



SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, December 2018

Summary

This is the third report for the 2018–19 influenza season. As of week 1/2019, 18 049 influenza detections across the WHO European Region had been reported. Detections were made up of 97.7% type A viruses, with A(H1N1)pdm09 prevailing over A(H3N2), and 2.3% type B viruses, with 16 (64%) of 25 ascribed to a lineage being B/Yamagata.

Since the November 2018 characterisation report¹, a further 12 EU/EEA countries have shared influenza-positive specimens with the London WHO CC, the Francis Crick Worldwide Influenza Centre (WIC). The great majority of these specimens are still in the characterisation process. Hence, the main focus of this report is phylogenetic analyses of HA gene sequences from recently collected seasonal influenza viruses that had been submitted by laboratories in any country globally to GISAID's EpiFlu database as of 3 January 2019.

As for previously characterised viruses, the single virus from Norway was antigenically similar to the vaccine virus, A/Michigan/45/2015, and the HA fell in genetic subclade 6B.1. The HA genes of all five viruses characterised to date have fallen within subclade 6B.1, defined by HA1 amino acid substitutions S84N, S162N and I216T, with all recently circulating viruses having additional substitutions of S74R, S164T and I295V compared to A/Michigan/45/2015.

Twenty-one of the 24 recently circulating A(H3N2) viruses characterised antigenically by HI assay have shown similarity to the A/Singapore/INFIMH-16-0019/2016 vaccine virus. HA sequences of these viruses are pending, but of seven viruses that could not be characterised antigenically due to a lack of HA activity, six had HAs falling in subgroup 3C.2a1b and one fell in subclade 3C.2a2, which reflects the dominance of subgroup 3C.2a1b viruses globally.

No B/Victoria-lineage viruses have been characterised antigenically. All viruses with recent collection dates carry HA genes that fall in clade 1A but encode HA1 amino acid substitutions of I117V, N129D and V146I compared to B/Brisbane/60/2008. Within this subclade, two groups of viruses defined by deletions of two (Δ 162-163) or three (Δ 162-164) amino acids in HA1, with the (Δ 162-164) group splitting into two subgroups (Asian and African), have emerged. Previous HI analyses with panels of post-infection ferret antisera have shown these virus groups and subgroups to be antigenically distinguishable. Δ 162-163 viruses have spread globally while Δ 162-164 viruses have not, though a virus of the African subgroup was detected recently in Sweden. B/Colorado/06/2017, a Δ 162-163 virus, has been recommended for use in trivalent vaccines for the current northern hemisphere influenza season.

All B/Yamagata-lineage viruses with recent collection dates have HA genes that fall within the B/Phuket/3073/2013 vaccine virus clade (clade 3) and encode HA1 amino acid substitutions of L172Q and M251V, with some having additional substitutions. HI analyses with post-infection ferret antisera raised against B/Phuket/3073/2013 have shown such viruses, including the single virus characterised here, to be antigenically similar to the vaccine virus which has been recommended for use in quadrivalent vaccines for the current northern hemisphere influenza season.

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Table 1 shows a summary of influenza virus detections in the WHO European Region reported to ECDC's TESSy database since the start of the 2018–19 season (weeks 40/2018–1/2019). Since week 48/2018, the cumulative number of detections has increased from 2 061 to 18 049, with type A (97.7%) predominating over type B (2.3%) viruses, which is a common pattern, unlike the 2017–18 season when type B predominated over type A at the start of the season and throughout most of it. Of the type A viruses subtyped ($n = 6\,278$) and the type B viruses ascribed to a lineage ($n = 25$), A(H1N1)pdm09 ($n = 4\,089$) are prevailing over A(H3N2) ($n = 2\,189$) viruses, and 16 of 25 B viruses have been B/Yamagata-lineage; these relative proportions have increased in favour of A(H1N1)pdm09 and B/Victoria-lineage viruses compared to the summary in the November 2018 characterisation report¹. Overall, the ratio of type A to type B detections is dramatically increased compared with the 2017–18 season (0.8:1 to 42.2:1), and of the influenza A viruses that have been subtyped, an increase in the proportion of A(H1N1)pdm09 has been seen (65.1% in 2018–19 compared with 50.6% in 2017–18).

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2018–19 season (weeks 40/2018–1/2019)^a

Virus type/subtype/lineage	Cumulative number of detections			Totals*		Totals for 2017-18 season*		
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Number	%	Ratios
Influenza A	1 941	15 690	17 631	97.7	42.2:1	106 003	44.1	0.8:1
A(H1N1)pdm09	800	3 289	4 089	65.1		23 121	50.6	
A(H3N2)	699	1 490	2 189	34.9	0.5:1	22 568	49.4	1:1
A not subtyped	442	10 911	11 353			60 314		
Influenza B	32	386	418	2.3		134 618	55.9	
Victoria lineage	3	6	9	36.9		301	1.9	
Yamagata lineage	9	7	16	64.0	1.8:1	15 701	98.1	52.2:1
Lineage not ascribed	20	373	393			118 616		
Total detections (total tested)	1 973 (13 201)	16 076 (223 326)	18 049 (236 527)			240 621 (903 182)		

* 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(H3N2):A(H1N1)pdm09 and Yamagata:Victoria lineages.

Since week 40/2018, shipments of specimens (virus isolates and/or clinical specimens) from 13 EU/EEA countries have been received at the Crick Worldwide Influenza Centre (WIC), and they have contained a total of 237 individual virus-related samples with collection dates after 31 August 2018 (Table 2). The received samples match closely the proportions as reported to TESSy (Table 1) in terms of virus type and virus subtype or lineage.

Analyses of viruses with collection dates from September 2018 to January 2019 will be considered at the upcoming WHO influenza vaccine composition meeting (17–21 February 2019). Since most of the shipments were received in 2019, limited antigenic characterisation data have been generated for viruses in EU/EEA countries, and this report focuses largely on haemagglutinin (HA) phylogenetic analyses for seasonal influenza viruses, with the most recent collection dates, that have been deposited in GISAID's EpiFlu database (as of 3 January 2019). These phylogenetic analyses serve to illustrate the recent global situation as an indicator of what may emerge in the WHO European Region.

¹ European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, November 2018. Stockholm: ECDC; 2018. Available from: <https://ecdc.europa.eu/sites/portal/files/documents/ECDCFlu-characterisation-rep-Nov-2018.pdf>

Table 2. Summary of clinical samples and virus isolates, contained in packages received from EU/EEA Member States since week 40/2018

MONTH	TOTAL RECEIVED	A		A(H1N1)pdm09		A(H3N2)		B		B Victoria lineage		B Yamagata lineage		
		Number received	Number propagated ¹	Number received	Number propagated ¹	Number received	Number propagated ²	Number received	Number propagated ¹	Number received	Number propagated ¹	Number received	Number propagated ¹	
2018														
SEPTEMBER														
France	7					6	in process					1	in process	
OCTOBER														
Czech Republic	2			2	in process									
Denmark	2					2	in process							
Estonia	3	1	in process	1	in process	1	in process							
France	9			3	in process	6	4	2						
Germany	1					1	0	1						
Iceland	2					1	in process	1	in process					
Ireland	3			2	in process	1	in process							
Latvia	1			1	in process									
Norway	29			12	in process	14	in process					3	1	
United Kingdom	3			1	in process	2	in process							
NOVEMBER														
Austria	4	1	in process	1	in process	2	1	1						
Bulgaria	1			1	in process									
Czech Republic	1			1	in process									
Denmark	12			8	in process	3	in process			1	in process			
Estonia	3			3	in process									
France	16			10	in process	6	4	2						
Germany	7			3	in process	4	0	1						
Iceland	15			4	in process	11	in process							
Ireland	17			12	in process	4	in process	1	in process					
Latvia	2			2	1	1	1	1						
Norway	15	1	in process	12	in process	2	0	1						
United Kingdom	8			5	in process	1	0	1		1	in process	1	in process	
DECEMBER														
Austria	1					1	0	1						
Bulgaria	9			5	in process	4	in process							
Denmark	7			5	in process	2	in process							
Estonia	18	5	in process	12	in process	1	in process							
France	5			2	in process	3	3							
Iceland	3			3	in process									
Ireland	3			3	in process									
Latvia	6			5	in process	1	1	0						
Norway	7			4	in process	3	1	2						
Poland	1			1	in process									
2019														
JANUARY														
Bulgaria	8			7	in process	1	in process							
Poland	6			6	in process									
13 Countries	237	8	0	135	0	85	15	13	2	0	2	0	5	1
						57.0%	35.9%				0.8%		2.1%	
						96.2%					3.8%			

1. Propagated to sufficient titre to perform HI assay (the totalled number does not include any from batches that are in process)
 2. Propagated to sufficient titre to perform HI assay in the presence of 20nM oseltamivir (the totalled number does not include any from batches that are in process)
 Numbers in red indicate viruses recovered but with insufficient HA titre to permit HI assay

As of 15 January 2019

Influenza A(H1N1)pdm09 virus analyses

A single virus has been characterised by haemagglutination inhibition (HI) assay since the November 2018 report (Table 3). A/Norway/3399/2018 was antigenically similar to the vaccine virus for the northern hemisphere 2018–19 influenza season, A/Michigan/45/2015 [1], being recognised at titres within twofold of the titre of the antiserum for the homologous virus. The test virus showed good reactivity with eight other antisera in the panel, all being recognised at titres within twofold of the respective homologous titres. The antiserum raised against cell culture-propagated A/Lviv/N6/2009 (which has HA1 amino acid substitutions of G155E and D222G) recognised the test virus less well at a titre eightfold reduced compared to the homologous titre. This is a pattern that has been seen with the great majority of test viruses in recent virus characterisation reports.

All viruses with collection dates from 1 September 2018, for which HA gene sequences have been submitted to GISAID's EpiFlu database, fall within clade 6B.1, the A/Michigan/45/2015 vaccine virus clade [1], but carry additional amino acid substitutions of S74R, S164T and I295V (Figure 1). A number of genetic subgroups defined by specific amino acid substitutions have emerged but the great majority of viruses in the various subgroups have remained antigenically similar to A/Michigan/45/2015 as shown in the November 2018 and earlier characterisation reports.

EU/EEA influenza viruses with collection dates from 1 September 2018 are distributed throughout the representative HA phylogeny, with a number of the subgroups being defined by at least one of the HA1 amino acid substitutions T120A, N129D, T185I or S183P, often in combination with additional substitutions (Figure 1).

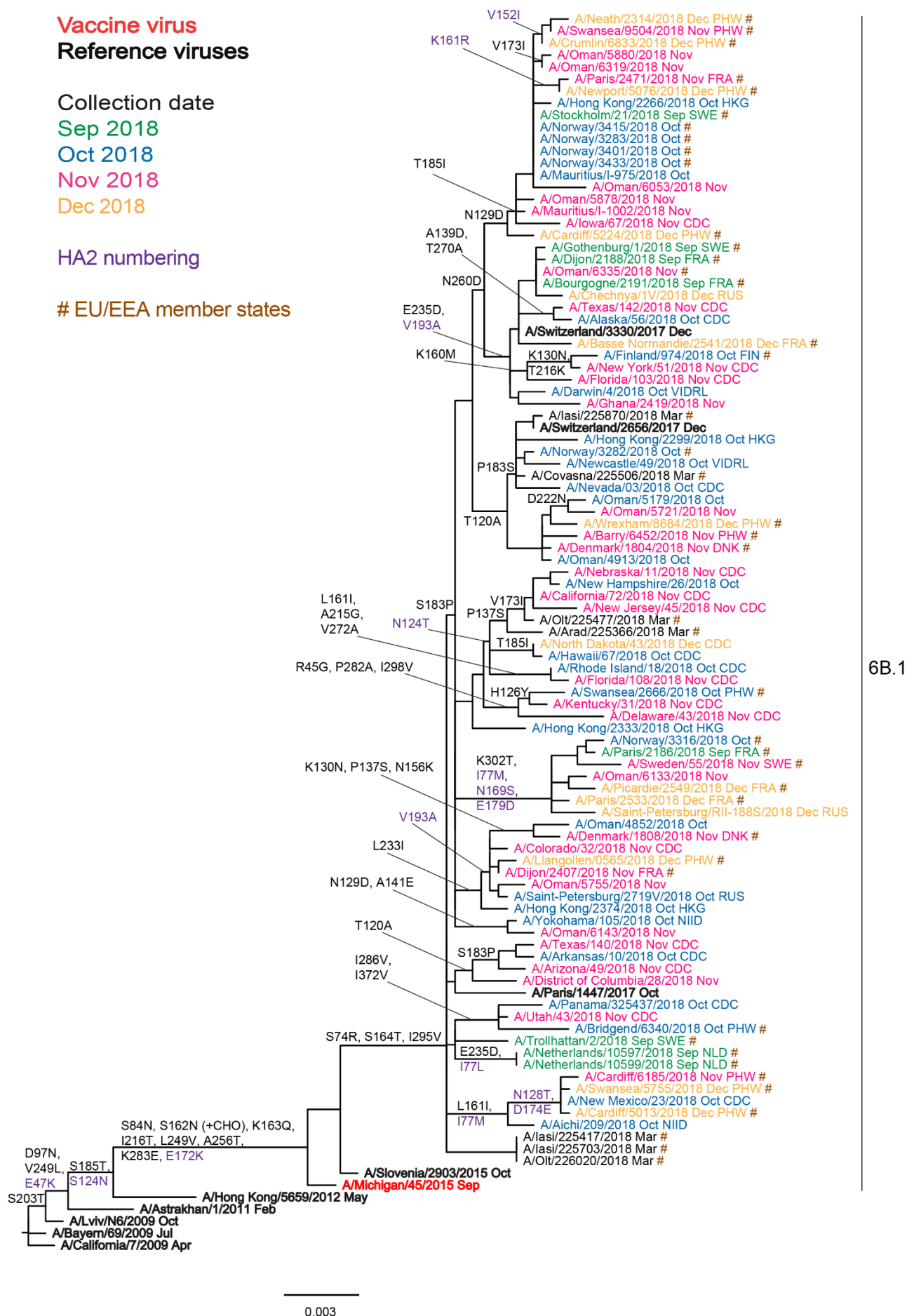
Table 3. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
				A/Mich 45/15 Egg NIB F-42/16 ^{*1} 6B.1	A/Cal 7/09 Egg F07/16 ^{*1} 6B.1	A/Bayern 69/09 MDCK F09/15 ^{*1}	ALviv N6/09 MDCK F14/13 ^{*1}	A/viv MDCK F22/13 ^{*1}	A/Astrak 1/11 MDCK F17/15 ^{*1}	A/HK 5659/12 MDCK F03/18 ^{*2}	A/Slov 2903/2015 Egg F02/16 ^{*1}	A/Paris 1447/17 MDCK F20/18 ^{*1}	A/Swit 2656/17 Egg F23/18 ^{*1}	
REFERENCE VIRUSES														
A/Michigan/45/2015		2015-09-07	E3/E3	640	640	320	320	320	640	640	1280	2560	640	320
A/California/7/2009	clone 38-32	2009-04-09	E3/E3	320	640	320	320	320	640	640	640	1280	640	320
A/Bayern/69/2009	G155E	2009-07-01	MDCK5/MDCK1	40	80	320	160	<	40	40	80	320	80	40
A/Lviv/N6/2009	G155E, D222G	2009-10-27	MDCK4/SIAT1/MDCK3	80	160	640	640	40	160	160	160	640	160	160
A/Astrakhan/1/2011		2011-02-28	MDCK1/MDCK5	640	1280	320	160	320	640	320	1280	2560	640	320
A/Hong Kong/5659/2012		2012-05-21	MDCK4/MDCK2	160	320	80	80	160	320	320	1280	640	160	80
A/Slovenia/2903/2015	clone 37	2015-10-26	MDCK1/MDCK3	320	1280	320	160	160	640	320	640	2560	640	320
A/Paris/1447/2017		2017-10-20	MDCK1/MDCK3	640	1280	640	320	320	640	320	1280	2560	640	320
A/Switzerland/2656/2017		2017-12-21	E5/E2	640	1280	640	320	320	640	320	1280	2560	640	320
A/Switzerland/3330/2017	clone 35	2017-12-20	E6/E2	320	640	160	160	160	320	320	640	1280	640	320
TEST VIRUSES														
A/Norway/3399/2018		2018-10-29	MDCK3	320	640	160	80	320	320	320	640	1280	640	160
				Vaccine										

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

1 < = <40; 2 < = <80

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



Influenza A(H3N2) virus analyses

As described in many previous reports², influenza A(H3N2) viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells (RBCs) from guinea pigs, turkeys and humans, often with the loss of ability to agglutinate any of these RBCs. As was highlighted first in the November 2014 report³, this is a particular problem for most viruses that fall in genetic clade 3C.2a.

Since the November 2018 report of the viruses recovered, based on positive neuraminidase activity, only 24 retained sufficient HA activity to allow antigenic analysis by HI (Tables 4-1 and 4-2). Of the 24 test viruses 21 and 6 were recognised at titres within fourfold and twofold, respectively, of the homologous titre by the antiserum raised against the currently used vaccine virus, egg-propagated A/Singapore/INFIMH-16-0019/2016 (subclade 3C.2a1). Test viruses were analysed with antisera raised against three cell culture-propagated subclade 3C.2a1 viruses for which no homologous titres are given, due to the inability of these cell culture-propagated reference viruses to agglutinate RBCs that reacted with the majority of reference viruses at titres of ≥ 160 . Those raised against A/Norway/4436/2016 and A/Greece/4/2017 (subgroup 3C.2a1a) each recognised 12 of the test viruses at titres of ≥ 160 , while those raised against A/La Rioja/2202/2018 (subgroup 3C.2a1b) recognised 16 test viruses at titres of ≥ 160 .

Antisera raised against subclade 3C.2a2 viruses generally recognised the test viruses poorly. Those raised against cell culture-propagated viruses, A/Bretagne/1413/2017 and A/Hong Kong/656/2018, recognised three and two viruses, respectively, at titres within twofold of the homologous titres, with all others having titres at least eightfold reduced. Antiserum raised against egg-propagated A/Switzerland/8060/2017, the vaccine virus recommended for use in the 2019 southern hemisphere season, recognised only one test virus at a titre within fourfold of the homologous titre.

Antiserum raised against a cell culture-propagated clade 3C.2a virus, A/Hong Kong/5738/2014, recognised all 24 test viruses at titres within fourfold of the homologous titre and 13 within twofold. An antiserum raised against the cell culture-propagated cultivar of A/Stockholm/6/2014, a clade 3C.3a virus, recognised 13 test viruses at titres within fourfold of the titre of the antiserum with the homologous virus and six within twofold.

HA gene sequences of the 24 test viruses are currently unavailable, hence phylogenetic analysis of HA genes of representative A(H3N2) viruses with collection dates from 1 September 2018, available in the GISAID EpiFlu database, is shown in Figure 2. Viruses in clades 3C.2a and 3C.3a have been in circulation since the 2013–14 northern hemisphere influenza season, with clade 3C.2a viruses having been dominant since the 2014–15 influenza season, notably subclade 3C.2a2 viruses, though subgroup 3C.2a1b viruses have predominated in recent months (Figure 2). The HA gene sequences of viruses in both clades continue to diverge. Notably, clade 3C.3a viruses have evolved to carry **HA1** amino acid substitutions of **L3I**, **S91N**, **N144K** (loss of an N-linked glycosylation motif at residues 144–146), **F193S** and **K326R**, compared to A/Stockholm/6/2014 (Figure 2), and new genetic groups have emerged among the clade 3C.2a viruses, designated as subclades/subgroups. Amino acid substitutions that define these subclades/subgroups are:

- Clade 3C.2a: **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) and **Q311H** in **HA1**, and **D160N** in **HA2**, e.g. A/Hong Kong/7295/2014 a cell culture-propagated surrogate for A/Hong Kong/4801/2014 (a former vaccine virus)
- Subclade 3C.2a1: Those in clade 3C.2a plus: **N171K** in **HA1** and **I77V** and **G155E** in **HA2**, most also carry **N121K** in **HA1**, e.g. A/Singapore/INFIMH-16-0019/2016 (2018–19 northern hemisphere vaccine virus)
- Subgroup 3C.2a1a: Those in subclade 3C.2a1 plus **T135K** in **HA1**, resulting in the loss of a potential glycosylation site, and also **G150E** in **HA2**, e.g. A/Greece/4/2017
- Subgroup 3C.2a1b: Those in subclade 3C.2a1 plus **K92R** and **H311Q** in **HA1**, e.g. A/La Rioja/2202/2018, with many viruses in this subgroup carrying additional HA1 amino acid substitutions
- Subclade 3C.2a2: Those in clade 3C.2a plus **T131K**, **R142K** and **R261Q** in **HA1**, e.g. A/Switzerland/8060/2017 (2019 southern hemisphere vaccine virus)
- Subclade 3C.2a3: Those in clade 3C.2a plus **N121K** and **S144K** in **HA1**, e.g. A/Cote d'Ivoire/544/2016
- Subclade 3C.2a4: Those in clade 3C.2a plus **N31S**, **D53N**, **R142G**, **S144R**, **N171K**, **I192T**, **Q197H** and **A304T** in **HA1** and **S113A** in **HA2**, e.g. A/Valladolid/182/2017
- Clade 3C.3a: **T128A** (resulting in the loss of a potential glycosylation site), **R142G** and **N145S** in **HA1** which defined clade 3C.3 plus **A138S**, **F159S** and **N225D** in **HA1**, many with **K326R**, e.g. A/England/538/2018.

² For example, the September 2013 report: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2013. Stockholm: ECDC; 2014. Available from: <https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/influenza-virus-characterisation-sep-2013.pdf>

³ 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

Globally, the great majority of viruses with collection dates from 9 January 2018 have HA genes that continue to fall into genetic groups within clade 3C.2a, notably the 3C.2a2 subclade and the 3C.2a1b subgroup, with a small number of viruses falling in clade 3C.3a (Figure 2). Viruses in subgroup 3C.2a1b have been more numerous than those in subclade 3C.2a2 for the period September to December 2018; notably, a significant number of the subgroup 3C.2a1b viruses have fallen in a recently emerged cluster defined by substitutions **T131K** and **K135T** (a reversion resulting in re-establishment of the **133-135** glycosylation sequon) in **HA1** with **V200I** in **HA2**.

The location of A/Singapore/INFIMH-16-0019/2016 (3C.2a1), the A(H3N2) virus recommended for inclusion in vaccines for the northern hemisphere 2018–2019 influenza seasons [1], is indicated in Figure 2, as is A/Switzerland/8060/2017 (3C.2a2) the A(H3N2) virus recommended for inclusion in vaccines for the southern hemisphere 2019 [2].

Table 4-1. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre													
				Post-infection ferret antisera													
				A/Stock 6/14 SIAT F14/14 ¹ 3C.3a	A/HK 5738/14 MDCK F30/14 ¹ 3C.2a	A/Bretagne 1413/17 SIAT F01/18 ¹ 3C.2a2	A/Nor 4436/16 SIAT F03/17 ¹ 3C.2a1	A/Greece 4/17 SIAT F27/17 ¹ 3C.2a1a	A/Singapore 0019/16 E99 10 ⁻⁴ F41/17 ¹ 3C.2a1	A/La Rioja 2202/18 SIAT F26/18 ¹ 3C.2a1b	A/HK 656/18 SIAT F25/18 ¹ 3C.2a2	A/Switzerland 8060/17 E99 F27/18 ¹ 3C.2a2					
REFERENCE VIRUSES																	
A/Stockholm/6/2014		2014-02-06	SIAT1/SIAT3	320	160	160	320	320	320	320	160	160	320	320			
A/Hong Kong/5738/2014	3C.3a	2014-04-30	MDCK1/MDCK2/SIAT2	160	160	160	320	320	320	320	320	320	320	320			
A/Bretagne/1413/2017	3C.2a2	2017-10-09	MDCK1/SIAT4	160	160	1280	320	320	320	320	160	160	160	1280			
A/Singapore/INF16-0019/2016	3C.2a1	2016-04-14	ESE3	<	40	40	160	160	640	640	40	40	80	80			
A/Hong Kong/656/2018	3C.2a2	2018-04-07	MDCK1/SIAT3	160	160	640	320	320	320	320	320	320	320	640			
A/Switzerland/8060/2017	clone 57	2017-12-12	E7/E1	40	160	2560	320	320	640	640	2560	160	160	2560			
TEST VIRUSES																	
A/Latvia/11-019324/2018		2018-11-07	MDCK2/SIAT1	160	160	640	320	320	160	160	640	80	160	640			
A/Clermont-Ferrand/2062/2018		2018-11-07	MDCK3/SIAT1	160	160	80	320	320	320	320	80	160	160	160			
A/Iceland/108/2018		2018-11-13	MDCK1/SIAT1	40	40	<	80	80	160	160	<	160	160	40			
A/Austria/1102969/2018		2018-11-16	SIAT1/SIAT1	160	80	40	320	320	320	320	40	320	320	80			
A/Lyon/2106/2018		2018-11-20	MDCK3/SIAT1	160	80	80	160	160	160	160	40	320	320	80			
A/Paris/2513/2018		2018-11-26	MDCK1/SIAT1	80	80	80	320	320	160	160	160	40	160	160			
A/Paris/2511/2018		2018-11-29	MDCK1/SIAT1	80	80	80	320	320	160	160	80	40	160	160			
A/Latvia/12-005233/2018		2018-12-03	MDCK1/SIAT1	160	160	640	320	320	320	320	640	160	160	320			
A/Paris/2538/2018		2018-12-04	MDCK1/SIAT1	80	80	80	320	320	160	160	160	40	160	80			
A/Paris/2544/2018		2018-12-04	MDCK1/SIAT1	80	80	80	320	320	160	160	80	80	160	160			
<table border="0" style="width:100%; border:none;"> <tr> <td style="width:50%;"></td> <td style="width:50%; text-align:right;">Vaccine SH 2018 NH 2018-19</td> </tr> <tr> <td style="width:50%;"></td> <td style="width:50%; text-align:right;">Vaccine SH 2019</td> </tr> </table>															Vaccine SH 2018 NH 2018-19		Vaccine SH 2019
	Vaccine SH 2018 NH 2018-19																
	Vaccine SH 2019																

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)¹ < = <40

Table 4-2. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre																
				Post-infection ferret antisera																
				A/Stock 6/14 SIAT F14/14 ¹ 3C.3a	A/HK 5738/14 MDCK F30/14 ¹ 3C.2a	A/Bretagne 1413/17 SIAT F01/18 ¹ 3C.2a2	A/Nor 4436/16 SIAT F03/17 ¹ 3C.2a1	A/Greece 4/17 SIAT F27/17 ¹ 3C.2a1a	A/Singapore 0019/16 Egg 10 ⁴ F15/18 ¹ 3C.2a1	A/HK 656/18 SIAT F25/18 ¹ 3C.2a2	A/La Rioja 2202/18 SIAT F26/18 ¹ 3C.2a1b	A/Switz 8060/17 Egg F27/18 ¹ 3C.2a2								
REFERENCE VIRUSES																				
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT1/SIAT3	160	160	160	320	160	160	160	160	160	160	160	160	160	160	160	160	160
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK2/SIAT2	160	160	160	320	160	160	160	160	160	160	160	160	160	160	160	160	160
A/Bretagne/1413/2017	3C.2a2	2017-10-09	MDCK1/SIAT4	160	160	640	320	160	160	160	160	160	160	160	160	160	160	160	160	160
A/Singapore/INF16-0019/2016	3C.2a1	2016-04-14	E5/E3	<	40	40	80	80	80	80	80	80	80	80	160	80	80	80	80	80
A/Hong Kong/656/2018	3C.2a2	2018-04-07	MDCK1/SIAT3	320	320	640	320	320	320	320	640	320	320	320	320	320	1280	160	640	640
A/Switzerland/8060/2017	clone 57	2017-12-12	E7/E1	40	160	1280	160	160	160	160	1280	160	160	160	160	1280	160	160	1280	1280
TEST VIRUSES																				
A/Saint-Etienne/1912/2018		2018-09-07	MDCK2/SIAT1	80	40	40	160	40	40	40	40	40	40	40	40	40	40	40	40	40
A/Poitiers/1978/2018		2018-10-08	MDCK2/SIAT1	40	40	<	80	<	80	<	<	80	80	80	80	<	160	160	160	160
A/Poitiers/1976/2018		2018-10-09	MDCK3/SIAT1	40	40	<	80	<	80	<	<	80	80	80	80	<	160	160	160	160
A/Poitiers/2003/2018		2018-10-20	MDCK2/SIAT1	40	80	40	80	40	40	40	40	40	40	40	40	40	160	320	320	40
A/Lorraine/2365/2018		2018-10-22	MDCK2/SIAT2	<	40	<	40	<	40	<	<	40	40	40	40	<	80	80	80	40
A/Iceland/101/2018		2018-11-01	MDCK1/SIAT1	<	40	<	40	<	40	<	<	40	40	40	40	<	80	80	80	<
A/Iceland/100/2018		2018-11-01	MDCK1/SIAT1	40	80	<	40	<	40	<	<	40	40	40	40	<	160	160	160	40
A/Iceland/102/2018		2018-11-02	MDCK1/SIAT1	40	40	<	40	<	40	<	<	40	40	40	40	<	160	160	160	<
A/Iceland/103/2018		2018-11-03	MDCK1/SIAT1	40	40	<	40	<	40	<	<	40	40	40	40	<	160	160	160	<
A/Iceland/104/2018		2018-11-05	MDCK1/SIAT1	<	40	<	40	<	40	<	<	40	40	40	40	<	160	160	160	<
A/Iceland/107/2018		2018-11-09	MDCK1/SIAT2	40	40	<	80	<	80	<	<	80	80	80	40	<	160	320	320	80
A/Norway/3668/2018		2018-11-29	SIAT1	80	80	80	80	80	80	80	80	80	80	80	160	40	80	80	80	80
A/Norway/3735/2018		2018-12-03	SIAT1	160	80	320	320	320	320	320	320	320	320	320	160	80	80	80	80	160
A/Paris/2572/2018		2018-12-06	SIAT1	80	40	<	160	<	160	<	<	160	40	40	80	80	80	40	40	80

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)¹ < = <40

Vaccine SH 2018-19
Vaccine SH 2019

Influenza B virus analyses

Influenza B – Victoria lineage

No B/Victoria-lineage viruses from EU/EEA countries, with collection dates from 01 September 2018 have been analysed antigenically at the WIC. A relatively small number of HA sequences for viruses collected since this date have been deposited in the EpiFlu database of GISAID and the great majority of these have been from China and the USA, with only two from an EU country (Sweden; Figure 3). All recently collected viruses continue to have HA genes that fall in the B/Brisbane/60/2008 clade (clade 1A; Figure 3), with all falling in a subclade defined by **HA1** amino acid substitutions **I117V**, **N129D** and **V146I** within clade 1A. Two new groups within this subclade have deletions in the HA gene. A major group seen in Europe, the Americas, Asia, Oceania and South Africa have HA genes encoding an HA with deletion of residues **K162** and **N163** of **HA1** (1A(Δ 2) in Figure 3). These viruses have additional substitutions of **D129G** and **I180V** in **HA1**, and **R151K** in **HA2**, with a recent cluster in China also carrying **N178S** in **HA2**. This group of viruses is more prevalent than the subclade viruses that show no deletions. Less common are viruses with HA genes encoding a deletion of three **HA1** amino acids, **K162**, **N163** and **D164** (1A(Δ 3) in Figure 3), which have been detected primarily in the Far East and Africa; this group splits into an Asian subgroup with viruses carrying additional substitutions of **I180T** and **K209N** in **HA1** and a West African subgroup with viruses carrying the **HA1** substitution **K136E**, often with additional HA1 substitutions of **K52N** and **E198G** (within the **197-199** glycosylation site). Viruses in the latter subgroup have been detected recently in China and one of the viruses from Sweden fell in this subgroup. It was noted in the September 2018 characterisation report⁴, and earlier ones, that the clade 1A viruses without deletions, the 1A(Δ 2) group and the 1A(Δ 3) subgroups are antigenically distinct from one another. Following the emergence and spread of viruses in the 1A(Δ 2) group a representative, B/Colorado/06/2017, has been recommended for use in trivalent influenza vaccines for both the 2018-19 northern hemisphere [1] and 2019 southern hemisphere [2] seasons.

Influenza B – Yamagata lineage

A single B/Yamagata-lineage virus, B/Norway/3367/2018, has been characterised by haemagglutination inhibition (HI) assay since the November 2018 report (Table 5). The antiserum raised against egg-propagated B/Phuket/3073/2013, recommended for inclusion in quadrivalent vaccines for the 2018–19 [1] northern hemisphere and the 2019 [2] southern hemisphere seasons, recognised the test virus at a titre within twofold of the titre of the antiserum with the homologous virus. An antiserum raised against the cell culture-propagated cultivar of B/Phuket/3073/2013 recognised the test virus at a titre within fourfold of the homologous titre of the antiserum. Antisera raised against two other egg-propagated clade 3 viruses, B/Wisconsin/1/2010 (a former vaccine virus) and B/Stockholm/12/2011, recognised the test virus at titres within twofold and fourfold, respectively, of the homologous titres. An antiserum raised against a recently circulating clade 3 cell culture-propagated virus, B/Mauritius/1791/2017, recognised the test virus at the same titre as the homologous virus.

Antisera raised against cell culture-propagated clade 2 viruses, B/Estonia/55669/2011 and B/Massachusetts/02/2012, recognised the test virus at titres within twofold of the homologous titres, while that raised against egg-propagated B/Massachusetts/02/2012 gave an eightfold reduction in titre with the test virus compared to the homologous titre.

The test virus carried an HA gene in genetic clade 3 (Tables 5). Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses, including recently circulating ones. Worldwide, all HA genes from viruses collected in the 2017–18 season and since have fallen in clade 3, the B/Wisconsin/1/2010–B/Phuket/3073/2013 clade. All viruses with collection dates after 31 August 2018, including two from EU/EEA countries, as deposited in the EpiFlu database of GISAID, fall in a subgroup defined by **HA1 L172Q** and **M251V** amino acid substitutions compared to B/Phuket/3073/2013. Some subclustering of sequences, defined by specific amino acid substitutions (e.g. HA1 S120T or D229N or D232N [introducing a potential N-linked glycosylation site]), is occurring. It has been noted in previous characterisation reports for 2018 that none of these amino acid substitutions have any obvious antigenic effects based on haemagglutination inhibition (HI) assays using post-infection ferret antisera raised against egg-propagated B/Phuket/3073/2013 which has been recommended for inclusion in quadrivalent vaccines for the 2018–19 [1] northern hemisphere and the 2019 [2] southern hemisphere seasons.

⁴ European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, November 2018. Stockholm: ECDC; 2018. Available from: <https://ecdc.europa.eu/sites/portal/files/documents/ECDC-Flu-Characterisation-Report-Sep-2018.pdf>

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

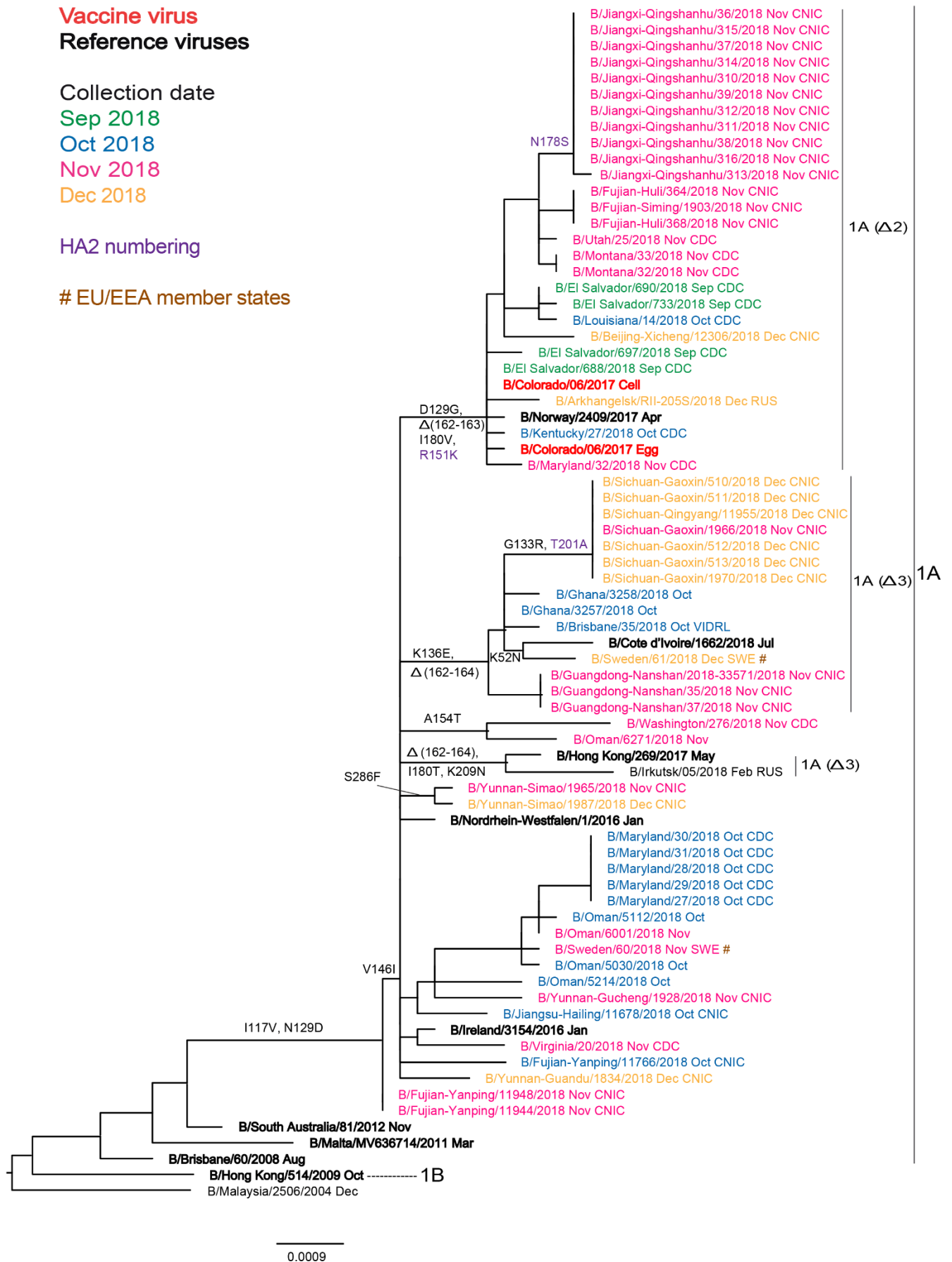


Table 5. Antigenic analysis of influenza B/Yamagata-lineage viruses by HI

Viruses	Other information	Passage history	Ferret number	Genetic Group	Collection date	Passage history	Haemagglutination inhibition titre																
							B/Phuket 3073/13 Egg SH614 ^{1,3}	B/Estonia 55669/11 MDCK F40/18 ²	B/Mass 02/12 MDCK F10/16 ²	B/Mass 02/12 Egg F16/14 ²	B/Wis 1/10 Egg F36/15 ²	B/Stock 12/11 Egg F05/17 ²	B/Phuket 3073/13 MDCK F27/15 ²	B/Phuket 3073/13 Egg F25/17 ²	B/Maur 1791/17 MDCK F04/18 ²								
REFERENCE VIRUSES																							
B/Estonia/55669/2011		2		2	2011-03-14	MDCK2/MDCK3	640	160	80	80	40	40	20	40	10								
B/Massachusetts/02/2012		2		2	2012-03-13	MDCK1/C2/MDCK3	640	160	80	320	160	80	40	80	10								
B/Massachusetts/02/2012		2		2	2012-03-13	E3/E4	640	80	20	320	80	20	20	80	<								
B/Wisconsin/1/2010		3		3	2010-02-20	E3/E2	1280	80	20	320	160	80	80	40	40								
B/Stockholm/12/2011		3		3	2011-03-28	E4/E1	1280	40	20	160	80	40	40	20	20								
B/Phuket/3073/2013		3		3	2013-11-21	MDCK2/MDCK3	2560	640	160	320	320	160	160	320	160								
B/Phuket/3073/2013		3		3	2013-11-21	E4/E3	1280	40	10	160	80	40	40	20	20								
B/Mauritius/1791/2017		3		3	2017-09-20	MDCK1/MDCK4	1280	160	40	80	80	80	80	80	80								
TEST VIRUSES																							
B/Norway/3367/2018		3		3	2018-10-19	MDCK1	1280	80	40	40	80	40	40	80	80								

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

1 < = <40; 2 < = <10; 3 hyperimmune sheep serum

B/Yamagata-lineage virus recommended for use in trivalent vaccines SH 2018 and quadrivalent vaccines NH 2017-18 & 2018-19

Sequences in phylogenetic trees

Vaccine#

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

Vaccine virus
Reference viruses

Collection date

Sep 2018

Oct 2018

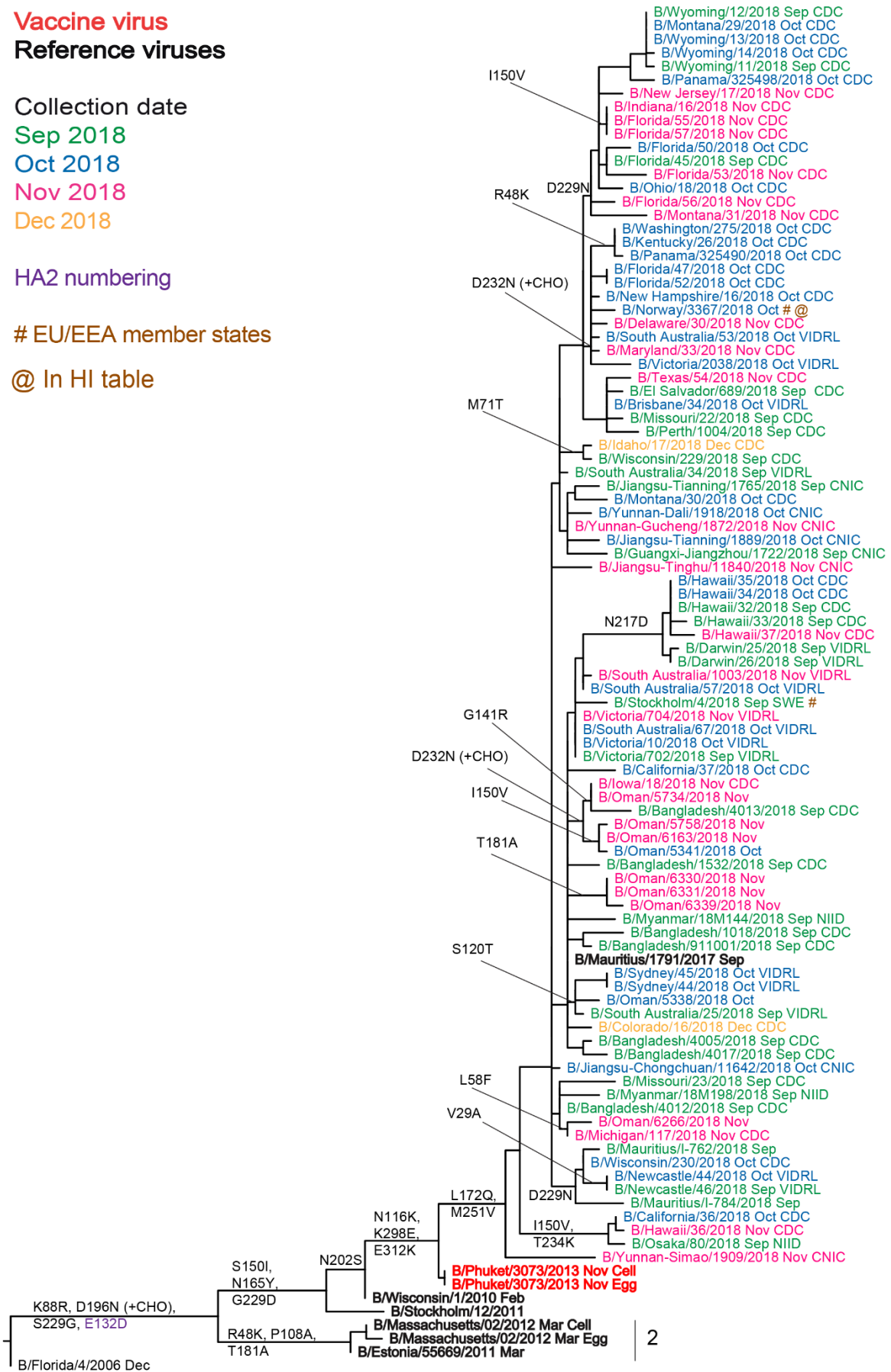
Nov 2018

Dec 2018

HA2 numbering

EU/EEA member states

@ In HI table



3

0.003

Summaries of data submitted to TESSy

Genetic characterisation

For the 2018–19 season, as of week 1/2019, 215 viruses had been characterised genetically and ascribed to a genetic clade:

- 151 A(H1N1)pdm09 were subclade 6B.1, represented by the vaccine virus A/Michigan/45/2015
- 62 were A(H3N2) viruses, with 44 being subgroup 3C.2a1b represented by A/Alsace/1746/2018, 3 being subclade 3C.2a2 represented by A/Switzerland/8060/2017, 4 being subclade 3C.2a3 represented by A/Cote d'Ivoire/544/2016, 8 being clade 3C.3a represented by A/England/538/2018, and 3 were attributed to a subgroup not listed in current TESSy reporting categories
- 1 was B/Yamagata-lineage clade 3 represented by the vaccine virus B/Phuket/3073/2013
- 1 was B/Victoria-lineage clade 1A represented by B/Brisbane/60/2008.

Antiviral susceptibility

For viruses collected in the course of the 2018–19 season, as of week 1/2019, 131 A(H1N1)pdm09, 27 A(H3N2) and 2 type B have been tested for susceptibility to neuraminidase inhibitors. One A(H1N1)pdm09 and 1 type B virus showed evidence of reduced inhibition (RI) by the inhibitors.

At the WIC for this season, 42 viruses from EU/EEA countries have been assessed against oseltamivir and zanamivir: 7 A(H1N1)pdm09, 34 A(H3N2) and 1 B/Yamagata-lineage. All showed normal inhibition (NI) by the two 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 WHO of three cases of human infection with influenza A(H7N9). 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 following seasons and cases were reported in 2017, including the fifth (2016–17) and largest wave to date, which included the emergence of highly pathogenic avian influenza (HPAI) strains that have caused some zoonoses, though few human cases were reported during the 2017–18 season [5]. WHO posted an analysis of information on A(H7N9) viruses on 10 February 2017 [6]; a summary and assessment of influenza viruses at the human-animal interface on 13 December 2018 indicates that A(H7N9) avian influenza viruses continue to be detected by agricultural authorities in China but at lower levels than before [7], with the latest human case having occurred early in February 2018 [8]. The latest overview of avian influenza by ECDC in collaboration with the European Food Safety Authority and the EU Reference Laboratory for Avian Influenza was published on 27 September 2018 and can be found on the ECDC website [9].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human–animal interface was published by WHO on 13 December 2018, indicating that various A(H5Nx) subtypes continue to be detected in birds in Africa, Europe and Asia, notably A(H5N6) viruses; no new human cases were detected since the two in China that were reported in the assessment published on 1 November 2018 [7]. By 13 December 2018, no cases of human infection by A(H5N1) viruses had been reported to WHO in 2018 [10]. On 18 November 2016, ECDC published a rapid risk assessment related to outbreaks of highly pathogenic avian influenza H5N8 viruses in Europe [11]. As described above, the EU Reference Laboratory for Avian Influenza, in collaboration with ECDC and the European Food Standards Agency, published on 27 September 2018 the latest overview of avian influenza, which can be found on the ECDC website [9].

WHO CC reports

A description of results generated by the London WHO CC at the WIC and used at WHO vaccine composition meetings held in 1) WHO Geneva, 19–21 February 2018, and 2) CDC Atlanta, 24–26 September 2018, can be found at:

https://www.crick.ac.uk/sites/default/files/2018-07/crick_feb2018_report_for_the_web.pdf [accessed 15 Jan 2019]

and

https://www.crick.ac.uk/sites/default/files/2018-10/September%202018%20interim%20report_opt.pdf [accessed 15 Jan 2019]

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 most viruses from non-EU/EEA countries were recovered from the EpiFlu database of GISAID; those that were generated by WHO CC London are indicated (@). 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 WHO CC London.

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