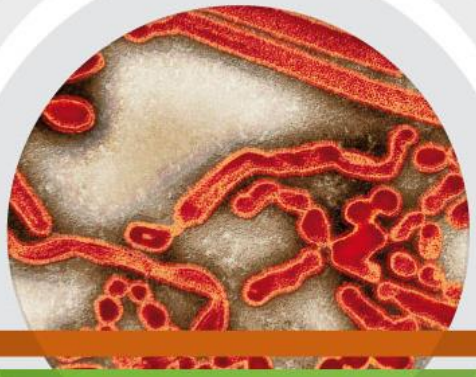


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, July 2016

Summary

Since week 40/2015, over 139 000 influenza detections across the Region have been reported. Influenza type A viruses have prevailed over type B, with A(H1N1)pdm09 viruses greatly outnumbering A(H3N2) and B/Victoria-lineage detections representing over 91% of the type B viruses assigned to a lineage.

Since 1 January 2016, EU/EEA countries have shared 511 influenza-positive specimens with the Francis Crick Institute, London, for detailed characterisation. Since the June report, 28 viruses have been characterised antigenically and genetic analyses are ongoing.

Of the 18 A(H1N1)pdm09 viruses characterised antigenically, all were similar to the vaccine virus A/California/7/2009. Worldwide new genetic subclusters of viruses within the 6B clade have emerged, with two being designated as subclades: 6B.1 defined by HA1 amino acid substitutions S162N and I216T and 6B.2 defined by HA1 amino acid substitutions V152T and V173I. Of the 460 viruses characterised genetically for the 2015–2016 season, 29 (6%) were clade 6B, 426 (92%) were subclade 6B.1 and 11 (2%) were subclade 6B.2.

The two A(H3N2) test viruses characterised by haemagglutination inhibition (HI) assay were poorly recognised 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 2016 southern hemisphere and 2016–2017 northern hemisphere influenza vaccines. Of 119 A(H3N2) viruses characterised genetically for the 2015–2016 season: two (2%) were clade 3C.3, 88 (74%) were subclade 3C.2a and 29 (24%) were subclade 3C.3a.

The seven B/Victoria-lineage viruses were antigenically similar to tissue culture-propagated surrogates of B/Brisbane/60/2008. All 161 viruses characterised genetically for the 2015–2016 season fell in genetic clade 1A, as do recently collected viruses worldwide.

One B/Yamagata virus has been characterised since the previous report; it 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 and for quadrivalent vaccines in the 2016 southern hemisphere and 2016–17 northern hemisphere seasons. Of the 25 viruses characterised genetically for the 2015–2016 season 24 fell in genetic clade 3 and one in clade 2.

Erratum: Table 5, page 11, was replaced on 17 October 2016.

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).

Suggested citation: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, July 2016. Stockholm: ECDC; 2016.

Table 1 shows a summary of influenza virus detections in the WHO European Region reported to TESSy for the weekly reporting period (weeks 40/2015–20/2016) of the 2015–16 season. A total of over 138 000 detections had been made with type A viruses prevailing over type B at a ratio of 2.3:1; this compares to a ratio of 5.8:1 to week 7/2016, indicating a surge in influenza type B circulation over the subsequent 13 weeks. As of week 20/2016, of the type A viruses subtyped (n = 66 707) and the type B viruses ascribed to lineage (n = 7 834), A(H1N1)pdm09 have prevailed over A(H3N2) and B/Victoria over B/Yamagata by ratios of 10.2:1 and 11.1:1, respectively. While relatively few influenza detections have been reported for weeks 21–30/2016, it is notable that that the ratios for type A:type B, A(H1N1)pdm09:A(H3N2) and B/Victoria:B/Yamagata have dropped to 0.4:1, 0.9:1 and 2.1:1, respectively.

Since 1 January 2016, 48 shipments of specimens have been received at the Crick Worldwide Influenza Centre (WIC), from 26 countries in the EU/EEA. These packages contained 511 specimens, a mix of clinical samples and virus isolates originating from 21 countries, with collection dates after 31 December 2015 (Table 2). The majority (71%) were type A viruses, and A(H1N1)pdm09 outnumbered A(H3N2) at a ratio of 4.3:1. Of the 149 type B specimens received (29% of the specimens), 128 were B/Victoria-lineage, 19 were B/Yamagata-lineage. A number of specimens are still in the process of being characterised. The antigenic and genetic properties of influenza virus isolates characterised since the June 2016 report[1] are presented and discussed in this report.

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

Virus type/subtype/lineage	Cumulative number of detections						Totals*			
	Sentinel sources		Non-sentinel sources		Totals		%		Ratios	
	Weeks 40/2015-20/2016	Weeks 21-30/2016	Weeks 40/2015-20/2016	Weeks 21-30/2016	Weeks 40/2015-20/2016	Weeks 21-30/2016	Weeks 40/2015-20/2016	Weeks 21-30/2016	Weeks 40/2015-20/2016	Weeks 21-30/2016
Influenza A	**10496**	**4**	**85919**	**217**	**96415**	**221**	**69.7**	**30.7**	**2.3:1**	**0.4:1**
A(H1N1)pdm09	8665	1	52083	47	60748	48	91.1	46.6	10.2:1	0.9:1
A(H3N2)	1365	2	4594	53	5959	55	8.9	53.4		
A not subtyped	466	1	29242	117	29708	118				
Influenza B	**8144**	**12**	**33791**	**486**	**41935**	**498**	**30.3**	**69.3**		
Victoria lineage	3974	2	3210	50	7184	52	91.7	67.5	11.1:1	2.1:1
Yamagata lineage	145	0	505	25	650	25	8.3	32.5		
Lineage not ascribed	4025	10	30076	411	34101	421				
Total detections (total tested)	**18 640 (50 861)**	**16 (870)**	**119 710 (536 625)**	**703 (24 477)**	**138 350 (587 486)**	**719 (25 347)**				

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

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, June 2016. Stockholm: ECDC; 2016. Available from http://ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-june-2016.pdf

Table 2. Summary of clinical samples and virus isolates, with collection dates after 31 December 2015, received from EU/EEA Member States

MONTH* / Country	TOTAL RECEIVED	H1N1pdm09		H3N2			B		B Victoria lineage		B Yamagata lineage	
		Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated	Number received	Number propagated[1]	Number received	Number propagated[1]
2016 JANUARY												
Bulgaria	18	18	18									
Cyprus	15	9	5	1	1	0			5	3		
Czech Republic	3	3	3									
Estonia	3	2	0						1	1		
Finland	1	1	1									
Germany	26	11	11	5	5	0			8	8	2	2
Greece	27	27	17									
Hungary	7	4	4						3	3		
Iceland	6	5	5								1	1
Ireland	10	9	9						1	1		
Italy	2	1	1	1	1	0						
Latvia	8	6	6						2	2		
Netherlands	2	2	2									
Portugal	6	6	6									
Romania	8	7	7	1	0	1						
Slovenia	8	3	3	3	0	3	2	0				
Spain	19	16	15	1	0	1			2	2		
2016 FEBRUARY												
Bulgaria	47	34	33	1	0	1			12	12		
Cyprus	9	8	8						1	1		
Czech Republic	8	8	8									
Finland	5	4	4	1	1	0						
Germany	13	6	6	2	2	0					5	5
Greece	4	4	2									
Iceland	3	2	2								1	1
Italy	22	7	7	10	7	3			4	4	1	1
Latvia	2	2	2									
Lithuania	12	10	10						2	2		
Netherlands	1	1	1									
Portugal	1	1	1									
Romania	6	3	3	2	2	0			1	1		
Slovakia	9	3	3	1	1	0			5	5		
Slovenia	17	5	5	9	4	4			3	3		
Spain	17	15	14						2	2		
Sweden	5	1	1	2	2	0			2	2		
2016 MARCH												
Bulgaria	16	6	6	2	0	2			8	8		
Czech Republic	11	2	2	1	1	0			8	8		
Estonia	10	7	7						3	3		
Finland	5	2	2						3	3		
Germany	9			1	0	1			7	7	1	1
Iceland	3	1	1	1	0	1			1	1		
Italy	10	1	1	5	4	1			4	4		
Norway	2	2	2									
Portugal	10	6	6						4	3		
Romania	9	6	6	1	1	0			1	1	1	1
Slovakia	10	3	3						5	5	2	2
Slovenia	14	3	3	6	3	3			5	5		
Sweden	5	1	1	1	1	0			2	2	1	1
2016 APRIL												
Iceland	8	2	2	2	0	1			3	3	1	1
Italy	3	1	1	1	0	1			1	1		
Portugal	6	1	1						5	5		
Romania	8	3	3						5	5		
Slovakia	2	1	1								1	1
Slovenia	4	1	1						2	2	1	1
Sweden	1			1	0	1						
2016 MAY												
Iceland	4			2	1	1			2	2		
Norway	5	1	1	1	1	0			2	2	1	1
Slovenia	2								2	2		
2016 JUNE												
Iceland	1								1	1		
Norway	3			3	in process							
21 Countries	511	294	273	68	38	25	2	0	128	125	19	19
		57.5%		13.3%					25.0%		3.7%	
		70.8%							29.2%			

*** 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 June 2016 report are shown in Table 3. Of the 18 A(H1N1)pdm09 viruses from EU/EEA countries antigenically characterised all were similar to the vaccine virus, A/California/7/2009, reacting with antiserum raised against the vaccine virus at titres within twofold of the homologous titre. 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 11/18 (61%) test viruses at a titre within fourfold of the titre for the homologous virus reference viruses carrying HA1 G155E amino acid substitutions, A/Bayern/69/2009 and A/Lviv/N6/2009, showed reduced recognition by the antisera raised against A/California/7/2009 and reference viruses in genetic clades 4, 5, 6, 7 and subclades 6A, 6B, 6B.1 and 6B.2.

HA gene sequencing is still in progress for all the test viruses shown in Table 3. Since 2009, the HA genes have evolved, and nine clades have been designated. For well over a year, viruses in clade 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 subclades emerging. All EU/EEA viruses characterised since the September 2014 report[2] carry HA genes in subclade 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. A number of virus clusters have emerged within clade 6B, and two of these have been designated as subclades: viruses in subclade 6B.1 are defined by **HA1** amino acid substitutions **S84N, S162N** (which results in the formation of a new potential glycosylation motif at residues 162-164 of HA1) and **I216T**, while those in subclade 6B.2 are defined by **HA1** amino acid substitutions **V152T** and **V173I** (Figure 1).

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

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

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre												
				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	A/Slov	A/Israel		
				7/09	69/09	N6/09	16/10	1/11	27/11	100/11	5659/12	3626/13	2903/2015	Q-504/15		
				Egg	MDCK	MDCK	Egg	MDCK	Egg	Egg	MDCK	Egg	Egg	MDCK		
				F06/16[1]	F09/15[1]	F14/13[1]	F15/14[1]	F22/13[1]	F26/14[1]	F24/11[1]	F30/12[1]	F03/14[1]	F02/16[2]	F08/16[2]		
	Passage history						4	5	6	7	6A	6B	6B.1	6B.2		
	Ferret number														G155E	
	Genetic group														G155E>G, D222G	
REFERENCE VIRUSES																
A/California/7/2009	clone38-32	2009-04-09	E3/E2	1280	640	640	640	1280	640	2560	2560	1280	2560	2560		
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK1	40	320	160	80	40	40	40	40	40	40	40		
A/Lviv/N6/2009		2009-10-27	MDCK4/SIAT1/MDCK3	160	1280	640	320	80	160	80	160	80	160	160		
A/Christchurch/16/2010	4	2010-07-12	E2/E2	1280	1280	1280	5120	2560	640	2560	2560	1280	2560	2560		
A/Astrakhan/1/2011	5	2011-02-28	MDCK1/MDCK5	1280	640	320	1280	1280	640	2560	1280	640	1280	1280		
A/St. Petersburg/27/2011	6	2011-02-14	E1/E4	2560	1280	640	1280	2560	1280	5120	2560	1280	2560	2560		
A/St. Petersburg/100/2011	7	2011-03-14	E1/E4	1280	640	640	1280	1280	1280	2560	2560	1280	2560	2560		
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	320	1280	80	160	320	320	640	640	320	640	320		
A/South Africa/3626/2013	6B	2013-06-06	E1/E3	1280	1280	640	640	1280	1280	1280	1280	1280	1280	1280		
A/Slovenia/2903/2015	6B.1	2015-10-26	E4/E1	2560	640	640	1280	1280	1280	2560	2560	1280	2560	1280		
A/Israel/Q-504/2015	6B.2	2015-12-15	C1/MDCK2	1280	640	320	640	1280	640	2560	2560	1280	2560	2560		
TEST VIRUSES																
A/Norway/2914/2015		2015-12-14	MDCK1/MDCK1	2560	1280	640	2560	2560	1280	5120	5120	2560	5120	5120		
A/Czech Republic/11/2016		2016-01-28	C3/MDCK1	2560	640	320	1280	1280	640	2560	2560	1280	2560	2560		
A/Estonia/98215/2016		2016-02-04	MDCK1/MDCK1	2560	1280	640	1280	1280	1280	2560	2560	1280	2560	2560		
A/Estonia/98192/2016		2016-02-04	MDCK2/MDCK1	2560	640	320	640	1280	640	2560	2560	1280	2560	2560		
A/Estonia/98290/2016		2016-02-08	MDCK1/MDCK1	1280	640	320	640	1280	640	2560	2560	1280	2560	2560		
A/Estonia/98475/2016		2016-02-12	MDCK1/MDCK1	1280	640	320	1280	1280	1280	2560	2560	1280	2560	2560		
A/Estonia/98636/2016		2016-02-19	MDCK1/MDCK1	1280	1280	640	1280	1280	1280	2560	2560	1280	2560	2560		
A/Estonia/98801/2016		2016-02-26	MDCK1/MDCK1	2560	2560	640	2560	2560	2560	5120	5120	2560	5120	5120		
A/Estonia/98923/2016		2016-03-04	MDCK1/MDCK1	1280	640	320	640	1280	640	2560	1280	1280	2560	2560		
A/Estonia/99005/2016		2016-03-08	MDCK2/MDCK1	640	640	640	640	640	640	1280	1280	1280	2560	2560		
A/Norway/2036/2016		2016-03-10	MDCK1/MDCK1	2560	1280	640	1280	2560	1280	5120	2560	2560	2560	2560		
A/Estonia/99218/2016		2016-03-17	MDCK1/MDCK1	1280	640	320	640	1280	640	2560	1280	1280	2560	2560		
A/Estonia/99242/2016		2016-03-21	MDCK1/MDCK1	1280	1280	640	1280	1280	640	2560	2560	1280	2560	2560		
A/Norway/2298/2016		2016-03-21	MDCK2/MDCK1	1280	640	320	640	640	640	1280	1280	1280	1280	2560		
A/Estonia/99288/2016		2016-03-22	MDCK1/MDCK1	1280	1280	640	1280	1280	1280	2560	2560	1280	2560	2560		
A/Estonia/99350/2016		2016-03-24	MDCK1/MDCK1	1280	640	320	640	640	640	1280	1280	1280	2560	2560		
A/Estonia/99352/2016		2016-03-24	MDCK1/MDCK1	2560	1280	640	1280	2560	1280	5120	2560	2560	2560	5120		
A/Norway/3591/2016		2016-05-21	MDCK1/MDCK1	2560	1280	640	1280	2560	1280	2560	2560	1280	2560	2560		

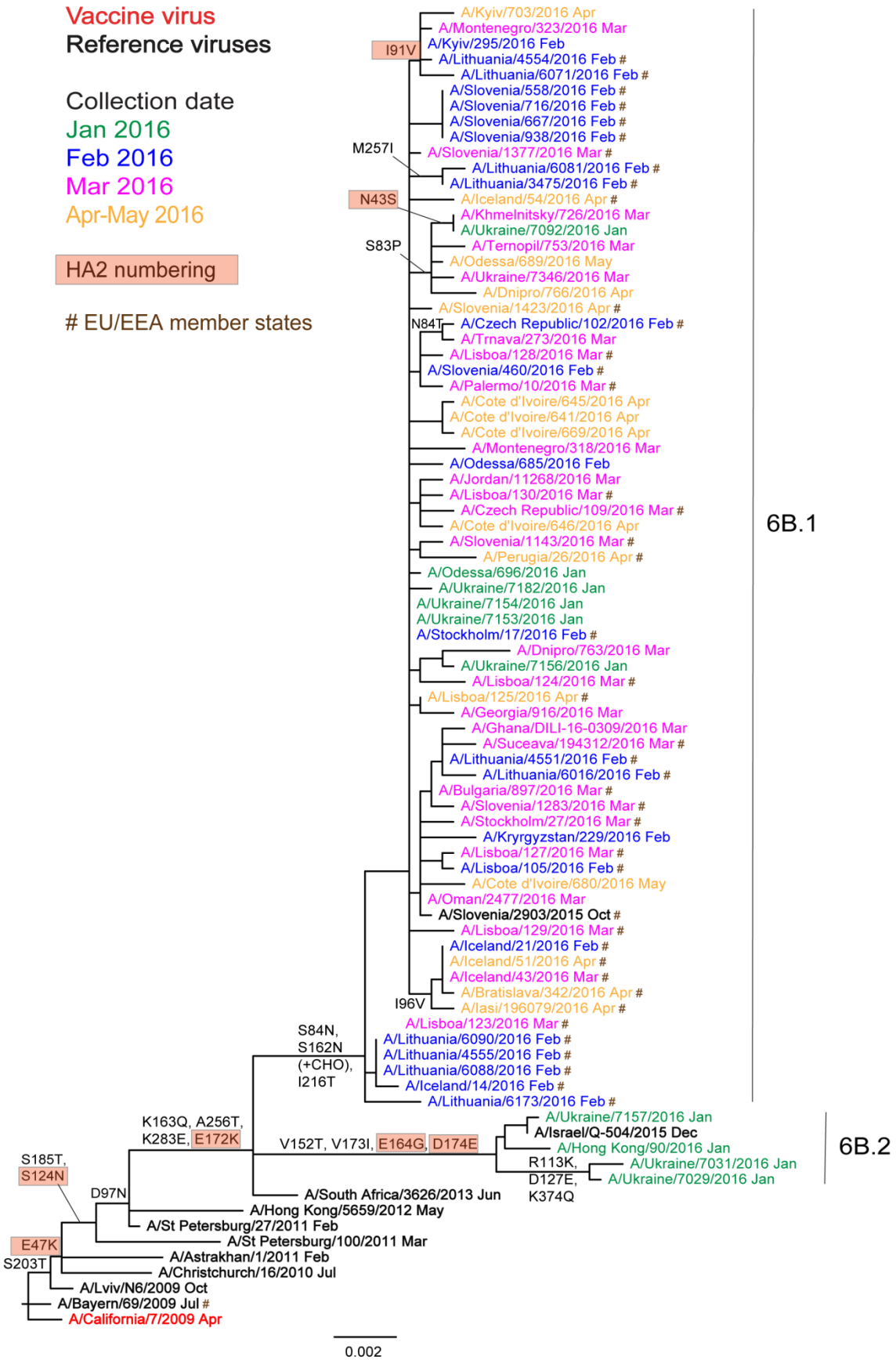
Vaccine

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

[1] < = <40

[2] < = <80

Figure 2. Phylogenetic comparison of influenza A(H1N1)pdm 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 these 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. Only two test viruses retained sufficient HA titre to be analysed by HI assay. One of these, A/Finland/586/2015, was characterised genetically and fell in subclade 3C.3a.

The antiserum raised against egg-propagated A/Switzerland/9715293/2013 (3C.3a), the northern hemisphere 2015–16 vaccine component, reacted with both test viruses at a titre of 80 (eightfold reduced compared to the homologous titre), while the antiserum raised against the cell culture-propagated cultivar of A/Switzerland/9715293/2013, which had a very low homologous titre, was able to recognise both test viruses at titres similar to the homologous titre of the antiserum. The antiserum raised against egg-propagated A/Hong Kong/4801/2014, the virus recommended for use in vaccines for the southern hemisphere 2016 and northern hemisphere 2016–17 influenza seasons, recognised the two test viruses at titres twofold or fourfold reduced compared to the homologous titre. Antisera raised against both tissue culture-propagated and egg-propagated A/Stockholm/6/2014 (3C.3a) and tissue culture-propagated A/Hong Kong/5738/2014 (3C.2a) recognised both test viruses at titres equal to, within twofold, or within fourfold of their homologous titres.

Since 2009, seven genetic groups based on the HA gene have been defined for A(H3N2) viruses. A 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 clade 3C. This clade 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 subclades emerged, one in subdivision 3C.2, 3C.2a, and two in 3C.3, 3C.3a and 3C.3b, with subclade 3C.2a viruses dominating in recent months (Figure 2). While viruses in subclades 3C.2a and 3C.3a are antigenic drift variants, those in 3C.3b (e.g. A/Netherlands/525/2014) have remained antigenically similar to previously circulating viruses in the 3C.3 subdivision. Amino acid substitutions that define these subdivisions and subclades 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/Netherlands/525/2014

Based on results available at the time of the February 2015 vaccine composition meeting showing cross-reactivity of antisera raised against subclade 3C.3a and 3C.2a viruses, but with changes acquired on egg-adaptation of genetic subgroup 3C.2a viruses and, at that time, 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, a new subclade designated 3C.3b emerged, these three subclades being antigenically distinguishable, but subclade 3C.2a viruses became prevalent and have remained so. While ferret antisera raised against 3C.3a and 3C.2a subclade viruses showed some cross-reactivity with viruses in all three subclades, antisera raised against 3C.3b viruses were subclade specific. With the availability of new subclade 3C.2a vaccine candidates and the continued cross-reactivity of antisera raised against viruses in subclades 3C.3a and 3C.2a viruses, the World Health Organization recommendation for the A(H3N2) component of influenza vaccines for the southern hemisphere 2016 [2] and northern hemisphere 2016–17 [3] influenza seasons was for an A/Hong Kong/4801/2014-like (3C.2a) virus.

[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

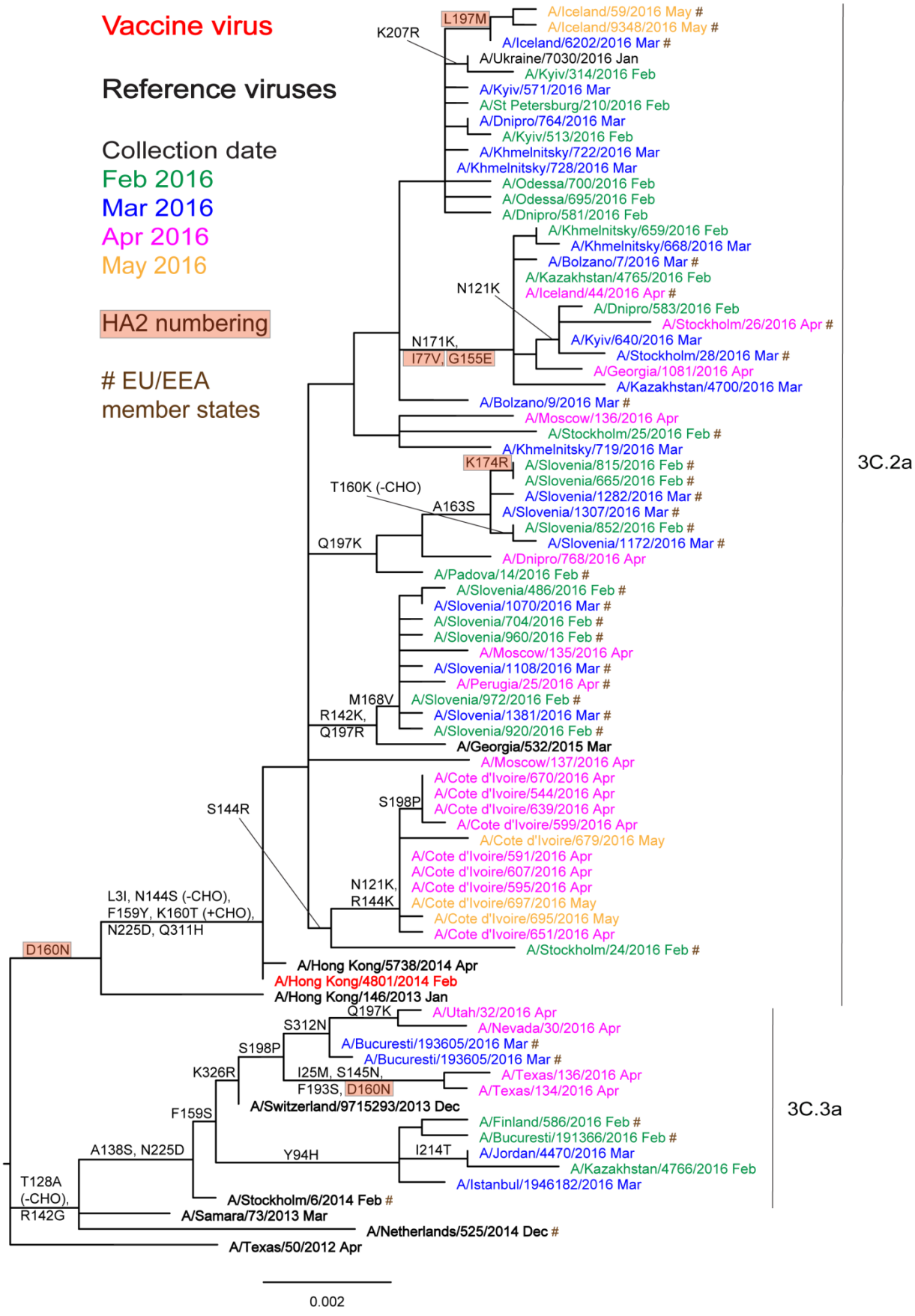
Table 4. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre												
				Post-infection ferret antisera												
	Passage history			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/Georgia
	Ferret number			50/12	73/13	6/14	6/14	9715293/13	9715293/13	525/14	146/13	5738/14	4801/14	4801/14	532/15	
				Egg	SIAT	SIAT	Egg	SIAT	Egg	SIAT	Egg	MDCK	MDCK	Egg	SIAT	
				F36/12[-1]	F35/15[-1]	F14/14[-1]	F20/14[-1]	F18/15[-1]	F32/14[-1]	F23/15[-1]	F10/15[-1]	F30/14[-1]	F43/15[-1]	F12/15[-1]	F33/15[-1]	
	Genetic group			3C.1	3C.3	3C.3a	3C.3a	3C.3a	3C.3a	3C.3b	3C.2	3C.2a	3C.2a	3C.2a	3C.2a	3C.2a
REFERENCE VIRUSES																
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	5120	1280	160	640	40	640	320	320	80	320	80	160	160
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT3	2560	640	320	640	80	640	320	640	320	640	160	320	320
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT1/SIAT2	160	40	320	160	160	160	80	80	160	160	160	160	160
A/Stockholm/6/2014 isolate 2	3C.3a	2014-02-06	E4/E1	640	80	80	320	40	320	80	40	160	320	40	40	80
A/Switzerland/9715293/2013	3C.3a	2013-12-06	SIAT1/SIAT2	40	<	160	80	40	40	40	40	80	80	40	40	80
A/Switzerland/9715293/2013 clone 123	3C.3a	2013-12-06	E4/E1	320	80	160	160	40	640	40	80	160	320	80	160	160
A/Netherlands/525/2014	3C.3b	2014-12-17	SIAT2/SIAT4	320	160	320	160	80	160	1280	160	160	320	80	160	160
A/Hong Kong/146/2013	3C.2	2013-01-11	E6	2560	640	80	640	40	640	320	1280	320	640	160	320	320
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK2/SIAT2	80	40	160	80	40	40	<	80	160	320	80	160	160
A/Hong Kong/4801/2014	3C.2a	2014-02-26	MDCK4/MDCK1/SIAT1	80	40	160	80	40	40	<	40	80	320	160	160	160
A/Hong Kong/4801/2014 isolate 1	3C.2a	2014-02-26	E6/E2	40	80	80	40	40	40	40	<	160	640	160	320	320
A/Georgia/532/2015 plaq 20	3C.2a	2015-03-09	SIAT1/SIAT3	160	80	160	80	40	80	40	80	160	640	160	640	640
TEST VIRUSES																
A/Finland/586/2016	3C.3a	2016-02-15	SIAT1/SIAT1	80	<	320	160	80	80	<	40	80	160	80	80	80
A/Norway/3545/2016		2016-05-13	SIAT1/SIAT1	40	<	320	80	40	80	<	<	80	40	40	40	40
									Vaccine NH2015-16					Vaccine SH 2016 NH2016-17		

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

[1] < = <40

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



Influenza B virus analyses

EU/EEA countries have provided 149 influenza type B-positive specimens with collection dates after 31 December 2015: 147 were ascribed to a lineage, 128 B/Victoria-lineage and 19 B/Yamagata-lineage (Table 2).

Influenza B – Victoria lineage

Since the June 2016 report, seven viruses of this lineage have been characterised antigenically. HI results are shown in Table 5. Genetic analysis is ongoing.

The test viruses showed similar HI reactivity patterns to those from the 2014–15 influenza season: none of the test viruses showed HI titres within fourfold of the titre for the homologous virus with post-infection ferret antisera raised against the recommended vaccine virus for quadrivalent live and inactivated vaccines for the northern hemisphere 2015–2016 influenza season, B/Brisbane/60/2008. Similarly, the test viruses were not recognised well by post-infection ferret antisera raised against reference viruses propagated in eggs (B/Malta/636714/2011, B/Johannesburg/3964/2012 and B/South Australia/81/2012) although the antiserum raised against B/Malta/636714/2011 recognised four of the seven test viruses at a titre within fourfold of its homologous titre. In contrast, all test viruses showed reactivity within fourfold – the majority within twofold – of the titres for the corresponding homologous viruses with antisera raised against viruses that are considered to be surrogate tissue culture-propagated antigens representing the egg-propagated B/Brisbane/60/2008 prototype virus. These antisera were raised against tissue culture-propagated viruses B/Hong Kong/514/2009 (clade 1B) and B/Formosa/V2367/2012 and recently circulating viruses B/Ireland/3154/2016 and B/Nordrhein-Westfalen/1/2016 (all clade 1A).

A phylogenetic analysis of the HA gene of representative B/Victoria lineage viruses is shown in Figure 3. Throughout the previous season, and this season to date, viruses from Europe and elsewhere, have HA genes that fall into the B/Brisbane/60/2008 clade (clade 1A) and remain antigenically similar to the vaccine virus B/Brisbane/60/2008. The great majority of viruses, with collection dates since October 2015, fall in a major subcluster defined by amino acid substitutions I117V, N129D and V146I within clade 1A.

These results, linked with the rise in the proportion of B/Victoria-lineage viruses seen in the 2015 southern hemisphere and 2015–2016 northern hemisphere influenza seasons, support the recommendations made to include B/Brisbane/60/2008 in trivalent influenza vaccines for the southern hemisphere 2016 [2] and northern hemisphere 2016–2017 [3] influenza seasons and in quadrivalent vaccines.

Influenza B – Yamagata lineage

HI results for one B/Yamagata-lineage test virus analysed since the June 2016 report are shown in Table 6. A genetic analysis of this virus is in progress.

The homologous titres of the 10 post-infection ferret antisera, shown in red, ranged from 80 to 1 280 and the test virus showed reactivity with nine of the antisera (Table 6).

Antisera raised against egg-propagated clade 3 viruses B/Phuket/3073/2013 (the virus recommended for inclusion in trivalent influenza vaccines for the northern hemisphere 2014–2015 season) and B/Hong Kong/3417/2014 recognised the test virus at titres within twofold of their respective homologous titres. B/Norway/3948/2016 showed a reduction in HI reactivity of eight-fold, compared to the homologous titre, with antiserum raised against egg-propagated B/Massachusetts/02/2012, the clade 2 vaccine virus recommended for use in the 2014–15 northern hemisphere 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 few viruses, annotated in the phylogenetic tree, are reassortants carrying NA genes normally associated with the B/Victoria-lineage.

Based on such results, a B/Phuket/3073/2013-like virus has been recommended for inclusion in quadrivalent vaccines for the 2016 southern hemisphere [2] and 2016–2017 northern hemisphere [3] influenza seasons.

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

Viruses		Collection date	Passage history	Haemagglutination inhibition titre									
				Post-infection ferret antisera									
				B/Bris 60/08	B/Mal 2506/04	B/Bris 60/08	B/Malta 636714/11	B/Jhb 3964/12	B/For V2367/12	B/Sth Aus 81/12	B/HK 514/09	B/Ireland 3154/16	B/Nord-West 1/16
	Passage history			Egg	Egg	Egg	Egg	Egg	MDCK	Egg	MDCK	MDCK	MDCK
	Ferret number			Sh 539,540,543,544, 570,571,574*[1]	F37/11*[2]	F26/13*[2]	F29/13*[2]	F01/13*[2]	F04/13*[2]	F41/13*[2]	F09/13*[2]	F15/16*[2]	F16/16*[2]
	Genetic group			1A		1A	1A	1A	1A	1A	1B	1A	1A
REFERENCE VIRUSES													
B/Malaysia/2506/2004		2004-12-06	E3/E6	2560	640	80	160	160	80	160	20	<	<
B/Brisbane/60/2008	1A	2008-08-04	E4/E4	2560	80	640	320	320	160	1280	80	40	20
B/Malta/636714/2011	1A	2011-03-07	E4/E1	2560	80	320	320	320	160	640	80	40	20
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	640	1280	1280	1280	1280	1280	320	160	160
B/Formosa/V2367/2012	1A	2012-08-06	MDCK1/MDCK3	5120	20	640	320	320	160	640	80	40	20
B/South Australia/81/2012	1A	2012-11-28	E4/E2	5120	80	640	320	640	320	1280	160	80	40
B/Hong Kong/514/2009	1B	2009-10-11	MDCK3	2560	<	80	40	40	20	80	80	80	40
B/Ireland/3154/2016	1A	2016-01-14	MDCK1/MDCK4	2560	<	80	20	20	40	40	40	40	20
B/Nordrhein-Westfalen/1/2016	1A	2016-01-04	C2/MDCK3	2560	<	80	40	20	40	80	40	80	40
TEST VIRUSES													
B/Estonia/98464/2016		2016-02-12	MDCK1/MDCK1	5120	40	80	80	80	80	160	80	80	40
B/Estonia/98655/2016		2016-02-19	MDCK2/MDCK1	2560	<	80	80	40	40	80	80	80	40
B/Estonia/98924/2016		2016-03-04	MDCK1/MDCK1	2560	<	80	40	40	40	80	40	80	40
B/Estonia/99079/2016		2016-03-11	MDCK1/MDCK1	5120	<	80	40	40	40	80	40	80	40
B/Estonia/99128/2016		2016-03-14	MDCK1/MDCK1	5120	<	80	40	40	40	80	80	80	40
B/Norway/3678/2016		2016-05-24	MDCK1/MDCK1	5120	10	80	80	80	80	160	160	160	80
B/Norway/3701/2016		2016-05-26	MDCK1/MDCK1	5120	20	80	80	40	80	160	80	80	80

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

[1] < = <40

[2] < = <10

[3] hyperimmune sheep serum

[4] < = <20

[#] B/Victoria-lineage virus recommended for use in quadravalent vaccines

Vaccine
NH 2015-16[#]

SH 2016

NH 2016-17

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

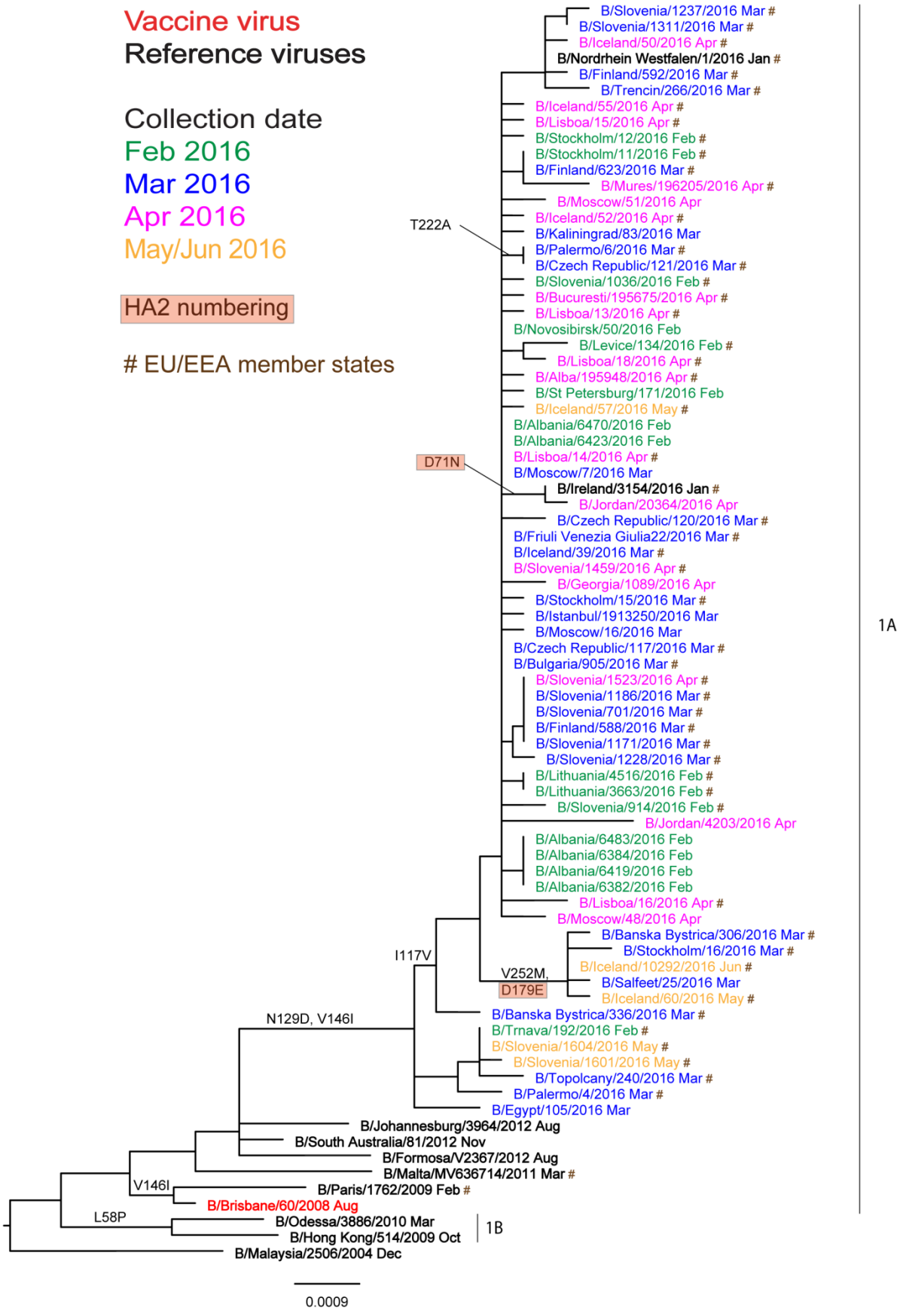


Table 6. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses		Collection date	Passage history	Haemagglutination inhibition titre										
				Post-infection ferret antisera										
				B/Phuket 3073/13	B/Fl 4/06	B/Bris 3/07	B/Estonia 55669/11	B/Mass 02/12	B/Mass 02/12	B/Wis 1/10	B/Stock 12/11	B/Phuket 3073/13	B/Phuket 3073/13	B/HK 3417/14
	Passage history			Egg	Egg	Egg	MDCK	MDCK	Egg	Egg	Egg	MDCK	Egg	Egg
	Ferret number			SH614[*1,3]	F17/13[*1]	F38/14[*2]	F32/12[*2]	F05/15[*2]	F42/14[*2]	F10/13[*2]	F06/15[*1]	F35/14[*2]	F36/14[*2]	St Judes F715/14[*2,4]
	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	1280	640	80	80	1280	160	160	20	160	160
B/Brisbane/3/2007	2	2007-09-03	E2/E3	1280	640	320	40	80	640	80	80	10	80	160
B/Estonia/55669/2011	2	2011-03-14	MDCK2/MDCK3	640	80	40	80	160	80	40	10	40	40	80
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	1280	640	320	320	320	640	160	80	80	160	160
B/Massachusetts/02/2012	2	2012-03-13	E3/E3	1280	640	640	80	160	640	160	160	20	160	160
B/Wisconsin/1/2010	3	2010-02-20	E3/E3	2560	320	160	10	10	160	160	80	20	160	160
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	160	80	10	<	80	80	80	20	80	80
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK2	5120	160	160	160	320	320	160	80	320	320	160
B/Phuket/3073/2013	3	2013-11-21	E4/E3	2560	320	160	20	<	320	320	160	40	160	320
B/Hong Kong/3417/2014	3	2014-06-04	E4/E3	1280	80	40	<	<	40	40	20	10	40	160
TEST VIRUSES														
B/Norway/3694/2016		2016-05-26	MDCK1/MDCK1	2560	80	80	40	<	80	160	40	80	80	160
													Vaccine NH 2015-16 SH2016[#] NH 2016-17[#]	

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

1 < = <40

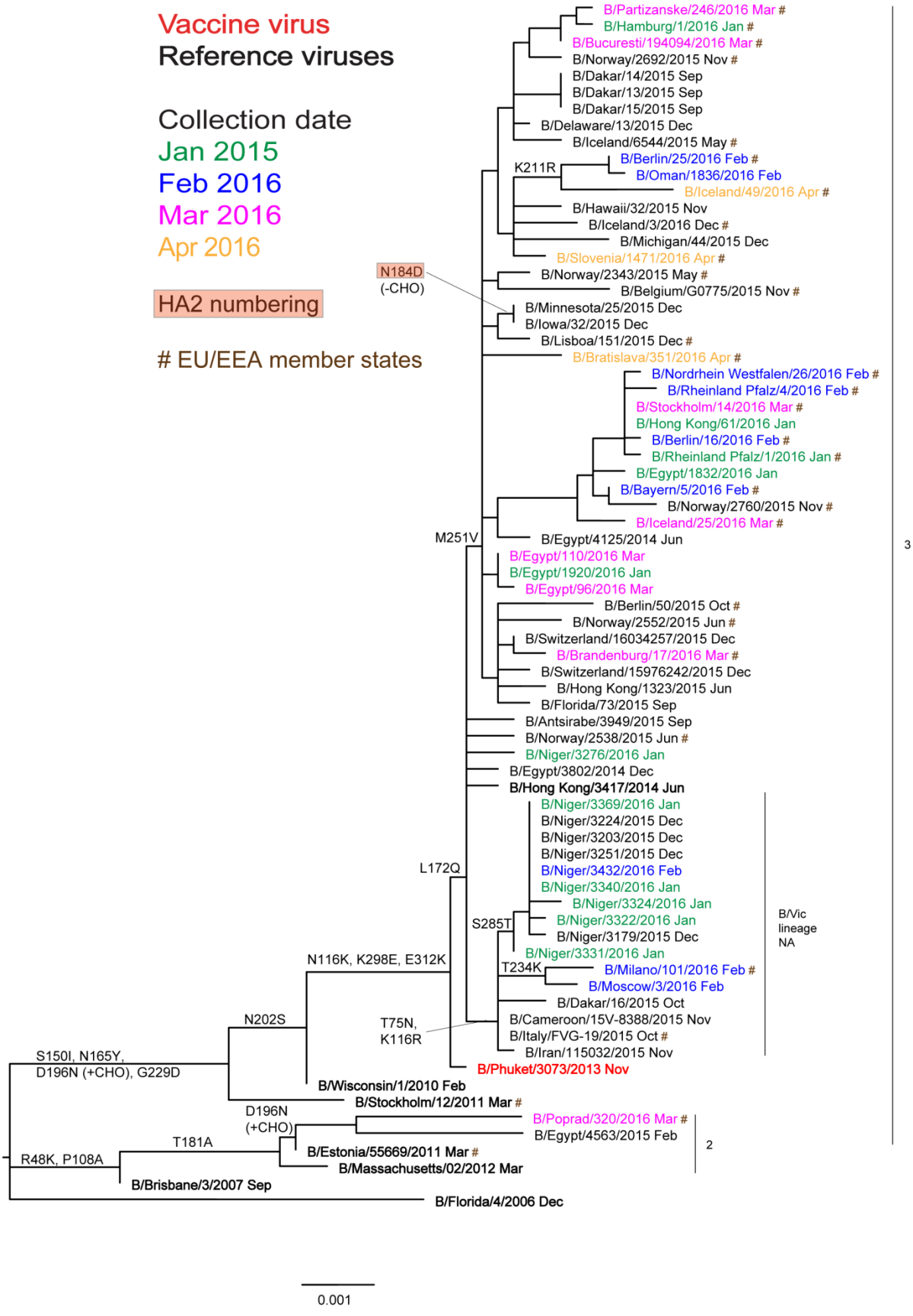
2 < = <10

3 hyperimmune sheep serum

4 RDE serum pre-absorbed with TRBC

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

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



Summary of genetic data submitted to TESSy

For the period covering weeks 40/2015–20/2016, 2601 viruses have been characterised genetically: 1770 A(H1N1)pdm09 clade 6B represented by A/South Africa/3626/2013 (6B.1 and 6B.2 subclade designations were not available as reporting categories at the start of the 2015–2016 influenza season); 211 A(H3N2) subclade 3C.2a represented by A/Hong Kong/4801/2014, 65 subclade 3C.3a represented by A/Switzerland/9715293/2013, two subclade 3C.3b represented by A/Stockholm/28/2014, and two subclade 3C.3 represented by A/Samara/73/2013; 496 B/Victoria-lineage clade 1A represented by B/Brisbane/60/2008; and 55 B/Yamagata-lineage clade 3 represented by B/Phuket/3073/2013.

Antiviral susceptibility

For weeks 40/2015–30/2016 of the 2015–2016 influenza season, countries reported on the antiviral susceptibility of 3042 A(H1N1)pdm09 viruses, 211 A(H3N2) viruses and 688 influenza type B viruses from sentinel and non-sentinel sources. All but 28 showed no molecular or phenotypic evidence of reduced inhibition (RI) by neuraminidase inhibitors (oseltamivir and zanamivir). Twenty-six A(H1N1)pdm09 viruses carried NA H275Y amino acid substitution associated with highly reduced inhibition (HRI) by oseltamivir, one A(H3N2) virus showed RI by oseltamivir associated with NA-E119V amino acid substitution and one B/Victoria-lineage viruses showed HRI by both drugs due to NA-D197N amino acid substitution.

Phenotypic testing for susceptibility to oseltamivir and zanamivir has been conducted on 736 viruses at the WIC: 424 A(H1N1)pdm09, 116 A(H3N2), 168 B/Victoria-lineage and 28 B/Yamagata-lineage viruses. All but five A(H1N1)pdm09 viruses showed normal inhibition (NI) by these neuraminidase inhibitors: A/Bayern/151/2015 showed RI by zanamivir and carried NA I117R amino acid substitution, while A/Norway/2036/2016, A/Norway/2298/2016, A/Norway/2914/2015 and A/Czech Republic/11/2016 showed HRI by oseltamivir and carried NA H275Y amino acid substitution.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [4] 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 [5]. Increased numbers of cases were reported over the course of the 2013–14, 2014–15 and 2015–16 seasons and cases have been reported recently [6]. A revised Rapid Risk Assessment [7] for these A(H7N9) viruses was carried out by ECDC and posted on 11 February 2015. WHO posted a summary of human infection on 31 January 2014 [8], updated on 19 July 2016 [9] with 12 new cases since the report of 13 June 2016, and conducted a risk assessment on 23 February 2015 [10]. 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 [11] and has provided subsequent situation updates, with the latest being on 22 July 2016 [6].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 19 July 2016 [9]. Since the last WHO Influenza update on 13 June 2016, three human cases of A(H5N1) infection in Egypt have been reported. ECDC published an updated rapid risk assessment on the situation in Egypt on 13 March 2015 [12] and an epidemiological update 10 April 2015 [13]. On 2 December 2015, ECDC published a rapid risk assessment related to identification highly pathogenic H5 viruses in poultry in France [14].

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 and used at the WHO Vaccine Composition Meetings held in Memphis, USA (21–23 September 2015) and at WHO Geneva (22–24 February 2016) can be found at:

https://www.crick.ac.uk/media/273950/crick_sep2015_vcm_report_to_post.pdf and
https://www.crick.ac.uk/media/286458/crick_feb2016_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 (#). Sequences for some viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu database which were downloaded for use in the preparation of this report (all submitters of data may be contacted directly via the GISAID website), along with all laboratories who submitted sequences directly to the London WHO Collaborating Centre.

References

1. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2015–2016 northern hemisphere influenza season. Weekly Epidemiological Record 90, 97-108.
2. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2016 southern hemisphere influenza season. Weekly Epidemiological Record 90, 545-558.
3. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2016–2017 northern hemisphere influenza season. Weekly Epidemiological Record 91, 121-132.
4. World Health Organization. Global alert and response: Human infection with influenza A(H7N9) virus in China. 1 April 2013. Available from: http://www.who.int/csr/don/2013_04_01/en/index.html
5. World Health Organization. Avian influenza A(H7N9) virus. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/
6. World Health Organization. Situation updates - avian influenza. Available from: http://www.who.int/csr/don/22-july-2016-ah7n9-china/en/
7. European Centre for Disease Prevention and Control. Updated rapid risk assessment. Human infection with avian influenza A(H7N9) virus. Fourth update. 2 February 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/RRA-Influenza-A-H7.pdf
8. World Health Organization. Background and summary of human infection with avian influenza A(H7N9) virus – as of 31 January 2014. Geneva: WHO; 2014. Available from: http://www.who.int/influenza/human_animal_interface/20140131_background_and_summary_H7N9_v1.pdf
9. World Health Organization. Influenza at the human-animal interface. Summary and assessment as of 19 July 2016. Available from: www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_07_19_2016.pdf
10. World Health Organization. WHO risk assessment: Human infections with avian influenza A(H7N9) virus, 23 February 2015. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/RiskAssessment_H7N9_23Feb20115.pdf
11. World Health Organization. Map and epidemiological curve of confirmed human cases of avian influenza A(H7N9). Report 18- data in WHO/HQ as of 14 July 2014. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/18_reportwebh7n9number_20140714.pdf
12. European Centre for Disease Prevention and Control. Rapid Risk Assessment. Human infection with avian influenza A(H5N1) virus, Egypt. Available from: http://ecdc.europa.eu/en/publications/Publications/Rapid-Risk-Assessment-Influenza-A-H5N1-Egypt-March-2015.pdf
13. European Centre for Disease Prevention and Control. Epidemiological update: increase in reporting of human cases of A(H5N1) influenza, Egypt. Available from: http://ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1199
14. European Centre for Disease Prevention and Control. Rapid Risk Assessment. Situation overview: highly pathogenic avian influenza virus A of H5 type. Available from: http://ecdc.europa.eu/en/publications/Publications/highly-pathogenic-avian-influenza-virus-A-H5-rapid-risk-assessment-2-dec-2015.pdf