5120	5120	
A/Parma/04/2013	2013-01-09	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	2560	5120	5120	
A/Trieste/02/2013	2013-01-10	Cx/MDCK1	1280	640	1280	1280	640	1280	1280	5120	5120	
A/Trieste/04/2013	2013-01-10	Cx/MDCK1	1280	640	1280	1280	2560	1280	2560	5120	2560	
A/Parma/03/2013	2013-01-14	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	5120	2560	
A/Trieste/03/2013	2013-01-14	Cx/MDCK1	640	1280	1280	1280	2560	1280	1280	5120	2560	
A/Dolj/131915/2013	2013-01-14	MDCK2/MDCK1	1280	1280	1280	1280	1280	1280	2560	2560	2560	
A/Estonia/74590/2013	2013-01-17	MDCK1/MDCK3	640	640	640	640	1280	1280	1280	2560	2560	
A/Iasi/132200/2013	2013-01-20	MDCK1/MDCK1	1280	1280	1280	1280	2560	1280	2560	2560	2560	
A/Dolj/132523/13	2013-01-21	MDCK1/MDCK1	1280	640	1280	1280	1280	1280	2560	2560	2560	
A/Dolj/132532/2013	2013-01-22	MDCK1/MDCK1	1280	640	1280	1280	2560	1280	1280	2560	2560	
A/Estonia/74833/2013	2013-01-25	SIAT2	320	640	320	160	80	160	320	160	320	G155X

1. < = <40

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 analysis of recently collected viruses is shown in Tables 7 to 9. HI assays were carried out using guinea pig red blood cells in the presence of 20nM oseltamivir, added to circumvent the NA-mediated binding of H3N2 viruses to the red blood cells (Lin et al. 2010). The vast majority of test viruses reacted poorly with post-infection ferret antiserum raised against the currently recommended egg-propagated vaccine virus, A/Victoria/361/2011, compared with the titre of 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 antisera raised against egg-propagated A/Texas/50/2012, compared with the titre of the antiserum to the homologous virus - in these tables antisera reacted with titres within four-fold of that for A/Texas/50/2012 in ~ 40% of the test viruses.

The test viruses, with very few exceptions, reacted well with antisera raised against reference viruses exclusively propagated in cells when compared with the titres of the homologous viruses. These antisera were raised against an isolate of A/Victoria/361/2011 cultivated in MDCK cells in parallel with the isolation of the egg-propagated virus, 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 the EU/EAA collected since 1 September 2012 have HA genes that fall into genetic groups 5 and 3C, with one exception in the figure, a virus from Sweden (A/Stockholm/39/2012) falling into group 3A.

The amino acid substitutions in the HA that are associated with each of 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, T48I, A198S, V223I** & **N312S,** e.g. the prototype vaccine virus A/Victoria/361/2011; the great majority of viruses also carry the substitutions **Q33R, N278K** (e.g. A/Berlin/93/2011) and **N145S**; an emerging sub-group also carries the substitutions **T128A** (resulting in the loss of a potential glycosylation site) and **R142G**;
  - Group 3A: **N144D** (resulting in the loss of a potential glycosylation site);
- Group 5 viruses: **K2E, N8D** (resulting in the loss of a potential glycosylation site), **D53N, Y94H, I230V** & **E280A** in combination with the substitution **D158N** in **HA2.**

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

Table 7 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	F36/12	F37/12
						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	320	160	160	320	320	640	640	160
A/Victoria/208/2009		2009-06-02	E3/E2	640	5120	2560	1280	2560	2560	2560	1280	2560	5120	2560
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT3	40	<	160	80	160	80	160	160	320	320	80
A/Stockholm/18/2011	3A	2011-03-28	MDCK2/SIAT5	80	80	40	320	160	160	320	320	640	640	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	320	640	640	1280	2560	640	2560	1280	2560	5120	1280
A/Victoria/361/2011	3C	2011-10-24	E3/E2	320	320	640	160	640	2560	640	320	320	5120	1280
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT3	320	160	80	160	160	160	640	640	640	1280	320
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT3	160	160	80	320	320	160	640	640	1280	1280	640
A/Athens/112/2012	3B	2012-02-01	SIAT5	80	160	80	320	160	320	640	320	1280	1280	320
A/Texas/50/2012	3C	2012-04-15	E5/E1	320	640	640	1280	1280	1280	2560	640	1280	5120	2560
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	320	640	640	640	1280	640	1280	640	2560	5120	5120
TEST VIRUSES														
A/Poitiers/2407/2012	3C	2012-10-24	MDCK2/SIAT1	<	40	<	80	40	40	160	160	160	320	80
A/Niedersachsen/31/2012		2012-11-21	C2/SIAT1	40	80	160	160	160	160	640	160	640	640	320
A/England/625/2012	3C	2012-12-04	MDCK1/SIAT1	<	40	80	320	160	160	640	320	640	640	320
A/Lyon/2623/2012		2012-12-05	MDCK3/SIAT1	<	40	80	160	160	80	320	160	320	320	160
A/Berlin/165/2012	3C	2012-12-05	C2/SIAT1	<	40	40	160	80	80	320	80	320	640	320
A/Poitiers/2745/2012	3C	2012-12-06	MDCK2/SIAT1	<	40	<	80	40	40	320	160	160	320	80
A/Lyon/2633/2012		2012-12-10	MDCK2/SIAT1	40	80	160	320	160	160	640	640	640	1280	320
A/Bremen/18/2012		2012-12-10	C2/SIAT1	80	80	320	160	320	160	640	160	640	1280	320
A/England/668/2012	3C	2012-12-17	MDCK1/SIAT1	<	40	80	160	80	80	320	320	320	640	320
A/Lyon/2743/2012		2012-12-17	MDCK2/SIAT1	<	40	80	160	80	80	320	160	640	640	320
A/Bremen/19/2012		2012-12-17	C2/SIAT1	80	80	320	320	320	160	640	160	640	1280	320
A/Schleswig Holstein/7/2012	3C	2012-12-17	C2/SIAT1	40	80	80	320	160	160	640	160	640	640	320
A/Mecklenburg Vorpommern/10/2012	5	2012-12-17	C2/SIAT1	40	40	160	160	320	160	640	160	320	640	320
A/England/670/2012	3C	2012-12-18	MDCK1/SIAT1	<	40	40	160	40	40	320	160	320	640	160
A/Lyon/2763/2012	3C	2012-12-19	MDCK2/SIAT1	<	40	40	160	80	80	320	160	320	320	160
A/Niedersachsen/33/2012		2012-12-19	C2/SIAT1	40	80	80	320	160	160	640	160	640	640	320
A/Berlin/170/2012		2012-12-20	C2/SIAT1	40	80	160	320	160	160	640	160	640	1280	320
A/Berlin/171/2012		2012-12-20	C2/SIAT1	80	80	160	320	160	160	640	160	640	1280	320
A/Thüringen/41/2012		2012-12-20	C2/SIAT1	40	80	80	320	160	160	640	160	640	640	320
A/Annecy/2809/2013		2012-12-21	MDCK2/SIAT1	<	40	40	160	80	80	320	320	320	640	160
A/Brandenburg/3/2012		2012-12-21	C2/SIAT1	40	80	160	320	160	160	640	160	640	1280	320
A/Annecy/2806/2012	3C	2012-12-22	MDCK2/SIAT1	<	40	80	160	80	80	640	320	640	640	320
A/Clermont-Ferrand/14/2013	3C	2012-12-23	MDCK2/SIAT1	<	40	40	160	40	80	320	320	320	640	160
A/Lyon-CHU/52.123/2012		2012-12-24	MDCK2/SIAT1	<	80	80	320	160	160	320	160	640	640	320
A/Lyon-CHU/52.131/2012	3C	2012-12-24	MDCK2/SIAT1	<	<	40	80	40	80	160	80	320	320	160
A/Mecklenburg Vorpommern/13/2012		2012-12-27	C2/SIAT1	160	160	320	320	640	320	640	320	1280	1280	640
A/Mecklenburg Vorpommern/12/2012	5	2012-12-27	C2/SIAT1	40	80	320	160	320	160	640	160	320	640	320
A/England/676/2012	3C	2012-12-29	SIAT1/SIAT1	<	40	40	160	40	80	320	320	320	640	160
A/Nordrhein-Westfalen/1/2013	3C	2013-01-03	C1/SIAT1	<	40	80	160	160	80	640	80	640	640	320
A/Sachsen/2/2013	3C	2013-01-07	C1/SIAT2	160	80	160	320	160	160	640	640	640	1280	640
Sequences in phylogenetic trees									Vaccine		Vaccine			

1. < = <40

Table 8 Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

				Haemagglutination inhibition titre[1]										
				Post infection ferret sera										
Viruses		Collection Date	Passage History	A/Perth 16/09	A/Vic 208/09	A/Ala 5/10	A/Stock 18/11	A/Iowa 18/10	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 F24/12	F16/12	F36/12	F37/12
Genetic group	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	1280	80	320	160	320	320	320	320	640	320	160
A/Victoria/208/2009		2009-06-02	E3/E2	1280	2560	1280	1280	2560	1280	2560	1280	2560	5120	5120
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT3	<	<	80	80	80	80	160	160	160	160	40
A/Stockholm/18/2011	3A	2011-03-23	SIAT4	80	160	160	640	320	160	640	640	640	640	320
A/Iowa/15/2010	6	2010-12-30	E3/E2	320	640	640	1280	1280	1280	1280	1280	1280	2560	1280
A/Victoria/36 1/2011	3C	2011-10-24	E3/E2	320	640	320	160	640	2560	640	320	320	2560	1280
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT3	160	160	320	320	320	320	640	640	640	1280	640
A/Victoria/36 1/2011	3C	2011-10-24	MDCK2/SIAT2	160	160	320	320	320	320	640	640	640	1280	640
A/Athens/112/2012	3B	2012-02-01	SIAT7	160	160	320	320	320	320	640	640	640	1280	640
A/Texas/50/2012	3C	2012-04-15	E5/E1	640	1280	640	1280	1280	640	1280	640	1280	5120	2560
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	320	1280	320	640	1280	640	1280	640	1280	2560	5120
TEST VIRUSES														
A/Norway/2102/2012		2012-10-15	SIAT1/SIAT1	80	160	160	320	160	160	640	640	640	640	320
A/Norway/2103/2012		2012-10-22	SIAT1/SIAT1	80	160	160	320	160	160	640	640	640	640	320
A/Paris/1654/2012		2012-10-26	MDCK2/SIAT1	80	160	160	320	160	160	640	320	320	640	320
A/Paris/1651/2012	3C	2012-10-29	MDCK2/SIAT2	80	160	80	320	160	160	640	640	640	1280	320
A/Acores/SU43/2012	5	2012-11-06	SIAT2	160	160	320	320	320	320	640	640	640	1280	320
A/Austria/705367/2012	5	2012-11-06	SIAT1/SIAT1	160	160	320	320	320	160	640	640	320	1280	320
A/Lyon/2456/2012	3C	2012-11-19	MDCK2/SIAT3	80	160	160	160	160	160	320	640	640	640	160
A/Norway/2423/2012		2012-11-26	MDCK1/SIAT1	80	160	160	320	160	160	640	320	640	1280	640
A/Austria/709773/2012	3C	2012-12-06	SIAT1/SIAT1	80	160	320	320	320	160	640	640	640	1280	640
A/Paris/1512/2012	5	2012-12-07	MDCK2/SIAT1	40	40	80	160	80	80	320	160	160	320	160
A/Norway/2456/2012	3C	2012-12-10	SIAT2	40	80	160	320	160	160	640	640	640	640	320
A/Austria/710638/2012	5	2012-12-11	SIAT1/SIAT1	160	160	640	640	640	320	1280	1280	1280	1280	640
A/Paris/1966/2012		2012-12-12	MDCK1/SIAT1	40	80	80	320	80	160	320	320	320	640	320
A/Paris/1938/2012		2012-12-18	MDCK1/SIAT1	80	80	160	320	160	160	640	640	320	1280	320
A/Nord Pas de Calais/2000/2012	3C	2012-12-19	MDCK1/SIAT1	40	80	40	320	160	160	320	320	640	640	320
A/Haute Normandie/2022/2012	3C	2012-12-19	MDCK1/SIAT1	<	80	80	160	80	160	320	160	320	640	160
A/Ireland/0046 2/2012		2012-12-20	MDCK1/SIAT1	80	80	160	320	320	320	640	320	640	1280	320
A/Belgium/S0304/2012		2012-12-21	SIAT2	640	320	320	640	640	320	1280	640	1280	2560	640
A/Belgium/G1010/2012		2012-12-24	SIAT2	40	160	160	320	320	160	640	320	640	640	320
A/Belgium/S0325/2012		2012-12-26	SIAT2	40	80	80	160	160	160	320	320	640	640	160
A/Lisboa/SU15/2012	3C	2013-01-04	SIAT2	40	80	80	320	160	80	640	320	640	640	320
A/Latvia/1-32593/2013		2013-01-06	MDCK1/SIAT1	40	160	160	320	160	320	640	320	640	640	320
A/Madeira/MS3/2012		2013-01-07	SIAT2	40	80	80	320	160	80	640	320	320	640	320
A/Belgium/G0044 /2013		2013-01-07	SIAT2	40	80	160	320	160	160	640	320	640	640	320
A/Athens GR/14/2013	3C	2013-01-08	SIAT2	<	80	40	160	80	80	320	160	320	640	160
A/Latvia/1-37023/2013		2013-01-13	MDCK1/SIAT1	40	160	160	320	160	160	640	320	640	1280	640
Sequences in phylogenetic trees									Vaccine		Vaccine			

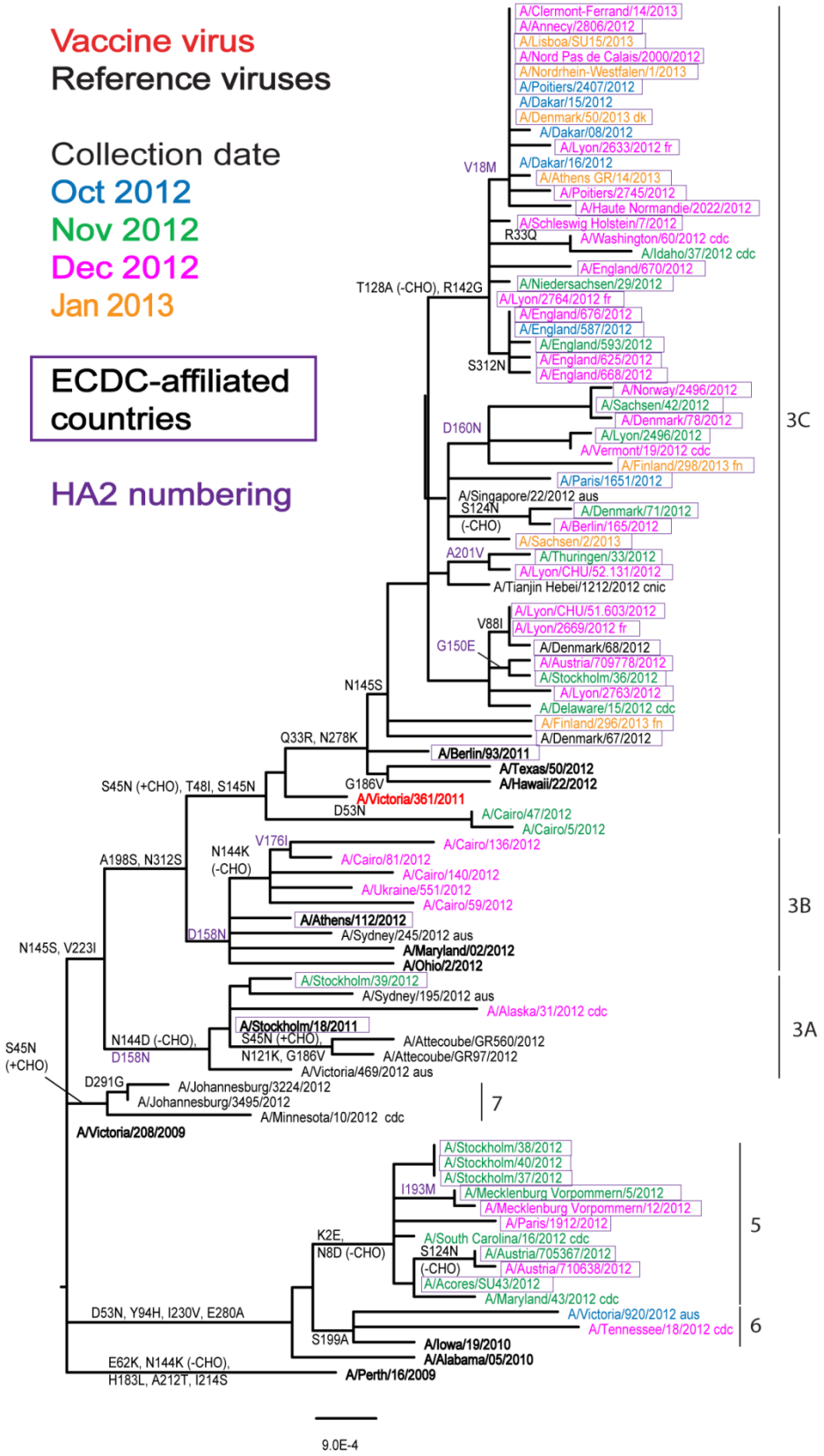
1. <= <40

Table 9 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/16/2009	A/Vic/208/2009	A/Ala/5/10	A/Stock/18/11	A/Iowa/15/11	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	F37/12
	Genetic group					group 5	group 3A	group 6	group 3C	group 3C	group 3C	group 3B	group 3C	group 3C
REFERENCE VIRUSES														
A/Perth/16/2009		2009-07-04	E3/E2	640	40	80	80	160	160	320	160	320	320	80
A/Victoria/208/2009		2009-06-02	E3/E2	640	2560	1280	2560	2560	2560	5120	1280	2560	5120	2560
A/Alabama/5/2010	5	2010-07-13	MK1/C2/SIAT2	<	<	80	80	80	80	160	160	160	160	40
A/Stockholm/13/2011	3A	2011-03-28	SIAT4	40	80	80	320	160	80	640	320	640	640	320
A/Iowa/15/2010	6	2010-12-30	E3/E2	320	640	640	1280	1280	640	1280	1280	2560	5120	1280
A/Victoria/361/2011	3C	2011-10-24	E3/E2	320	640	160	80	640	2560	1280	320	160	2560	1280
A/Berlin/93/2011	3C	2011-12-07	NVD3/SIAT6	80	80	160	320	320	320	640	320	640	2560	320
A/Victoria/361/2011	3C	2011-10-24	MDCK2/SIAT2	80	80	160	320	320	160	640	640	640	640	320
A/Athens/112/2012	3B	2012-02-01	SIAT8	40	80	160	160	160	160	320	320	640	640	320
A/Texas/50/2012	3C	2012-04-15	E5/E1	320	1280	320	1280	1280	1280	2560	640	1280	5120	1280
A/Hawaii/22/2012	3C	2012-07-09	E4/E1	320	640	320	640	1280	640	1280	640	1280	5120	2560
TEST VIRUSES														
A/Gallicia/15/2012		2012-02-07	SIAT2/SIAT1	80	80	160	320	320	160	640	320	640	1280	320
A/Gallicia/RR5908/2012		2012-03-09	SIAT2/SIAT1	80	80	320	160	320	320	640	320	320	1280	320
A/Gallicia/RR5910/2012		2012-03-08	SIAT1/SIAT3	320	320	640	640	640	640	1280	1280	1280	2560	320
A/Gallicia/RR5907/2012		2012-03-09	SIAT1/SIAT2	160	160	320	320	320	320	1280	320	640	1280	640
A/Gallicia/RR5911/2012		2012-04-18	SIAT2/SIAT2	640	640	640	1280	1280	640	2560	1280	1280	2560	1280
A/Arges/126697/2012		2012-10-29	MDCK3/SIAT1	<	80	40	160	160	80	640	320	320	640	320
A/Trieste/65/2012		2012-11-26	Cx/SIAT1	80	160	160	320	320	320	640	640	640	1280	640
A/Madrid/323/2012		2012-11-30	SIAT1/SIAT1	40	80	80	320	320	160	640	320	640	640	320
A/Lisboa/SU51/2012		2012-12-11	SIAT3	40	40	160	160	160	80	320	160	320	320	160
A/Netherlands/555/2012		2012-12-17	MDCK2/SIAT1	40	160	160	320	160	40	320	320	320	640	320
A/Netherlands/533/2012		2012-12-20	MDCK2/SIAT1	40	160	160	320	320	160	640	320	640	640	320
A/Roma/02/2013		2012-12-31	Cx/SIAT1	<	<	<	80	40	40	320	80	160	320	80
A/Roma/03/2013		2012-12-31	Cx/SIAT1	<	40	40	160	80	80	320	160	320	640	80
A/Roma/04/2013		2012-12-31	Cx/SIAT1	<	40	<	160	160	80	320	320	320	640	160
A/Latvia/1-3002 1/2013		2013-01-01	MDCK1/SIAT3	80	160	160	160	160	160	640	320	640	640	640
A/Ireland/00367/2013		2013-01-02	MDCK2/SIAT2	160	160	160	320	320	320	1280	640	640	1280	640
A/Trieste/01/2013		2013-01-04	Cx/SIAT1	80	160	160	640	320	320	1280	320	640	1280	640
A/Denmark/60/2013		2013-01-07	SIAT2/SIAT1	<	40	40	160	160	160	640	320	640	640	320
A/Denmark/20/2013		2013-01-10	SIAT2/SIAT1	<	80	80	320	160	160	640	320	320	640	160
A/Israel/24/2013		2013-01-14	P1/SIAT1	40	80	80	160	80	160	640	320	320	640	320
A/Finland/311/2013		2013-01-23	SIAT1/SIAT1	40	80	80	320	160	160	640	320	640	640	320
A/Finland/296/2012	3C	2013-01-24	SIAT1MDCK1SIAT1/SIAT1	40	80	80	160	160	80	640	320	320	320	320
A/Finland/293/2012	3C	2013-01-24	SIAT1MDCK1SIAT1/SIAT1	40	80	40	160	80	80	640	320	640	640	160
Sequences in phylogenetic trees									Vaccine		Vaccine			

1 <= <40

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



Influenza B virus analyses

B/Victoria-lineage virus

Tables 10-12 show the results of antigenic analyses for viruses of the B/Victoria lineage. The majority of 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 2010-2011 season and a recommended component of quadrivalent vaccines for the 2013/2014 northern hemisphere influenza season. The test viruses showed a similar reduction in reactivity with antiserum 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. The vast majority of recently collected viruses received from EU and EAA laboratories carried HA genes that fell into clade 1A with a small number falling into clade 1B. The amino acid substitution associated with the separation of clade 1 into clades 1A and 1B, L58P, has no discernible effect on antigenicity. The HA of recent viruses show only a small number of amino acid substitutions compared with that of B/Brisbane/60/2008.

Table 10 Antigenic analysis of influenza B viruses (Victoria lineage) by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1]							
					Post infection ferret sera						
				B/Bris[2] 60/08 Sh 523	B/Mal 2506/04 F28/05	B/Eng 393/08 F05/11	B/Bris 60/08 F22/12	B/Paris 1762/08 F17/11	B/HK 514/09 F13/10	B/Odessa 3886/10 F19/11	B/Malta 636714/11 F33/11
	1A			1A		1A	1A	1A	1B	1B	1A
REFERENCE VIRUSES											
B/Malaysia/2506/2004		2004-12-06	E3/E6	640	640	40	80	<	<	<	80
B/England/393/2008	1A	2008-08-29	E1/E2	2560	80	160	320	40	20	40	320
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	80	160	320	40	20	40	320
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	10	20	40	40	80	40	20
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	2560	<	10	20	80	80	160	10
B/Odessa/3886/2010	1B	2010-03-19	MDCK2/MDCK4	2560	<	20	20	80	160	160	20
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	160	320	40	20	40	320
TEST VIRUSES											
B/England/626/2012	1A	2012-12-06	SIAT1/MDCK1	2560	20	20	40	80	80	80	20
B/Lyon/2696/2012		2012-12-06	MDCK3/MDCK1	2560	10	20	40	80	80	80	40
B/Lyon/2664/2012		2012-12-11	MDCK2/MDCK1	2560	20	20	40	80	80	80	20
B/Poitiers/2821/2012	1A	2012-12-12	MDCK1/MDCK1	2560	<	20	40	80	80	80	40
B/Lyon/2673/2012		2012-12-13	MDCK2/MDCK1	2560	20	80	80	40	40	40	80
B/Lyon-CHU/50.569/2012		2012-12-13	MDCK2/MDCK1	2560	<	20	40	80	80	80	20
B/Lyon/2760/2012	1A	2012-12-17	MDCK2/MDCK1	1280	<	20	40	80	40	80	20
B/Sachsen/26/2012	1A	2012-12-18	C2/MDCK1	2560	20	40	40	80	160	80	40
B/LisboaPT/8/2012	1A	2012-12-18	SIAT1/MDCK1	2560	80	40	80	80	160	160	80
B/Valladolid/96/2012	1A	2012-12-26	MDCK1/MDCK1	2560	10	10	20	80	40	80	20
B/Bayern/1/2013	1A	2013-01-03	C2/MDCK1	1280	<	10	20	40	40	40	10
Sequences in phylogenetic trees											

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

Table 11 Antigenic analysis of influenza B viruses (Victoria lineage) by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1]									
				Post infection ferret sera									
				B/Bris[2] 60/08 Sh 523	B/Mal 2506/05 F28/05	B/Eng 393/08 F05/11 1A	B/Bris 60/08 F22/12 1A	B/Paris 1762/08 F17/11 1A	B/HK 514/09 F13/10 1B	B/Odessa 3886/10 F19/11 1B	B/Malta 636714/11 F33/11 1A	B/Jhb 3964/12 F01/13 1A	B/For V2367/12 F04/13 1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	1280	40	80	<	<	<	80	160	80
B/England/393/2008	1A	2008-08-29	E1/E2	2560	80	160	160	20	20	20	160	160	160
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	80	160	320	40	40	40	160	320	320
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	1280	10	10	20	20	40	40	20	40	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	<	10	40	40	80	80	20	40	80
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	1280	20	40	80	20	40	40	80	80	160
B/Malta/636714/2011	1A	2011-03-07	E4	1280	80	160	320	40	40	40	320	640	640
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E1	1280	320	320	640	40	40	40	320	640	640
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	2560	10	20	80	40	80	40	40	160	160
TEST VIRUSES													
B/Belgium/G886/2012		2012-11-12	MDCK2	2560	<	20	40	40	40	40	40	80	160
B/Burgos/95/2012	1A	2012-11-14	MDCK2	2560	10	10	20	40	40	40	20	40	80
B/Finland/293/2012	1A	2012-11-20	MDCK2/MDCK1	2560	<	20	20	40	40	40	40	80	80
B/Andalucia/359/2012		2012-12-03	SIAT1/MDCK1	2560	<	20	20	40	80	40	40	80	80
B/Andalucia/358/2012		2012-12-04	SIAT1/MDCK1	2560	<	10	20	40	80	40	20	80	80
B/Valladolid/99/2012	1A	2012-12-20	MDCK2	2560	10	20	40	40	40	40	40	80	80
B/Ireland/00125/2013		2013-01-03	MDCK1/MDCK1	2560	<	10	20	40	40	40	40	80	80
B/Roma/01/2013		2013-01-07	Cx/MDCK1	2560	10	20	40	40	80	40	40	80	160
B/Finland/310/2013		2013-01-23	MDCK1/MDCK1	2560	<	20	40	40	40	40	40	80	80

Sequences in phylogenetic trees

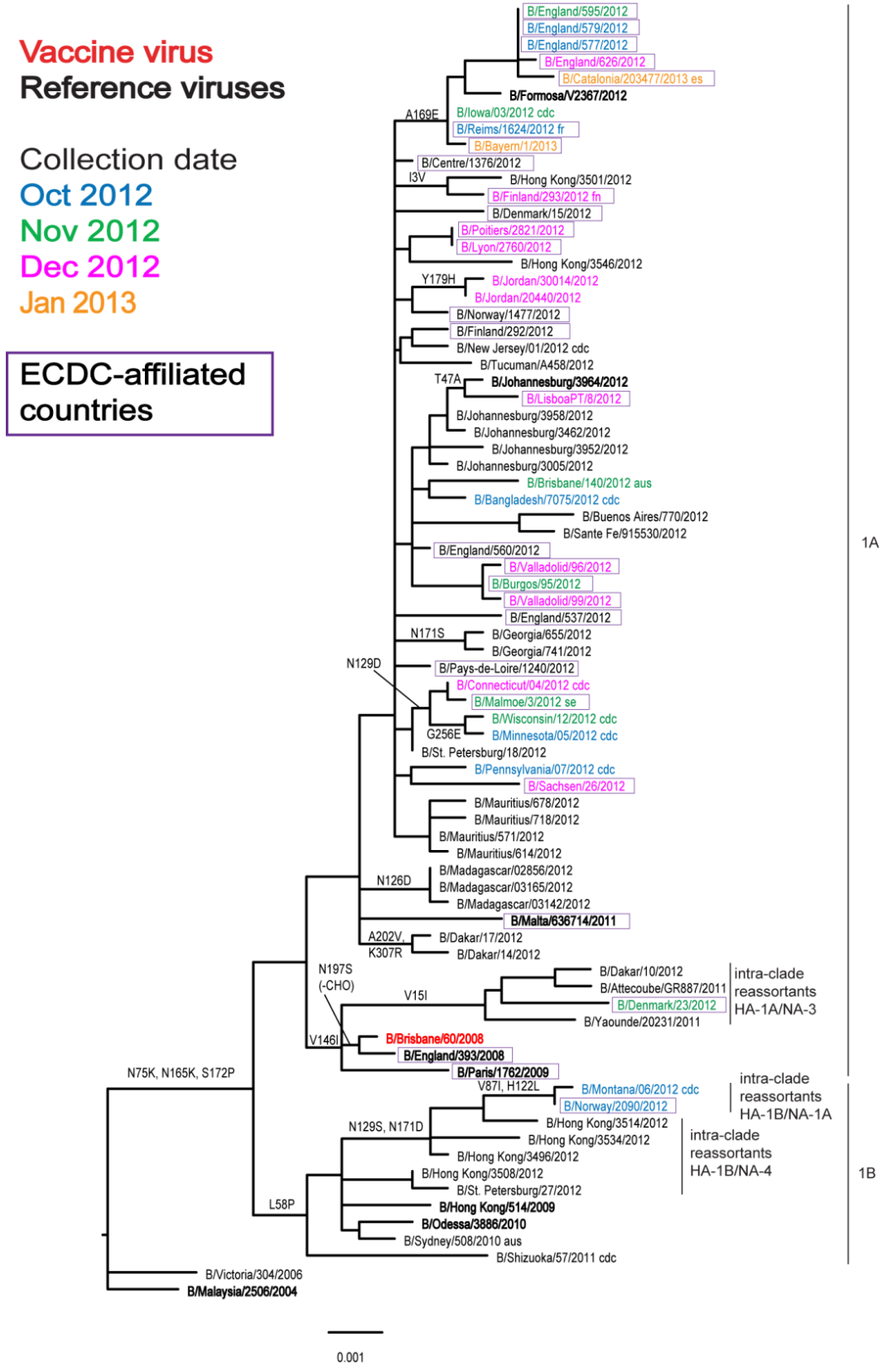
1. < = <10; 2. hyperimmune sheep serum

Table 12 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 523	B/Mal[1] 2506/05 F28/05	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 3964/12 F01/13 1A	B/For V2367/12 F04/13 1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	640	640	40	80	<	<	<	80	160	80
B/England/393/2008	1A	2008-08-29	E1/E2	1280	80	320	320	80	40	40	320	320	320
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	2560	160	320	320	80	40	80	320	64	320
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	10	20	40	40	40	40	20	80	160
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	10	10	20	80	160	160	20	8	160
B/Odessa/3886/2010	1B	2010-03-19	MDCK2/MDCK2	2560	10	10	20	80	80	160	20	40	80
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	320	320	80	40	40	320	320	320
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E1	2560	320	320	640	80	80	80	320	1280	640
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	2560	20	40	80	40	80	40	80	160	320
TEST VIRUSES													
B/Reims/1624/2012		2012-10-22	MDCK2/MDCK1	2560	<	10	20	40	40	40	20	40	160
B/Caen/1623/2012		2012-10-24	MDCK2/MDCK1	2560	10	20	40	80	80	80	40	80	160
B/Norway/2402/2012		2012-11-27	MDCK1/MDCK1	2560	<	20	20	40	40	40	20	40	160
B/Norway/2389/2012		2012-11-28	MDCK1/MDCK1	2560	80	160	160	40	40	40	160	640	320
B/Andalucia/359/2012		2012-12-03	E1/E2	1280	80	160	320	80	40	40	160	320	320
B/Andalucia/358/2012		2012-12-04	E1/E1	2560	320	160	320	40	40	80	160	320	320
B/Iasi/131732/13		2013-01-15	MDCK2/MDCK1	2560	10	20	20	40	80	80	40	80	160

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

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



B/Yamagata-lineage viruses

Tables 13 to 15 show the results of HI analyses of B/Yamagata lineage viruses, virus sequences included in the phylogenetic trees are highlighted. Where known, the clade into which the virus HA gene falls is shown: 25 test viruses had HA genes that fell into clade 2 and 18 had HA genes that fell into clade 3.

The vast majority of viruses in clade 2 (24 out of 25) showed good reactivity with antisera raised against B/Estonia/55669/2011 and B/Hong Kong/3577/2012, whereas these antisera recognised only six of 18 test viruses carrying an HA gene of clade 3 within four-fold of the titre of the homologous viruses. Similarly, all viruses in clade 3 reacted well with antiserum raised against the clade 3 virus A/Novosibirsk/1/2012, as did all but two (16 of 18) with antiserum raised against a second clade 3 virus propagated in cells, A/Stockholm/12/2011. There were a slightly reduced number of viruses in clade 2 showed that reacted well with these two antisera (17 of 25 and 18 of 25, respectively).

Antiserum raised against egg-propagated B/Wisconsin/1/2010 showed low reactivity to viruses with HA genes in clade 2, with only three viruses of 25 showing reactivity within 4-fold of the homologous titre; viruses with HA genes from clade 3 showed somewhat better reactivity with this antiserum (8 of 18 with titres within 4-fold of the titre of the serum against the homologous virus). In contrast, antisera raised against B/Massachusetts/02/2012 reacted within 4-fold of the titre of the homologous virus with all but one of the viruses tested (60 of 61), irrespective of the HA clade of the test virus or whether the antiserum was raised against the reference virus propagated in eggs or exclusively in tissue culture cells.

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/Bangladesh/3333/2007, B/Serbia/1994/2011, 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 13 Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

Viruses	Genetic group	Collection date	Passage History	B/FI[3] 4/06 SH479	Haemagglution Inhibition Titre: Post infection ferret sera							
					B/FI[1] 4/06 F21/07	B/Bris[2] 3/07 F21/12	B/Wis[2] 1/10 F26/10	B/Stock[2] 12/11 F12/12	B/Estonia[2] 55669/11 F26/11	B/Stock[2] 12/11 T/C F8/12	B/Novo[2] 1/12 F31/12	B/HK[2] 3577/12 F33/12
					Group 1	Group 2	Group 3	Group 3	Group 2	Group 3	Group 3	Group 2
REFERENCE VIRUSES												
B/Florida/4/2006	1	2006-12-15	E3/E3	5120	640	640	320	640	160	640	40	640
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	640	640	320	640	160	640	40	640
B/Wisconsin/1/2010	3	2010-02-20	E3/E2	2560	160	320	320	640	40	640	80	160
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	40	80	80	320	10	160	40	40
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	40	80	40	40	640	20	80	640
B/Stockholm/12/2011	3	2011-03-28	Cx/MDCK2	640	<	80	40	80	40	160	160	160
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	640	<	80	80	160	40	80	160	160
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK1	1280	40	80	20	80	640	20	40	640
TEST VIRUSES												
B/LisboaPT/7/2012	3	2012-11-26	SIAT1/MDCK1	1280	<	80	80	160	40	40	160	160
B/Baden Württemberg/41/2012	3	2012-11-26	C2/MDCK1	320	<	40	10	20	10	20	40	40
B/Bayern/57/2012		2012-11-28	C2/MDCK1	640	<	40	40	40	20	40	80	80
B/Baden Württemberg/42/2012		2012-12-03	C2/MDCK1	640	<	40	20	40	10	20	80	80
B/England/708/2012		2012-12-10	SIAT1/MDCK1	640	<	20	10	20	10	20	40	40
B/Lyon/2771/2012	2	2012-12-17	MDCK2/MDCK1	640	40	40	10	20	320	10	40	320
B/Lyon/2749/2012		2012-12-17	MDCK2/MDCK1	320	<	40	10	20	320	10	20	320
B/England/706/2012	2	2012-12-17	MDCK1/MDCK1	640	<	40	10	20	40	10	20	80
B/Saarland/2/2012	2	2012-12-17	C2/MDCK1	1280	<	40	10	160	640	10	20	640
B/Brandenburg/2/2012		2012-12-19	C2/MDCK1	640	<	40	20	20	10	40	80	80
B/BragaPT/11/2012		2012-12-21	SIAT1/MDCK1	2560	160	40	80	160	40	40	160	160
B/BragaPT/12/2012	3	2012-12-21	SIAT1/MDCK1	2560	80	40	80	160	320	80	160	320
B/BragaPT/10/2012	3	2012-12-23	SIAT1/MDCK1	2560	40	40	80	160	40	40	160	160
B/LisboaPT/9/2012	2	2012-12-27	SIAT1/MDCK1	2560	80	40	40	160	640	40	40	640
B/BragaPT/13/2012		2012-12-27	SIAT1/MDCK1	2560	80	40	80	160	40	40	160	160
B/PortoPT/14/2012	3	2012-12-31	SIAT1/MDCK1	2560	80	40	80	160	40	40	160	160
B/LyonCHU/51.410/2012		2012-12-19	MDCK2/MDCK1	640	40	40	10	20	320	10	20	320
B/Nordrhein-Westfalen/5/2012	3	2012-12-20	C2/MDCK1	320	<	40	20	40	20	20	40	80
B/Lyon/2791/2012		2012-12-20	MDCK2/MDCK1	640	<	40	10	20	320	10	20	320
B/England/709/2012	3	2012-12-20	MDCK1/MDCK1	640	<	40	20	40	10	40	40	80
B/LyonCHU/51.559/2012	2	2012-12-21	MDCK2/MDCK1	640	40	40	10	20	320	10	20	320
B/England/707/2012	2	2012-12-29	SIAT1/MDCK1	640	40	80	10	40	320	20	40	640
B/Rheinland-Pfalz/3/2013		2013-01-03	C2/MDCK1	640	<	40	10	20	320	10	20	320
B/Rheinland-Pfalz/4/2013	3	2013-01-04	C2/MDCK1	640	<	40	20	40	20	40	40	80
B/Thüringen/1/2013	2	2013-01-07	C1/MDCK1	640	<	40	10	40	320	10	20	320
B/Rheinland-Pfalz/2/2013		2013-01-07	C1/MDCK1	640	<	40	10	40	320	10	20	320

Sequences in phylogenetic trees

Vaccine

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

Table 14 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 F21/07	B/Bris[2] 3/07 F24/07	B/Wis[1] 1/10 F26/10	B/Stock[2] 12/11 F12/12	B/Estonia[2] 55669/11 F26/11	B/Serbia[2] 1894/11 F25/11	B/Stock[2] 12/11 T/C F8/12	B/Novo[2] 1/12 F31/12	B/HK[2] 3577/12 F33/12	B/Mass[2] 2/12 Egg F02/13	B/Mass[2] 2/12 T/C F03/13
	Genetic group				Group 1	Group 2	Group 3	Group 3	Group 2	Group 3	Group 3	Group 3	Group 2	Group 2	Group 2
REFERENCE VIRUSES															
B/Florida/4/2006	1	2006-12-15	E3/E3	5120	1280	1280	160	640	160	20	640	80	640	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	640	320	80	320	160	<	320	40	320	640	80
B/Wisconsin/1/2010	3	2010-02-20	E3/E2	1280	160	80	80	640	10	20	640	80	80	640	40
B/Stockholm/12/2011	3	2011-03-28	E4/E2	1280	40	40	<	320	<	10	320	40	40	160	20
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	40	40	80	40	320	20	40	80	1280	160	160
B/Serbia/1894/2011	3	2011-03-08	MDCK1/MDCK4	1280	<	40	40	80	80	80	160	160	320	160	160
B/Stockholm/12/2011	3	2011-03-28	Cx/MDCK2	2560	80	40	40	160	160	160	160	320	320	320	320
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	1280	<	40	40	80	40	40	80	160	320	160	80
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK1	1280	80	40	40	40	320	40	40	80	640	160	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E2	2560	320	160	40	320	40	<	320	20	320	320	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK2	1280	40	40	<	40	160	<	40	40	320	160	160
TEST VIRUSES															
B/Norway/2256/2012	2	2012-11-01	MDCK2/MDCK1	1280	80	40	40	80	320	<	40	40	1280	160	160
B/Navarra/303/2012		2012-11-05	SIAT1/MDCK1	1280	80	40	<	40	640	20	80	80	640	160	160
B/Norway/2182/2012		2012-11-07	MDCK1/MDCK1	1280	40	40	40	80	320	10	40	40	640	160	160
B/PaisVasco/284/2012	3	2012-11-07	SIAT1/MDCK1	2560	80	80	40	160	160	160	160	320	320	160	320
B/Norway/2250/2012		2012-11-09	MDCK2/MDCK1	1280	80	40	40	80	320	10	40	40	320	160	160
B/Navarra/304/2012	2	2012-11-12	SIAT1/MDCK1	1280	40	40	<	40	640	20	40	80	640	160	320
B/Norway/2281/2012	2	2012-11-14	MDCK1/MDCK1	2560	80	40	80	160	640	20	80	80	640	320	160
B/Navarra/300/2012		2012-11-16	SIAT1/MDCK1	2560	80	40	<	80	640	40	80	160	640	160	320
B/Norway/2276/2012		2012-11-19	MDCK1/MDCK1	1280	40	40	40	320	40	80	160	160	320	160	80
B/Navarra/299/2012		2012-11-20	SIAT1/MDCK1	1280	80	40	<	40	640	20	40	80	640	160	160
B/Ireland/88486/2012	2	2012-11-21	MDCK1/MDCK1	1280	40	40	<	80	320	10	20	40	640	160	160
B/Ireland/89450/2012		2012-11-22	MDCK1/MDCK1	2560	80	40	<	80	320	10	40	40	640	160	80
B/Ireland/90999/2012	2	2012-11-25	MDCK1/MDCK1	2560	80	40	<	80	320	10	40	40	640	320	160
B/Norway/2349/2012	2	2012-11-26	MDCK1/MDCK1	1280	40	40	<	80	320	10	80	40	640	160	160
B/Ireland/90800/2012		2012-11-26	MDCK1/MDCK1	1280	40	40	<	80	320	<	20	20	640	160	160
B/Milano/37/2012		2012-11-26	MDCK1/MDCK1	2560	80	40	80	320	40	80	320	160	320	320	80
B/Milano/38/2012		2012-11-26	MDCK1/MDCK1	1280	<	20	<	80	40	80	160	160	160	160	80
B/Belgium/G901/2012	3	2012-11-28	MDCK3	1280	<	20	<	160	20	40	160	80	80	160	40
B/Ireland/91035/2012		2012-11-28	MDCK1/MDCK1	1280	40	40	<	80	320	<	20	40	640	160	160
B/Latvia/12-32427p/2012	3	2012-12-03	MDCK2/MDCK1	1280	80	40	40	160	80	80	160	160	160	160	160
B/Belgium/G908/2012		2012-12-04	MDCK2	2560	80	40	<	80	320	10	40	40	640	160	160
B/Milano/36/2012	3	2012-12-04	MDCK1/MDCK1	1280	40	20	40	160	40	80	160	160	160	160	80
B/Belgium/G922/2012	2	2012-12-07	MDCK3	1280	40	40	<	80	320	10	40	20	320	160	160
B/Roma/02/2012	3	2012-12-10	Cx/MDCK1	2560	160	80	160	320	160	320	320	320	320	160	320
B/Roma/03/2012		2012-12-10	Cx/MDCK1	2560	160	80	80	640	320	320	640	320	160	320	160
B/Milano/42/2012		2012-12-11	MDCK1/MDCK1	2560	80	80	<	160	640	20	80	40	640	320	160
B/Parma/02/2012		2012-12-14	MDCK1/MDCK1	1280	80	40	<	80	320	10	40	40	640	160	160
B/Milano/44/2012	2	2012-12-17	MDCK1/MDCK1	2560	80	40	<	80	320	20	40	40	640	320	160
B/Parma/03/2012	3	2012-12-19	MDCK2/MDCK1	1280	<	<	<	160	20	40	80	80	80	160	40
B/Torino/01/2012		2012-12-19	MDCK1/MDCK1	1280	80	40	<	80	320	10	40	20	640	160	160
B/Parma/04/2012		2012-12-27	MDCK1/MDCK1	2560	80	80	80	160	640	10	40	40	1280	320	160
B/Torino/06/2012		2012-12-31	Cx/MDCK1	2560	80	40	<	80	320	10	40	40	640	160	160
B/PaisVasco/13005/2012		2012-12-31	SIAT1/MDCK1	2560	40	40	40	160	80	320	160	160	320	160	160
B/Braga/SU17/2012		2013-01-02	MDCK2	1280	<	20	40	160	20	40	160	80	80	160	80
B/Ireland/00329/2013		2013-01-02	MDCK1/MDCK1	1280	80	40	<	80	320	10	40	40	640	160	160
B/Porto/EuroEva38/2012	3	2013-01-04	MDCK2	2560	<	20	<	160	10	40	160	80	80	160	40
B/Ireland/02490/2013	2	2013-01-08	MDCK1/MDCK1	640	40	40	<	40	160	<	20	20	160	80	80
B/Estonia/74419/2013	3	2013-01-10	MDCK1/MDCK1	1280	40	40	40	160	80	80	160	160	160	160	160
B/Latvia/1-40275p/2013	2	2013-01-15	MDCK1/MDCK1	1280	40	40	<	80	320	10	40	20	640	160	160

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

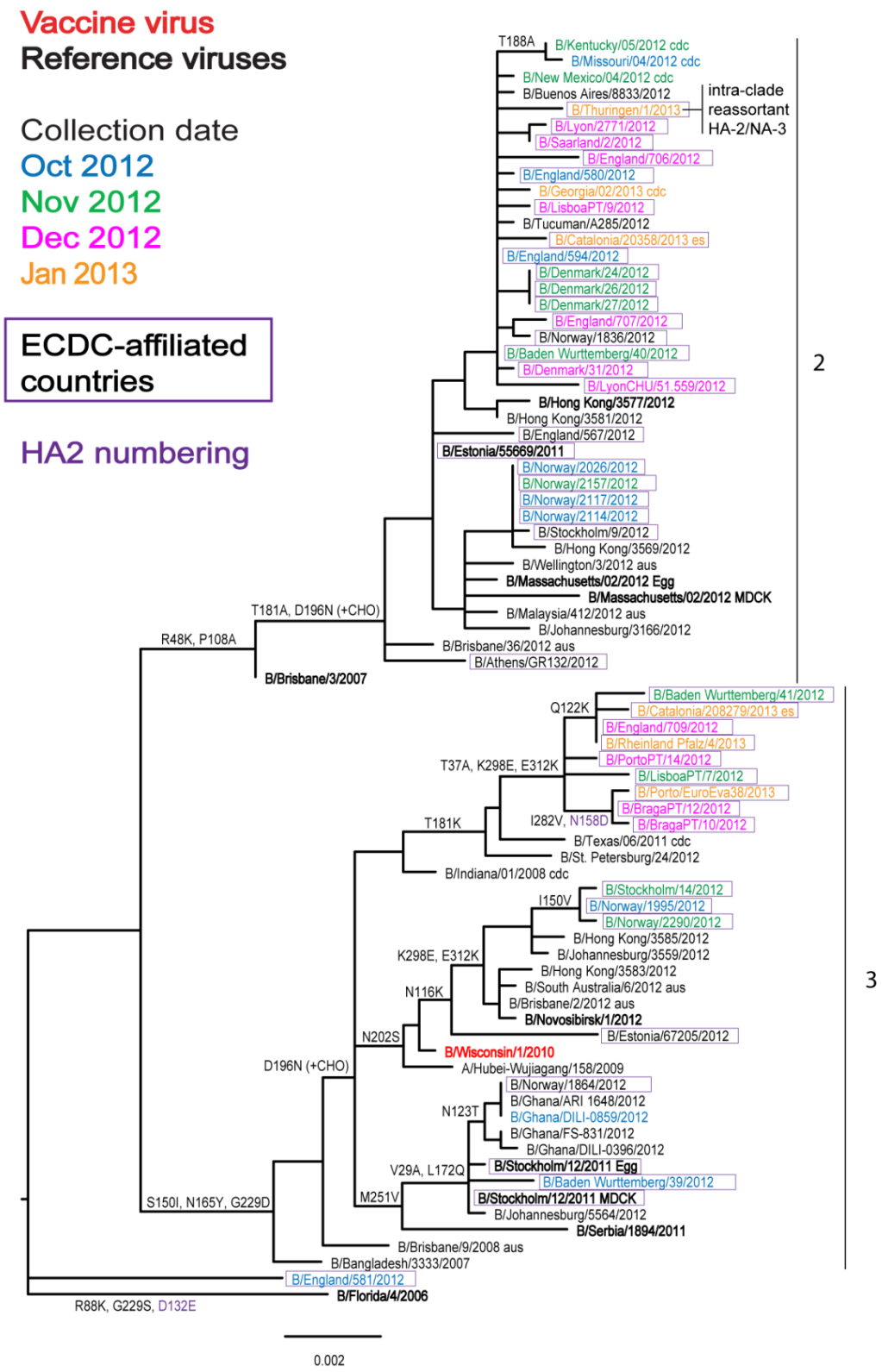
Vaccine

Table 15 Antigenic analysis of influenza B viruses (Yamagata lineage) by HI

Viruses	Genetic group	Collection date	Passage History	B/FP 4/08 BH479	Haemagglutination Inhibition Titre: Post infection ferret sera										
					B/FI[1] 4/08 F21/07 Group 1	B/Bris[2] 3/07 F21/12 Group 2	B/Wis 1/10 F28/10 Group 3	B/Stock[2] 12/2011 F12/12 Group 3	B/Estonia[1] 55669/2011 F26/11 Group 2	B/Serbia[1] 1894/2011 F26/11 Group 3	B/Stock[2] 12/2011 T/C F8/12 Group 3	B/Novo 1/2012 F3/1/12 Group 3	B/HK 3677/2012 F33/12 Group 2	B/Mass 2/12 Egg F02/13 Group 2	B/Mass 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	20	640	40	320	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E1	5120	640	640	160	640	320	<	320	80	320	1280	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	2560	320	320	640	640	40	40	640	160	160	640	80
B/Stockholm/12/2011	3	2007-08-07	E4/E1	1280	80	160	160	320	10	20	80	40	40	320	40
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	2560	40	160	40	160	1280	80	80	160	1280	320	640
B/Serbia/1894/2011	3	2011-03-08	MDCK1/MDCK4	2560	<	160	160	320	320	320	320	320	640	320	320
B/Stockholm/12/2011	3	2011-03-28	Cx/MDCK2	2560	40	80	80	160	80	160	160	320	320	320	80
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK2	1280	40	160	80	320	160	160	160	320	320	320	160
B/Hong Kong/3577/2012	2	2012-06-13	MDCK2/MDCK3	5120	320	320	160	320	1280	320	320	320	1280	640	640
B/Massachusetts/02/2012	2	2012-03-13	E3/E2	2560	320	320	160	320	640	<	320	20	160	640	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK2	2560	160	320	40	160	320	40	80	40	1280	640	320
TEST VIRUSES															
B/Paris/1443/2012		2012-04-16	MDCK2/MDCK1	1280	80	40	160	320	40	80	320	320	320	320	80
B/Paris/1448/2012		2012-07-13	MDCK2/MDCK1	2560	160	80	40	160	640	20	80	40	640	320	160
B/Paris/1466/2012		2012-09-24	MDCK2/MDCK1	2560	160	80	80	160	640	40	160	80	640	320	160
B/Caen/1808/2012	3	2012-11-16	MDCK1/MDCK1	2560	40	40	80	320	20	40	160	80	160	160	80
B/Pays de Loire/1791/2012	2	2012-11-19	MDCK1/MDCK1	2560	160	160	80	320	640	80	80	80	640	320	320
B/Athens GR/11/2013	2	2013-01-08	MDCK4	5120	640	320	160	640	1280	640	320	320	1280	640	1280
B/Norway/2363/2012		2012-11-10	MDCK1/MDCK1	5120	40	160	80	320	640	80	80	80	1280	320	320
B/Norway/2365/2012		2012-11-30	MDCK2/MDCK1	2560	40	160	80	320	640	40	80	80	1280	320	320
B/Norway/2387/2012		2012-11-24	MDCK1/MDCK1	2560	20	160	40	320	640	40	80	80	640	320	320
B/Norway/2453/2012		2012-12-03	LLC-MK2 2/MDCK1/MDCK1	2560	40	160	80	160	1280	160	80	160	1280	320	320
B/Norway/2476/2012	2	2012-12-10	MDCK1/MDCK1	2560	40	160	40	320	640	80	80	80	640	320	320
B/Pays de Loire/1825/2012		2012-11-27	MDCK1/MDCK1	2560	40	320	80	320	640	80	80	80	640	640	320
B/Paris/1828/2012	3	2012-11-29	MDCK1/MDCK1	2560	20	160	160	640	160	320	320	320	320	640	320
B/Parma/01/2013	2	2013-01-09	MDCK1/MDCK1	5120	80	320	80	320	1280	80	160	80	640	640	640
B/Parma/02/2013		2012-12-27	MDCK1/MDCK1	2560	40	160	80	320	1280	40	80	80	640	320	320
B/Trieste/01/2013	2	2013-01-14	Cx/MDCK1	2560	320	160	80	320	1280	40	80	80	640	640	320
B/Trieste/02/2012		2012-12-11	Cx/MDCK1	2560	40	160	160	640	80	160	320	160	160	640	160
B/Bucuresti/131574/2013	2	2013-01-14	MDCK1/MDCK1	5120	320	320	80	320	1280	40	80	80	1280	640	320
B/Iasi/131604/2013		2013-01-12	MDCK1/MDCK1	2560	160	160	40	160	640	40	80	80	640	320	320
B/Netherlands/521/12	2	2012-11-29	MDCK2/MDCK1	5120	320	320	80	320	1280	80	80	80	1280	640	640
B/Netherlands/039/13	2	2013-01-04	MDCK2/MDCK1	2560	160	320	80	320	640	40	80	80	1280	640	320
B/PaisVasco/13005/2012		2012-12-31	E1/E2	320	<	20	20	40	20	40	40	40	40	40	40
							Vaccine								

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

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



Influenza A(H3N2)v virus

On 3 August 2012, the United States CDC issued a Health Advisory describing an increase in the number of influenza A(H3N2)v infections in three US states and CDC have prepared further background information and provided updated case counts. As at 1 December 2012 there were 308 confirmed cases. Antigenic and genetic characterisation of H3N2v viruses has been described by Lindstrom et al., 2012. The virus was characterised as being antigenically distinct from currently circulating human seasonal influenza viruses and to be a reassortant virus with seven genes from swine influenza 'triple reassortant' H3N2 viruses and the M-gene from an influenza A(H1N1)pdm09 virus.

Risk assessments for these A(H3N2)v viruses, as a risk to public health, have been posted by the United States CDC and ECDC.

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza, based 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 in the sequence. 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/EAA countries were recovered from GISAID and we acknowledge all laboratories who submitted sequences directly to the London WHO CC.