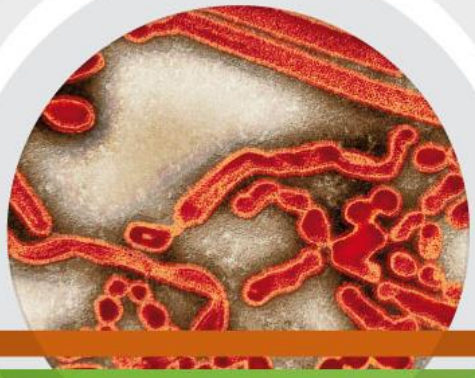

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, September 2015

Summary

Over the course of the 2014–2015 influenza season, influenza A(H3N2), A(H1N1)pdm09 and type B viruses have co-circulated in EU/EEA countries. To date, 28 EU/EEA countries have shared 1 160 influenza-positive specimens with the WHO Collaborating Centre in London for detailed characterisation. Since the July 2015 report [1], 102 viruses have been characterised antigenically and 60 genetically.

The 30 A(H1N1)pdm09 viruses characterised antigenically were similar to the vaccine virus A/California/07/2009; all 26 characterised genetically had HA genes belonging to genetic subgroup 6B, as observed worldwide.

All 24 A(H3N2) test viruses characterised by haemagglutination inhibition (HI) assay were poorly recognised (titres at least fourfold reduced compared with the homologous titre) by reference antiserum raised against A/Texas/50/2012, the vaccine virus recommended for use in the 2014–2015 northern hemisphere influenza season. The test viruses were relatively well recognised by antisera raised against cell-culture-propagated A/Switzerland/9715293/2013 and some other cell-culture-propagated cultivars of genetic subgroup 3C.3a viruses; however, they were not recognised well, relative to the homologous titre of the antiserum, by post-infection ferret antiserum raised against the egg-propagated cultivar of A/Switzerland/9715293/2013, the prototype virus recommended for vaccines to be used in the 2015–2016 northern hemisphere influenza season. The 401 (60 since the July 2015 report) viruses, with collection dates after 31 August 2014, characterised genetically this season fell in genetic group/subgroups 3C.3 (46), 3C.3b (98), 3C.3a (41) and 3C.2a (216). Viruses in genetic group 3C.3, excluding those in subgroup 3C.3a, were antigenically similar to reference viruses closely related to A/Texas/50/2012, while those in subgroups 3C.3a and 3C.2a were antigenically distinct.

The three B/Victoria-lineage viruses characterised since the July 2015 report were antigenically similar to B/Brisbane/60/2008 and all fell in genetic clade 1A. All 45 B/Yamagata-lineage test viruses characterised antigenically 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 seasonal vaccine) did not recognise test viruses as well as antisera raised against B/Phuket/3073/2013. The 37 viruses characterised genetically all fell in clade 3, represented by B/Phuket/3073/2013.

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Influenza-positive samples (1 160 viruses or clinical specimens: 153 being received since the July 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 28 countries in the EU/EEA. Overall, the majority (76.6%) were type A viruses, and A(H3N2) outnumbered A(H1N1)pdm09 at a ratio of approximately 2.9:1 (Table 1). Of the 272 type B specimens received (23.4% of the specimens), 223 were of the B/Yamagata lineage, 36 were not ascribed to a lineage, and only 13 were of the B/Victoria lineage. A few 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 July 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 / Country	TOTAL RECEIVED	A	H1N1pdm09		H3N2			B		B Victoria lineage		B Yamagata lineage	
		Number received	Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated	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	3				1	1						2	2
France	6				5	1	4					1	1
Germany	6		3	3	2	2				1	1		
Malta	4				4	3	1						
Netherlands	7				6	2	4					1	1
Norway	8		5	3	3	1							
Slovenia	2				1	1						1	0
Spain	13				10	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	4				4	1	3						
Norway	11				3	2	1					8	3
Portugal	2											2	2
Slovenia	1		1	1									
Spain	10				9	6	3					1	1
Sweden	4				4	4							
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	0			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	27		2	2	23	6	12			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	0				
Malta	4				4	1	2						
Netherlands	7		2	2	5	2	3						
Norway	26		4	4	15	7	5					7	4
Portugal	10				3	1	2					7	7
Slovenia	19		17	14	1	0		1	0				
Spain	48		2	2	40	9	26					6	6
Sweden	9		2	2	5	5						2	2
United Kingdom	12		1	1	10	2	5					1	1
2015													
JANUARY													
Bulgaria	11				11	2	9						
Croatia	1				1	0							
Cyprus	8				8	6	2						
Denmark	2				2	2							
Estonia	29		1	0	27	2	16					1	0
Finland	1		1	1									
Germany	33		5	5	22	12	10					6	6
Greece	61		13	6	25	4	14	15	0	1	0	7	7
Italy	17		8	7	7	3	4					2	2
Latvia	2				2	0	2						
Luxembourg	1							1	0				
Malta	5				5	1	1						
Netherlands	1				1	1							
Norway	8				7	4	2					1	1
Portugal	7		2	1	3	0	2					2	1
Romania	6		4	4	1	1						1	1
Slovakia	9		5	5	4	4							
Slovenia	25		11	11	10	3	7	2	0			2	2
Spain	47		11	10	25	14	11	1	0			10	10
Sweden	8		3	3	4	3	1					1	1
United Kingdom	27		2	2	25	7	8						
FEBRUARY													
Bulgaria	26		3	3	19	1	15					4	4
Cyprus	12				11	1	9					1	1
Estonia	20		1	1	17	0	17					2	2
Finland	2				2	2							
France	1		1	1									
Greece	13		3	0	4	0		4	0			2	1
Hungary	7		1	1	3	3						3	1
Iceland	8		1	1	5	5	0					2	2
Italy	41		12	12	15	5	10	1	0			13	13
Netherlands	11		2	2	7	5	2					2	2
Norway	1				1	1							
Poland	3	2										1	1
Romania	8		2	2	4	3	1					2	2
Slovakia	15		5	5	8	7	1					2	2
Slovenia	12		4	3	3	2	1	1	0			4	4
Spain	58	5	1	1	38	16	16	3	0			11	10
Sweden	13		5	5	6	6						2	2
MARCH													
Bulgaria	3				2	0	2					1	1
Estonia	4				2	0	2					2	2
Finland	1									1	1		
France	20		6	6	6	4	2			2	2	6	6
Iceland	7				3	3				1	1	3	3
Italy	1							1	0				
Netherlands	2				1	0	1					1	1
Norway	11		4	2	4	2	1			1	1	2	2
Poland	10	1	3	3	4	0	0					2	2
Romania	17		5	5	2	2						10	10
Slovakia	6		1	1								5	5
Slovenia	10		5	5	2	1	1					3	3
Sweden	4		3	3	1	1							
APRIL													
Estonia	5				2	0	2					3	3
Finland	4				2	1	1			1	1	1	1
France	6		1	1	1	1	0					4	4
Norway	14		5	3	2	0	1			2	2	5	2
Poland	2				2	0	1						
Slovakia	1											1	1
Slovenia	10				2	2		1	0			7	7
MAY													
Iceland	4				2	2	0	1	in process			1	1
Norway	11		4	4	3	2	1			2	0	2	2
Poland	1		1	0									
JUNE													
Iceland	1				1	0	0						
Norway	17		2	0	10	4	6					5	3
JULY													
Iceland	2				2	0	1						
Norway	5				4	0	4					1	0
	1160	8	228	198	652	261	307	36		13	10	223	201
				19.7%		56.2%				1.1%		19.2%	
28 Countries				76.6%						23.4%			

1. Propagated to sufficient titre to perform HI assay
2. Propagated to sufficient titre to perform HI assay in presence of 20nM oseltamivir; numbers in red indicate viruses recovered but with insufficient HA titre to permit HI assay

Influenza A(H1N1)pdm09 virus analyses

Haemagglutination inhibition (HI) analyses of viruses that have been performed since the July 2015 report are shown in Tables 2-1 and 2-2. All 30 A(H1N1)pdm09 viruses (from Estonia, France, Hungary, Iceland, Norway, Poland and Slovenia) were antigenically similar to the vaccine virus, A/California/7/2009, with only one virus (A/Paris/1686/2015) showing a more than twofold reduction in HI titre compared to that for the homologous virus. Generally, the test viruses were recognised by the 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 73% (22/30) of the test viruses at a titre within fourfold of the titre for the homologous virus. 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 fourfold to sixteenfold reduced compared with 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 twelve months, 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.

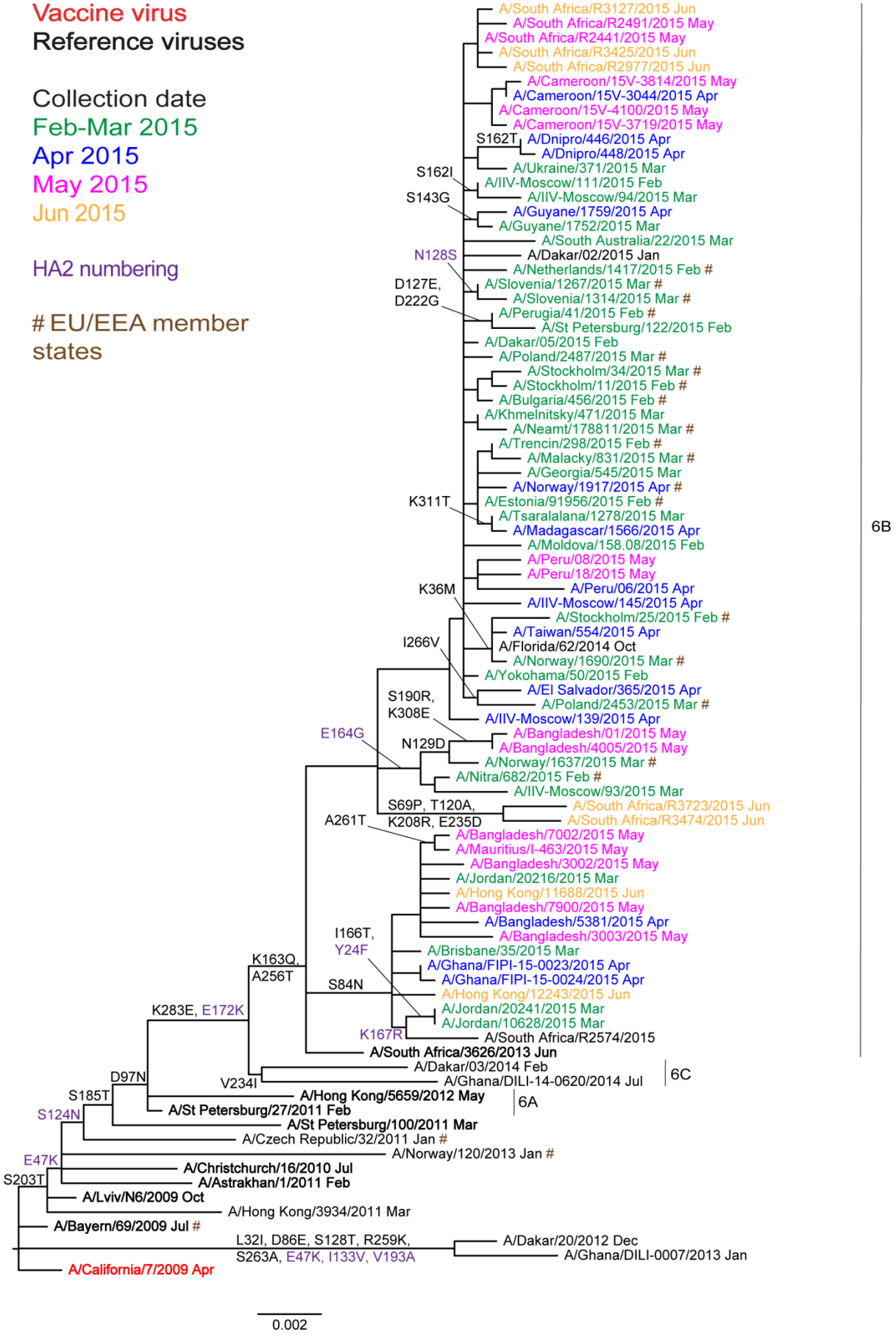
Table 2-1. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre									
				Post-infection ferret antisera									
				A/Cal 7/09 F05/14	A/Bayern 69/09 F11/11	A/Lviv N6/09 F14/13	A/Chch 16/10 F15/14	A/Astrak 1/11 F22/13	A/St. P 27/11 F26/14	A/St. P 100/11 F24/11	A/HK 5659/12 F30/12	A/Sth Afr 3626/13 F3/14	
							4	5	6	7	6A	6B	
REFERENCE VIRUSES													
A/California/7/2009		2009-04-09	E1/E3	1280	640	640	160	160	160	320	160	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	80	640	160	80	40	40	40	40	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	640	1280	320	80	80	80	320	80	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	1280	2560	1280	640	2560	2560	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	320	320	640	1280	640	2560	1280	640	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	1280	1280	640	2560	640	5120	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	1280	1280	1280	640	2560	1280	5120	25620	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	160	80	80	80	320	160	640	640	160	
A/South Africa/3626/2013	6B	2013-06-06	E1/E3	640	640	640	320	640	640	1280	640	1280	
TEST VIRUSES													
A/Slovenia/153/2015		2015-01-11	MDCKx/MDCK1	640	640	320	640	1280	640	2560	1280	1280	
A/Slovenia/367/2015		2015-01-20	MDCK1/MDCK1	640	320	320	320	1280	640	1280	1280	1280	
A/Slovenia/596/2015		2015-01-26	MDCK1/MDCK1	640	320	320	320	640	320	1280	1280	640	
A/Slovenia/839/15	6B	2015-02-05	MDCK1/MDCK1	1280	640	640	640	1280	640	2560	2560	1280	
A/Slovenia/826/15	6B	2015-02-06	MDCK1/MDCK1	640	640	640	640	1280	640	2560	1280	1280	
A/Estonia/91956/2015	6B	2015-02-11	SIAT1/SIAT1	1280	640	640	640	2560	640	2560	2560	1280	
A/Slovenia/1079/15	6B	2015-02-20	MDCK1/MDCK1	640	320	320	320	640	640	2560	1280	640	
A/Slovenia/1267/2015	6B	2015-03-03	MDCK1/MDCK1	640	320	320	320	1280	640	2560	1280	640	
A/Slovenia/1314/15	6B	2015-03-05	MDCK1/MDCK1	640	320	320	320	640	640	2560	1280	640	
A/Poland/2453/2015	6B	2015-03-10	MDCK1/MDCK1	1280	640	640	640	2560	640	2560	2560	1280	
A/Poland/2456/2015	6B	2015-03-10	MDCK1/MDCK1	1280	1280	640	640	1280	640	2560	2560	1280	
A/Slovenia/1413/15	6B	2015-03-11	MDCKx/MDCK1	640	640	320	640	1280	640	2560	2560	1280	
A/Slovenia/1541/15	6B	2015-03-17	MDCK1/MDCK1	640	320	320	640	1280	640	2560	1280	1280	
A/Slovenia/1550/15	6B	2015-03-17	MDCK1/MDCK1	640	640	320	640	1280	640	2560	1280	1280	
A/Poland/2487/2015	6B	2015-03-19	MDCK1/MDCK1	1280	1280	640	1280	2560	1280	2560	2560	1280	
				Vaccine									

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

Viruses	Collection date	Genetic group	Passage History	Haemagglutination inhibition titre											
				Post-infection ferret antisera											
				A/Cal 7/09 F05/14	A/Bayern 69/09 F09/15	A/Lviv N6/09 F14/13	A/Chch 16/10 F15/14	A/Astrak 1/11 F22/13	A/St. P 27/11 F26/14	A/St. P 100/11 F24/11	A/HK 5659/12 F30/12	A/Sth Afr 3626/13 F3/14		
							4	5	6	7	6A	6B		
REFERENCE VIRUSES														
A/California/7/2009	2009-04-09		E1/E3	1280	1280	640	320	320	320	320	320	320		
A/Bayern/69/2009	2009-07-01		MDCK5/MDCK1	160	640	320	80	80	40	80	80	80	G155E	
A/Lviv/N6/2009	2009-10-27		MDCK4/SIAT1/MDCK3	320	1280	1280	320	160	160	160	320	80	G155E>G, D222G	
A/Christchurch/16/2010	2010-07-12	4	E1/E3	1280	2560	1280	2560	2560	1280	5120	2560	1280		
A/Astrakhan/1/2011	2011-02-28	5	MDCK1/MDCK5	1280	1280	640	1280	2560	1280	2560	2560	1280		
A/St. Petersburg/27/2011	2011-02-14	6	E1/E3	1280	1280	1280	640	2560	1280	5120	2560	1280		
A/St. Petersburg/100/2011	2011-03-14	7	E1/E3	1280	1280	1280	1280	2560	1280	5120	5120	1280		
A/Hong Kong/5659/2012	2012-05-21	6A	MDCK4/MDCK2	320	320	160	320	640	320	1280	1280	640		
A/South Africa/3626/2013	2013-06-06	6B	E1/E3	1280	1280	1280	640	1280	640	2560	1280	1280		
TEST VIRUSES														
A/Hungary/ 419/2015	2015-02-09	6B	MDCK3/MDCK1	640	640	320	320	640	640	1280	1280	1280		
A/Paris/1686/2015	2015-02-16	6B	MDCK1/MDCK1	320	320	160	320	640	320	640	640	320		
A/Iceland/39/2015	2015-02-28		MDCKx/MDCK1	640	320	320	640	1280	640	1280	1280	1280		
A/Paris/1453/2015	2015-03-10	6B	MDCK1/MDCK1	1280	1280	640	640	1280	640	2560	2560	1280		
A/Nord Pas de Calais/1462/2015	2015-03-10	6B	MDCK1/MDCK1	1280	1280	640	1280	1280	640	5120	2560	2560		
A/Picardie/1479/2015	2015-03-12	6B	MDCK1/MDCK1	1280	1280	640	1280	2560	1280	2560	2560	2560		
A/Alsace/1504/2015	2015-03-14	6B	MDCK1/MDCK1	1280	1280	640	640	1280	1280	2560	2560	1280		
A/Alsace/1532/2015	2015-03-17	6B	MDCK1/MDCK1	1280	1280	640	1280	2560	1280	5120	2560	2560		
A/Lorraine/1590/2015	2015-03-26	6B	MDCK1/MDCK1	1280	640	640	640	1280	640	2560	2560	1280		
A/Champagne Ardenne/1666/2015	2015-04-02	6B	MDCK1/MDCK1	1280	1280	640	1280	2560	1280	5120	2560	2560		
A/Norway/2330/2015	2015-04-29	6B	MDCK1/MDCK1	1280	640	640	640	1280	640	2560	2560	1280		
A/Norway/2335/2015	2015-05-05	6B	MDCK1	640	320	160	320	1280	640	1280	1280	640		
A/Norway/2227/2015	2015-05-06	6B	MDCK1/MDCK1	1280	1280	640	1280	2560	1280	5120	5120	2560		
A/Norway/2320/2015	2015-05-13	6B	MDCK1	1280	1280	640	640	2560	1280	2560	2560	2560		
A/Norway/2443/2015	2015-05-27	6B	MDCK1/MDCK1	1280	1280	1280	1280	2560	1280	5120	5120	2560		
			Vaccine											

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



Influenza A(H3N2) virus analyses

As described in many previous reports[1], 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 July 2015 report are shown in Tables 3-1 and 3-2. Viruses were received from France, Hungary, Iceland and Norway, and the HA genetic group is indicated for those viruses that have been sequenced. Of the 71 successfully propagated viruses, 24 (34%) retained sufficient HA titre to be analysed by HI assay. The remainder (n = 47) were either unable to agglutinate guinea pig RBCs at all or were unable to agglutinate RBCs in the presence of 20nM oseltamivir. The majority of viruses unable to be characterised by HI assay that were subjected to genetic analysis belonged to genetic subgroup 3C.2a.

Generally, the 24 test viruses, propagated in MDCK-SIAT1 cells, reacted poorly in HI assays 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: five showed fourfold, two showed eightfold and 17 greater than eightfold reductions in titre compared with the titre of the antiserum with the homologous virus. Ten of the test viruses were sequenced with eight falling in the 3C.3b subgroup and showing eightfold to sixteenfold reductions in HI titre, with the remaining two being 3C.2a viruses and showing sixty-fourfold reductions. Low levels of reactivity were also seen with antiserum raised against the egg-propagated reference virus A/Hong Kong/146/2013: only five 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 22 (92%) of the 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, gave a homologous titre of 640 and recognised only six of the test viruses at titres within fourfold of the homologous titre. Better reactivity of an antiserum raised against the egg-propagated 3C.2a virus, A/Hong Kong/4801/2014, was observed with 18 of the 24 test viruses being recognised at titres within fourfold of the titre of the antiserum for the homologous virus.

Ferret antisera raised against reference viruses propagated in tissue culture cells recognised the test viruses more effectively. Of the antisera raised against 3C.3a reference viruses propagated exclusively in cell culture, the antiserum raised against A/Switzerland/9715293/2013 recognised 16/24 (66%) test viruses at titres within twofold of that with the homologous virus, while the antiserum raised against A/Stockholm/6/2014 recognised 22/24 (92%) within twofold of the low homologous titres. 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 19/24 (79%) test viruses at titres within twofold of that for the homologous virus and 23/24 (96%) within fourfold. The antiserum raised against A/Samara/73/2013, a genetic group 3C.3 virus, recognised 79% (19/24) of test viruses at a titre within fourfold of the titre for the homologous virus. An antiserum raised against A/Netherlands/525/2014, a cell-culture-propagated virus in genetic subgroup 3C.3b, recognised 20 of the 24 (83%) test viruses at titres within fourfold of the titre for the homologous antiserum. However, this antiserum raised did not recognise any test viruses known to be in the 3C.2a genetic subgroup at a titre of 40 or over (Tables 3-1 and 3-2), but recognised all 12 viruses tested known to belong to genetic group 3C.3b at titres within fourfold of the titre of the antiserum for the homologous virus.

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 vaccine virus recommended for use in 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. 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**[2] 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**,

[1] For example, the September 2013 report [4].

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

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/Stockholm/28/2014

Of the A(H3N2) viruses received from EU/EEA countries, with collection dates since 31 August 2014, 341 have been characterised genetically. These have fallen in HA genetic subgroups 3C.2a (n = 216; 54%), 3C.3a (n = 41; 10%) and 3C.3b (n = 98; 24%), with the remainder (n = 46; 12%) being in subdivision 3C.3. This is indicative of 66% of recently circulating A(H3N2) viruses being antigenic drift variants compared with A/Texas/50/2012, the virus recommended for use in northern hemisphere 2014–15 vaccines.

Based on results available at the time of the February 2015 vaccine composition meeting, 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 at that time and the lack of a suitable 3C.2a vaccine candidate, 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. After February 2015, 3C.2a viruses became prevalent and a new genetic subgroup designated 3C.3b emerged, with these three subgroups being antigenically distinguishable. While ferret antisera raised against 3C.3a and 3C.2a viruses showed some cross-reactivity with viruses in all three genetic subgroups, antiserum raised against 3C.3b viruses were subgroup specific. Therefore, with the availability of new genetic subgroup 3C.2a vaccine candidates and the continued cross-reactivity of antisera raised against genetic subgroup 3C.3a and 3C.2a viruses, in September 2015 the World Health Organization recommendation for the A(H3N2) component of the influenza vaccine for the southern hemisphere 2016 influenza season was for an A/Hong Kong/4801/2014-like (3C.2a) virus.

Table 3-1. 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/Texas 50/12 Egg F36/12	A/Samara 73/13 F24/13	A/HK 146/13 F10/15	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 4801/14 F12/15	A/Neth 525/14 F23/15
	Genetic group			3C.1	3C.3	3C.2	3C.3a	3C.3a isolate 2	3C.3a	3C.3a cl123	3C.2a	3C.2a	3C.3b
REFERENCE VIRUSES													
A/Perth/16/2009	2009-07-04	E3/E3	640	320	160	80	40	160	<	80	<	40	80
A/Texas/50/2012	2012-04-15	E5/E2	320	5120	640	320	160	640	80	640	160	80	320
A/Samara/73/2013	2013-03-12	C1/SIAT3	160	1280	640	640	320	640	160	320	320	160	320
A/Hong Kong/146/2013	2013-01-11	E3/E3	640	2560	640	1280	160	640	80	640	320	160	320
A/Stockholm/6/2014	2014-02-06	SIAT1/SIAT2	<	160	320	160	640	320	320	160	160	160	80
A/Stockholm/6/2014	2014-02-06	E4/E1 isolate 2	80	640	80	80	160	320	160	640	160	40	80
A/Switzerland/9715293/2013	2013-12-06	SIAT1/SIAT2	<	80	160	40	640	160	80	160	160	80	40
A/Switzerland/9715293/2013	2013-12-06	E4/E1 clone 123	40	640	160	80	160	320	80	640	160	80	40
A/Hong Kong/5738/2014	2014-04-30	MDCK1/MDCK3	<	160	160	80	320	160	80	80	160	160	40
A/Hong Kong/4801/2014	2014-02-26	E6/E1 isolate 1	40	80	160	40	160	160	40	40	320	320	80
A/Netherlands/525/2014	2014-12-17	SIAT2/SIAT3	160	640	320	160	320	160	80	160	80	80	1280
TEST VIRUSES													
A/Dijon/1484/2015	2015-03-09	MDCK1/SIAT1	80	320	320	80	320	160	40	80	80	80	640
A/Bretagne/1564/2015	2015-03-19	MDCK2/SIAT1	80	320	320	80	160	80	40	80	80	80	640
A/Alsace/1571/2015	2015-03-21	MDCK2/SIAT1	80	640	320	80	320	320	40	160	80	160	1280
A/Lorraine/1641/2015	2015-03-30	MDCK1/SIAT1	160	320	160	80	320	160	40	80	80	80	640
A/Bretagne/1661/2015	2015-04-01	MDCK2/SIAT1	80	320	160	80	320	160	40	80	80	80	640
A/Norway/2326/2015	2015-05-10	SIAT1/SIAT1	40	320	160	80	320	160	40	40	80	40	320
A/Norway/2506/2015	2015-06-05	SIAT1/SIAT2	<	80	80	40	160	80	<	40	80	80	<
A/Norway/2504/2015	2015-06-07	SIAT1/SIAT2	40	320	160	80	320	160	40	80	80	80	640
A/Norway/2475/2015	2015-06-09	SIAT1/SIAT1	<	80	80	<	160	80	<	80	80	80	<
				Vaccine NH 2014-15						Vaccine SH 2015 NH 2015-16		Vaccine SH 2016	

1. < = <40

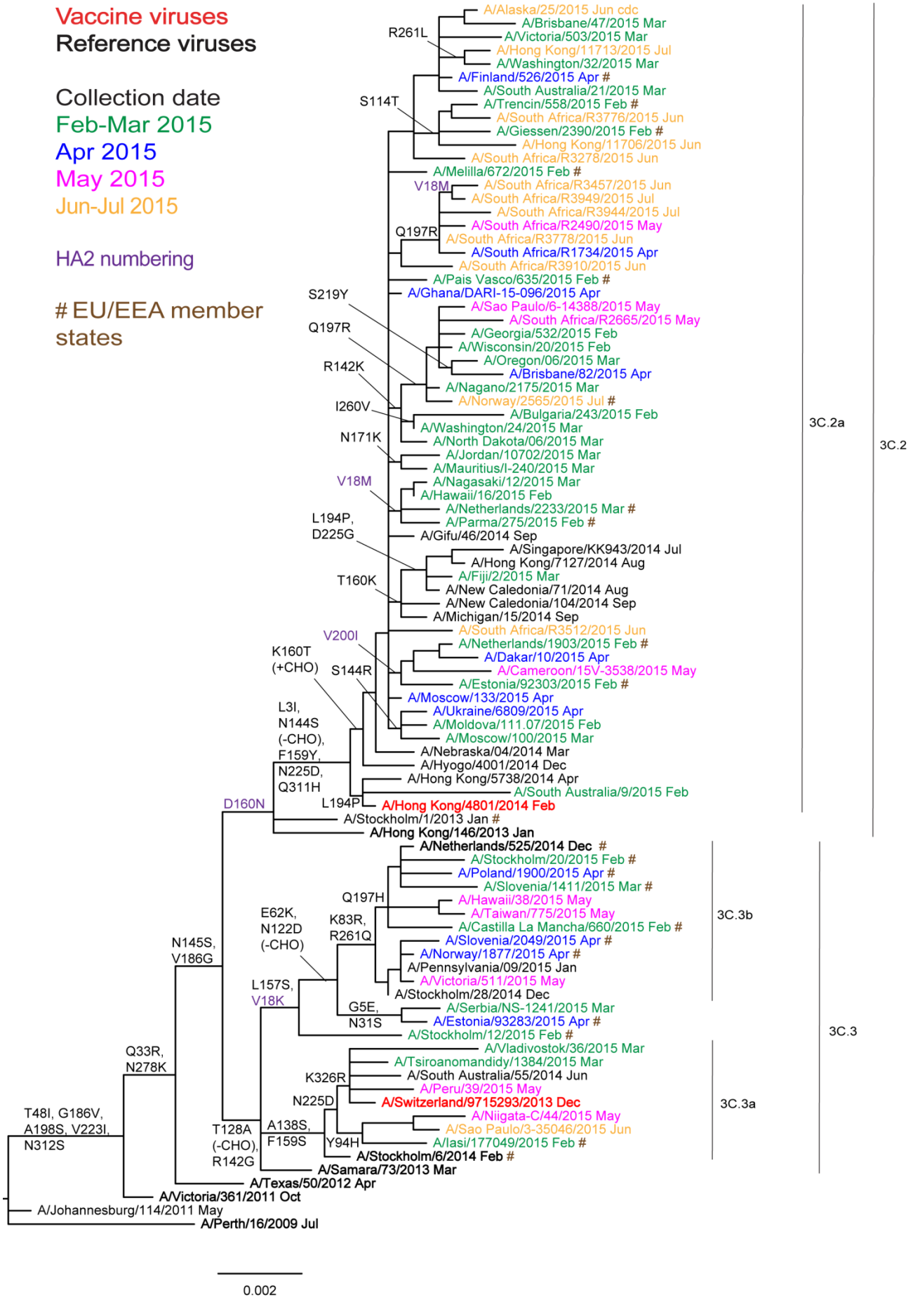
Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-09-15

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1] — Post-infection ferret antisera											
				A/Texas 50/12 Egg F36/12	A/Samara 73/13 F24/13	A/HK 146/13 F10/15	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 4801/14 F12/15	Georgia 532 pl 20 F33/15	A/Neth 525/14 F23/15
				3C.1	3C.3	3C.2	3C.3a	3C.3a isolate 2	3C.3a	3C.3a cl123	3C.2a	3C.2a	3C.2a	3C.3b
REFERENCE VIRUSES														
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	5120	640	320	160	640	40	640	160	80	160	320
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT3	1280	640	320	320	320	80	320	320	160	320	320
A/Hong Kong/146/2013	3C.2	2013-01-11	E3/E3	2560	640	640	80	640	40	640	320	160	320	320
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT1/SIAT2	160	320	80	320	160	160	160	80	80	80	40
A/Stockholm/6/2014	3C.3a	2014-02-06	E4/E1 isolate 2	640	80	80	160	320	80	640	160	40	80	40
A/Switzerland/9715293/2013	3C.3a	2013-12-06	SIAT1/SIAT2	40	80	<	320	160	80	80	80	40	40	<
A/Switzerland/9715293/2013	3C.3a	2013-12-06	E4/E1 clone 123	640	160	80	320	320	80	640	160	80	160	40
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK3	80	160	80	320	160	40	80	160	160	160	40
A/Hong Kong/4801/2014	3C.2a	2014-02-26	E6/E1 isolate 1	80	160	40	160	160	40	40	320	320	320	80
A/Georgia/532/2015 plaq 20	3C.2a	2015-03-09	SIAT2	160	320	80	320	160	40	80	160	160	640	80
A/Netherlands/525/2014	3C.3b	2014-12-17	SIAT2/SIAT3	640	320	160	320	160	80	160	80	160	160	1280
TEST VIRUSES														
A/Iceland/32/2015		2015-02-06	MDCK1/SIAT1	1280	320	160	160	160	40	160	160	80	160	1280
A/Hungary/423/2015	3C.2a	2015-02-09	MDCK3/SIAT2	80	160	40	160	80	<	40	160	80	160	<
A/Hungary/438/2015	3C.3b	2015-02-09	MDCK3/SIAT2	160	160	40	160	80	<	40	40	40	40	320
A/Hungary/436/2015	3C.3b	2015-02-09	MDCK3/SIAT3	160	80	<	160	40	<	40	40	40	<	640
A/Iceland/34/2015		2015-02-09	MDCKx/SIAT1	1280	320	160	160	160	40	320	160	80	160	1280
A/Iceland/35/2015		2015-02-10	MDCKx/SIAT1	40	80	40	160	40	<	40	160	80	320	<
A/Iceland/37/2015		2015-02-20	MDCKx/SIAT1	1280	640	160	320	160	40	320	160	80	160	1280
A/Iceland/38/2015	3C.3b	2015-02-24	MDCK1/SIAT1	320	160	80	160	160	<	80	40	40	40	640
A/Iceland/41/2015	3C.3b	2015-03-02	MDCKx/SIAT1	640	320	80	320	160	40	80	80	80	80	1280
A/Iceland/43/2015	3C.3	2015-03-06	MDCKx/SIAT1	1280	640	320	320	160	40	160	160	160	160	2560
A/Iceland/45/2015	3C.3	2015-03-13	MDCKx/SIAT1	1280	640	320	320	320	40	320	160	160	160	1280
A/Norway/2319/2015		2015-05-13	SIAT2/SIAT2	320	160	80	160	80	40	40	40	80	80	640
A/Iceland/6879/2015		2015-05-13	SIAT1	160	80	40	80	80	<	40	<	<	<	320
A/Iceland/7018/2015		2015-05-17	SIAT2	320	320	80	160	80	40	80	80	40	80	640
A/Norway/2501/2015	3C.3b	2015-06-01	SIAT2/SIAT2	320	320	80	320	80	40	80	80	80	40	640
				Vaccine NH 2014-15						Vaccine SH 2015 NH 2015-16		Vaccine SH 2016		

1. < = <40

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

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



Influenza B virus analyses

Influenza B viruses account for approximately 23% of samples received from EU/EEA countries with collection dates after 31 August 2014 (Table 1). Of the 236 viruses ascribed to a lineage, B/Yamagata viruses predominated over those of B/Victoria at a ratio of 17:1.

Influenza B – Victoria lineage

Since the July 2015 report, three viruses of this lineage have been received, two from France and one from Iceland. HI results are shown in Table 4 and, as observed throughout the season, the three test viruses carried HA genes of genetic group 1A.

The three test viruses showed greater than eightfold reductions in HI titres compared with the titre for the homologous virus with post-infection ferret antisera raised against the recommended vaccine virus for quadrivalent live and inactivated vaccines, B/Brisbane/60/2008. Similarly, they were poorly recognised by post-infection ferret antisera raised against the egg-propagated reference viruses B/Malta/636714/2011, B/Johannesburg/3964/2013 and B/South Australia/81/2012. In contrast, all test viruses showed reactivity within twofold of the titres for the corresponding homologous viruses with antisera raised against viruses that are considered to be surrogate cell-propagated antigens representing the egg-propagated B/Brisbane/60/2008 prototype virus; these antisera were raised against B/Paris/1762/2009, B/Hong Kong/514/2009 and B/Odessa/3886/2010. Similarly, the test viruses reacted at titres within fourfold of the homologous titre with antiserum raised against cell-propagated B/Formosa/V2367/2012.

Phylogenetic analysis of the HA gene of representative B/Victoria lineage viruses, based on sequences available in GISAID with collection dates since 1 January 2015, is shown in Figure 3. There are nine sequences available for viruses collected in EU/EEA countries during 2015. 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. Compared with the phylogenetic analysis presented in the April 2015 report [6], covering viruses with collection dates up to the end of December 2014, there has been a change in prevalence of genetic subgroup from that defined by HA1 amino acid substitution K209N (e.g. B/Baden-Württemberg/3/2014) to that defined by N129D substitution (e.g. B/Moscow/113/2015) based on viruses collected in the USA and Japan. Of the nine viruses collected in EU/EEA countries in 2015, four fell in the group defined by N129D substitution and three in the group defined by K209N substitution, while two viruses from Finland fell in a separate group defined by amino acid substitutions K56N and V124A in HA1 with D179E in HA2.

Influenza B – Yamagata lineage

HI results for the 45 B/Yamagata-lineage test viruses (from Estonia, France, Hungary, Iceland, Norway, Poland and Slovenia) analysed since the July 2015 report are shown in Tables 5-1 to 5-4. All 37 test viruses for which HA gene sequencing was performed fell in genetic clade 3.

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 36% (16/45) 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 58% (26/45) of test viruses at titres within fourfold of its titre with the homologous virus. Antisera raised against cell culture-propagated B/Estonia/55669/2011, a virus belonging to the B/Massachusetts/02/2012 clade (clade 2), recognised 80% (36/45) of test viruses at titres within fourfold of the titres of the antisera with the homologous virus.

Antisera raised against a previously recommended vaccine virus (B/Wisconsin/1/2010) and an egg-propagated reference virus (B/Stockholm/12/2011), both belonging to clade 3 represented by B/Wisconsin/1/2010 and B/Phuket/3073/2013, recognised all and 39/45 (87%) test viruses, respectively, at titres within fourfold of the titres with the homologous viruses. Similarly, test viruses were recognised well by antisera raised against recent egg-propagated viruses compared with their respective homologous titres: all test viruses reacted within twofold with antisera raised against B/Phuket/3073/2013 (the virus recommended as a vaccine virus for the southern hemisphere 2015 and northern hemisphere 2015–16 influenza seasons) and B/Hong Kong/3417/2014 reference virus. Antiserum raised against a cell-culture-propagated cultivar of B/Phuket/3073/2013 recognised 82% (37/45) of 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 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) with the great majority falling in a subgroup defined by HA1 L172Q amino acid substitution. A small proportion of viruses, detected in many parts of the world, have HA genes of clade 3 of the B/Yamagata lineage, in a genetic group defined by M251V amino acid substitution, combined with NA genes of the B/Victoria lineage.

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 antisera									
				B/Bris[1,3] 60/08 Sh 522	B/Mal[2] 2506/04 F37/11	B/Bris[2] 60/08 F22/12	B/Paris[2] 1762/09 F07/11	B/HK[2] 514/09 F19/13	B/Odessa[2] 3886/10 F13/15	B/Malta[2] 636714/11 F33/11	B/Jhb[2] 3964/12 F01/13	B/For[4] V2367/12 F04/13	B/Sth Aus[2] 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/E7	1280	640	40	<	10	10	80	160	80	160
B/Brisbane/60/2008	1A	2008-08-04	E4/E5	1280	80	320	40	80	160	320	320	320	640
B/Paris/1762/2009	1A	2009-02-09	C2/MDCK2	1280	<	20	80	80	160	10	20	80	80
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	640	<	<	80	80	160	<	10	40	80
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	1280	<	10	40	80	160	10	20	80	80
B/Malta/636714/2011	1A	2011-03-07	E4/E1	640	80	160	40	80	80	320	320	160	640
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	2560	640	640	160	320	160	640	1280	1280	1280
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	1280	40	160	40	80	160	160	160	320	640
B/South Australia/81/2012	1A	2012-11-28	E4/E1	1280	80	320	40	80	160	320	320	640	1280
TEST VIRUSES													
B/Alsace/1443/2015	1A	2015-03-09	MDCK1/MDCK1	1280	<	<	80	80	320	20	40	80	80
B/Paris/1566/2015	1A	2015-03-23	MDCK1/MDCK1	1280	<	20	80	80	320	20	40	80	80
B/Iceland/48/2015	1A	2015-03-25	MDCKx/MDCK1	640	<	<	80	80	80	40	<	80	80
						Vaccine* SH 2016							

1. < = <40; 2. < = <10; 3. hyperimmune sheep serum; 4. <=<20

* B/Victoria-lineage virus recommended for use in quadravalent vaccines (NH 2014-15, SH 2015, NH 2015-16)

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

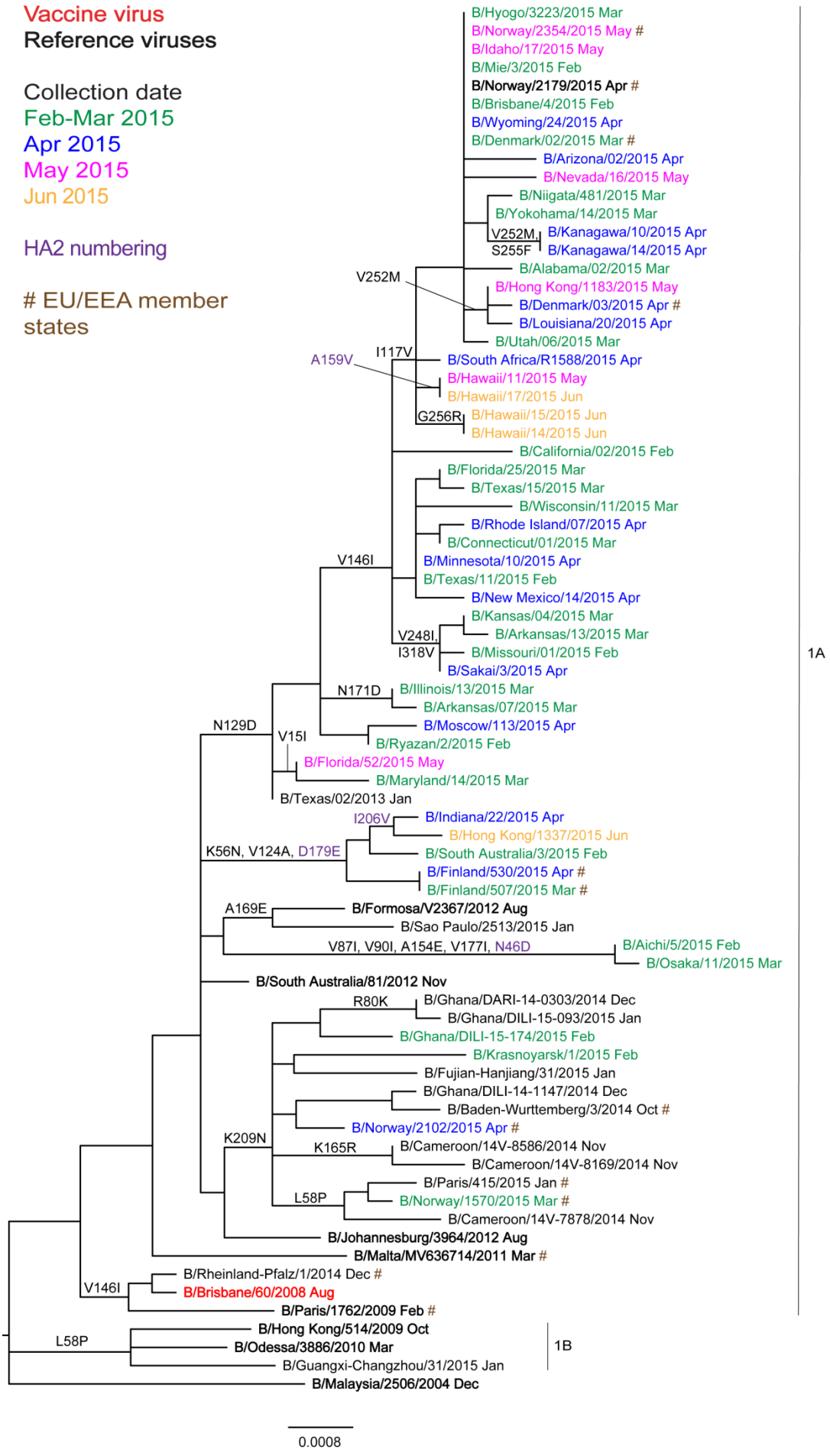


Table 5-1. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses	Collection date	Passage History	Haemagglutination inhibition titre											
			Post-infection ferret antisera											
			B/Phuket[1,3]	B/Fl[1]	B/Bris[1]	B/Wis[2]	B/Stock[2]	B/Estonia[2]	B/Mass[2]	B/Phuket[2]	B/Mass[2]	B/Phuket[2]	B/Phuket[2]	B/HK[4]
			3073/13	4/06	3/07	1/10	12/11	55669/11	02/12	3073/13	02/12	3073/13	3073/13	3417/14
			SH614	F1/10	F38/14	F10/13	F06/15	F32/12	T/C F15/13	Egg F36/14	T/C F15/13	T/C F35/14	Egg St Judes F715/14	
	Genetic Group			1	2	3	3	2	2	3	2	3	3	3
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15 E7/E1	2560	640	1280	160	320	80	1280	320	160	40	320	
B/Brisbane/3/2007	2	2007-09-03 E2/E3	1280	320	640	80	160	40	640	160	80	20	160	
B/Wisconsin/1/2010	3	2010-02-20 E3/E3	2560	160	160	160	80	10	160	160	40	20	160	
B/Stockholm/12/2011	3	2011-03-28 E4/E1	1280	80	80	80	80	10	160	80	20	20	80	
B/Estonia/55669/2011	2	2011-03-14 MDCK2/MDCK3	5120	80	160	160	80	320	320	320	640	320	160	
B/Massachusetts/02/2012	2	2012-03-13 E3/E3	1280	320	640	80	160	80	1280	160	160	20	160	
B/Massachusetts/02/2012	2	2012-03-13 MDCK1/C2/MDCK3	1280	320	320	160	80	160	320	160	320	40	160	
B/Phuket/3073/2013	3	2013-11-21 E4/E2	2560	160	160	160	160	20	320	160	40	40	160	
B/Phuket/3073/2013	3	2013-11-21 MDCK2/MDCK2	5120	160	160	320	80	320	320	320	320	640	160	
B/Hong Kong/3417/2014	3	2014-06-04 E4/E1	1280	80	40	40	20	10	40	40	20	10	160	
TEST VIRUSES														
B/Slovenia/765/2015	3	2015-02-03 MDCK1/MDCK1	5120	80	160	160	40	40	160	160	80	160	160	
B/Slovenia/935/2015		2015-02-11 MDCKx/MDCK1	2560	80	80	80	20	20	80	80	40	80	80	
B/Slovenia/1069/2015	3	2015-02-19 MDCK1/MDCK1	1280	80	80	80	20	20	80	80	40	80	80	
B/Slovenia/1143/2015		2015-02-24 MDCK1/MDCK1	2560	80	80	160	40	40	160	160	80	160	160	
B/Slovenia/1312/2015	3	2015-03-04 MDCK1/MDCK1	2560	40	80	80	20	20	80	80	40	80	80	
B/Slovenia/1736/2015	3	2015-03-25 MDCK1/MDCK1	2560	40	80	80	20	20	40	80	40	80	40	
B/Slovenia/1750/2015		2015-03-25 MDCK1/MDCK1	1280	40	80	80	20	20	40	80	40	80	40	
B/Slovenia/1900/2015		2015-04-01 MDCKx/MDCK1	1280	40	40	40	20	20	40	80	40	80	40	
B/Slovenia/2006/2015	3	2015-04-08 MDCK1/MDCK1	2560	80	80	80	40	40	80	160	40	160	160	

Vaccine NH2014-15

Vaccine* SH2015 NH2015-16

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

* B/Yamagata-lineage virus recommended for use in quadravalent vaccines (SH 2016)

Table 5-2. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses	Collection date	Passage History	Haemagglutination inhibition titre											
			Post-infection ferret antisera											
			B/Phuket[1,3] 3073/13 SH614	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 F06/15 3	B/Estonia[2] 55669/11 F32/12 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
Genetic Group														
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	1280	320	640	160	160	40	640	160	160	20	160
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	320	320	80	160	40	640	80	160	10	160
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	2560	160	160	320	160	20	320	40	160	40	160
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	160	160	80	160	10	160	40	80	20	160
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	160	160	160	40	160	1280	320	160	40	160
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	1280	320	640	80	160	40	160	160	160	10	160
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	1280	640	320	160	160	160	640	320	160	40	320
B/Phuket/3073/2013	3	2013-11-21	E4/E3	2560	320	320	320	160	20	320	40	320	40	160
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	160	320	160	320	320	320	640	640	320
B/Hong Kong/3417/2014	3	2014-06-04	E4/E1	1280	80	80	80	20	10	80	40	80	10	160
TEST VIRUSES														
B/Estonia/91207/2015		2015-01-21	SIAT2/MDCK1	2560	160	160	320	80	80	320	160	160	160	160
B/Estonia/92315/2015		2015-02-20	SIAT2/MDCK1	5120	160	160	320	160	160	320	320	320	640	320
B/Poland/1062/2015	3	2015-02-26	MDCK1/MDCK1	5120	160	320	320	160	160	640	320	640	640	320
B/Estonia/92621/2015	3	2015-02-27	SIAT1/MDCK1	2560	80	160	320	80	160	320	160	320	320	160
B/Estonia/92596/2015	3	2015-03-05	SIAT1/MDCK1	2560	80	160	160	40	40	160	80	160	160	160
B/Poland/2477/2015	3	2015-03-16	MDCK1/MDCK1	5120	160	320	320	160	320	320	320	640	640	320
B/Estonia/93061/2015	3	2015-03-27	SIAT1/MDCK1	5120	160	320	320	160	160	320	160	320	320	320
B/Poland/1054/2015	3	2015-03-27	MDCK1/MDCK1	2560	160	160	160	80	40	160	80	160	160	160
B/Estonia/93249/2015	3	2015-04-07	SIAT2/MDCK1	5120	160	320	320	160	320	640	320	640	640	320
B/Estonia/93274/2015	3	2015-04-09	SIAT2/MDCK1	2560	160	160	160	80	80	160	80	160	160	320
B/Estonia/93275/2015	3	2015-04-09	SIAT2/SIAT2	5120	640	640	640	320	640	640	640	1280	1280	320

Vaccine NH2014-15

Vaccine* SH2015 NH2015-16

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

* B/Yamagata-lineage virus recommended for use in quadravalent vaccines (SH 2016)

Table 5-3. 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/Phuket[1,3]	B/Fl[1]	B/Bris[1]	B/Wis[2]	B/Stock[2]	B/Estonia[2]	B/Mass[2]	B/Mass[2]	B/Phuket[2]	B/Phuket[2]	B/HK[4]
				3073/13	4/06	3/07	1/10	12/11	55669/11	02/12	02/12	3073/13	3073/13	3417/14
				SH614	F1/10	F38/14	F10/13	F06/15	F32/12	Egg F42/14	T/C F15/13	Egg F36/14	T/C F35/14	St Judes F715/14
				1		2	3	3	2	2	2	3	3	3
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	2560	640	1280	160	160	80	1280	160	320	40	320
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	320	640	80	160	40	640	160	160	20	160
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	2560	320	320	320	160	20	320	40	160	40	320
B/Stockholm/12/2011	3	2011-03-28	E4/E1	2560	160	160	80	160	20	160	40	160	40	320
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	80	160	40	40	80	160	320	80	40	320
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	1280	320	640	160	160	80	1280	160	320	20	320
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK4	5120	640	640	640	160	320	1280	640	640	320	640
B/Phuket/3073/2013	3	2013-11-21	E4/E2	2560	160	320	320	160	20	320	40	320	80	320
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	160	320	160	160	320	320	640	640	320
B/Hong Kong/3417/2014	3	2014-06-04	E4/E1	1280	80	80	80	20	10	80	40	80	20	160
TEST VIRUSES														
B/Paris/1553/2015	3	2015-03-15	MDCK1/MDCK1	5120	160	160	320	40	80	160	80	320	320	320
B/Lorraine/1556/2015	3	2015-03-17	MDCK1/MDCK1	5120	160	160	320	80	80	160	80	160	320	320
B/Norway/1564/2015	3	2015-03-17	MDCK1/MDCK1	5120	160	160	320	40	160	320	160	320	320	320
B/Champagne Ardenne/1563/2015	3	2015-03-18	MDCK1/MDCK1	2560	160	160	320	80	160	160	160	320	320	320
B/Franche Comte/1596/2015	3	2015-03-23	MDCK1/MDCK1	5120	320	320	320	160	160	320	160	320	320	320
B/Paris/1632/2015	3	2015-03-30	MDCK1/MDCK1	2560	160	160	160	80	80	160	80	160	160	320
B/Bretagne/1659/2015	3	2015-03-31	MDCK1/MDCK1	2560	160	160	160	40	40	80	80	160	80	160
B/Paris/1669/2015	3	2015-04-01	MDCK1/MDCK1	5120	320	320	320	80	160	320	160	320	160	320
B/Bretagne/1668/2015	3	2015-04-04	MDCK1/MDCK1	5120	160	160	160	80	80	160	80	160	160	320
B/Nord Pas de Calais/1681/2015	3	2015-04-07	MDCK1/MDCK1	5120	160	160	160	40	80	160	80	160	160	320
B/Pays de Loire/1692/2015	3	2015-04-09	MDCK1/MDCK1	5120	160	160	320	80	80	160	160	320	160	320
B/Norway/2343/2015	3	2015-05-02	MDCK1/MDCK1	5120	160	160	160	40	160	320	160	320	640	320
B/Norway/2444/2015	3	2015-05-30	MDCK1/MDCK1	5120	160	160	160	20	160	160	160	320	320	320
B/Norway/2538/2015	3	2015-06-22	MDCK1/MDCK1	5120	160	160	320	20	80	160	160	320	320	320
B/Norway/2545/2015	3	2015-06-24	MDCK1/MDCK1	5120	160	160	160	40	80	160	160	320	320	320
B/Norway/2552/2015	3	2015-06-25	MDCK1/MDCK1	5120	160	160	320	20	160	320	320	320	640	320
										Vaccine NH 2014-15			Vaccine* SH 2015 NH 2015-16	

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

* B/Yamagata-lineage virus recommended for use in quadravalent vaccines (SH 2016)

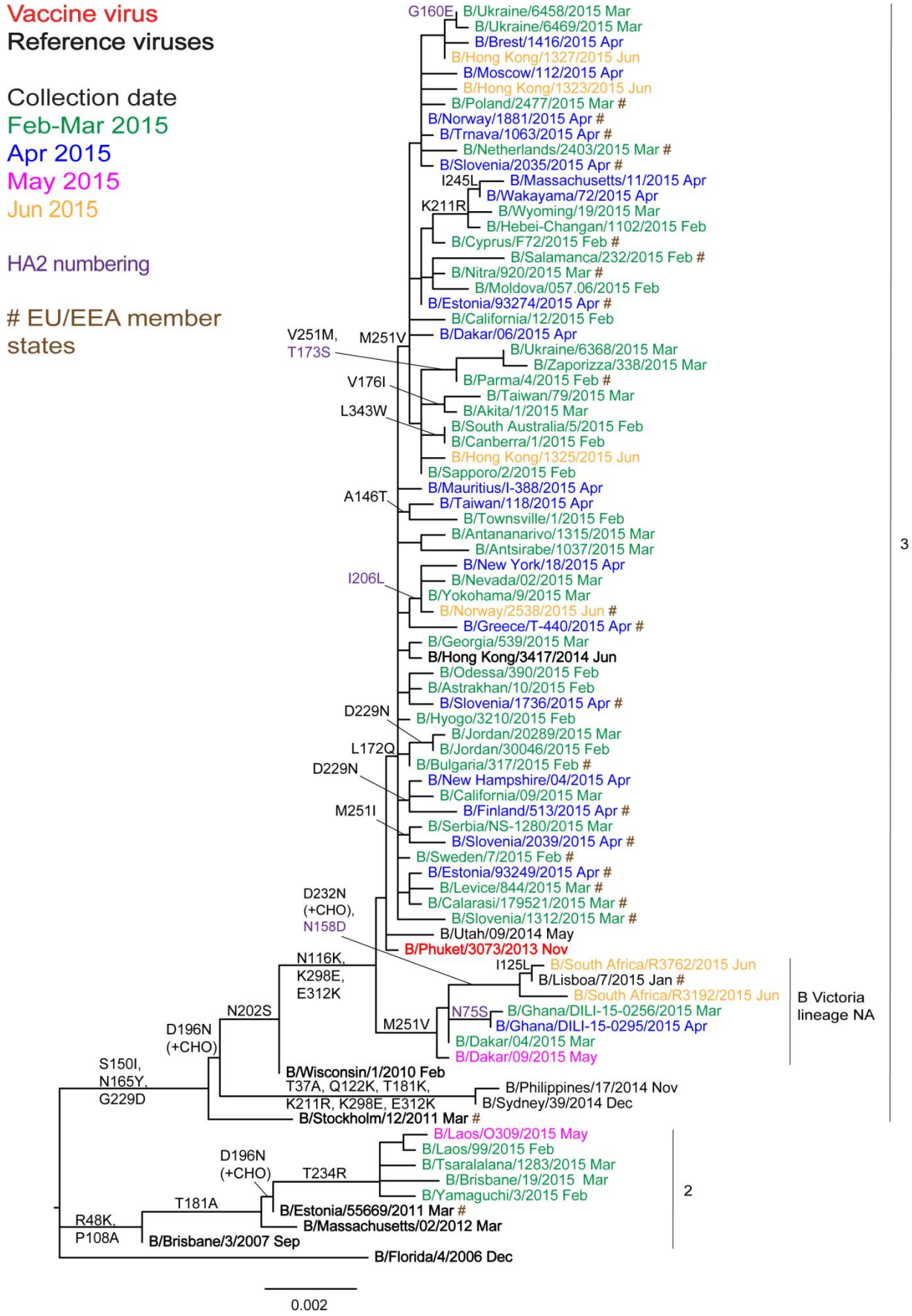
Table 5-4. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses	Genetic Group	Collection date	Passage History	Haemagglutination inhibition titre															
				Post-infection ferret antisera															
				B/Phuket[1,3]	B/Fl[1]	B/Bris[1]	B/Wis[2]	B/Stock[2]	B/Estonia[2]	B/Mass[2]	B/Mass[2]	B/Phuket[2]	B/Phuket[2]	B/Phuket[2]	B/HK[4]				
				3073/13	4/06	3/07	1/10	12/11	55669/11	02/12	02/12	3073/13	3073/13	3073/13	3417/14				
				SH614	F1/10	F38/14	F10/13	F06/15	F32/12	Egg F42/14	T/C F15/13	Egg F36/14	T/C F35/14	St Judes F715/14					
					1	2	3	3	2	2	2	3	3	3					
REFERENCE VIRUSES																			
B/Florida/4/2006	1	2006-12-15	E7/E1	1280	320	640	160	160	40	640	80	160	40	320					
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	320	640	80	160	40	640	80	160	20	160					
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	2560	160	160	160	80	10	160	20	160	20	160					
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	160	80	80	160	10	80	20	80	20	160					
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	80	80	40	20	80	40	320	80	40	160					
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	1280	160	320	80	80	40	640	160	80	20	160					
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	1280	320	640	160	80	160	640	320	160	80	640					
B/Phuket/3073/2013	3	2013-11-21	E4/E2	2560	160	160	160	80	20	160	40	160	40	160					
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	160	160	80	160	160	160	320	320	320					
B/Hong Kong/3417/2014	3	2014-06-04	E4/E1	1280	80	80	80	20	10	40	20	40	10	320					
TEST VIRUSES																			
B/Iceland/28/2015	3	2015-02-05	MDCKx/MDCK1	1280	80	40	40	20	20	40	40	80	80	160					
B/Hungary/437/2015	3	2015-02-09	MDCK3/SIAT2	2560	160	80	160	40	40	80	80	160	160	160					
B/Iceland/33/2015	3	2015-02-09	MDCKx/MDCK1	1280	80	80	40	40	20	40	40	80	20	160					
B/Iceland/42/2015	3	2015-03-03	MDCKx/MDCK1	2560	80	80	80	40	40	80	40	80	80	320					
B/Iceland/44/2015	3	2015-03-12	MDCKx/MDCK1	1280	80	40	40	40	20	40	40	80	80	160					
B/Iceland/47/2015	3	2015-03-16	MDCKx/MDCK1	2560	160	160	160	80	40	160	80	160	160	320					
B/Norway/1859/2015	3	2015-03-28	MDCK1	2560	160	80	160	<	40	160	80	160	160	320					
B/Norway/1921/2015		2015-04-13	MDCK1	2560	160	80	160	<	40	160	80	160	160	320					
B/Iceland/6544/2015		2015-05-05	MDCK1	2560	80	80	160	40	40	80	40	160	160	160					
										Vaccine NH 2014-15					Vaccine* SH 2015 NH 2015-16				

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

* B/Yamagata-lineage virus recommended for use in quadravalent vaccines (SH 2016)

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



Summary of genetic data submitted to TESSy

As of 17 May 2015 (to week 20/2015), the majority of influenza viruses identified genetically since week 40/2014 were A(H3N2) viruses (60%), with lower numbers of influenza B viruses (23%) and A(H1N1)pdm09 viruses (17%) being reported. 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 over 40 to 1. The majority of influenza A(H3N2) viruses belonged to genetic subgroup 3C.2a (62%), represented by A/Hong Kong/5738/2014; smaller proportions were in genetic group 3C.3 (30%), represented by A/Samara/73/2013, genetic subgroup 3C.3a (< 7%), 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.

Over the period of weeks 21–39/2015, influenza B has dominated with 574 detections (142 B/Yamagata, 4 B/Victoria and 428 not assigned to lineage) compared with 379 influenza A detections (164 H3N2, 82 H1N1pdm09 and 133 not subtyped). Of these, 76 have been characterised genetically: 18 H1N1pdm09 (all subgroup 6B), 45 H3N2 (42 3C.2a and three 3C.3) and 13 influenza B viruses (all B/Yamagata lineage clade 3).

Antiviral susceptibility

Between weeks 40/2014 and 39/2015, based on reports to TESSy, 2 675 influenza viruses (1 574 A(H3N2), 569 A(H1N1)pdm09 and 532 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 959 viruses, with collection dates after 31 August 2014, from EU/EEA countries have been assessed phenotypically for NAI susceptibility at the London WHO CC: 220 influenza B, 197 A(H1N1)pdm09 and 542 A(H3N2) inclusive of many 3C.2a genetic subgroup viruses that could not be analysed by HI assay. All but one of the viruses tested were susceptible to oseltamivir and zanamivir. The B/Yamagata-lineage virus, B/Extremadura/728/2015, showed reduced inhibition by oseltamivir and zanamivir, and carried NA amino acid substitution D197G. In addition, one H1N1pdm09 virus not tested in the sialidase inhibition assay, A/Norway/2227/2015, carried an H275Y substitution in the NA, associated with highly reduced inhibition by oseltamivir.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [7] reported that the China Health and Family Planning Commission notified 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 [8]. Increased numbers of cases were reported over the course of the 2013–14 and 2014–15 seasons. A revised Rapid Risk Assessment [9] 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 [10], updated on 15 October 2015 [11], and conducted a new risk assessment on 23 February 2015 [12]. 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 [13] and has provided subsequent situation updates, with the latest being on 18 July 2015 [14].

Influenza A(H5N1) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 4 September 2015 [11]. No new laboratory-confirmed human cases of H5Nx infection had been reported to WHO since the update on 17 July 2015. ECDC published an updated rapid risk assessment on the situation in Egypt on 13 March 2015 [15] and an epidemiological update 10 April 2015 [16]. On 14 July 2015, WHO reported on a recent fatal case of human infection with avian A(H5N6) virus in China [17].

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, formerly the MRC National

Instuite for Medical Research, 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 (#) as are those viruses for which data is presented in the HI tables (@). 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.

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