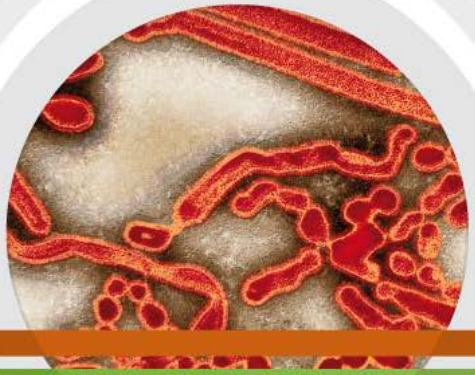


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, April 2015

Summary

Over the course of the 2014–15 influenza season influenza A(H3N2), A(H1N1)pdm09 and type B viruses have co-circulated in EU/EEA countries. To date, 23 EU/EEA countries have shared 805 influenza-positive specimens with the WHO Collaborating Centre in London for detailed characterisation. Since the March 2015 report[1], 41 viruses have been characterised antigenically and 83 genetically.

The 10 A(H1N1)pdm09 viruses characterised antigenically were similar to the vaccine virus A/California/07/2009; all those characterised genetically had HA genes belonging to genetic subgroup 6B, as observed worldwide.

Many of the 21 A(H3N2) viruses characterised by haemagglutination inhibition (HI) assay were poorly recognised by antisera raised against the A/Texas/50/2012 vaccine virus but relatively well recognised by antisera raised against cell-propagated genetic subgroup 3C.3a viruses. The 267 (59 since the March 2015 report) viruses, with collection dates after 31 August 2014, characterised genetically this season fell in genetic group/subgroups 3C.3 (30), 3C.3b (58), 3C.3a (24) and 3C.2a (155). Viruses in genetic group 3C.3 and subgroup 3C.3b were antigenically similar to A/Texas/50/2012, while those in subgroups 3C.2a and 3C.3a were antigenically distinct, and the two subgroups were antigenically distinguishable.

No B/Victoria-lineage viruses were received since the March 2015 report.

The 10 characterised B/Yamagata-lineage test viruses fell in genetic clade 3 and showed good reactivity with antisera raised against B/Phuket/3073/2013 (the clade 3 virus recommended for the southern hemisphere 2015 and northern hemisphere 2015–16 vaccines). Antisera raised against B/Massachusetts/02/2012 (the clade 2 virus recommended for the 2014–15 northern hemisphere season vaccine) did not recognise test viruses as well as antisera raised against B/Phuket/3073/2013.

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, March 2015. Stockholm: ECDC; 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/Influenza-virus-characterisation-March-2015.pdf

This report was prepared by Rod Daniels, Burcu Ermetal, Aine Rattigan 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, April 2015. Stockholm: ECDC; 2015.

Influenza-positive samples (805 viruses or clinical specimens: 157 being received since the March 2015 report) with collection dates after 31 August 2014 were received at the Crick Worldwide Influenza Centre, the Francis Crick Institute, Mill Hill Laboratory, from 23 countries in the EU/EEA. Overall, the majority (~80%) were type A viruses, and A(H3N2) outnumbered A(H1N1)pdm09 at a ratio of 3:1 (Table 1). Of the 168 type B specimens received (~20% of the specimens), 113 were of the B/Yamagata-lineage, 52 were not ascribed to a lineage, and only three were of the B/Victoria lineage. Some of these samples, mostly received as clinical specimens, are still in the process of being characterised. The antigenic and genetic properties of influenza virus isolates characterised since the March 2015 report are presented and discussed in this report.

Table 1. Summary of clinical samples and virus isolates received from EU/EEA Member States, with collection dates after 31 August 2014

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
Country		Number received	Number received	Number propagated[1]	Number received	Number propagated[2]	Number received	Number received	Number propagated[1]	Number received	Number propagated[1]
2014											
SEPTEMBER											
Belgium	1				1	1					
France	2				1	1				1	1
Spain	1				1	0 (1)					
Sweden	3				3	2 (1)					
OCTOBER											
Belgium	5				5	1 (4)					
Denmark	2				2	2					
Finland	1				1	1					
France	6				5	1 (4)				1	1
Germany	6		3	3	2	2		1	1		
Malta	4				4	3 (1)					
Netherlands	6				5	3 (2)				1	1
Norway	8		5	3	3	1					
Slovenia	2				1	1				1	0
Spain	10				7	5 (1)				3	3
Sweden	2				2	1 (1)					
United Kingdom	2				1	1				1	1
NOVEMBER											
Belgium	4		1	1	1	0 (1)				2	1
Denmark	1				1	1					
Finland	2				2	0 (2)					
France	6		1	1	3	0 (3)				2	2
Germany	8		2	2	5	3 (2)				1	1
Latvia	1		1	1							
Luxembourg	1		1	1							
Netherlands	3				3	0 (3)					
Norway	10				2	2				8	3
Portugal	2									2	2
Slovenia	1		1	1							
Spain	10				9	6 (3)				1	1
Sweden	3				3	3					
United Kingdom	7				6	3 (2)				1	1
DECEMBER											
Austria	8				7	1 (6)				1	1
Belgium	5		3	3	1	1				1	1
Croatia	10		4	4	2	1	2			2	2
Czech Rep	7				7	1 (6)					
Denmark	5		2	2	3	0					
Estonia	1				1	0 (1)					
Finland	5		2	2	1	0 (1)				2	2
France	37		4	4	26	19 (7)				7	7
Germany	18		2	2	14	6 (6)		1	1	1	1
Greece	3				2	1 (1)				1	1
Italy	30		14	14	9	4 (5)				7	7
Latvia	8		1	1	5	3 (2)				2	2
Luxembourg	11		6	3	3	1 (2)	2				
Malta	4				4	1 (2)					
Netherlands	5		1	1	4	1 (3)					
Norway	25		4	4	14	6 (5)				7	4
Portugal	10				3	1 (2)				7	7
Slovenia	19		17	14	1	0	1				
Spain*	43	1	2	2	35	7 (21)				5	5
United Kingdom	12		1	1	10	2 (5)				1	1
2015											
JANUARY											
Bulgaria	11				11	in process					
Croatia	1				1	0					
Cyprus	8				8	6 (2)					
Denmark	2				2	2					
Estonia	24		1	0	22	2 (11)				1	0
Germany	33		5	5	22	12 (10)				6	6
Greece	61		12	5	25	4 (14)	16	1	in process	7	7
Italy*	17		8	1	7	in process	1			1	in process
Latvia	2				2	0 (2)					
Luxembourg	1						1				
Malta	5				5	1 (1)					
Norway	4				4	1 (2)					
Portugal	7		2	1	3	0 (2)				2	1
Slovenia	15		8	8	3	0 (3)	2			2	2
Spain*	47		11	10	25	10 (6)	4			7	7
United Kingdom	27		2	2	25	7 (8)					
FEBRUARY											
Bulgaria	26		3	3	19	in process				4	4
Cyprus	12				11	1 (9)				1	1
Greece	13		3	in process	4	in process	4			2	in process
Italy*	41		12	in process	15	in process	8			6	in process
Spain*	58	8	1	1	35	12 (15)	10			4	4
MARCH											
Bulgaria	3				2	0 (2)				1	1
Italy*	1						1				
23 Countries	805	9	146	106	482	158 (193)	52	3	2	113	92
			18.1%		59.9%			0.4%		14.0%	
			79.1%				20.9%				

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)
Numbers in parentheses indicate viruses recovered but with insufficient HA titre to permit HI assay
* Packages received from these countries in April contained specimens with collection dates from December onwards, overlapping with earlier packages: a number of specimens are in process

Influenza A(H1N1)pdm09 virus analyses

Haemagglutination inhibition (HI) analyses of viruses that have been performed since the March 2015 report are shown in Table 2. Nine of ten recovered A(H1N1)pdm09 viruses were antigenically similar to the vaccine virus, A/California/7/2009, showing no more than twofold reduction in HI titre compared to that for the homologous virus, while A/Castilla La Mancha/666/2015 showed a fourfold reduction and carried HA1 P271S/P amino acid polymorphism. All viruses but A/Thessaloniki/93/2015 were recognised by the extended panel of antisera at titres within fourfold of the titres for the homologous viruses, with the exception of the antiserum raised against A/Christchurch/16/2010. This antiserum recognised 60% (6/10) of the test viruses at a titre within fourfold of the titre for the homologous virus. The A/Thessaloniki/93/2015 egg-propagated virus showed somewhat reduced titres, compared to the homologous titres, with all antisera in the panel and carried HA1 K153E/K polymorphism with S190R substitution. It is also noteworthy that all antisera raised against viruses falling outside of genetic group 1, the A/California/7/2009 group, recognised the egg-propagated vaccine virus at titres at least fourfold reduced compared to the titres of the antisera with their homologous viruses.

Figure 1 shows a phylogenetic tree for the HA genes of representative, recently circulating A(H1N1)pdm09 viruses. Since 2009, the HA genes have evolved, and eight genetic groups have been designated. Over the last two seasons viruses in genetic group 6, represented by A/St Petersburg/27/2011 and carrying amino acid substitutions of **D97N**, **S185T** and **S203T** in **HA1** and **E47K** and **S124N** in **HA2** compared with A/California/7/2009, have predominated worldwide with a number of subgroups emerging. All EU/EEA viruses characterised since the September 2014 report[2] carry HA genes in genetic subgroup 6B, which is characterised by additional amino acid substitutions of **K163Q, A256T** and **K283E** in **HA1** and **E172K** in **HA2** compared with A/California/7/2009, e.g. A/South Africa/3626/2013.

These results are compatible with those that contributed to the World Health Organization recommendation[3] to retain the A/California/7/2009 vaccine virus for the northern hemisphere 2015–16 influenza season.

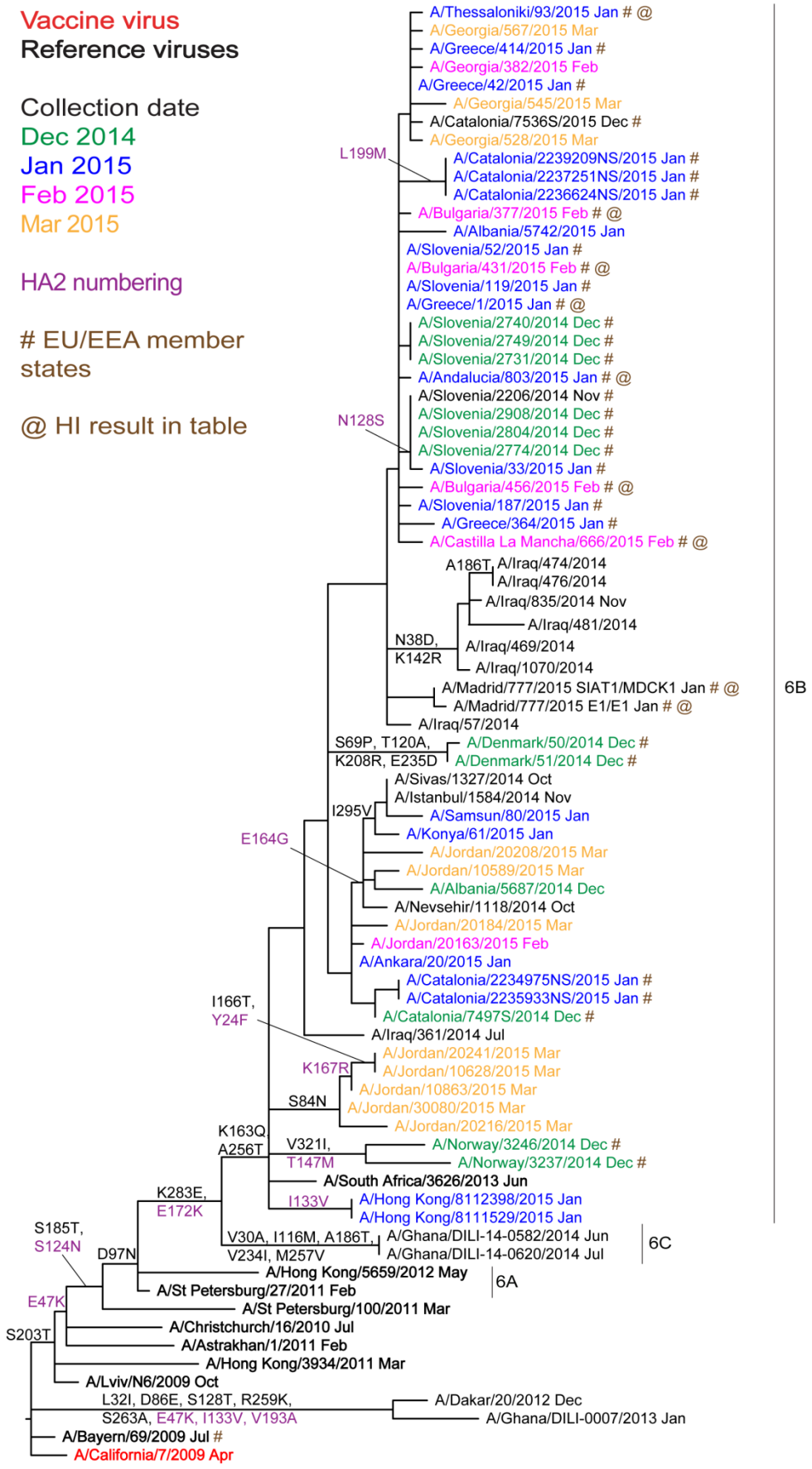
[2] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/Influenza-ERLI-Net-report-Sept-2014.pdf

[3] Recommended composition of influenza virus vaccines for use in the 2015–2016 northern hemisphere influenza season. Weekly Epidemiological Record Vol 90, p 97-108

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

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre										
				Post infection ferret antisera										
				A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 F14/13	A/Chch 16/10 F15/14 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/Sth Afr 3626/13 F3/14 6B	
REFERENCE VIRUSES														
A/California/7/2009		2009-04-09	E1/E3	640	640	1280	160	160	160	160	320	160	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	320	640	640	160	40	80	160	80	160	80	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/SIAT1/MDCK3	320	1280	1280	320	80	80	160	160	160	80	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	2560	5120	1280	2560	1280	5120	2560	1280	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	160	320	640	1280	1280	640	2560	1280	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	640	640	640	1280	1280	1280	2560	1280	640	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	1280	1280	640	1280	1280	1280	2560	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	1280	640	640	640	1280	1280	1280	5120	2560	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	320	160	160	160	640	640	640	1280	640	320	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	320	640	640	320	640	640	640	1280	640	640	
TEST VIRUSES														
A/Greece/1/2015	6B	2015-01-01	MDCK1	640	640	640	640	2560	1280	1280	2560	2560	1280	
A/Madrid/777/2015	6B	2015-01-23	SIAT1/MDCK1	1280	640	1280	1280	2560	2560	5120	5120	2560	1280	
A/Madrid/777/2015	6B	2015-01-23	E1/E2	640	640	1280	1280	1280	1280	1280	2560	2560	1280	
A/Thessaloniki/93/2015	6B	2015-01-23	Ex/E2	320	320	320	160	320	320	320	640	320	320	K153E/K, S190R
A/Andalucia/803/2015	6B	2015-01-27	SIAT2/MDCK1	1280	640	1280	2560	2560	5120	5120	5120	5120	2560	
A/Castilla La Mancha/666/2015	6B	2015-02-02	SIAT1/MDCK1	160	320	320	320	640	640	320	2560	640	640	P271S/P
A/Bulgaria/377/2015	6B	2015-02-18	SIAT2/MDCK1	1280	640	1280	1280	2560	2560	5120	5120	5120	2560	
A/Bulgaria/431/2015	6B	2015-02-24	SIAT2/MDCK1	1280	1280	1280	1280	2560	2560	2560	5120	2560	1280	
A/Bulgaria/456/2015	6B	2015-02-24	SIAT2/MDCK1	1280	1280	1280	2560	2560	5120	5120	5120	5120	2560	
A/Bulgaria/438/2015	6B	2015-02-24	SIAT2/MDCK2	640	640	640	640	1280	2560	2560	5120	2560	1280	
Sequences in phylogenetic tree				Vaccine										

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



Influenza A(H3N2) virus analyses

As described in many previous reports[4], influenza A(H3N2) viruses continue to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells (RBCs) from guinea pigs, turkeys and humans or the loss of the ability of viruses to agglutinate any of the RBCs. This is a particular problem for most viruses that fall in genetic subgroup 3C.2a as was highlighted in the November 2014 report[5].

Results of HI tests performed with guinea pig RBCs in the presence of 20nM oseltamivir, added to circumvent any NA-mediated binding of A(H3N2) viruses to the RBCs, conducted since the March 2015 report are shown in Table 3. The HA genetic group is indicated for those viruses that have been sequenced, and those included in the HA phylogenetic tree (Figure 2) are highlighted. Of the 78 successfully propagated viruses, only 21 (~27%) retained sufficient HA titre to be analysed by HI assay, showing a decrease from ~42% in the March 2015 report. The remainder (n = 57) were either unable to agglutinate guinea pig RBCs at all or were unable to agglutinate RBCs in the presence of 20nM oseltamivir. The vast majority of viruses unable to be titred by HI that were subjected to genetic analysis belonged to genetic subgroup 3C.2a. Viruses in genetic subgroup 3C.2a have acquired a glycosylation motif at positions 158 to 160 in HA1; those viruses in genetic subgroup 3C.2a that were able to bind guinea pig RBCs in the presence of oseltamivir (and hence were analysed by HI assay) had either lost, or were polymorphic for, this glycosylation motif.

All 21 test viruses, propagated in MDCK-SIAT1 cells, reacted poorly in HI assays ($\geq$ eightfold decrease) with post-infection ferret antiserum raised against the egg-propagated vaccine virus, A/Texas/50/2012, compared to the titre of the antiserum with the homologous virus. Similarly, low levels of reactivity were seen with antisera raised against the egg-propagated reference virus A/Hong Kong/146/2013: only three test viruses reacted within fourfold of the titre with the homologous egg-propagated virus. Better reactivity was seen with test viruses when analysed with an antiserum raised against the exclusively egg-propagated A/Stockholm/6/2014 isolate 2, a virus belonging to genetic subgroup 3C.3a. This antiserum showed a low titre for the homologous virus but recognised over 80% of test viruses at titres within fourfold of the homologous titre. Antiserum raised against egg-propagated A/Switzerland/9715293/2013, the virus in genetic subgroup 3C.3a recommended for the southern hemisphere 2015 and northern hemisphere 2015–16 vaccines, had a homologous titre of 640 and recognised only 5% of the test viruses at titres within fourfold of the homologous titre. Antiserum raised against egg-propagated A/Hong Kong/5738/2014 clone 121, a virus in genetic subgroup 3C.2a, had a homologous titre of 640 and failed to recognise any of the test viruses at titres within fourfold of the homologous titre.

Ferret antisera raised against reference viruses propagated in tissue culture cells, A/Victoria/361/2011 and A/Samara/73/2013, recognised the test viruses somewhat more effectively. The antiserum raised against A/Victoria/361/2011 recognised ~81% of the test viruses at a titre within fourfold of the antiserum for the homologous virus, but the antiserum raised against A/Samara/73/2013 recognised only ~29% of test viruses at a titre within fourfold of the titre for the homologous virus. These reference viruses have HA genes from genetic groups 3C.1 and 3C.3, respectively. Antisera raised against reference viruses belonging to genetic subgroup 3C.3a that had been exclusively propagated in cell culture, A/Stockholm/6/2014 and A/Switzerland/9715293/2013, recognised 95% and 81%, respectively, of test viruses at titres within fourfold of those with the corresponding homologous viruses. An antiserum raised against a reference virus belonging to genetic subgroup 3C.2a that had been exclusively propagated in cell culture, A/Hong Kong/5738/2014, recognised 95% of test viruses at titres within fourfold of that for the homologous virus.

[4] 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

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

Since 2009, seven genetic groups based on the HA gene have been defined for A(H3N2) viruses. Phylogenetic analysis of the HA genes of representative, recently circulating A(H3N2) viruses is shown in Figure 2. The HA genes fall within genetic group 3C. This group has three subdivisions: 3C.1 (to which the recommended vaccine virus for the 2014–15 northern hemisphere season, A/Texas/50/2012, belongs), 3C.2 and 3C.3. Viruses in these three subdivisions have been antigenically similar. However, in 2014 three new genetic subgroups emerged, one in subdivision 3C.2, 3C.2a, and two in 3C.3, 3C.3a and 3C.3b (Figure 2). While viruses in genetic subgroups 3C.2a and 3C.3a are antigenic drift variants, those in 3C.3b have remained antigenically similar to previously circulating viruses in the 3C.3 subdivision. Amino acid substitutions that define these subdivisions and subgroups compared with A/Texas/50/2012 are:

- (3C.2) **N145S** and **V186G**[6] in **HA1**, and **D160N** in **HA2**, e.g. A/Hong Kong/146/2013
- (3C.2a) Those in 3C.2 plus **L3I**, **N144S** (resulting in the loss of a potential glycosylation site), **F159Y**, **K160T** (in the majority of viruses, resulting in the gain of a potential glycosylation site), **N225D** and **Q311H** in **HA1**, e.g. A/Hong Kong/5738/2014
- (3C.3) **T128A** (resulting in the loss of a potential glycosylation site), **R142G**, **N145S** and **V186G** in **HA1**, e.g. A/Samara/73/2013
- (3C.3a) those in 3C.3 plus **A138S**, **F159S** and **N225D** in **HA1**, many with **K326R**, e.g. A/Switzerland/9715293/2013
- (3C.3b) those in 3C.3 plus **E62K, K83R, N122D** (resulting in the loss of a potential glycosylation site), **L157S** and **R261Q** in **HA1** with **M18K** in **HA2**, e.g. A/Newcastle/22/2014

Of the A(H3N2) viruses received from EU/EEA countries, with collection dates since 31 August 2014, 267 have been characterised genetically. These have fallen in HA genetic subgroups 3C.2a (n = 155; 58%), 3C.3a (n = 24; 9%) and 3C.3b (n = 58; 22%), with the remainder (n = 30; 11%) being in subdivision 3C.3. This is indicative of 67% of recently circulating A(H3N2) viruses being antigenic drift variants compared to A/Texas/50/2012, the virus recommended for use in northern hemisphere 2014–15 vaccines.

Based on results that showed cross-reactivity of antisera raised against genetic subgroup 3C.3a and 3C.2a viruses but with issues of antigenic changes on egg-adaptation of genetic subgroup 3C.2a viruses, the World Health Organization recommendation was to use an A/Switzerland/9715293/2013-like virus as the A(H3N2) component of vaccines for the northern hemisphere 2015–16 influenza season.

[6] Note: the G186V substitution in HA1 occurred during adaptation of A/Texas/50/2012 to propagation in hens' eggs.

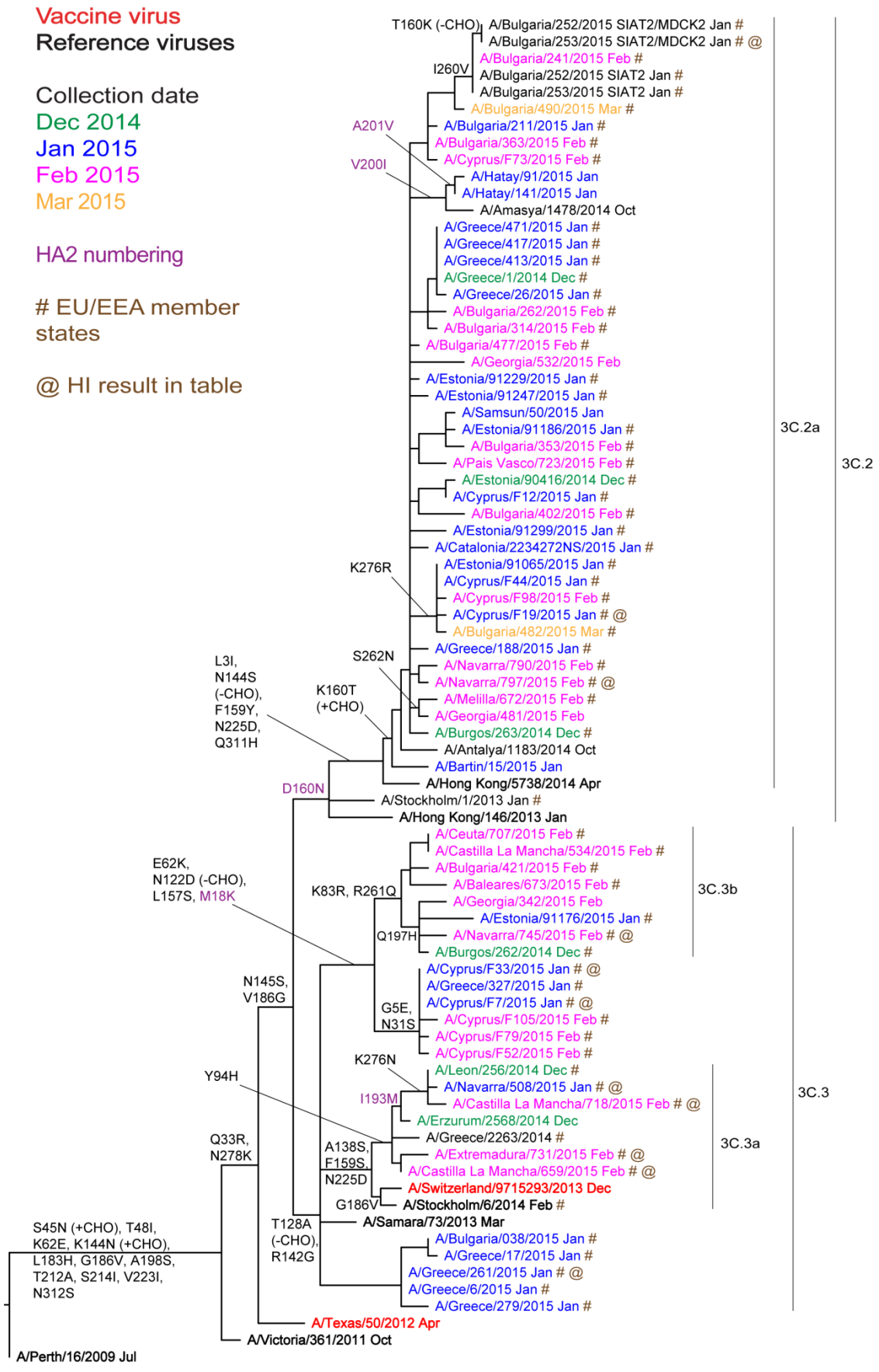
Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

Viruses	Collection Date	Passage History	Haemagglutination inhibition titre[1]											
			Post infection ferret antisera											
			A/Perth 16/09 F18/11	A/Vic 361/11 T/C F09/12	A/Texas 50/12 Egg F05/13	A/Samara 73/13 F24/13	A/HK 146/13 F40/13	A/Stock 6/14 T/C F14/14	A/Stock 6/14 Egg F20/14	A/Switz 9715293/13 T/C NIB F13/14	A/Switz 9715293/13 Egg F32/14	A/HK 5738/14 T/C F30/14	A/HK 5738/14 Egg NIB F53/14
	Genetic group			3C.1	3C.1	3C.3	3C.2	3C.3a	3C.3a isolate 2	3C.3a	3C.3a cl123	3C.2a	3C.2a cl 121
REFERENCE VIRUSES													
A/Perth/16/2009		E3/E2	640	160	640	160	320	40	80	<	40	<	<
A/Victoria/361/2011	3C.1	MDCK4/SIAT1	40	160	160	160	80	160	160	40	40	40	40
A/Texas/50/2012	3C.1	E5/E2	640	1280	5120	640	640	160	640	40	640	160	80
A/Samara/73/2013	3C.3	C1/SIAT4	320	320	1280	1280	1280	640	640	80	640	320	80
A/Hong Kong/146/2013	3C.2	E3/E3	320	320	640	640	1280	160	640	40	640	320	40
A/Stockholm/6/2014	3C.3a	SIAT1/SIAT2	<	80	160	320	160	640	160	160	80	160	80
A/Stockholm/6/2014	3C.3a	E4/E1 isolate 2	80	320	320	80	160	160	320	80	640	160	40
A/Switzerland/9715293/2013	3C.3a	SIAT1/SIAT3	<	40	160	160	80	320	160	160	80	80	40
A/Switzerland/9715293/2013	3C.3a	E4/E2 clone 123	40	320	320	160	320	320	320	160	640	320	80
A/Hong Kong/5738/2014	3C.2a	MDCK3/SIAT1	<	80	160	160	80	320	80	40	40	160	40
A/Hong Kong/5738/2014	3C.2a	E5/E1 clone121	40	40	160	80	80	160	40	40	40	320	640
TEST VIRUSES													
A/Cyprus/F7/2015	3C.3	SIAT1	40	80	160	160	80	160	80	<	40	40	<
A/Cyprus/F16/2015		SIAT1	80	160	320	320	160	320	160	40	80	80	<
A/Greece/261/2015	3C.3	SIAT3	80	320	640	320	320	320	320	40	80	160	40
A/Cyprus/F19/2015	3C.2a	SIAT1	<	<	<	40	40	40	<	<	<	40	<
A/Cyprus/F34/2015		SIAT1	80	160	320	320	160	320	160	40	80	80	<
A/Cyprus/F33/2015	3C.3	SIAT1	80	160	320	160	160	320	160	40	40	80	<
A/Bulgaria/253/2015	3C.2a	SIAT2/MDCK3	<	80	160	160	80	320	80	40	40	160	40
A/Castilla La Mancha/513/2015		SIAT1/SIAT1	<	<	40	80	80	160	40	<	<	80	<
A/Navarra/508/2015	3C.3a	SIAT2/SIAT1	<	<	<	<	<	160	40	40	<	40	<
A/Castilla La Mancha/523/2015		SIAT1/SIAT1	<	40	80	160	160	640	160	80	40	160	<
A/Castilla La Mancha/532/2015		SIAT1/SIAT1	<	80	160	160	160	1280	320	80	40	160	<
A/Extremadura/731/2015	3C.3a	SIAT2/SIAT1	<	40	160	160	80	640	160	80	40	160	40
A/Castilla La Mancha/660/2015		SIAT1/SIAT1	320	160	640	640	320	640	160	40	80	160	<
A/Castilla La Mancha/658/2015		SIAT1/SIAT1	<	80	160	160	80	320	80	<	<	80	<
A/Pais Vasco/722/2015		SIAT2/SIAT1	<	40	80	80	80	640	160	40	40	160	<
A/Castilla La Mancha/659/2015	3C.3a	SIAT1/SIAT1	<	40	80	80	80	640	160	80	40	160	<
A/Castilla La Mancha/646/2015		SIAT1/SIAT1	<	40	40	80	80	320	80	40	<	80	<
A/Navarra/745/2015	3C.3b	SIAT2/SIAT1	160	160	640	1280	640	320	320	80	320	320	40
A/Navarra/642/2015		SIAT2/SIAT1	<	40	80	80	80	640	160	80	40	160	<
A/Castilla La Mancha/718/2015	3C.3a	SIAT1/SIAT1	<	<	<	<	<	160	40	40	40	<	<
A/Navarra/797/2015	3C.2a	SIAT2/SIAT1	40	40	320	320	160	320	320	40	40	320	40
				Vaccine NH2014-15							Vaccine SH2015 NH2015-16		

1. < = <40

Sequences in phylogenetic tree

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



Influenza B virus analyses

Influenza B viruses represented just over 20% of samples received from EU/EEA countries with collection dates after 31 August 2014 (Table 1). Of the 116 viruses ascribed to a lineage, B/Yamagata viruses predominated over those of B/Victoria at a ratio of 38:1.

Influenza B – Victoria lineage

No viruses of this lineage have been received since the March 2015 report.

Phylogenetic analysis of the HA gene of representative, recently collected B/Victoria lineage viruses is shown in Figure 3. Worldwide, recent viruses have HA genes that fall into the B/Brisbane/60/2008 genetic clade (clade 1A) and remain antigenically similar to the recommended vaccine virus, B/Brisbane/60/2008, for use in quadrivalent vaccines. B/Rheinland-Pfalz/1/2014, which clusters closely with B/Brisbane/60/2008 in the phylogenetic tree, was isolated from a child recently vaccinated with a Live Attenuated Influenza Vaccine.

Influenza B – Yamagata lineage

HI results for the 32 B/Yamagata-lineage test viruses analysed since the March 2015 report are shown in Table 4. All eight test viruses for which HA gene sequencing has been completed fell in genetic clade 3 and viruses for which gene sequences are included in the phylogenetic tree are highlighted in the Table.

Post-infection ferret antiserum raised against the egg-propagated vaccine virus B/Massachusetts/02/2012, recommended for use in the 2014–15 northern hemisphere influenza season, recognised 20% (2/10) of test viruses at titres within fourfold of the titre with the homologous virus. A ferret antiserum raised against a cell culture-propagated cultivar of B/Massachusetts/02/2012 recognised 90% (9/10) of test viruses at titres within fourfold of its titre with the homologous virus. Antisera raised against cell culture-propagated B/Estonia/55669/2011 and belonging to the B/Massachusetts/02/2012 clade (clade 2) recognised 20% (2/10) of the test viruses at titres within fourfold of the titres of the antisera with the homologous virus.

An antiserum raised against a previously recommended vaccine virus, B/Wisconsin/1/2010, recognised all 10 test viruses at titres within fourfold of the titre with the homologous virus, as was the case with an antiserum raised against egg-propagated B/Stockholm/12/2011, a virus also belonging to clade 3 represented by B/Wisconsin/1/2010 and B/Phuket/3073/2013. Similarly, all test viruses were recognised at titres within twofold by antisera raised against egg-propagated B/Phuket/3073/2013, the virus recommended as a vaccine virus for the southern hemisphere 2015 and northern hemisphere 2015–16 influenza seasons, and egg-propagated B/Hong Kong/3417/2014. Antiserum raised against a cell culture-propagated cultivar of B/Phuket/3073/2013 recognised all 10 test viruses at titres within fourfold of the titre for the homologous virus. Based on HI titre fold-drop, antisera raised against the egg-propagated clade 3 viruses, which include a previous (A/Wisconsin/1/2010) and recently recommended (A/Phuket/3073/2013) vaccine viruses, are more reactive with currently circulating clade 3 viruses than antisera raised against the egg-propagated clade 2 B/Massachusetts/02/2012 vaccine virus used in the northern hemisphere 2014–15 influenza season.

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. Worldwide, the vast majority of HA genes from recently collected viruses have fallen in the B/Wisconsin/1/2010–B/Phuket/3073/2013 clade (clade 3). A small proportion of viruses, detected in many parts of the world, have HA genes of clade 3 of the B/Yamagata lineage combined with NA genes of the B/Victoria lineage. A small group of viruses that are antigenically distinguishable from the great majority of clade 3 viruses, designated as clade 3a, have been detected in Australia.

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

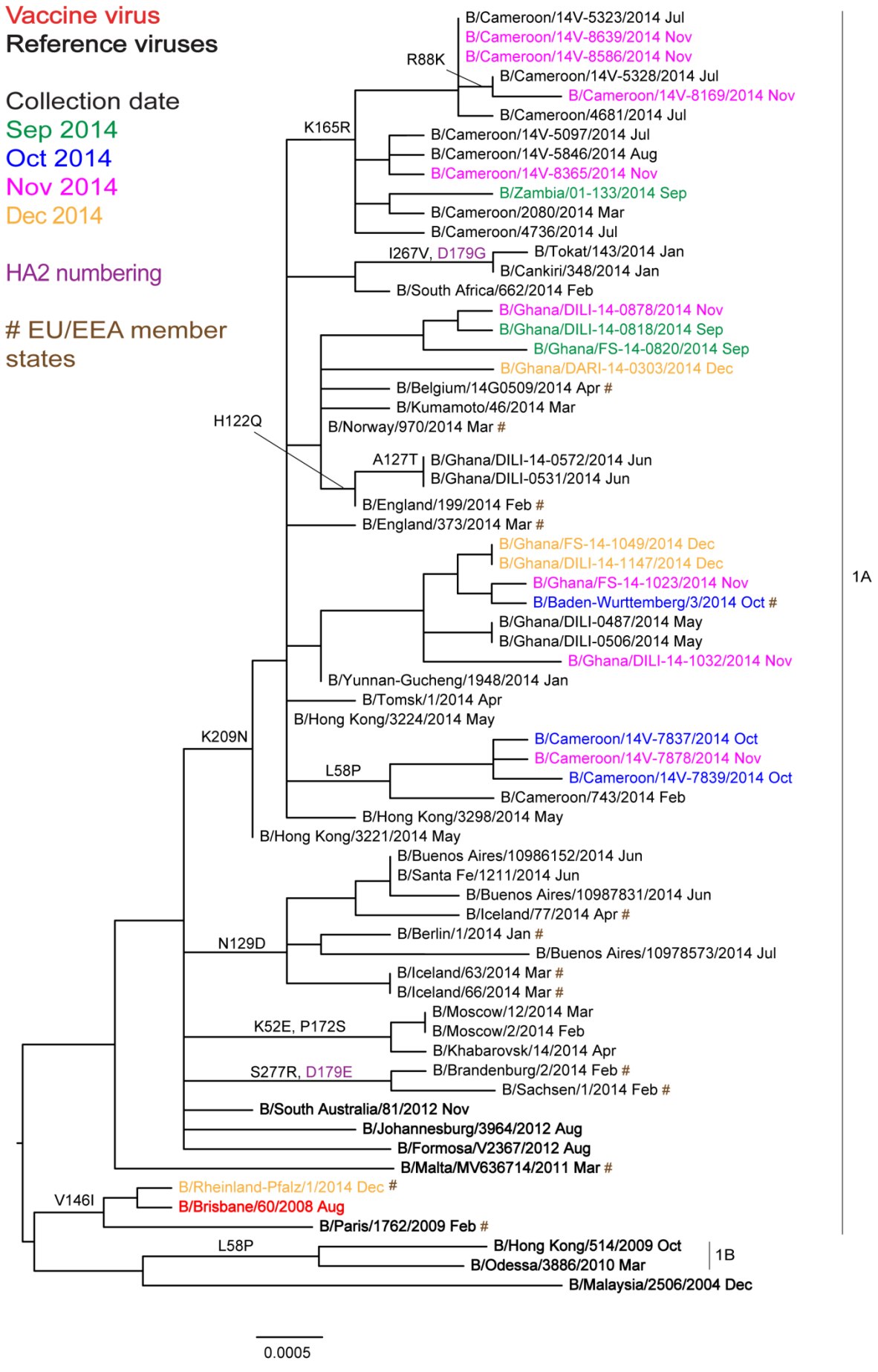


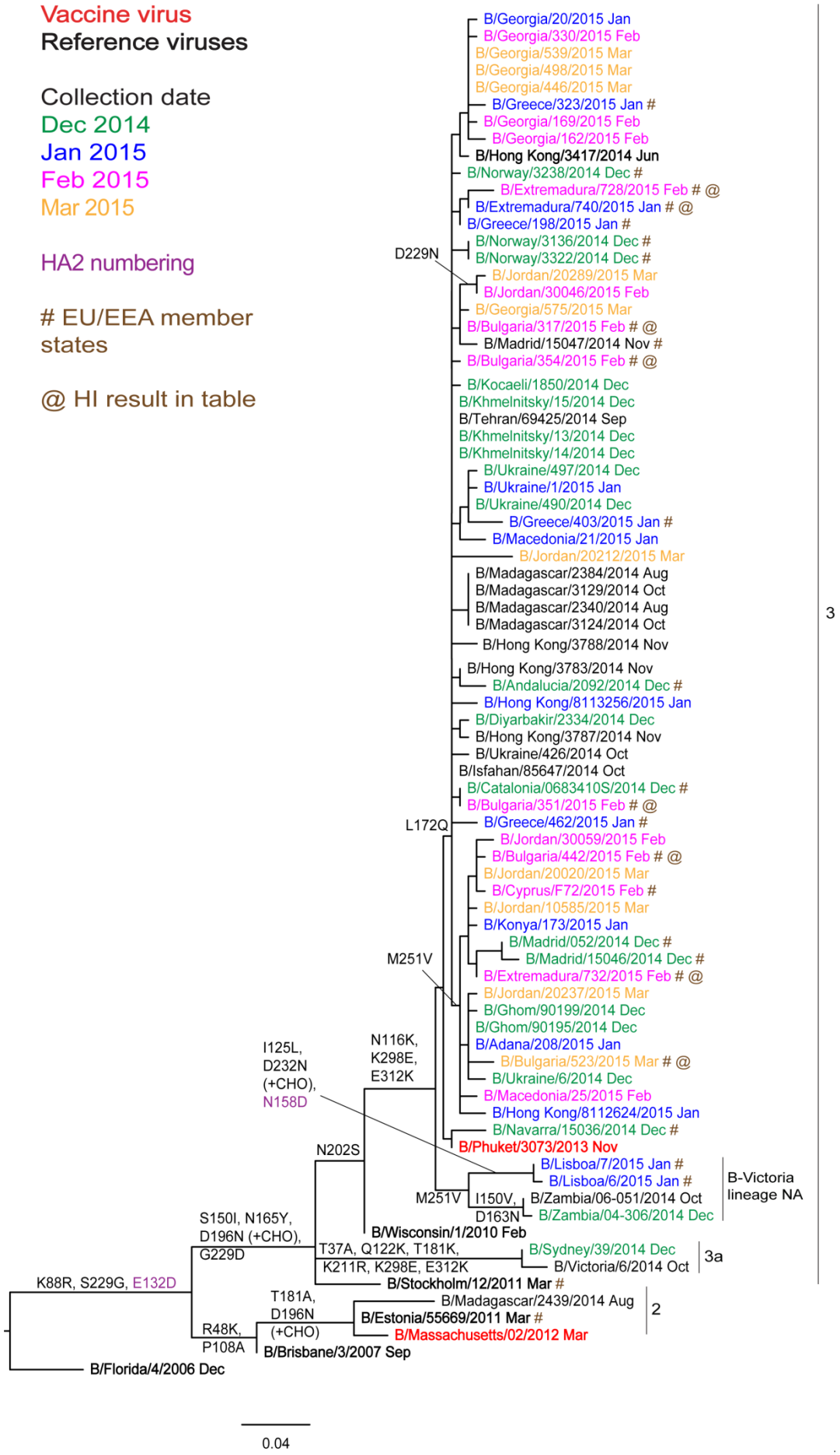
Table 4. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses	Genetic Group	Collection date	Passage History	Haemagglutination inhibition titre											
				Post infection ferret antisera											
				B/Fl[1,3] 4/06 SH479 1	B/Fl[1] 4/06 F1/10 1	B/Bris[1] 3/07 F38/14 2	B/Wis[2] 1/10 F10/13 3	B/Stock[2] 12/11 F12/12 3	B/Estonia[2] 55669/11 F27/13 2	B/Mass[2] 02/12 Egg F42/14 2	B/Mass[2] 02/12 T/C F15/13 2	B/Phuket[2] 3073/13 Egg F36/14 3	B/Phuket[2] 3073/13 T/C F35/14 3	B/HK[4] 3417/14 Egg St Judes F715/14 3
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	2560	640	1280	160	640	160	1280	160	320	20	320
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	320	320	80	160	80	320	80	80	<	160
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	320	80	80	160	320	20	80	20	80	<	160
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	320	160	160	320	40	160	40	160	20	320
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	640	80	40	40	40	640	40	320	40	40	160
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	2560	640	640	160	320	160	640	160	160	<	160
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	2560	640	320	160	320	320	320	320	160	40	320
B/Phuket/3073/2013	3	2013-11-21	E4/E3	640	160	80	160	320	20	80	40	160	20	160
B/Phuket/3073/2013	3	2013-11-21	M2/M2	1280	160	160	320	320	160	160	320	320	320	320
B/Hong Kong/3417/2014	3	2014-06-04	E5/E2	160	80	40	80	80	20	40	40	40	20	160
TEST VIRUSES														
B/Greece/5/2015		2015-01-02	MDCK2	320	80	<	80	80	40	20	40	80	80	160
B/Extremadura/740/2015	3	2015-01-28	SIAT2/SIAT1	640	80	80	160	160	80	80	80	160	160	320
B/Extremadura/732/2015	3	2015-02-02	SIAT2/SIAT1	320	80	80	160	80	80	80	80	80	80	320
B/Extremadura/728/2015	3	2015-02-03	SIAT2/SIAT1	640	160	160	160	160	160	160	320	320	160	320
B/Extremadura/725/2015		2015-02-03	SIAT2/SIAT1	640	160	160	320	320	160	160	320	320	160	160
B/Bulgaria/317/2015	3	2015-02-10	SIAT1/SIAT1	640	80	80	160	160	80	80	80	160	160	160
B/Bulgaria/351/2015	3	2015-02-13	SIAT2/SIAT1	320	160	80	160	160	80	80	80	160	640	320
B/Bulgaria/354/2015	3	2015-02-13	SIAT1/SIAT1	320	80	80	160	80	80	40	80	160	640	320
B/Bulgaria/442/2015	3	2015-02-24	SIAT2/SIAT1	320	160	80	160	160	80	80	80	160	160	160
B/Bulgaria/523/2015	3	2015-03-06	SIAT2/SIAT1	320	80	80	80	160	80	80	80	160	160	320
										Vaccine NH2014-15			Vaccine SH2015 NH2015-16	

1. < = <40; 2. < = <10; 3. hyperimmune sheep serum; 4. RDE serum pre-absorbed with TRBC.

Sequences in phylogenetic tree

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



Summary of genetic data submitted to TESSy

As of 3 May 2015 (to week 18/2015), the majority of influenza viruses identified genetically since week 40/2014 were A(H3N2) viruses (61%), with lower numbers of influenza B viruses (22%) and A(H1N1)pdm09 viruses (17%) being reported. This compares to proportions of 62%, 22% and 16%, respectively, as of 10 April 2015 (see March 2015 report).

All influenza A(H1N1)pdm09 viruses fell into genetic clade 6 with the great majority (98%) falling in genetic subgroup 6B, represented by A/South Africa/3626/2013. Influenza B viruses of the B/Yamagata lineage outnumbered those of the B/Victoria lineage by 52 to 1. The majority of influenza A(H3N2) viruses belonged to genetic subgroup 3C.2a (63%), represented by A/Hong Kong/5738/2014; smaller proportions were in genetic group 3C.3 (28%), represented by A/Samara/73/2013, genetic subgroup 3C.3a (< 8%), represented by A/Switzerland/9715293/2013, genetic group 3C.2, represented by A/Stockholm/1/2013 (< 1%), and genetic subgroup 3C.1 (1%), represented by A/Texas/50/2012, the vaccine virus for the 2014–15 northern hemisphere influenza season. For EU/EEA countries, similar proportions have been observed among the influenza-positive samples shared with the WHO CC, except for the small number of A(H3N2) subgroup 3C.1 detections.

Antiviral susceptibility

Between weeks 40/2014–18/2015, based on reports to TESSy, 2 160 influenza viruses (1360 A(H3N2), 452 A(H1N1)pdm09 and 348 type B) were subjected to phenotypic or genotypic testing for neuraminidase inhibitor (NAI) susceptibility. Four A(H3N2) viruses showed reduced susceptibility to oseltamivir with three viruses carrying NA E119V amino acid substitution and one carrying NA R292K substitution. The latter virus showed reduced susceptibility to zanamivir. Two A(H1N1)pdm09 viruses showed reduced susceptibility to oseltamivir.

A total of 628 viruses, with collection dates after 31 August 2014, from EU/EEA countries have been assessed phenotypically for NAI susceptibility at the London WHO CC: 121 influenza B, 115 A(H1N1)pdm09 and 392 A(H3N2) inclusive of many 3C.2a genetic subgroup viruses that could not be analysed by HI assay. All but one influenza B/Yamagata-lineage virus were susceptible to oseltamivir and zanamivir. The B/Yamagata-lineage virus showed reduced inhibition by oseltamivir and zanamivir, and carried NA amino acid substitution D197G.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [1] reported that the China Health and Family Planning Commission notified the 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 were reported over the course of the 2013–14 and 2013–14 seasons. A revised Rapid Risk Assessment [3] for these A(H7N9) viruses was carried out by ECDC and posted on 2 February 2015. WHO posted a summary of human infection on 31 January 2014 [4] and conducted a new risk assessment on 23 February 2015 [5]. In light of the assessment, WHO advised that countries continue to strengthen influenza surveillance. WHO last summarised the numbers of cases of human infection related to their geographic location on 14 July 2014 [6] and has provided subsequent situation updates with the latest being on 15 April 2015 [7].

Influenza A(H5N1) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 31 March 2015 [8]. The assessment included a description of a continuing rise in cases in Egypt with 42 new laboratory-confirmed human cases, including 11 fatal cases, of avian influenza A(H5N1) virus infection, three new cases in China and two fatal cases reported by Indonesia. ECDC published an updated rapid risk assessment on the situation in Egypt on 13 March 2015 [9] and an epidemiological update 10 April 2015 [10].

WHO CC reports

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza at the Crick Worldwide Influenza Centre, the Francis Crick Institute, Mill Hill Laboratory in London, and used at the WHO Vaccine Composition Meetings held at WHO Geneva on 22–24 September 2014 and 23–25 February 2015, can be found at:

http://crick.ac.uk/media/221823/nimr-vcm-report-sep-14-web.pdf

http://crick.ac.uk/media/221813/nimr-report-feb2015-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 EU/EEA countries are marked (#). Sequences for some 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.

References

1. World Health Organization. Global alert and response: Human infection with influenza A(H7N9) virus in China. 1 April 2013. Available from: http://www.who.int/csr/don/2013_04_01/en/index.html
2. World Health Organization. Avian influenza A(H7N9) virus. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/
3. European Centre for Disease Prevention and Control. Updated rapid risk assessment. Human infection with avian influenza A(H7N9) virus. Fourth update. 2 February 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/RRA-Influenza-A-H7N9-update-four.pdf
4. World Health Organization. Background and summary of human infection with avian influenza A(H7N9) virus – as of 31 January 2014. Geneva: WHO; 2014. Available from: http://www.who.int/influenza/human_animal_interface/20140131_background_and_summary_H7N9_v1.pdf
5. World Health Organization. WHO risk assessment: Human infections with avian influenza A(H7N9) virus, 2 October 2014. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/RiskAssessment_H7N9_23Feb20115.pdf
6. World Health Organization. Map and epidemiological curve of confirmed human cases of avian influenza A(H7N9). Report 18- data in WHO/HQ as of 14 July 2014. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/18_reportwebh7n9number_20140714.pdf
7. World Health Organization. Situation updates - avian influenza. Available from: http://www.who.int/csr/don/14-April-2015-avian-influenza-china/en/
8. World Health Organization. Influenza at the human-animal interface. Summary and assessment as of 31 March 2015. Available from: http://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_31_March_2015.pdf
9. European Centre for Disease Prevention and Control. Rapid Risk Assessment. Human infection with avian influenza A(H5N1) virus, Egypt. Available from: http://ecdc.europa.eu/en/publications/Publications/Rapid-Risk-Assessment-Influenza-A-H5N1-Egypt-March-2015.pdf
10. European Centre for Disease Prevention and Control. Epidemiological update: increase in reporting of human cases of A(H5N1) influenza, Egypt. Available from: http://ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1199