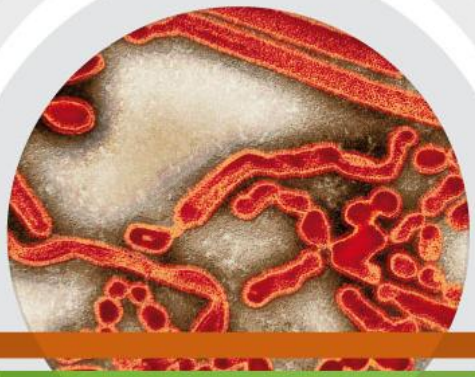


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, March 2014

Summary

During the 2013–14 season A(H1N1)pdm09, A(H3N2) and B/Victoria- and B/Yamagata-lineage influenza viruses have continued to cocirculate in ECDC-affiliated countries. The relative prevalence has varied between countries. Of the viruses received by the WHO Collaborating Centre in London:

- Type A and type B viruses have been received at a ratio of over 20:1.
- A(H3N2) and A(H1N1)pdm09 viruses have been received in similar numbers.
- Recently circulating A(H1N1)pdm09 viruses belonged to genetic subgroups 6B and 6C, with viruses in genetic subgroup 6B predominating. Viruses in subgroups 6B and 6C are antigenically similar to the vaccine virus, A/California/07/2009.
- Recently circulating A(H3N2) viruses have fallen within genetic group 3C represented by the recommended vaccine virus for the 2013–14 and 2014–15 seasons, A/Texas/50/2012, with viruses of genetic subgroup 3C.3 predominating. Antigenic analysis using antisera raised against cell-propagated H3N2 viruses indicates that the circulating viruses are antigenically similar to those in circulation in the 2012–13 and 2013–14 influenza seasons.
- Two genetic clades of B/Yamagata-lineage viruses continue to circulate: clade 3 represented by B/Wisconsin/1/2010 and clade 2 represented by B/Massachusetts/02/2012 (the recommended vaccine component for the 2013–14 and 2014–15 influenza seasons). Viruses in each clade have been received in similar numbers but with viruses in clade 3 predominating in those samples collected in 2014.
- Few B/Victoria-lineage viruses have been received, and phylogenetic analysis revealed that all were in genetic clade 1A. Antigenically the viruses were similar to the prototype virus B/Brisbane/60/2008 and viruses genetically similar to this prototype virus. B/Brisbane/60/2008 has been recommended by WHO as a component in quadrivalent influenza vaccines for 2013–14 and 2014–15 influenza seasons.

Influenza-positive samples, viruses or clinical specimens, with collection dates after 30 November 2013 (with week 40, the start of weekly monitoring of influenza activity for the 2013–14 influenza season, commencing on 30 September 2013) have been received at the MRC National Institute for Medical Research, WHO Collaborating Centre for Reference and Research on Influenza (WHO CC), from 21 countries in the EU/EEA region. The large majority (96%) were type A viruses, with A(H3N2) viruses and A(H1N1)pdm09 viruses approximately equally represented (Table 1). Of the small number of type B viruses received (4% of the specimens), B/Yamagata-lineage viruses predominated over those of the Victoria-lineage by a ratio of 5:1. Some samples have yet to be fully processed (in process: Table 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).

Suggested citation: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, March 2014. Stockholm: ECDC; 2014.

Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, with collection dates after 30 November 2013

MONTH / Country	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
			Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]
DECEMBER											
Belgium	8		2	0	5	5				1	0
Denmark	7		4	2	2	2				1	0
Finland	4		1	1	3	3					
France	33		17	17	14	14				2	2
Germany	3				3	3					
Iceland	2		1	1						1	1
Ireland	6		3	0	2	2				1	1
Italy	15		1	1	14	14					
Latvia	3		2	2	1	1					
Malta	1		1	1							
Netherlands	1				1	1					
Norway	13		3	3	6	6				4	4
Portugal	9		6	5	3	2					
Romania	1		1	0							
Slovenia	4				4	4					
Spain	39		20	17	19	16					
Sweden	6		1	1	4	4				1	1
United Kingdom	5		3	3	2	2					
2014											
JANUARY											
Belgium	3		2	2	1	1					
Bulgaria	32		24	in process	8	in process					
Cyprus	13	4	9	6							
Germany	22		4	4	17	17		1	1		
Greece	35		32	15	3	in process					
Iceland	4		4	4							
Ireland	3		1	1	2	2					
Italy	20		6	in process	12	in process	1			1	1
Latvia	1		1	1							
Malta	4		4	4							
Norway	30				30	in process					
Portugal	11		8	6	3	3					
Romania	13		5	0	8	4					
Slovenia	5				5	2					
Spain	52		38	29	13	11				1	1
United Kingdom	4		2	2	2	2					
FEBRUARY											
Bulgaria	27		20	in progress	7	in process					
Cyprus	12	1	11	11							
Germany	11		4	4	3	3		2	2	2	2
Italy	28		12	in progress	14	in process	2				
Sweden	2				2	in process					
MARCH											
Bulgaria	1		1	in process							
	493	5	254	143	213	124	3	3	3	15	13
			51.5%		43.2%			0.6%		3.0%	
		96%					4%				

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

All 42 H1N1 viruses analysed since the February 2014 report[1] were antigenically similar to the vaccine virus as assessed by haemagglutination inhibition (HI) assay (Tables 2–1 and 2–2). Only one test virus, A/Lamia.GR/77/2014 was recognised by antiserum raised against the vaccine virus (A/California/7/2009) at a titre fourfold reduced, compared with the titre for the vaccine virus. Another virus, A/Catalonia/2142245NS/2013, although recognised by antiserum raised against the vaccine virus at a titre within twofold of the titre of the antiserum for the homologous virus, was not recognised well by many of the antisera raised against other reference viruses. Viruses for which gene sequences are included in the phylogenetic tree are highlighted and, where known, the HA genetic group is indicated. Both viruses that showed unusual HI results had amino acid substitution or polymorphism at position 155 in HA1; A/Lamia.GR/77/2014

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, February 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-feb-2014.pdf

carried a G155E substitution and A/Catalonia/2142245NS/2013 carried a G155G/E polymorphism. Substitutions and polymorphism observed in the 153–157 region of HA1 are frequently associated with adaptation on culture. One virus, A/Catalonia/6634S/2013 showed highly reduced inhibition by oseltamivir in sialidase assays. The NA gene sequence of A/Catalonia/6634S/2013 encoded a H275Y substitution (H275Y in Figure 1), the most common substitution associated with highly reduced inhibition by oseltamivir in H1N1 viruses.

Figure 1 shows a phylogenetic tree for the HA genes of representative H1N1 viruses. The HA genes cluster into eight designated genetic groups, of which seven are indicated, with A/California/7/2009 representing group 1. Viruses collected after 30 November 2013, and since the February 2014 report, fell into genetic group 6 and clustered within subgroups 6B and 6C, with those in genetic subgroup 6B predominating for viruses collected in 2014 (Figure 1). Genetic subgroup 6B carries the substitutions **D97N**, **K163Q, S185T, S203T, A256T** and **K283E** in **HA1** and **E47K, S124N and E172K** in **HA2** compared with A/California/7/2009.

Table 2–1. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1] — Post infection ferret antisera: A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 4	A/HK 3934/11 F21/11 3	A/Astra 1/11 F22/13 5	A/St. P 27/11 F23/11 6	A/St. P 100/11 F24/11 7	A/HK 5659/12 F30/12 6A	A/SA 3626/13 F3/14 6B	
REFERENCE VIRUSES														
A/California/7/2009		2009-04-09	E1/E2	1280	1280	1280	640	640	640	640	640	640	640	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	160	320	160	80	40	80	80	80	80	80	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	1280	1280	1280	320	160	160	320	160	640	160	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	5120	2560	5120	5120	5120	5120	5120	5120	5120	5120	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK4	1280	320	1280	640	2560	2560	1280	2560	2560	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	2560	640	1280	1280	2560	2560	2560	5120	2560	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	2560	1280	2560	1280	2560	2560	2560	5120	5120	2560	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	1280	640	1280	1280	1280	2560	2560	5120	2560	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	5120	1280	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	1280	1280	1280	1280	1280	2560	2560	2560	2560	1280	
TEST VIRUSES														
A/Catalonia/6590S/2013	6B	2013-11-29	C0/MDCK1	640	160	320	640	1280	640	640	1280	1280	640	
A/Catalonia/6634S/2013	6B	2013-12-12	C0/MDCK1	1280	1280	2560	2560	5120	2560	5120	5120	5120	2560	*
A/Catalonia/2142245NS/2013	6C	2013-12-20	C0/MDCK2	640	640	320	160	80	160	320	160	320	160	G155E=G
A/Valladolid/145/2013		2013-12-23	MDCK1/SIAT1/EGG2/MDCK1	2560	2560	5120	2560	5120	5120	5120	5120	5120	5120	
A/Catalonia/2145903NS/2013		2013-12-30	C0/MDCK1	1280	640	1280	1280	5120	2560	2560	5120	2560	2560	
A/Valencia/621679S/2014		2014-01-02	C0/MDCK1	1280	1280	2560	2560	5120	2560	2560	5120	2560	2560	
A/Burgos/11/2014	6B	2014-01-08	MDCK1/SIAT1/MDCK1	5120	2560	5120	2560	5120	5120	5120	5120	5120	5120	
A/Athens.GR/14/2014		2014-01-10	MDCK2	2560	2560	2560	5120	5120	5120	5120	5120	5120	2560	
A/Athens.GR/18/2014		2014-01-10	MDCK2	1280	640	1280	2560	2560	2560	2560	5120	2560	1280	
A/Catalonia/2148757NS/2014		2014-01-11	C0/MDCK1	1280	640	1280	1280	5120	1280	2560	5120	1280	1280	
A/Astros.GR/39/2014		2014-01-12	MDCK3	2560	2560	2560	5120	5120	5120	5120	5120	5120	2560	
A/Astros.GR/42/2014	6B	2014-01-12	MDCK3	1280	640	1280	2560	2560	2560	2560	5120	5120	2560	
A/Arkadia.GR/55/2014	6B	2014-01-14	MDCK3	1280	640	1280	1280	2560	1280	2560	2560	2560	1280	
A/Athens.GR/71/2014	6B	2014-01-14	MDCK3	640	320	640	1280	1280	1280	1280	2560	2560	640	
A/Lamia.GR/77/2014	6B	2014-01-14	MDCK3	320	640	320	320	40	80	320	160	320	160	G155E
A/Athens.GR/68/2014		2014-01-15	MDCK3	1280	2560	2560	2560	2560	2560	5120	5120	5120	2560	
A/Athens.GR/70/2014		2014-01-15	MDCK3	2560	2560	2560	2560	2560	5120	5120	5120	5120	2560	
A/Lamia.GR/80/2014		2014-01-15	MDCK2	1280	1280	2560	2560	2560	2560	5120	5120	5120	2560	
A/Valladolid/46/2014		2014-01-15	MDCK1/SIAT1/MDCK1	2560	1280	2560	2560	5120	2560	5120	5120	2560	2560	
A/Palencia/55/2014		2014-01-15	MDCK1/SIAT1/MDCK1	2560	1280	2560	2560	5120	2560	5120	5120	2560	2560	
A/Athens.GR/89/2014		2014-01-16	MDCK2	2560	2560	2560	2560	2560	5120	5120	5120	5120	2560	
A/Catalonia/2150477NS/2014		2014-01-17	C0/MDCK1	2560	1280	2560	2560	5120	2560	2560	5120	2560	2560	
A/Valencia/624382S/2014	6B	2014-01-17	C0/MDCK1	1280	640	1280	1280	5120	1280	2560	5120	2560	2560	
A/Athens.GR/109/2014		2014-01-19	MDCK2	1280	640	640	1280	1280	2560	1280	5120	2560	1280	
A/Athens.GR/116/2014	6B	2014-01-20	MDCK2	5120	1280	2560	2560	2560	5120	2560	5120	5120	2560	
A/Burgos/80/2014		2014-01-21	MDCK1/SIAT1/MDCK1	5120	2560	5120	2560	5120	5120	5120	5120	5120	5120	
A/Leon/10020/2014	6B	2014-01-22	MDCK1/SIAT1/MDCK1	2560	1280	2560	2560	5120	2560	5120	5120	2560	2560	
A/Berlin/7/2014	6B	2014-01-22	C2/MDCK1	1280	1280	1280	1280	2560	2560	2560	5120	5120	2560	
A/Berlin/11/2014		2014-01-22	C2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	2560	
A/Valladolid/116/2014		2014-01-27	MDCK1/SIAT1/MDCK1	2560	1280	2560	5120	5120	5120	5120	5120	5120	2560	
A/Hessen/5/2014	6B	2014-02-03	C2/MDCK1	1280	1280	1280	1280	5120	2560	2560	5120	5120	2560	
A/Hamburg/2/2014	6B	2014-02-04	C2/MDCK1	2560	1280	2560	2560	5120	2560	2560	5120	5120	2560	
A/Brandenburg/2/2014		2014-02-04	C2/MDCK1	1280	1280	1280	1280	5120	2560	2560	5120	5120	2560	
A/Sachsen/1/2014	6B	2014-02-05	C1/MDCK1	2560	1280	2560	2560	5120	2560	5120	5120	5120	2560	

1. < = <40

Vaccine

Sequences in phylogenetic tree

* Highly reduced inhibition by oseltamivir

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

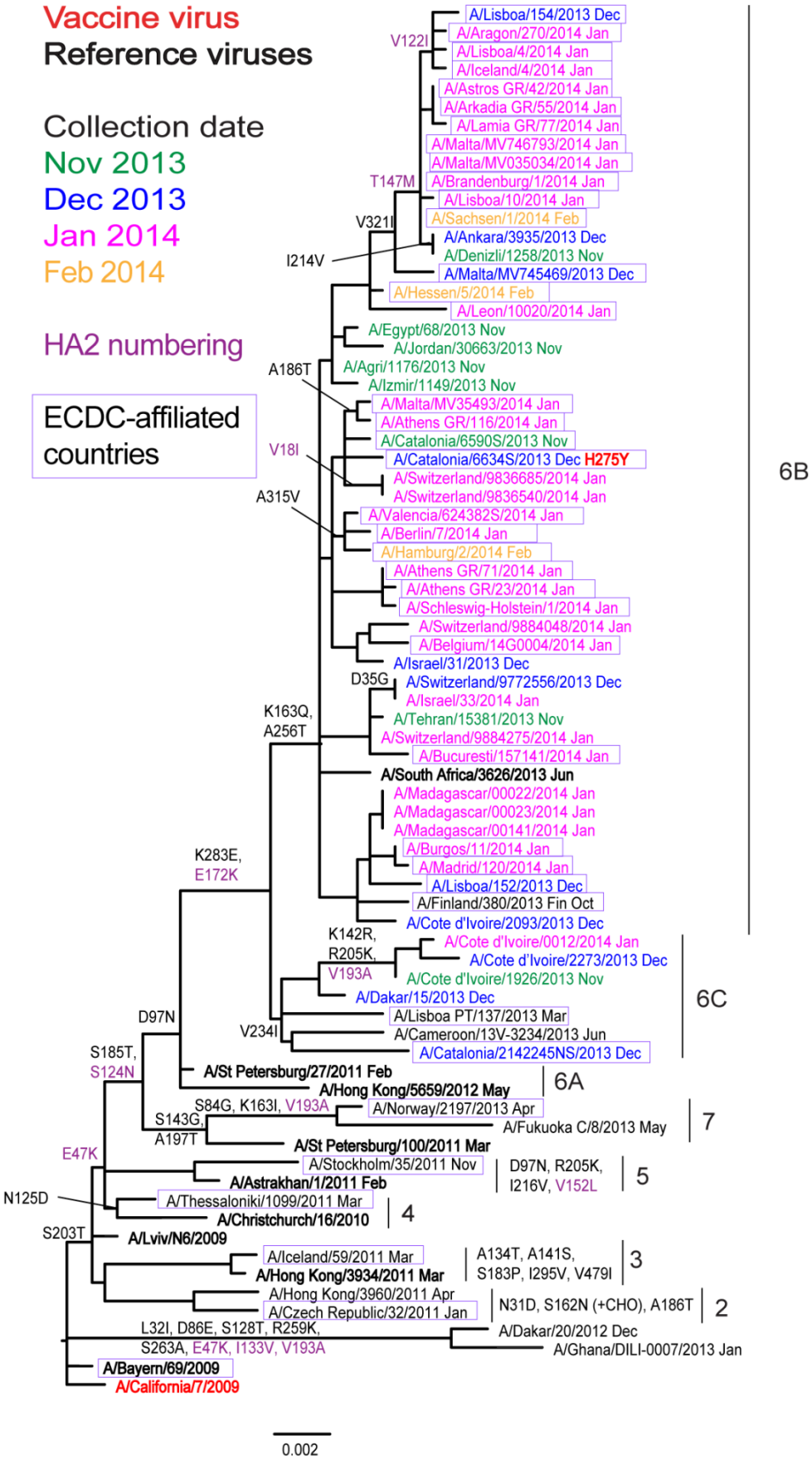
Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1] — Post infection ferret antisera: A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 4	A/HK 3934/11 F21/11 3	A/Astrak 1/11 F22/13 5	A/St. P 27/11 F23/11 6	A/St. P 100/11 F24/11 7	A/HK 5659/12 F30/12 6A	A/SA 3626/13 F3/14 6B	
REFERENCE VIRUSES														
A/California/7/2009		2009-04-09	E1/E2	1280	640	1280	320	320	320	320	640	320	320	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	320	320	160	40	40	40	80	80	80	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	1280	160	80	160	320	160	320	160	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	2560	5120	1280	2560	2560	2560	2560	1280	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK4	640	320	640	640	1280	2560	1280	2560	2560	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	1280	640	2560	1280	2560	5120	5120	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	640	1280	1280	1280	2560	2560	5120	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	320	640	320	640	1280	1280	2560	1280	640	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	320	160	320	160	640	640	640	1280	1280	320	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	640	640	640	640	640	1280	1280	1280	1280	1280	
TEST VIRUSES														
A/Malta/MV745469/2013	6B	2013-12-30	MDCK2	2560	2560	2560	2560	5120	2560	2560	5120	5120	2560	
A/Bucuresti/157141/2014	6B	2013-01-06	MDCK1/MDCK2	1280	640	1280	1280	2560	2560	2560	2560	2560	2560	
A/Malta/MV035034/2014	6B	2014-01-07	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	2560	1280	
A/Athens.GR/23/2014	6B	2014-01-07	MDCK2	1280	1280	1280	1280	5120	2560	2560	5120	2560	2560	
A/Athens.GR/19/2014		2014-01-08	MDCK3	1280	1280	1280	1280	5120	5120	2560	5120	2560	2560	
A/Malta/MV35493/2014	6B	2014-01-14	MDCK2	1280	320	640	640	1280	2560	1280	2560	2560	1280	
A/Malta/MV35488/2014		2014-01-14	MDCK2	1280	640	1280	1280	2560	1280	1280	2560	2560	1280	
A/Malta/MV746793/2014	6B	2014-01-19	MDCK2	2560	1280	2560	2560	5120	5120	5120	5120	5120	2560	

1. < = <40

Vaccine

Sequences in phylogenetic tree

Figure 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes



Influenza A(H3N2) virus analyses

As described in previous reports[2], 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. All viruses isolated since the February 2014 report had sufficient HA titre in assays conducted using guinea pig red blood cells in the presence of 20nM oseltamivir (added to circumvent any NA-mediated binding of H3N2 viruses to red blood cells) to be analysed by HI assay.

HI results are shown in Tables 3–1 and 3–2. Viruses for which gene sequences are included in the phylogenetic tree are highlighted and, where known, the HA genetic group is indicated.

All 22 test viruses analysed since the February 2014 report reacted poorly in HI assays (≥eightfold decrease) with post-infection ferret antiserum raised against the egg-propagated vaccine virus, A/Texas/50/2012, compared with the titre of the antiserum with the homologous virus. Test viruses examined with antisera raised against three other egg-propagated reference viruses – A/Serbia/NS-210/2013, A/Hong Kong/146/2013 and A/Almaty/2958/2013 (represented by the high-growth reassortant NIB-85) – showed similar results. Compared with the corresponding homologous titres, six test viruses reacted within fourfold of the titre against the homologous egg-propagated virus for antisera raised against A/Serbia/NS-210/2013, and seven were recognised at titres within fourfold of the titre of the homologous virus with the antiserum raised against A/Almaty/2958/2013. Ferret antisera raised against reference viruses exclusively propagated in tissue culture cells – A/Stockholm/18/2011, A/Athens/112/2012, A/Samara/73/2013 and A/Victoria/361/2011 – recognised the test viruses more effectively; all test viruses analysed since the February 2014 report were recognised at titres within fourfold of those for the antisera with their corresponding homologous viruses.

Since 2009, seven genetic groups based on the HA gene have been defined for H3N2 viruses. Phylogenetic analysis of the HA genes of representative, recently circulating H3N2 viruses is shown in Figure 2. The HA genes of viruses received at NIMR from EU/EEA countries since the February 2014 report fell in genetic group 3, within subgroup 3C. This subgroup has three subdivisions: 3C.1, 3C.2 and 3C.3.

The vaccine virus A/Texas/50/2012 belongs to genetic subgroup 3C.1. All of the viruses received and analysed since the February 2014 report fall into subgroups 3C.2 and 3C.3. Amino acid substitutions that define subgroups 3C.2 and 3C.3 are:

- 3C.2 **N145S** in **HA1**, and **D160N** in **HA2**, e.g. A/Hong Kong/146/2013; and
- 3C.3 **T128A** (resulting in the loss of a potential glycosylation site), **R142G,** and **N145S** in **HA1**, e.g. A/Samara/73/2013.

Table 3–1. 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 antisera									
				A/Perth 16/09	A/Stock 18/11	A/Iowa 19/10	A/Vic 361/11	A/Athens 112/12	A/Texas 50/12	A/Samara 73/13	A/Serbia NS-210/13	A/HK 146/13	NIB-85
				F35/11	F28/11	F15/11	T/C F11/13	F16/12	Egg F42/13	F24/13	F39/13	F40/13	F45/13
					3A	6	3C.1	3B	3C.1	3C.3	3C.3	3C.2	3C.3
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E3	1280	160	320	160	320	160	80	80	160	160
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	40	160	160	320	640	160	640	160	320	160
A/Iowa/19/2010	6	2010-12-30	E3/E2	640	1280	2560	2560	2560	1280	1280	1280	1280	1280
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT3	80	160	320	320	640	320	640	320	320	320
A/Athens/112/2012	3B	2012-02-01	SIAT4	80	160	160	320	640	160	320	160	320	160
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	640	1280	1280	1280	1280	1280	640	1280	1280	1280
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	160	320	320	640	640	320	640	640	640	640
A/Serbia/NS-210/2013	3C.3	2013-01-18	E5/E1	320	640	1280	640	1280	640	640	1280	640	640
A/Hong Kong/146/2013	3C.2	2013-01-11	E5/E1	320	1280	1280	1280	1280	640	640	640	2560	640
NIB-85 (A/Almaty/2958/2013)	3C.3	2013-01-27	E5/E1	640	1280	2560	1280	1280	1280	1280	1280	1280	1280
TEST VIRUSES													
A/Catalonia/2141012NS/2013	3C.3	2013-12-06	C0/SIAT1	<	80	160	160	320	80	320	160	160	80
A/Catalonia/6623S/2013	3C.3	2013-12-10	C0/SIAT1	40	160	160	320	640	160	640	320	320	320
A/Catalonia/2141628NS/2013		2013-12-10	C0/SIAT1	<	160	160	160	320	160	320	160	160	160
A/Catalonia/2143786NS/2013		2013-12-19	C0/SIAT1	<	160	160	160	320	160	320	160	160	160
A/Catalonia/2144572NS/2014		2013-12-22	C0/SIAT1	<	80	40	160	320	80	320	160	160	160
A/Valencia/624026S/2014	3C.3	2014-01-06	C0/SIAT1	<	160	160	160	320	160	320	160	160	160
A/Salamanca/27/2014		2014-01-10	MDCK1/SIAT1/SIAT1	40	160	160	320	640	160	640	320	320	160
A/Catalonia/6095851NS/2014		2014-01-13	C0/SIAT1	<	80	160	160	320	80	320	160	160	160
A/Salamanca/44/2014	3C.3	2014-01-14	MDCK1/SIAT1/SIAT1	40	160	160	160	320	160	320	160	320	160
A/Segovia/81/2014	3C.3	2014-01-16	MDCK1/SIAT1/SIAT1	40	160	160	320	320	160	320	320	320	320
A/Salamanca/92/2014	3C.3	2014-01-22	MDCK1/SIAT1/SIAT1	40	160	160	320	640	160	640	320	320	320
A/Catalonia/2152420NS/2014		2014-01-25	C0/SIAT1	40	80	80	160	320	80	320	160	160	160

1. < = <40

Vaccine

Sequences in phylogenetic tree

[2] For example, the September 2013 report: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2013. Stockholm: ECDC; 2013. Available from http://www.ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-sep-2013.pdf

Table 3–2. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

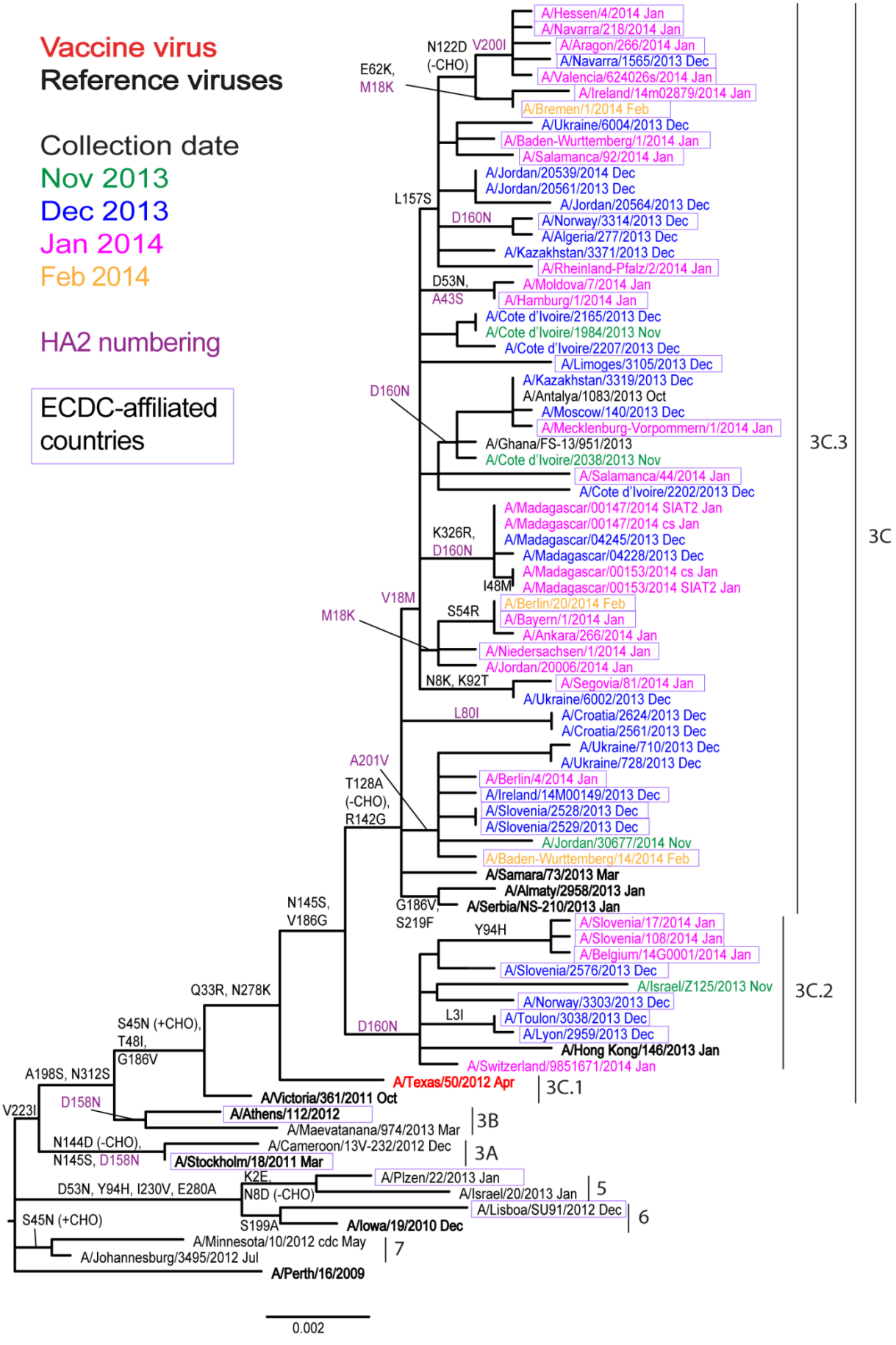
Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1] Post infection ferret antisera A/Perth 16/09 F35/11	A/Stock 18/11 F28/11 3A	A/Iowa 19/10 F15/11 6	A/Vic 361/11 T/C F11/13 3C.1	A/Athens 112/12 F16/12 3B	A/Texas 50/12 Egg F42/13 3C.1	A/Samara 73/13 F24/13 3C.3	A/Serbia NS-210/13 F39/13 3C.3	A/HK 146/13 F40/13 3C.2	NIB-85 F45/13 3C.3
REFERENCE VIRUSES													
A/Perth/16/2009		2009-07-04	E3/E3	1280	160	320	320	640	160	160	160	320	160
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	<	160	160	160	320	80	320	160	160	160
A/Iowa/19/2010	6	2010-12-30	E3/E2	640	1280	2560	2560	2560	1280	1280	1280	1280	1280
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT3	80	160	320	320	640	320	640	320	320	320
A/Athens/112/2012	3B	2012-02-01	SIAT4	80	320	320	320	640	160	640	320	320	160
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	640	1280	1280	1280	1280	1280	640	1280	640	1280
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	80	320	320	320	640	320	640	320	640	640
A/Serbia/NS-210/2013	3C.3	2013-01-18	E5/E1	320	640	1280	1280	1280	1280	640	1280	1280	1280
A/Hong Kong/146/2013	3C.2	2013-01-11	E5/E1	640	1280	1280	1280	1280	640	1280	640	2560	1280
NIB-85 (A/Almaty/2958/2013)	3C.3	2013-01-27	E5/E1	640	1280	2560	1280	1280	1280	1280	1280	2560	1280
TEST VIRUSES													
A/Hamburg/1/2014	3C.3	2014-01-20	C2/SIAT1	<	80	80	80	160	80	160	80	80	80
A/Berlin/8/2014		2014-01-21	C2/SIAT1	<	160	160	320	640	160	640	320	320	320
A/Hessen/4/2014	3C.3	2014-01-27	C2/SIAT1	<	40	80	80	160	80	160	80	80	80
A/Berlin/12/2014		2014-01-27	C2/SIAT1	<	80	80	160	160	80	160	80	80	80
A/Hessen/1/2014		2014-01-27	C2/SIAT1	40	160	160	320	320	160	320	160	160	160
A/Baden-Wurttemberg/7/2014		2014-01-29	C2/SIAT1	40	160	160	160	320	160	320	160	160	160
A/Bayern/3/2014		2014-01-30	C2/SIAT1	<	160	160	320	640	160	320	320	320	320
A/Berlin/20/2014	3C.3	2014-02-04	C2/SIAT1	<	80	80	160	320	80	160	80	80	80
A/Bremen/1/2014	3C.3	2014-02-07	C2/SIAT1	<	80	160	160	320	80	160	160	80	80
A/Baden-Wurttemberg/14/2014	3C.3	2014-02-10	C2/SIAT1	<	160	160	320	640	160	320	160	160	160

1. < = <40

Vaccine

Sequences in phylogenetic tree

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



Influenza B virus analyses

B/Victoria-lineage viruses

Only three viruses of the B/Victoria-lineage, from EU/EEA countries, were analysed at the WHO CC since the February 2014 report. HI results are shown in Table 4. All cell-propagated test viruses reacted with post-infection ferret antisera raised against the recommended vaccine virus for quadrivalent vaccines, B/Brisbane/60/2008, at titres within fourfold of the titre with the homologous virus. The test viruses were poorly recognised by post-infection ferret antisera raised against other egg-propagated reference viruses, B/Malta/636714/2011, B/Johannesburg/3964/2012 and B/South Australia/81/2012. In contrast, all viruses showed reactivity within fourfold of the titre for the homologous virus with antisera raised against viruses genetically closely related to B/Brisbane/60/2008 but propagated in cells. These antisera were raised against B/Paris/1762/2008, B/Hong Kong/514/2009, B/Odessa/3886/2010 and B/Formosa/V2367/2012. These viruses are considered to be surrogate cell-propagated antigens representing the egg-propagated prototype B/Brisbane/60/2008.

Phylogenetic analysis of the HA genes of representative B/Victoria-lineage viruses is shown in Figure 3. The HA genes of all recently collected viruses from EU/EEA countries sent to NIMR since the February 2014 report fell within the B/Brisbane/60/2008 genetic clade in subgroup 1A.

Table 4. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

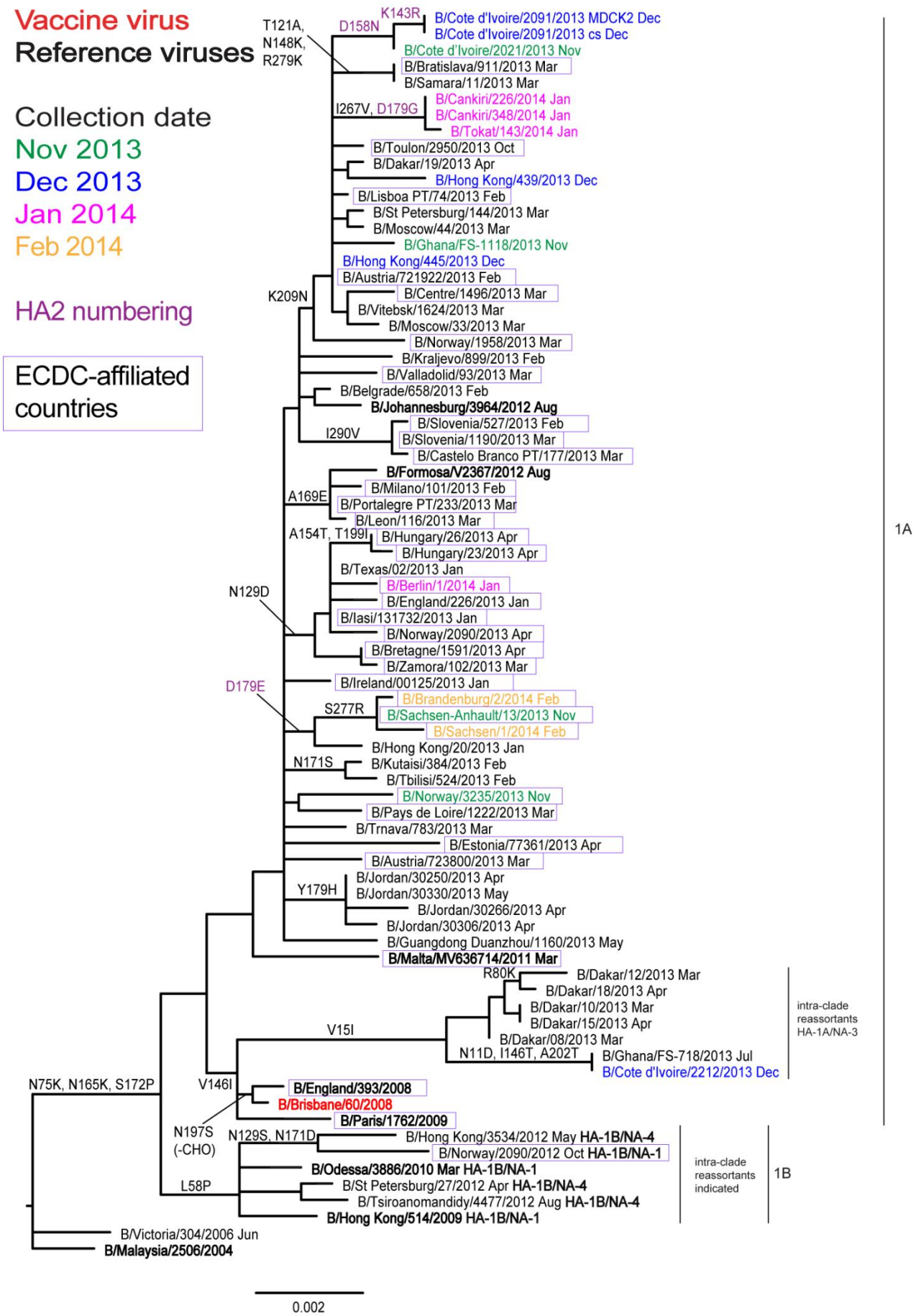
Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre: Post infection ferret sera[1]									
				B/Bris[2] 60/08 Sh 522	B/Mal 2506/05 F37/11	B/Bris 60/08 F26/13	B/Paris 1762/08 F7/11	B/HK 514/09 F9/13	B/Odessa 3886/10 F19/11	B/Malta 636714/11 F29/13	B/Jhb 3964/12 F1/13	B/For V2367/12 F4/13	B/Sth Aus 81/12 F41/13
	Genetic group			1A		1A	1A	1B	1B	1A	1A	1A	1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	2560	640	20	<	<	<	80	160	80	160
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	2560	80	320	40	80	80	320	320	320	1280
B/Paris/1762/2008	1A	2009-02-09	C2/MDCK2	2560	10	40	40	40	80	<	40	80	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK4	2560	10	40	80	80	160	<	40	80	160
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	2560	40	80	40	80	160	80	160	160	640
B/Malta/636714/2011	1A	2011-03-07	E4/E1	2560	80	320	40	80	80	320	320	320	640
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	640	1280	80	160	160	640	1280	1280	1280
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK2	1280	20	160	40	40	80	80	80	160	640
B/South Australia/81/2012	1A	2012-11-28	E4/E1	5120	80	1280	40	80	80	320	320	320	1280
TEST VIRUSES													
B/Toulon/2950/2013	1A	2013-10-23	MDCK2/MDCK1	5120	10	160	40	80	80	40	40	80	160
B/Sachsen Anhalt/13/2013	1A	2013-11-29	MDCK1	2560	40	80	40	40	80	20	<	40	80
B/Norway/3235/2013	1A	2013-11-30	MDCK1	2560	10	80	40	40	80	40	20	40	80
						Vaccine*							

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

Sequence in phylogenetic trees

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

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



B/Yamagata-lineage viruses

The results of HI analyses for propagated viruses of the B/Yamagata-lineage from EU/EEA countries, analysed since the February 2014 report, are shown in Table 5. The clades into which the HAs fall are shown. Post-infection ferret antiserum raised against the current, egg-propagated vaccine virus B/Massachusetts/02/2012 recognised all three test viruses at titres eightfold reduced, compared to the titre with the homologous virus. A ferret antiserum raised against a cell-propagated cultivar of B/Massachusetts/02/2012 recognised all three test viruses at titres within fourfold of its titre with the homologous virus. Two of the test viruses were recognised by ferret antisera raised against two other viruses with HA genes belonging to the B/Massachusetts/02/2012 clade (Clade 2), B/Estonia/55669/2011 and B/Hong Kong/3577/2012, at titres within fourfold of the titres of the antisera with their homologous viruses. All three test viruses were recognised well by an antiserum raised against the previous vaccine virus B/Wisconsin/1/2010, and all three were recognised at titres within fourfold of the homologous titre by antiserum raised against cell-propagated B/Novosibirsk/1/2012, a virus belonging to the B/Wisconsin/1/2010 clade, Clade 3.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. The HA genes of viruses collected since 31 October 2013 fell into the B/Massachusetts/02/2012 clade (Clade 2) and the B/Wisconsin/1/2010 clade (Clade 3) in approximately equal proportions. However, four of the five EU/EEA viruses collected in 2014 and received at the WHO CC, London had HA genes that clustered in Clade 3, the B/Wisconsin/1/2010 genetic clade.

Table 5. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

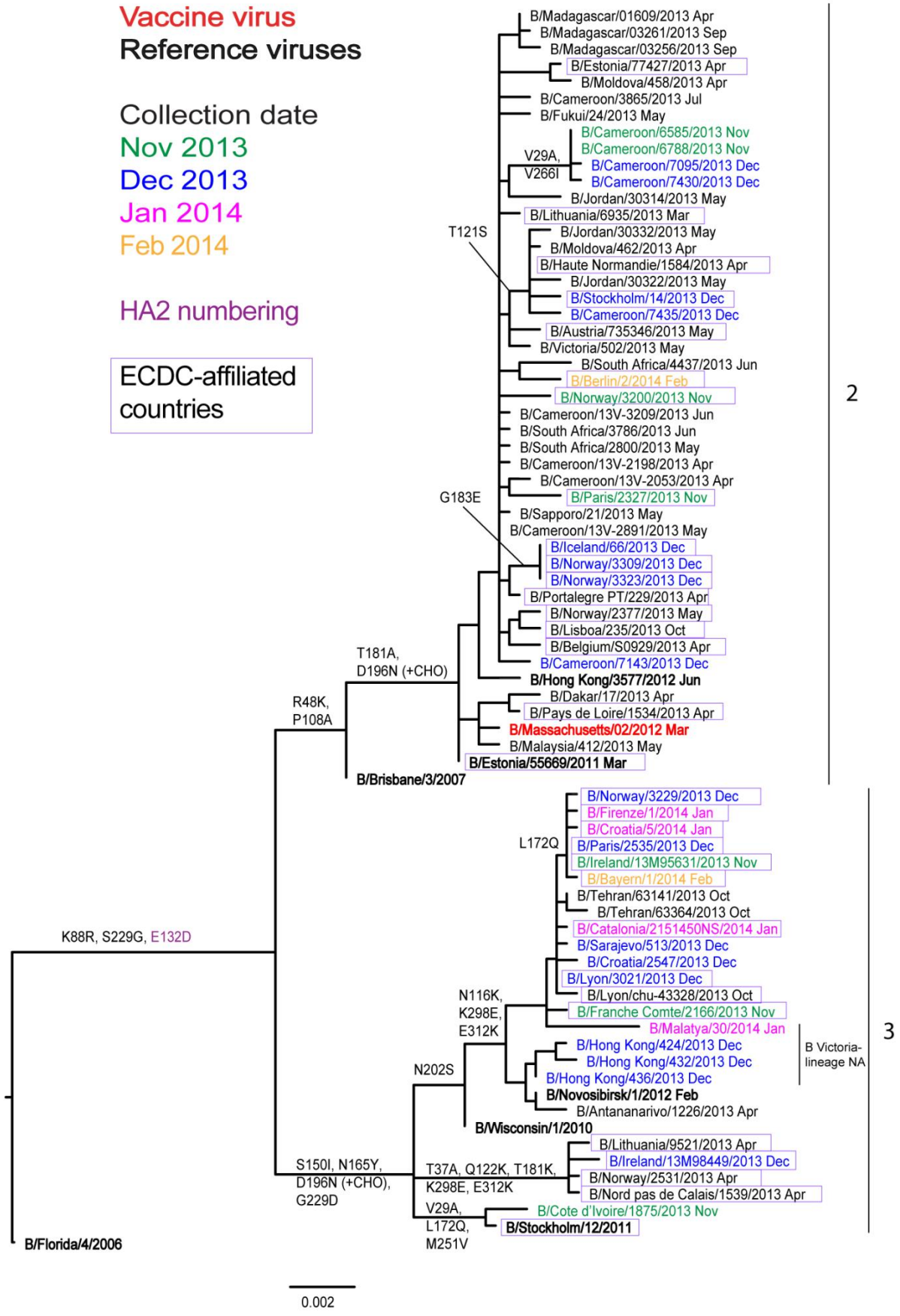
				Haemagglution Inhibition Titre									
				Post infection ferret sera									
Viruses	Genetic Group	Collection date	Passage History	B/Fl[1,3] 4/06 SH479 1	B/Fl[1] 4/06 F1/10 1	B/Bris[2] 3/07 F21/12 2	B/Wis[2] 1/10 F10/13 3	B/Stock[2] 12/11 F12/12 3	B/Estonia[2] 55669/11 F26/11 2	B/Novo[2] 1/12 F31/12 3	B/HK[2] 3577/12 F33/12 2	B/Mass[2] 02/12 Egg F2/13 2	B/Mass[2] 02/12 T/C F15/13 2
REFERENCE VIRUSES													
B/Florida/4/2006	1	2006-12-15	E7	5120	1280	1280	320	640	160	80	320	1280	160
B/Brisbane/3/2007	2	2007-09-03	E2/E2	5120	640	640	320	640	160	80	320	640	160
B/Wisconsin/1/2010	3	2007-08-07	E3/E2	640	160	160	160	320	<	40	40	320	40
B/Stockholm/12/2011	3	2007-08-07	E4/E1	2560	160	80	80	160	10	40	40	160	20
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	160	80	80	80	640	80	640	160	640
B/Novosibirsk/1/2012	3	2012-02-14	C2/MDCK3	2560	160	160	160	160	160	320	320	160	640
B/Hong Kong/3577/2012	2	2012-06-13	MDCK4	1280	80	80	80	80	640	80	640	160	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E4	5120	1280	1280	640	640	80	20	640	1280	320
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	2560	320	320	160	320	320	80	320	640	320
TEST VIRUSES													
B/Catalonia/2151450NS/2014	3	2014-01-21	C0/MDCK1	2560	160	160	320	640	160	160	160	160	320
B/Berlin/2/2014	2	2014-02-06	C2/MDCK1	5120	160	160	160	320	160	80	640	160	640
B/Bayern/1/2014	3	2014-02-10	C2/MDCK1	1280	160	80	160	320	40	80	80	160	80

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

Sequences in phylogenetic tree

Vaccine

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 [1] 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 2013 by the Chinese CDC. A description of the characteristics of H7N9 viruses can be found on the WHO website [2]. Increased numbers of cases have been reported over the course of the 2013–14 season, continuing into April 2014. A revised Rapid Risk Assessment [3] for these A(H7N9) viruses was carried out by ECDC and posted on 27 January 2014, and an updated summary of human infection was posted by WHO on 31 January 2014 [4] followed by an updated risk assessment on 28 February 2014 [5]. The most recent update of the epidemiological situation published by WHO was posted on 16 April 2014.

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva on 23–25 September 2013 and 17–19 February 2014, can be found at:

http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Sep2013final.pdf

http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Feb2014-web.pdf

Note on the figures

The phylogenetic trees were constructed using RAxML, drawn using FigTree and annotated using Adobe Illustrator. 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 many viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu database which were downloaded for use in the preparation of this report (all submitters of data may be contacted directly via the GISAID website), along with all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

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3 European Centre for Disease Prevention and Control. Updated rapid risk assessment. Human infection with a novel avian influenza A(H7N9) virus, China. Third update. 27 January 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/influenza-AH7N9-China-rapid-risk-assessment-27-January-2014.pdf

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