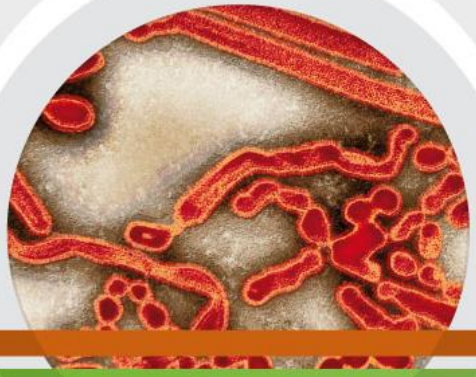

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, December 2015

Summary

From week 40/2015, the start of weekly reporting on influenza activity in the WHO European Region, to week 53/2015, 4 579 influenza detections across the Region have been reported. Influenza type A viruses are prevailing over type B, but, unlike the situation in the 2014–15 season, A(H1N1)pdm09 viruses are prevailing over A(H3N2), and the proportion of B/Victoria-lineage detections has risen substantially to represent ~62% of the type B detections.

To date, seven EU/EEA countries have shared 94 influenza-positive specimens with the Francis Crick Institute, London, for detailed characterisation. This includes three additional countries and 47 additional specimens since the November 2015 report. Since the latter report, 29 viruses have been characterised antigenically, and genetic analyses are ongoing.

The 23 A(H1N1)pdm09 viruses characterised antigenically were similar to the vaccine virus A/California/07/2009. Worldwide a new genetic subcluster of viruses within the 6B subgroup has emerged, defined by HA1 amino acid substitutions S162N and I216T, and 50% of the test viruses characterised in this report fall in this subcluster.

The three A(H3N2) test viruses characterised by haemagglutination inhibition (HI) assay were poorly recognised (titres at least thirty-twofold reduced compared to the homologous titre) by reference antiserum raised against egg-propagated A/Switzerland/9715293/2013, the vaccine virus recommended for use in the 2015–2016 northern hemisphere influenza season. The test viruses were recognised somewhat better by antisera raised against egg-propagated A/Hong Kong/4801/2014, the virus recommended for use in southern hemisphere 2016 influenza vaccines.

The B/Victoria-lineage virus was antigenically similar to B/Brisbane/60/2008 and fell in genetic clade 1A, as do recently collected viruses worldwide.

The two B/Yamagata viruses characterised fell in genetic clade 3 and reacted well with post-infection ferret antiserum raised against egg-propagated B/Phuket/3073/2013, the recommended vaccine virus for the northern hemisphere 2015–16 influenza season.

This report was prepared by Rod Daniels, Vicki Gregory, 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).

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Table 1 shows a summary of influenza virus detections in the WHO European Region reported to TESSy for the first 14 weeks (weeks 40–53/2015) of reporting for the 2015–16 season. A total of 4 579 detections had been made with type A viruses prevailing over type B at a ratio of 5.3:1. So far, of the type A viruses subtyped (n = 2348) and the type B viruses ascribed to a lineage (n = 107), A(H1N1)pdm09 have prevailed over A(H3N2) and B/Victoria over B/Yamagata by ratios of 5.8:1 and 1.6:1, respectively.

Since the start of weekly reporting for the 2015–16 influenza season (week 40/2015), ten shipments of specimens from countries in the EU/EEA (Belgium, England, Germany, the Netherlands, Norway, Portugal and Slovenia) have been received at the Crick Worldwide Influenza Centre (WIC). Of the 94 specimens received – a mix of clinical samples and virus isolates – the majority (84%) were type A viruses, and A(H1N1)pdm09 outnumbered A(H3N2) at a ratio of approximately 3.9:1 (Table 2). Of the 15 type B specimens received (16% of the specimens), ten were B/Victoria-lineage and five B/Yamagata-lineage. Many specimens are still in the process of being characterised. The antigenic and genetic properties of influenza virus isolates characterised since the November 2015 report[1] are presented and discussed in this report.

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

Table 1. Influenza virus detections in the WHO European Region since the start of reporting for the 2015–16 season (weeks 40–53/2015)

Virus type/subtype	Cumulative number of detections			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**544**	**4035**	**4579**	**84.0**	**5.3:1**
A(H1N1)pdm09	402	1599	2001	85.2	5.8:1
A(H3N2)	86	261	347	14.8	
A not subtyped	56	2175	2231		
Influenza B	**212**	**663**	**875**	**16.0**	
Victoria lineage	31	35	66	61.7	1.6:1
Yamagata lineage	6	35	41	38.3	
Lineage not ascribed	175	593	768		
Total detections (total tested)	**756 (10752)**	**4698 (119137)**	**5454 (129889)**		

*** Percentages are shown for total detections (types A & B, and for viruses ascribed to subtype/lineage). Ratios are given for type A:B, A(H1N1)pdm09: A(H3N2) and Victoria:Yamagata lineages.**

Table 2. Summary of clinical samples and virus isolates received from EU/EEA Member States: packages received since the start of the 2015–16 reporting period (week 40/2015)

MONTH*	TOTAL RECEIVED	A		H1N1pdm09		H3N2			B		B Victoria lineage		B Yamagata lineage	
Country		Number received	Number propagated	Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated	Number received	Number propagated[1]	Number received	Number propagated[1]
2015														
AUGUST														
Norway	3			1	1	2	1	1						
SEPTEMBER														
United Kingdom	3					3	3							
OCTOBER														
Belgium	1			1	in process									
Germany	2			1	1								1	1
Netherlands	1			1	in process									
Norway	11			8	6	2	2				1	1		
Portugal	1			1	in process									
Slovenia	2	1	in process	1	1									
United Kingdom	7			6	6						1	1		
NOVEMBER														
Belgium	11			2	in process	1	in process				6	in process	2	in process
Germany	12			10	10	2	in process							
Netherlands	4			3	in process						1	in process		
Norway	13			9	in process	1	in process				1	in process	2	in process
Portugal	8			8	in process									
Slovenia	2					2	in process							
United Kingdom	3			3	in process									
DECEMBER														
Norway	5			3	in process	2	in process							
Portugal	4			4	in process									
Slovenia	1					1	in process							
	94	1		62	25	16	6	1	0		10	2	5	1
					66.0%		17.0%					10.6%		5.3%
7 Countries					84.0%							16.0%		

* Month indicates the months in which the clinical specimens were collected

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 November 2015 report are shown in Tables 3-1 to 3-3. The 23 A(H1N1)pdm09 viruses from Germany, Norway and Slovenia were antigenically similar to the vaccine virus, A/California/07/2009. 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 14/23 (61%) 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/07/2009 group, recognised the egg-propagated vaccine virus at titres fourfold to sixteenfold reduced compared with the titres of the antisera with their homologous viruses. Fold reductions were greater for MDCK-isolated viruses from 2009 that carried an amino acid substitution at HA1 position 155 (G155E).

All test viruses for which HA sequences were available fell in genetic subgroup 6B (Tables 3-1 to 3-3 and Figure 1). Since 2009, the HA genes have evolved, and eight genetic groups have been designated. For over a year, 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/07/2009, 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/07/2009, e.g. A/South Africa/3626/2013. Notably, a new subcluster of viruses, defined by HA1 amino acid substitutions **S162N** (which results in the formation of a new potential glycosylation motif at residues 162–164 of HA1) and **I216T** in **HA1,** in the 6B genetic subgroup, has emerged worldwide, and 11/22 (50%) test viruses characterised genetically fell in this subcluster. All but one of the 11 test viruses in this genetic subcluster yielded HI titres within fourfold of the homologous titre with the ferret antiserum raised against A/California/07/2009.

[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

Table 3-1. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History	Ferret number	Haemagglutination inhibition titre[1]											
					Post-infection ferret antisera											
					A/Cal	A/Bayern	A/Lviv	A/Chch	A/Astrak	A/St. P	A/St. P	A/HK	A/Sth Afr			
					7/09	69/09	N6/09	16/10	1/11	27/11	100/11	5659/12	3626/13			
					Egg	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK	Egg			
					F01/15	F09/15	F34/09 C4	F15/14	F22/13	F26/14	F24/11	F30/12	F3/14			
					1	1	1	4	5	6	7	6A	6B			
REFERENCE VIRUSES																
A/California/7/2009	1	2009-04-09		E1/E3	640	640	640	160	160	160	160	160	160			
A/Bayern/69/2009	1	2009-07-01		MDCK5/MDCK1	80	320	160	80	40	40	40	40	40	G155E		
A/Lviv/N6/2009	1	2009-10-27		MDCK4/S1/MDCK2	640	1280	640	320	160	160	160	320	160	G155E>G, D222G		
A/Christchurch/16/2010	4	2010-07-12		E1/E3	640	1280	1280	2560	1280	320	2560	1280	1280			
A/Astrakhan/1/2011	5	2011-02-28		MDCK1/MDCK5	640	640	2560	640	1280	640	2560	2560	1280			
A/St. Petersburg/27/2011	6	2011-02-14		E1/E3	640	640	2560	640	1280	640	2560	2560	1280			
A/St. Petersburg/100/2011	7	2011-03-14		E1/E2	640	640	160	640	1280	640	2560	1280	640			
A/Hong Kong/5659/2012	6A	2012-05-21		MDCK4/MDCK1	160	160	640	160	320	160	640	640	320			
A/South Africa/3626/2013	6B	2013-06-06		E1/E3	320	320	320	320	640	320	640	640	640			
TEST VIRUSES																
A/Norway/2624/2015	6B	2015-10-20		MDCK1/MDCK1	640	640	1280	640	1280	640	2560	2560	1280			
A/Norway/2626/2015	6B	2015-10-22		MDCK1/MDCK1	320	320	640	320	1280	640	2560	1280	1280	S162N (+CHO), I216T		
A/Norway/2628/2015	6B	2015-10-23		MDCK1/MDCK1	320	640	1280	640	1280	640	2560	2560	1280			
A/Norway/2633/2015	6B	2015-10-27		MDCK1/MDCK1	640	640	1280	640	1280	640	2560	2560	1280			
A/Norway/2634/2015	6B	2015-10-27		MDCK1/MDCK1	320	640	1280	640	1280	640	2560	1280	1280			
A/Norway/2646/2015	6B	2015-11-03		MDCK1/MDCK1	320	640	640	320	1280	320	1280	1280	1280			
A/Norway/2647/2015	6B	2015-11-03		MDCK1/MDCK1	320	640	1280	640	2560	640	2560	2560	1280			
A/Norway/2650/2015	6B	2015-11-03		MDCK1/MDCK1	320	640	1280	640	1280	640	2560	2560	1280	S162N (+CHO), I216T		
A/Norway/2685/2015		2015-11-13		MDCK1	160	320	640	320	640	320	1280	1280	640			
									Vaccine							

[1] < = <40

Table 3-2. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1] Post-infection ferret antisera									
				A/Cal	A/Bayern	A/Lviv	A/Chch	A/Astrak	A/St. P	A/St. P	A/HK	A/Sth Afr	
				7/09	69/09	N6/09	16/10	1/11	27/11	100/11	5659/12	3626/13	
				Egg	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK	Egg	
			Ferret number	F01/15	F09/15	F14/13	F15/14	F22/13	F26/14	F24/11	F30/12	F3/14	
	Genetic group			1	1	1	4	5	6	7	6A	6B	
REFERENCE VIRUSES													
A/California/7/2009	1	2009-04-09	EP1/E3	1280	640	640	160	160	160	320	160	160	
A/Bayern/69/2009	1	2009-07-01	MDCK5/MDCK1	40	160	80	40	<	<	<	<	40	G155E
A/Lviv/N6/2009	1	2009-10-27	MDCK4/S1/MDCK3	320	640	640	160	80	80	80	160	80	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	640	1280	2560	1280	640	1280	1280	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	320	640	320	320	1280	640	1280	1280	640	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	640	640	640	320	1280	640	2560	1280	640	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	640	640	320	1280	640	2560	1280	640	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK1	80	80	160	80	320	160	640	640	160	
A/South Africa/3626/2013	6B	2013-06-06	E1/E3	320	320	320	160	320	320	640	640	640	
TEST VIRUSES													
A/Norway/2592/2015	6B	2015-08-25	MDCK1/MDCK3	320	640	320	320	1280	320	1280	1280	1280	
A/Slovenia/2903/2015	6B	2015-10-12	MDCKx/MDCK1	320	640	320	640	1280	640	5120	2560	2560	S162N (+CHO), I216T
A/Berlin/166/2015	6B	2015-10-21	C2/MDCK1	320	320	320	320	640	320	1280	1280	640	
A/Norway/2674/2015	6B	2015-11-04	MDCK2	320	640	320	320	1280	640	1280	1280	1280	
A/Berlin/167/2015	6B	2015-11-13	C2/MDCK1	320	640	320	640	1280	640	1280	1280	1280	S162N (+CHO), I216T
A/Sachsen//102/2015	6B	2015-11-13	C2/MDCK1	320	320	320	320	640	320	1280	1280	640	S162N (+CHO), I216T
A/Bayern/147/2015	6B	2015-11-19	C2/MDCK1	320	640	320	640	1280	640	1280	1280	640	S162N (+CHO), I216T
A/Hamburg/24/2015	6B	2015-11-23	C2/MDCK1	320	640	320	640	1280	640	1280	1280	1280	S162N (+CHO), I216T
A/Nordrhein-Westfalen/102/2015	6B	2015-11-23	C2/MDCK1	320	640	320	640	1280	640	1280	1280	1280	S162N (+CHO), I216T
A/Bayern/146/2015	6B	2015-11-30	C2/MDCK1	160	320	320	320	640	320	1280	1280	640	S162N (+CHO), I216T
				Vaccine									

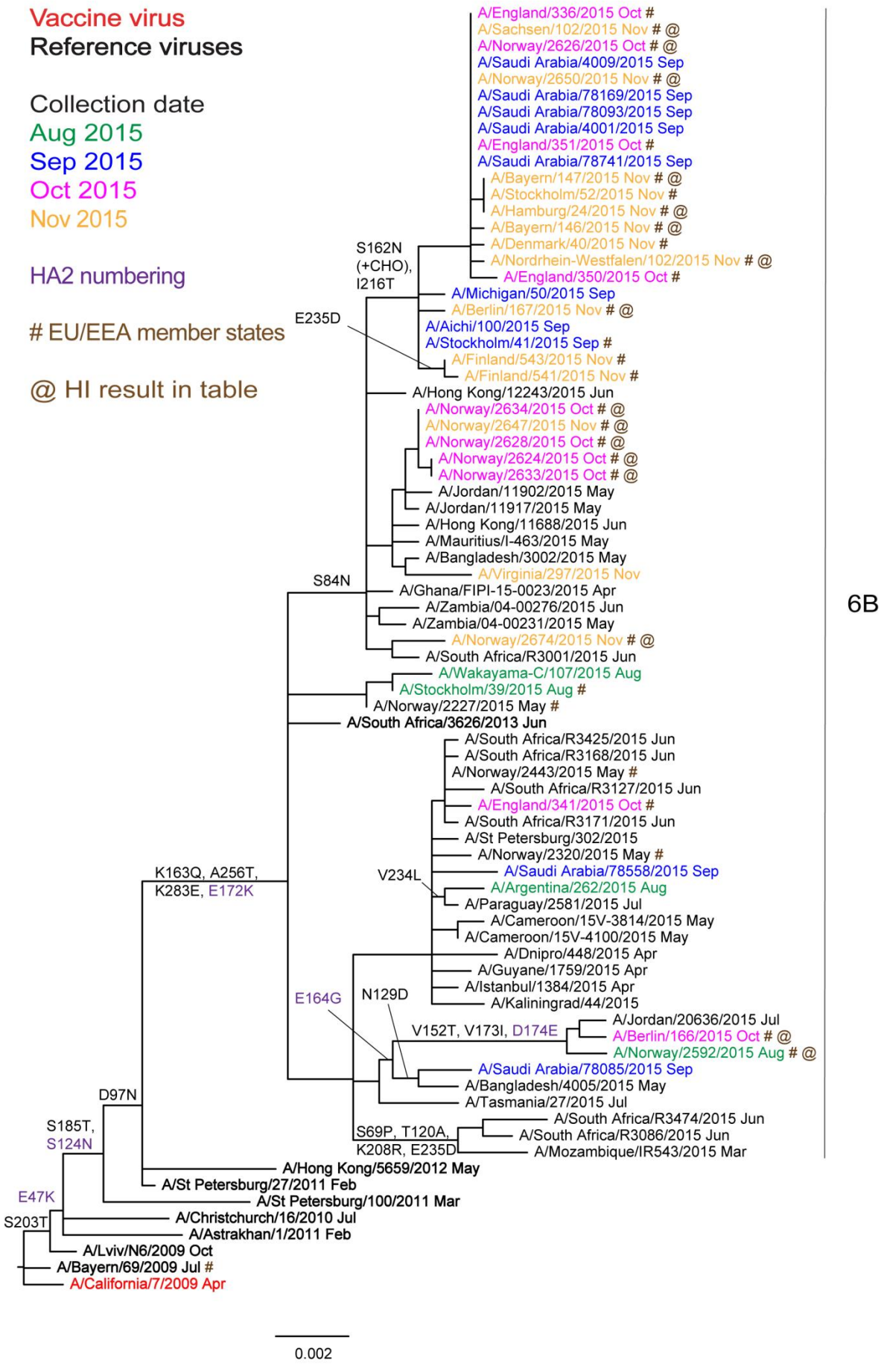
[1] < = <40

Table 3-3. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History / Ferret number	Haemagglutination inhibition titre[1] — Post-infection ferret antisera									
				A/Cal	A/Bayern	A/Lviv	A/Chch	A/Astrak	A/St. P	A/St. P	A/HK	A/Sth Afr	
				7/09	69/09	N6/09	16/10	1/11	27/11	100/11	5659/12	3626/13	
				Egg	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK	Egg	
				F01/15	F09/15	F14/13	F15/14	F22/13	F26/14	F24/11	F30/12	F3/14	
	1			1	1	1	4	5	6	7	6A	6B	
REFERENCE VIRUSES													
A/California/7/2009	1	2009-04-09	EP1/E3	640	640	640	160	160	160	160	80	160	
A/Bayern/69/2009	1	2009-07-01	MDCK5/MDCK1	40	160	80	40	<	<	<	<	40	G155E
A/Lviv/N6/2009	1	2009-10-27	MDCK4/S1/MDCK2	320	640	640	160	80	80	80	160	80	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	1280	2560	1280	640	2560	1280	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	640	1280	640	640	1280	640	2560	2560	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	640	640	640	320	1280	640	2560	1280	640	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E2	640	640	640	640	1280	640	2560	1280	640	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK1	160	160	160	160	640	160	1280	640	320	
A/South Africa/3626/2013	6B	2013-06-06	E1/E3	640	640	640	320	640	320	1280	640	640	
TEST VIRUSES													
A/Bremen/25/2015	6B	2015-11-09	C2/MDCK1	320	640	320	640	1280	320	2560	1280	640	
A/Berlin/169/2015	6B	2015-11-09	C2/MDCK1	640	640	640	640	1280	640	2560	1280	1280	
A/Berlin/168/2015	6B	2015-11-10	C2/MDCK1	320	640	320	320	1280	320	1280	1280	1280	S162N (+CHO), I216T
A/Nordrhein-Westfalen/103/2015	6B	2015-11-13	C2/MDCK1	320	640	320	640	1280	640	1280	1280	1280	S162N (+CHO), I216T
				Vaccine									

[1] < = <40

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



Influenza A(H3N2) virus analyses

As described in many previous reports[3], 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 first in the November 2014 report[4].

Results of HI tests performed with guinea pig RBCs in the presence of 20nM oseltamivir, added to circumvent NA-mediated binding of A(H3N2) viruses to the RBCs, are shown in Table 4. The three test viruses from Norway retained sufficient HA titre to be analysed by HI assay, with one falling in genetic group 3C.3 and two in genetic subgroup 3C.2a.

The test viruses, propagated in MDCK-SIAT1 cells, reacted poorly in HI assays, with the panel of post-infection ferret antisera relative to the titres of the antisera with their respective homologous viruses (shown in red: Table 4). However, in terms of absolute titres, antisera raised against A/Stockholm/6/2014 (3C.3a: cell- and egg-propagated), cell-propagated A/Hong Kong/5738/2014 (3C.2a) and the vaccine virus recommended for the southern hemisphere 2016 influenza season, A/Hong Kong/4801/2014 (3C.2a: cell- and egg-propagated), gave consistent reactivity with all three test viruses. Antisera raised against the northern hemisphere 2015–16 vaccine component, egg-propagated A/Switzerland/9715293/2013 (3C.3a) and egg-propagated A/Hong Kong/146/2013 (3C.2), reacted with all three test viruses at titres of 40 (the cut-off for the HI assay).

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 A(H3N2) viruses with recent collection dates is shown in Figure 2. The HA genes fall within genetic group 3C. This group has three subdivisions: 3C.1 (represented by A/Texas/50/2012, the vaccine virus recommended for use in the 2014–15 northern hemisphere season), 3C.2 and 3C.3. Viruses in these three subdivisions had 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 are:

- (3C.2) **N145S** 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** and **N145S** 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.

Figure 2 shows that genetic subgroup 3C.2a viruses have dominated in recent months, and viruses from Denmark, England, Norway and Sweden have been characterised. Gene sequences of a 3C.3 genetic subgroup virus have also been reported (A/Norway/2605/2015).

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 [1]. 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, antisera 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 influenza vaccines for the southern hemisphere 2016 influenza season was for an A/Hong Kong/4801/2014-like (3C.2a) virus [2].

[3] 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

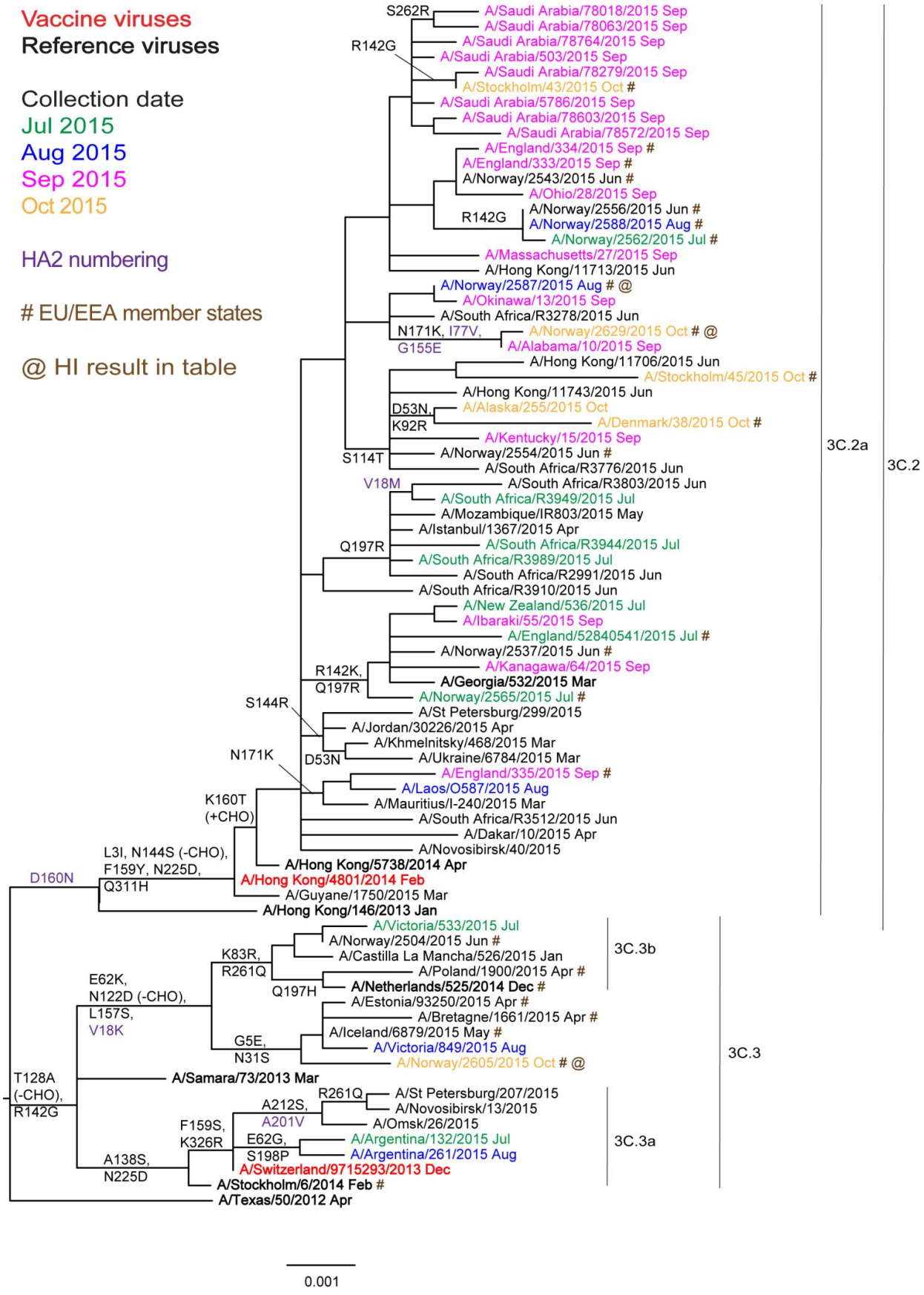
[4] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, November 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/ERLI-Net report November 2014.pdf

Table 4. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

Viruses	Collection Date	Genetic group	Passage History	Ferret number	Haemagglutination inhibition titre[1]													
					Post-infection ferret antisera													
			A/Texas	A/Samara	A/Stock	A/Stock	A/Switz	A/Switz	A/Neth	A/HK	A/HK	A/HK	A/HK	A/HK	A/HK	A/HK	A/HK	A/HK
			50/12	73/13	6/14	6/14	9715293/13	9715293/13	525/14	146/13	5738/14	4801/14	4801/14	4801/14	4801/14	4801/14	4801/14	4801/14
			Egg	SIAT	SIAT	Egg	SIAT	Egg	SIAT	Egg	MDCK	MDCK	Egg	Egg	Egg	Egg	Egg	Egg
			F36/12	F24/13	F14/14	F20/14	F18/15	F32/14	F23/15	F10/15	F30/14	F43/15	F12/15	F12/15	F12/15	F12/15	F12/15	F12/15
			3C.1	3C.3	3C.3a	3C.3a isolate 2	3C.3a	3C.3a cl123	3c.3b	3C.2	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a
REFERENCE VIRUSES																		
A/Texas/50/2012	2012-04-15	3C.1	E5/E2	5120	640	640	40	640	320	320	160	320	80	80	80	80	80	80
A/Samara/73/2013	2013-03-12	3C.3	C1/SIAT3	2560	1280	640	160	640	640	640	640	640	320	320	320	320	320	320
A/Stockholm/6/2014	2014-02-06	3C.3a	SIAT1/SIAT3	160	160	640	80	160	40	80	160	160	160	160	160	160	160	160
A/Stockholm/6/2014	2014-02-06	3C.3a	E4/E1 isolate 2	640	80	160	80	640	80	80	160	320	40	40	40	40	40	40
A/Switzerland/9715293/2013	2013-12-06	3C.3a	SIAT1/SIAT2	80	160	640	80	80	40	40	80	40	80	80	80	80	80	80
A/Switzerland/9715293/2013	2013-12-06	3C.3a	E4/E1 clone 123	640	160	320	80	1280	40	80	160	160	80	80	80	80	80	80
A/Netherlands/525/2014	2014-12-17	3C.3b	SIAT2/SIAT3	1280	320	320	160	160	1280	160	80	160	160	160	160	160	160	160
A/Hong Kong/146/2013	2013-01-11	3C.2	E3/E3	2560	640	80	640	640	320	640	320	640	160	160	160	160	160	160
A/Hong Kong/5738/2014	2014-04-30	3C.2a	MDCK1/MDCK3	160	160	320	160	80	40	80	160	320	160	160	160	160	160	160
A/Hong Kong/4801/2014	2014-02-26	3C.2a	MDCK4/MDCK2	160	160	320	160	80	40	80	160	320	160	160	160	160	160	160
A/Hong Kong/4801/2014	2014-02-26	3C.2a	E6/E2 isolate 1	80	160	160	80	80	80	40	320	640	320	320	320	320	320	320
TEST VIRUSES																		
A/Norway/2587/2015	2015-08-07	3C.2a	MDCK/SIAT1/SIAT1	40	40	80	80	<	40	<	40	40	80	80	80	80	80	80
A/Norway/2605/2015	2015-10-02	3C.3	MDCK/SIAT1/SIAT1	320	80	160	80	<	40	320	40	40	40	40	40	40	40	40
A/Norway/2629/2015	2015-10-22	3C.2a	MDCK/SIAT1/SIAT3	40	<	80	40	40	40	<	40	40	40	40	40	40	40	40
								Vaccine NH 2015-16										Vaccine SH 2016

[1] < = <40

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



Influenza B virus analyses

Fifteen influenza type B viruses have been received from EU/EEA countries: ten B/Victoria-lineage and five B/Yamagata-lineage (Table 2).

Influenza B – Victoria lineage

Since the November 2015 report only one virus of this lineage from an EU/EEA country has been characterised antigenically. HI results are shown in Table 5 and, as observed throughout the previous season, the test virus carried an HA gene of genetic group 1A.

This test virus showed a similar HI reactivity pattern to those from the 2014–15 influenza season: 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, it was 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, it 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.

Phylogenetic analysis of the HA gene of representative B/Victoria lineage viruses, based largely on sequences available in GISAID with collection dates from July 2015, 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. Viruses with collection dates in October of 2015, including viruses from Denmark, England and Norway, fall in a major subcluster defined by amino acid substitutions N129D, V146I and I117V within genetic clade 1A.

These results, linked with the rise in the proportion of B/Victoria-lineage viruses, support the recommendation made in September 2015 to include B/Brisbane/60/2008 in trivalent influenza vaccines for the southern hemisphere 2016 influenza season [2].

Influenza B – Yamagata lineage

HI results for two B/Yamagata-lineage test viruses analysed since the November 2015 report are shown in Table 6. Both test viruses fell in genetic clade 3.

The homologous titres of the ten post-infection ferret antisera, shown in red, ranged from 40 to 1 280, and the two test viruses show similar reactivity patterns, although B/Berlin/50/2015 was slightly less well recognised by the antisera than was B/Norway/2692/2015 (Table 6). Based on the criterion of 'fold-drop compared to homologous titre', antisera raised against the four genetic clade 2 viruses were slightly less cross-reactive with the test viruses, notably so for the antiserum raised against egg-propagated B/Massachusetts/02/2012, the vaccine virus recommended for use in the 2014–15 northern hemisphere influenza season, compared to the five antisera raised against genetic clade 3 viruses.

Of the five antisera raised against genetic clade 3 viruses, those raised against viruses carrying N116K, N202S, K298E and E312K HA1 amino acid substitutions (Figure 4), cell- and egg-propagated B/Phuket/3073/2013 and egg-propagated B/Hong Kong/3417/2014, showed somewhat higher titres with the test viruses compared with the antisera raised against egg-propagated B/Stockholm/12/2011 and B/Wisconsin/1/2010 (a previous vaccine virus). Antisera raised against egg-propagated B/Phuket/3073/2013 (the current vaccine virus) and B/Hong Kong/3417/2014 recognised both test viruses at titres within twofold of their respective homologous titres.

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 and L172Q amino acid substitution in HA1. A number of viruses, including the two test viruses characterised here, form a subcluster defined by HA1 M251V amino acid substitution. A few viruses are reassortants carrying NA genes normally associated with the B/Victoria-lineage.

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

Viruses	Genetic clade	Collection date	Passage History	Haemagglutination inhibition titre									
				Post infection ferret antisera									
				B/Bris[1,3] 60/08 Egg Sh 539/540 /543/544	B/Mal[2] 2506/04 Egg F37/11	B/Bris[2] 60/08 Egg F22/12	B/Paris[2] 1762/09 MDCK F07/11	B/Malta[2] 636714/11 Egg F29/13	B/Jhb[2] 3964/12 Egg F01/13	B/For[4] V2367/12 MDCK F04/13	B/Sth Aus[2] 81/12 Egg F41/13	B/HK[2] 514/09 MDCK F19/13	B/Odessa[2] 3886/10 MDCK F19/11
	1A			1A		1A	1A	1A	1A	1A	1A	1B	1B
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	640	80	<	160	160	80	160	20	<
B/Brisbane/60/2008	1A	2008-08-04	E4/E5	640	80	160	40	320	320	320	640	80	40
B/Paris/1762/2009	1A	2009-02-09	C2/MDCK2	640	<	10	40	20	20	40	40	40	40
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	320	<	10	40	40	10	40	40	40	80
B/Odessa/3886/2010	1B	2010-03-19	C2/MDCK2	320	<	10	20	40	20	40	40	40	80
B/Malta/636714/2011	1A	2011-03-07	E4/E1	640	80	160	40	320	320	320	640	80	40
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	2560	640	1280	160	1280	1280	1280	1280	320	160
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	1280	40	160	40	160	160	160	320	40	40
B/South Australia/81/2012	1A	2012-11-28	E4/E1	640	80	160	40	320	160	320	640	80	40
TEST VIRUSES													
B/Norway/2622/2015	1A	2015-10-21	MDCK1/MDCK1	640	<	<	40	40	<	40	80	40	80
						Vaccine NH 2015-16* SH 2016							

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

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

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

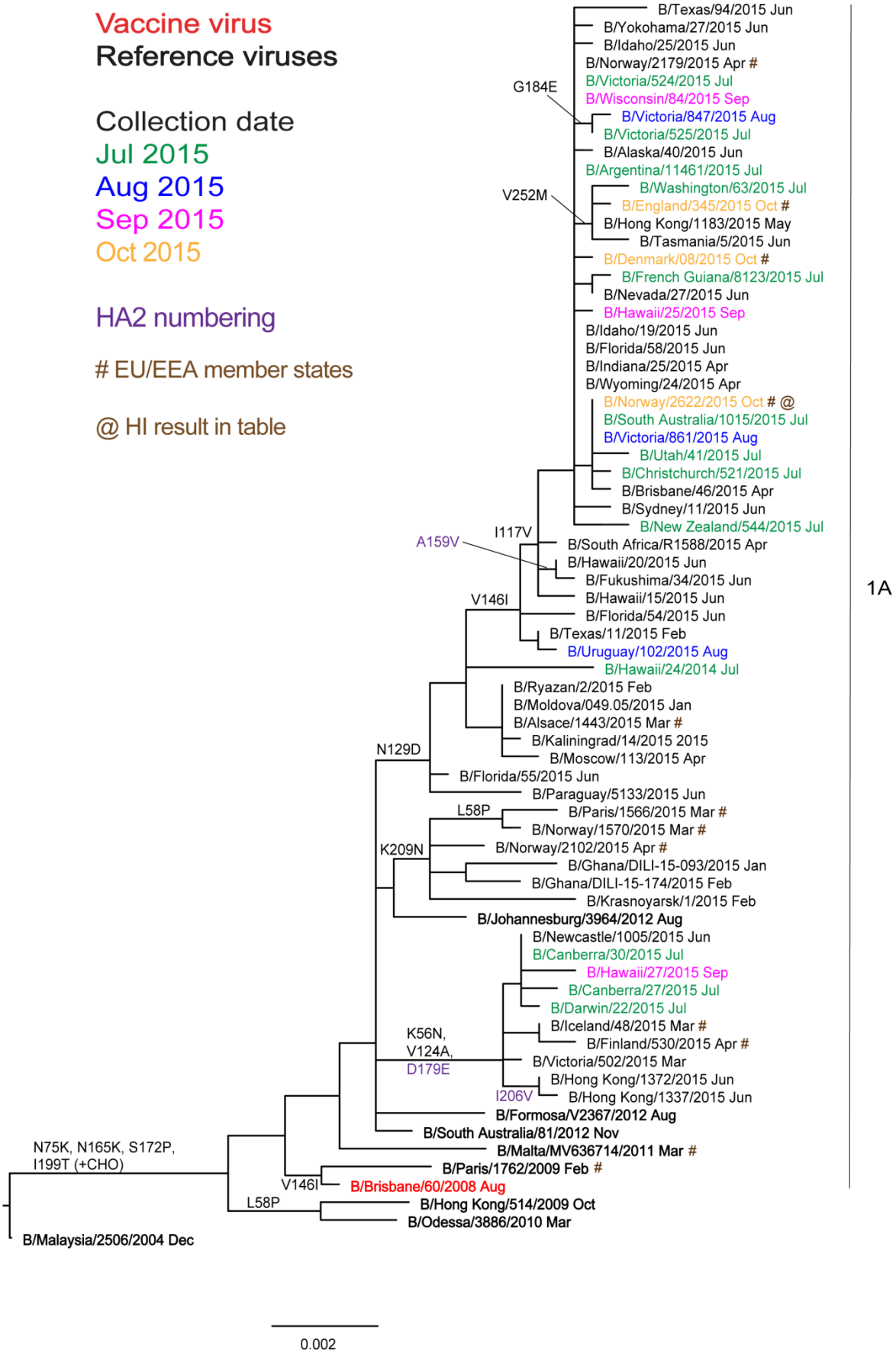


Table 6. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

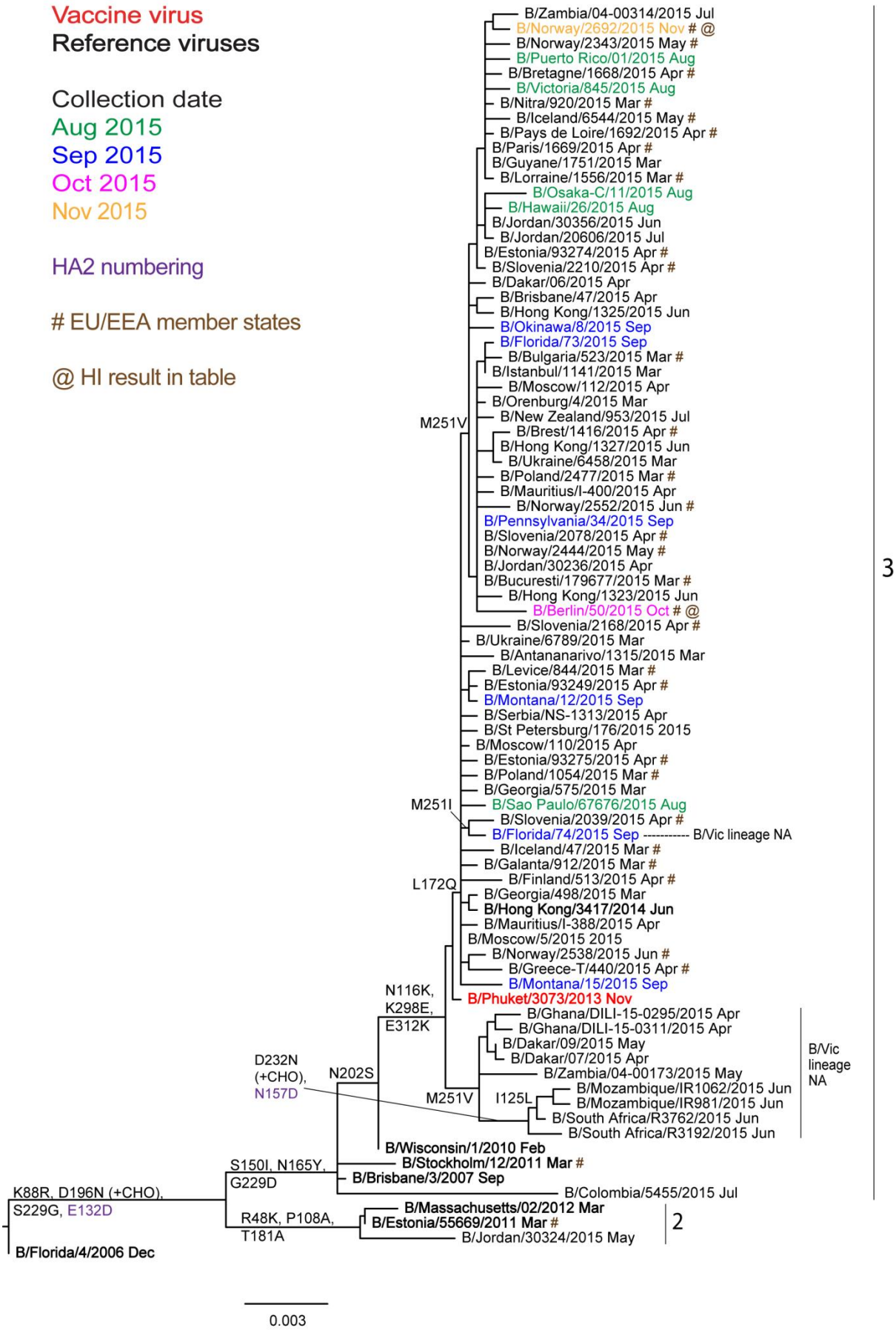
Viruses	Genetic Group	Collection date	Passage History / Ferret number	Haemagglutination inhibition titre: Post-infection ferret antisera										
				B/Phuket[1,3]	B/Fl[1]	B/Bris[2]	B/Estonia[2]	B/Mass[2]	B/Mass[2]	B/Wis[2]	B/Stock[1]	B/Phuket[2]	B/Phuket[2]	B/HK[4]
				3073/13	4/06	3/07	55669/11	02/12	02/12	1/10	12/11	3073/13	3073/13	3417/14
				Egg	Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	Egg
				SH614	F1/10	F38/14	F32/12	F15/13	F42/14	F10/13	F06/15	F35/14	F36/14	St Judes F715/14
	Genetic Group			3	1	2	2	2	2	3	3	3	3	3
REFERENCE VIRUSES														
B/Florida/4/2006	1	2006-12-15	E7/E1	1280	320	640	80	160	640	160	160	20	160	320
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	320	320	40	80	640	80	80	20	160	160
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	1280	80	80	40	80	80	40	20	40	80	160
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	2560	640	640	160	320	640	320	80	80	320	640
B/Massachusetts/02/2012	2	2012-03-13	E3/E4	2560	640	1280	80	160	1280	320	160	40	320	320
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	2560	160	160	10	40	160	160	80	20	160	160
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	160	80	10	20	80	80	80	20	80	160
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	320	160	160	320	320	80	640	640	320
B/Phuket/3073/2013	3	2013-11-21	E4/E2	2560	160	160	20	40	320	320	80	40	160	160
B/Hong Kong/3417/2014	3	2014-06-04	E4/E1	1280	80	80	10	40	80	80	20	20	80	160
TEST VIRUSES														
B/Berlin/50/2015	3	2015-10-12	C2/MDCK1	1280	<	40	10	40	40	40	<	80	80	80
B/Norway/2692/2015	3	2015-11-12	MDCK1	2560	80	80	20	40	80	80	40	80	80	160

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

Vaccine NH 2015-16 SH 2016*

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

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



Summary of genetic data submitted to TESSy

For the period covering weeks 40–53/2015, 254 viruses have been characterised genetically: 170 A(H1N1)pdm09 subgroup 6B represented by A/South Africa/3626/2013; A(H3N2) 38 subgroup 3C.2a represented by A/Hong Kong/4801/2014, six subgroup 3C.3a represented by A/Switzerland/9715293/2013 and two subgroup 3C.3 represented by A/Samara/73/2013; 28 B/Victoria-lineage clade 1A represented by B/Brisbane/60/2008; and 10 B/Yamagata-lineage clade 3 represented by B/Phuket/3073/2013.

Antiviral susceptibility

For weeks 40–53/2015 of the 2015–2016 influenza season, countries reported on the antiviral susceptibility of 18 A(H3N2) viruses, 108 A(H1N1)pdm09 viruses, four B/Victoria-lineage viruses, one B/Yamagata-lineage virus and one B virus not ascribed to a lineage from sentinel and non-sentinel sources. All but one showed no molecular or phenotypic evidence of reduced inhibition (RI) by neuraminidase inhibitors. One A(H1N1)pdm09 virus, detected in a specimen from an oseltamivir-treated immunocompromised hospitalised patient, carried NA H275Y amino acid substitution associated with highly reduced inhibition (HRI) by oseltamivir.

Phenotypic testing for susceptibility to oseltamivir and zanamivir has been conducted on 33 viruses at the WIC: 23 A(H1N1)pdm09, seven A(H3N2), one B/Victoria-lineage and two B/Yamagata-lineage viruses. All showed normal inhibition (NI) by these neuraminidase inhibitors.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [3] 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 [4]. Increased numbers of cases were reported over the course of the 2013–14 and 2014–15 seasons and cases have been reported recently [5]. A revised Rapid Risk Assessment [6] 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 [7], updated on 14 December 2015 [8], and conducted a new risk assessment on 23 February 2015 [9]. 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 [10] and has provided subsequent situation updates, with the latest being on 17 December 2015 [5].

Influenza A(H5N1) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 14 December 2015 [8]. ECDC published a rapid risk assessment on highly pathogenic avian influenza virus A of H5 type on 4 December 2015 [14].

No new laboratory-confirmed human cases of H5Nx infection have 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 [11] and an epidemiological update 10 April 2015 [12]. On 4 January 2016, WHO reported two recent cases of human infection with avian A(H5N6) virus in China [13].

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 Institute for Medical Research) and used at the WHO Vaccine Composition Meetings held at WHO Geneva 23–25 February 2015, and in Memphis, USA, 21–23 September 2015, can be found at:

https://www.crick.ac.uk/media/221813/nimr-report-feb2015-web.pdf and
https://www.crick.ac.uk/media/273950/crick_sep2015_vcm_report_to_post.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 are presented in the HI tables (@). Sequences for some viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors and originating/submitting laboratories of the sequences in 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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