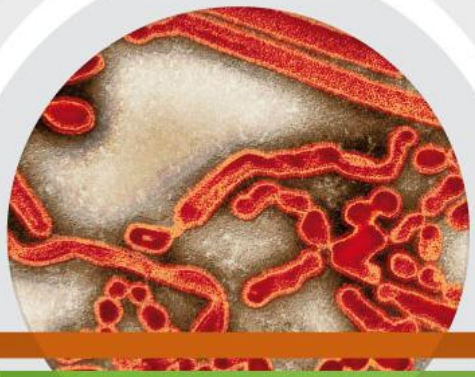

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, April 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. The viruses were antigenically 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 31 December 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 18 countries in the EU/EEA. 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- and B/Victoria-lineages were approximately equally represented. 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).

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Table 1. Summary of clinical samples and isolates received from ECDC-affiliated countries, with collection dates after 31 December 2013

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]
2014											
JANUARY											
Belgium	3		2	2	1	1					
Bulgaria	32		24	in process	8	in process					
Cyprus	13	4	9	7							
Germany	22		4	4	17	17		1	1		
Greece	35		32	15	3	2					
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					
Poland	2		1	in process	1	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	5		2	2	2	2				1	in process
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				
Latvia	1		1	in progress							
Norway	8				8	in process					
Poland	9		2	in progress	7	in process					
Sweden	2				2	in process					
United Kingdom	3				2	in process		1	in process		
MARCH											
Bulgaria	1		1	in process							
Italy	3		2	in process	1	in process					
Latvia	11		7	in process	3	in process				1	in process
Norway	6				6	in process					
Poland	26	2	2	in process	22	in process					
United Kingdom	8		4	in process	2	in process		1	in process	1	in process
APRIL											
Poland	3		2	in process	1	in process					
	414	7	209	90	183	47	3	5	3	7	4
				50.5%		44.2%			1.2%		1.7%
18 Countries			96.4%				3.6%				

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 18 H1N1 viruses analysed since the March 2014 report[1] were received from Cyprus. Antigenically, test viruses were similar to the vaccine virus as assessed by haemagglutination inhibition (HI) assay; all showed no more than twofold reduced HI titres with antiserum raised against the vaccine virus (A/California/7/2009), compared with the titre for the vaccine virus (Table 2). One virus, A/Cyprus/F24/2014, was recognised less well by a number of antisera raised against other reference viruses but there were no amino acid substitutions in the HA of this virus that would account for this. Viruses for which gene sequences are included in the phylogenetic tree are highlighted and, where known, the HA genetic group is indicated. The test virus, A/Cyprus/F79/2014 showed reduced inhibition (RI) by oseltamivir in sialidase assays. The NA gene sequence of A/Cyprus/F79/2014 encoded a S247N substitution, also present in the clinical specimen, that has been associated with RI by oseltamivir previously.

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

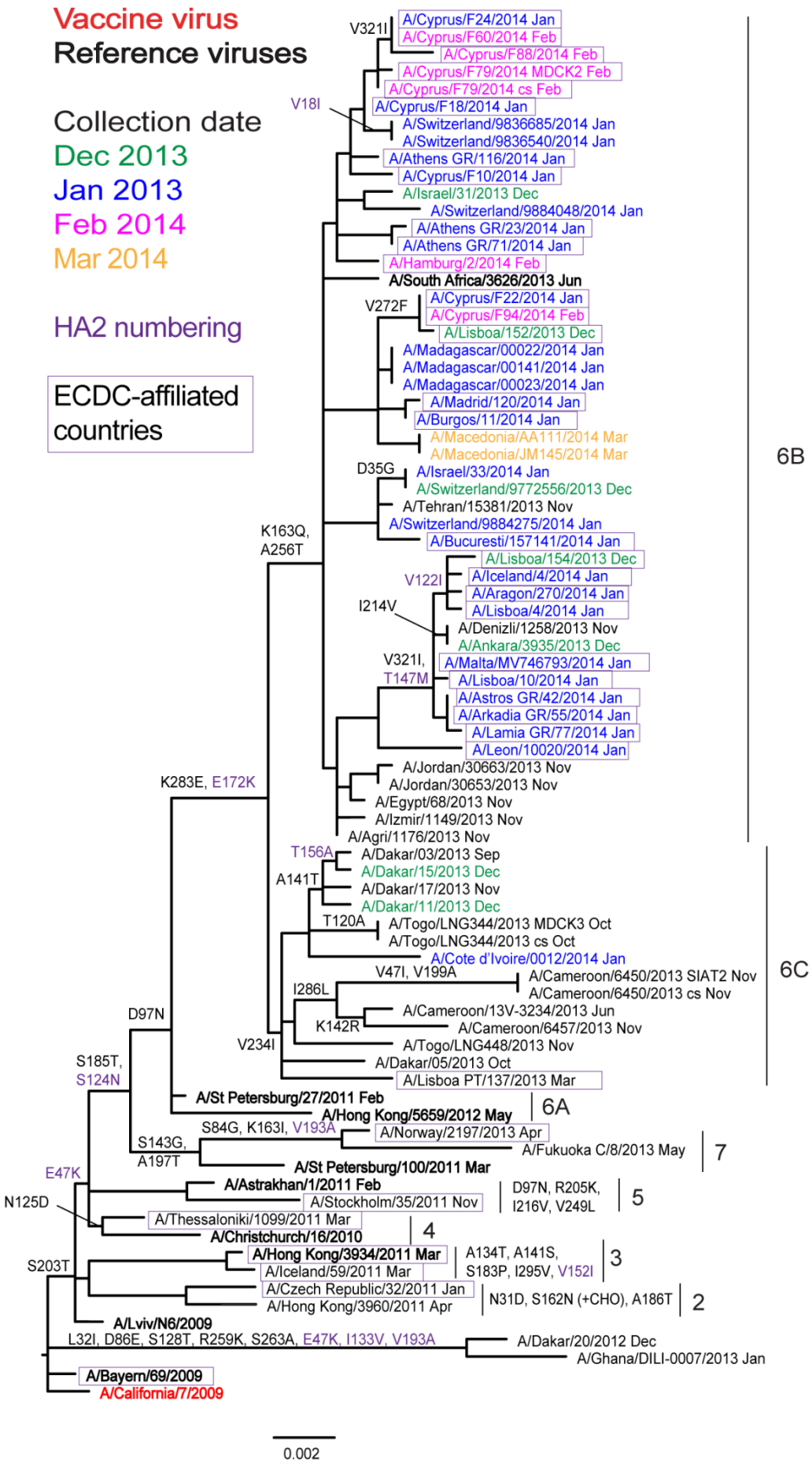
Figure 1 shows a phylogenetic tree for the HA genes of representative H1N1 viruses. The HA genes cluster into eight designated genetic groups, of which seven are indicated, with A/California/7/2009 representing group 1. Viruses collected after 31 December 2013 in the EU/EEA, and those characterised since the March 2014 report, fell into genetic subgroup 6B (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. Antigenic analysis of A(H1N1)pdm09 viruses by HI

				Haemagglutination inhibition titre										
				Post infection ferret antisera										
Viruses	Genetic group	Collection date	Passage History	A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 C4/09/34	A/Chch 16/10 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	1280	1280	320	320	320	320	640	640	320	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK2	320	320	160	80	40	80	80	80	80	80	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	640	160	80	160	160	160	320	160	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	2560	5120	2560	2560	2560	5120	5120	1280	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	160	640	320	1280	1280	640	1280	1280	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	1280	1280	1280	1280	1280	2560	2560	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	5120	2560	5120	5120	5120	5120	5120	5120	5120	5120	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	1280	640	1280	1280	1280	1280	2560	5120	2560	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK1	640	320	640	640	1280	1280	1280	5120	5120	1280	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	640	640	640	640	640	1280	640	2560	1280	1280	
TEST VIRUSES														
A/Cyprus/F5/2014		2014-01-21	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	2560	1280	
A/Cyprus/F10/2014	6B	2014-01-23	MDCK2	640	320	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F18/2014	6B	2014-01-27	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	2560	2560	
A/Cyprus/F19/2014		2014-01-27	MDCK2	1280	320	1280	1280	2560	2560	5120	5120	2560	1280	
A/Cyprus/F20/2014		2014-01-27	MDCK2	640	320	640	640	1280	1280	640	2560	2560	1280	
A/Cyprus/F22/2014	6B	2014-01-27	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	5120	1280	
A/Cyprus/F24/2014	6B	2014-01-27	MDCK3	640	320	640	320	640	640	640	2560	1280	640	
A/Cyprus/F37/2014		2014-02-03	MDCK2	640	320	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F38/2014		2014-02-03	MDCK2	1280	640	1280	1280	1280	1280	1280	2560	2560	1280	
A/Cyprus/F39/2014		2014-02-03	MDCK2	1280	640	1280	1280	1280	1280	1280	2560	2560	1280	
A/Cyprus/F45/2014		2014-02-04	MDCK2	640	320	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F51/2014		2014-02-05	MDCK2	640	320	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F60/2014	6B	2014-02-10	MDCK2	1280	640	1280	1280	2560	2560	1280	5120	5120	1280	
A/Cyprus/F76/2014		2014-02-12	MDCK2	1280	640	1280	1280	2560	2560	2560	5120	5120	1280	
A/Cyprus/F79/2014	6B	2014-02-17	MDCK2	640	640	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F81/2014		2014-02-17	MDCK2	640	640	640	640	1280	1280	1280	2560	2560	1280	
A/Cyprus/F88/2014	6B	2014-02-18	MDCK2	640	640	1280	1280	2560	2560	1280	5120	2560	1280	
A/Cyprus/F94/2014	6B	2014-02-20	MDCK3	640	320	640	640	1280	1280	1280	2560	2560	1280	
				Vaccine										

Sequences in phylogentic 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 March 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 Table 3. Viruses for which gene sequences are included in the phylogenetic tree are highlighted and, where known, the HA genetic group is indicated.

All seven test viruses analysed since the March 2014 report reacted poorly in HI assays (≥sixteenfold 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. 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 but one of the test viruses analysed since the March 2014 report were recognised at titres within fourfold of those for the antisera with their corresponding homologous viruses. The exception, A/Greece/12/2014, showed an eightfold reduction with the antiserum raised against A/Samara/73/2013, however, there were no amino acid substitutions in the HA of A/Greece/12/2014 that might explain this reduction in titre.

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 characterised at NIMR from EU/EEA countries since the March 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. The four viruses characterised antigenically and genetically since the March 2014 report fall into subgroup 3C.3 (Table 3, Figure 2). 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. 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 F12/13	A/Iowa 19/10 F15/11 3A*	A/Vic 361/11 T/C F11/13 6	A/Athens 112/12 F16/12 3B	A/Texas 50/12 Egg F42/13 3C.1	A/Samara 73/13 F24/13 3C.3	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	320	320	320	640	160	160	160	160	160
A/Stockholm/18/2011	3A	2011-03-28	SIAT5	40	640	160	320	640	160	640	320	320	320
A/Iowa/19/2010	6	2010-12-30	E3/E2	640	1280	5120	2560	2560	1280	1280	1280	1280	1280
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT3	80	320	160	320	640	160	640	160	320	640
A/Athens/112/2012	3B	2012-02-01	SIAT5	80	320	160	320	640	160	640	160	320	640
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	1280	2560	2560	2560	2560	1280	1280	2560	1280	2560
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	80	640	320	320	640	320	1280	320	640	640
A/Serbia/NS-210/2013	3C.3	2013-01-18	E5/E1	640	1280	1280	1280	2560	2560	1280	1280	1280	2560
A/Hong Kong/146/2013	3C.2	2013-01-11	E5/E1	640	1280	2560	1280	1280	640	1280	640	2560	1280
NIB-85 (A/Almaty/2958/2013)	3C.3	2013-01-27	E5/E1	640	2560	2560	1280	2560	2560	1280	2560	2560	2560
TEST VIRUSES													
A/Greece/12/2014	3C.3	2014-01-05	SIAT4	<	160	40	80	160	40	160	80	80	80
A/Slovenia/87/2014		2014-01-09	SIAT3	<	320	80	160	320	80	320	160	80	160
A/Lamia_GR/79/2014	3C.3	2014-01-15	SIAT4	<	320	80	160	320	80	640	160	160	160
A/Cyprus/F4/2014	3C.3	2014-01-21	SIAT3	<	160	160	80	320	80	320	160	160	80
A/Cyprus/F15/2014		2014-01-24	SIAT2	<	160	80	160	320	80	320	160	160	160
A/Cyprus/F30/2014	3C.3	2014-01-28	SIAT2	<	160	80	160	640	80	320	160	160	160
A/Cyprus/F31/2014		2014-01-28	SIAT2	<	320	80	160	320	80	320	160	160	160

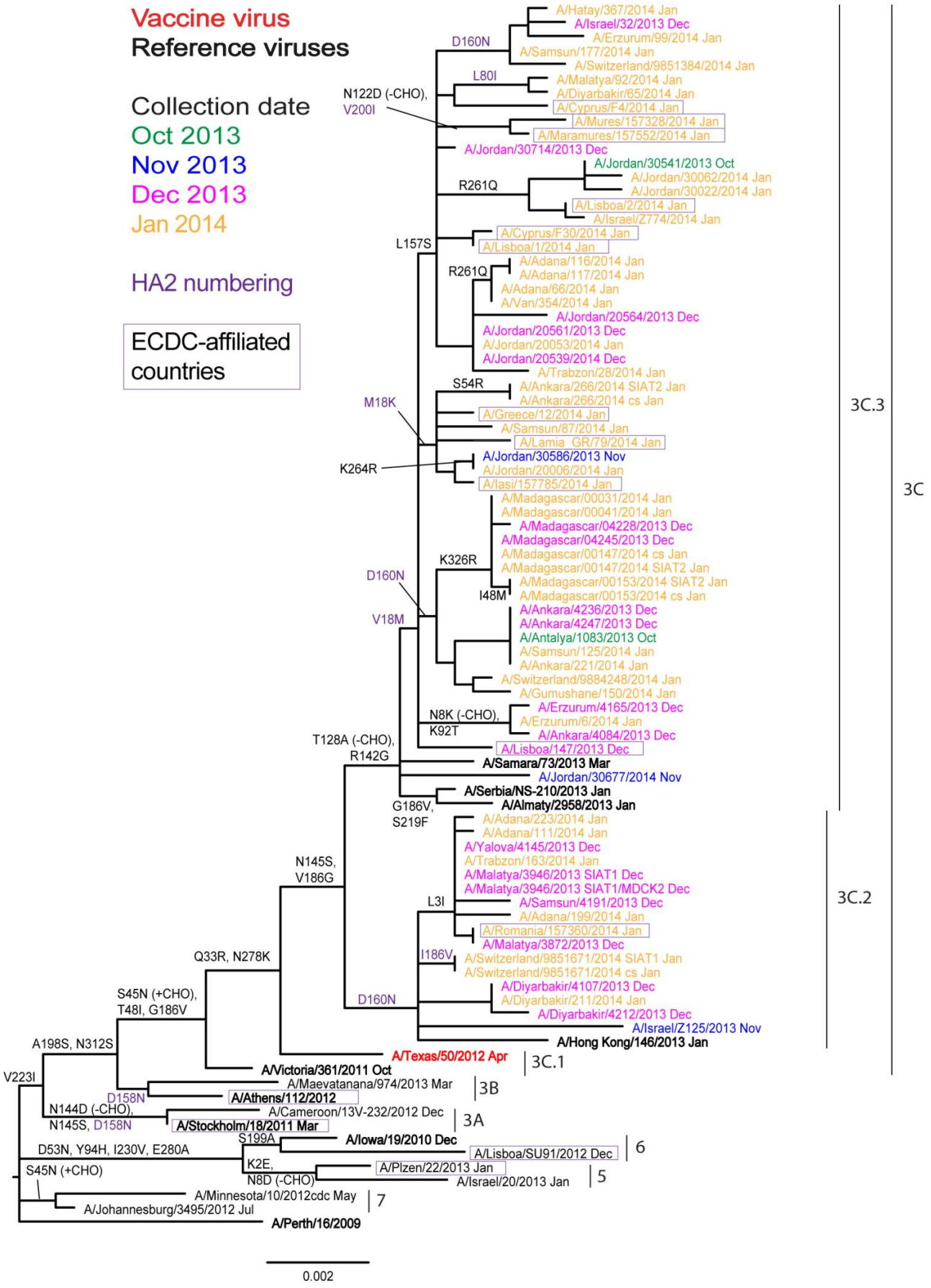
1. < = <40

Vaccine

Sequences in phylogentic 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

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



Influenza B virus analyses

No influenza B viruses from EU/EEA countries have been characterised since the March 2014 report.

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 May 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 15 May 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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5 World Health Organization. WHO risk assessment: Human infections with avian influenza A(H7N9) virus, 28 February 2014. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/140225_H7N9RA_for_web_20140306FM.pdf