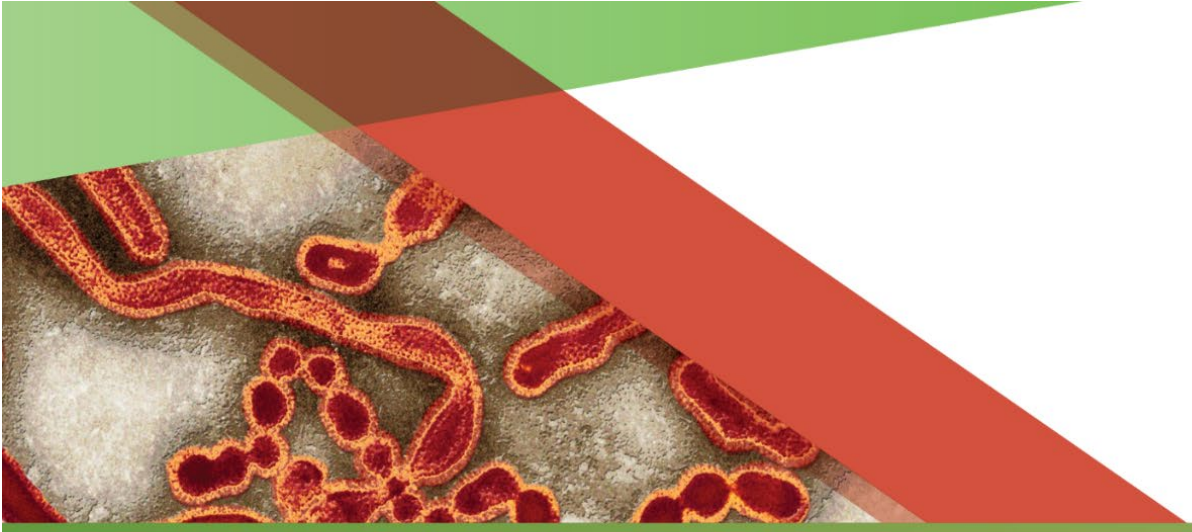


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, June 2020

Summary

This is the eighth report for the 2019–20 influenza season. As of week 25/2020, 164 883 influenza detections across the WHO European Region had been reported; 73% type A viruses, with A(H1N1)pdm09 prevailing over A(H3N2), and 27% type B viruses, with 4 479 (98%) of 4 568 ascribed to a lineage being B/Victoria.

Since the May 2020 characterisation report[1], nine shipments of influenza-positive specimens from EU/EEA countries have been received at the London WHO CC, the Francis Crick Worldwide Influenza Centre (WIC). In total, 1 608 virus specimens, with collection dates after 31 August 2019, have been received.

Of the 99 A(H1N1)pdm09 viruses from EU/EEA countries characterised antigenically since the May report, 77 were well recognised by antisera raised against the 2019–20 vaccine virus, A/Brisbane/02/2018. Of those viruses, 22, that showed poor reactivity generally carried amino acid substitutions (notably N156K) in the HA1 150-loop region. The 397 EU/EEA test viruses with collection dates from week 40/2019 genetically characterised at the WIC have fallen within subclades of clade 6B.1A: 358 6B.1A5A, 29 6B.1A5B, 1 6B.1A6 and 9 6B.1A7.

The majority (52) of the 68 A(H3N2) viruses from EU/EEA countries characterised antigenically in June were clade 3C.3a and were well recognised by antiserum raised against egg-propagated A/Kansas/14/2017, the current vaccine virus. Globally, approximately equal proportions of clade 3C.3a and subgroups 3C.2a1b+T131K and 3C.2a1b+T135K viruses have been detected, but for viruses detected since 1 February 2020, subgroups 3c.2a1b+T135KA/B have prevailed in the USA while those of clade 3C.3a and subgroup 3C.2a1b+T131K have dominated in Europe. In total, 438 viruses from EU/EEA countries have been characterised genetically at the WIC: 245 clade 3C.3a, 126 3C.2a1b+T131K, 48 3C.2a1b+T135K-A and 19 3C.2a1b+T135K-B.

Sixty-nine B/Victoria-lineage viruses from EU/EEA countries were antigenically characterised in June, 63 subclade 1A(Δ3)B, one subclade 1A(Δ2) and five not sequenced. Approximately 25% of the subclade 1A(Δ3)B viruses were not recognised well by antiserum raised against B/Washington/02/2019, the vaccine virus for the 2020–2021 northern hemisphere influenza season. Poor recognition was generally associated with HA1 amino acid substitutions of either N126K or E128K. In total, 269 EU/EEA viruses have been characterised genetically at the WIC: 255 subclade 1A(Δ3)B and 14 subclade 1A(Δ2).

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, April 2020. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/influenza-characterisation-report-may-2020.pdf

This report was prepared by Rod Daniels, Burcu Ermetal, Aine Rattigan and John McCauley (Crick Worldwide Influenza Centre) for the European Centre for Disease Prevention and Control under an ECDC framework contract.

Suggested citation: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, June 2020. Stockholm: ECDC; 2020.

All eight EU/EEA viruses characterised genetically at the WIC since week 40/2019, as all recently circulating B/Yamagata-lineage viruses, belong to genetic clade 3 and contain at least two HA amino acid substitutions (HA1 L172Q and M251V) compared to B/Phuket/3073/2013, the antigenic effects of which have been minimal as assessed in earlier reports.

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 2019–20 season (weeks 40/2019–25/2020), with a total of 164 883 detections over this period, of which 15 were detected over weeks 21-25/2020. Of the type B viruses ascribed to a lineage (n = 4 568) B/Victoria-lineage viruses (n = 4 479) have predominated over B/Yamagata-lineage viruses (n = 89) by a large margin, while for type A viruses subtyped (n = 47 195) A(H1N1)pdm09 viruses (56.0%) have predominated over A(H3N2) viruses (44.0%). Overall, there have been 41 064 (19.9%) less influenza detections reported than in 2018–19, but this is probably due to the increasing number of countries that either stopped influenza surveillance or stopped reporting (or reported sporadically) to TESSy from week 5/2020, due to responses to COVID–19 which WHO declared a pandemic on 11 March 2020 (week 11/2020). With this caveat, the ratio of type A to type B detections is dramatically reduced compared with the 2018–19 season (86:1 to 2.7:1), and while proportions of influenza A subtypes are similar, B/Victoria-lineage viruses have predominated among the type B viruses compared to near equivalence with B/Yamagata-lineage viruses in the 2018–19 season.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2019–20 season (weeks 40/2019–25/2020)[a]

	Cumulative number of detections			Totals*		Totals for 2018-19 season*		
Virus type/subtype/lineage	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Number	%	Ratios
Influenza A	**11302**	**108948**	**120250**	**72.9**	**2.7:1**	**203564**	**98.8**	**86:1**
A(H1N1)pdm09	6126	20302	26428	56.0		44179	57.2	
A(H3N2)	4174	16593	20767	44.0	0.79:1	33117	42.8	0.7:1
A not subtyped	1002	72053	73055			126271		
Influenza B	**6325**	**38308**	**44633**	**27.1**		**2380**	**1.2**	
Victoria lineage	2449	2030	4479	98.1		79	47.9	
Yamagata lineage	23	66	89	1.9	0.02:1	86	52.1	1.1:1
Lineage not ascribed	3853	36212	40065			2215		
Total detections (total tested)	**17627 (51946)**	**147256 (>874433)**	**164883 (>926379)**			**205947 (>849439)**		

[a] Numbers taken from Flu News Europe week 20/2020 and weeks 21-25/2020 reports

*** 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 40/2019, 61 shipments of specimens (virus isolates and/or clinical specimens) have been received at the Crick Worldwide Influenza Centre (WIC), from 27 EU/EEA countries, nine of which were received in June 2020 (from Cyprus, Denmark, Estonia, Finland, France, Ireland, Italy, Norway and the UK-Scotland). The packages contained 1 608 virus-related samples with collection dates after 31 August 2019 and were made up of 1 134 type A viruses, with 523 and 597 subtyped as A(H1N1)pdm09 and A(H3N2), respectively, and 474 type B viruses, with 371 and 18 ascribed to B/Victoria and B/Yamagata lineages, respectively (Tables 2a/b). Genetic and antigenic characterisation data generated at the WIC, for viruses with collection dates after 31 August 2019 and until 31 January 2020, contributed to the WIC virus characterisation report (the deadline for the report was 21 February 2020) that was presented at the WHO influenza vaccine composition meeting (VCM) in February 2020, when recommendations were made for the northern hemisphere 2020–21 season. Data on viruses with collection dates after 31 January 2020 will contribute to the WIC VCM report in September for recommendations for the southern hemisphere 2021 season. Recommendations for the current 2019–20 northern hemisphere and the subsequent 2020 southern hemisphere and 2020–21 northern hemisphere seasons, have been published [1, 2, 3].

We (WIC) thank those nine WHO-recognised national influenza centres (NICs) that have responded to messages requesting sharing of influenza-positive samples with recent collection dates. We encourage other NICs and laboratories that share influenza-positive samples with the WIC to do so in coming months to allow virus characterisation in time for the September 2020 WHO VCM. Please note that to inform the September 2020 southern hemisphere influenza VCM we focus on samples with collection dates from 1 February 2020 on, and more than one shipment from a country is encouraged to ensure that we capture any 'end-of-season' samples from countries in the northern hemisphere.

During the lockdown imposed by the UK Government due to the COVID-19 pandemic, WIC has been operating with reduced staff numbers. Consequently, only gene sequencing was performed to assess the emergence of any new genetic groups during March to May. Virus isolation and propagation for phenotypic analyses was re-instated in June following relaxation of lockdown restrictions in the UK. Therefore, this report is based mainly on phylogenetic analyses of complete HA gene sequences submitted to the EpiFlu™ database of GISAID during the month of June (inclusive of sequences generated at the WIC) with those from EU/EEA countries highlighted, together with a limited amount of antigenic and antiviral susceptibility data.

Table 2a. Summary of clinical samples and virus isolates*, with collection dates from 1 September 2019, contained in packages received from EU/EEA Member States since week 40/2019: September to December 2019.

MONTH / Country	TOTAL RECEIVED Seasonal viruses	A Number received	A Number propagated[1]	H1N1pdm09 Number received	H1N1pdm09 Number propagated[1]	H3N2 Number received	H3N2 Number propagated[2]		B Number received	B Number propagated[1]	B Victoria lineage Number received	B Victoria lineage Number propagated[1]	B Yamagata lineage Number received	B Yamagata lineage Number propagated[1]
2019														
SEPTEMBER														
Czech Republic	1					1	1							
Finland	1					1	1							
France	6			1	1	3	2	1			2	2		
Norway	7			1	1	4	1	3			2	2		
Romania	1					1	0	1						
Sweden	3			2	2	1	1							
United Kingdom	4			2	2	2	0							
OCTOBER														
Denmark	3			2	2	1	1							
Finland	2			1	1	1	1							
France	5			3	3						2	2		
Germany	6			2	2	4	2	1						
Greece	1					1	1							
Iceland	9					8	4	4			1	1		
Ireland	11			1	1	9	1	1					1	0
Latvia	3			1	1						2	2		
Lithuania	1										1	1		
Netherlands	3			1	1	2	0	2						
Norway	28			5	4	19	3	15			3	2	1	0
Poland	1	1	0											
Portugal	7			2	2	2	1		3	0				
Spain	5			3	3						2	2		
Sweden	3					2	2				1	1		
United Kingdom	29			5	2	21	11	6			3	0		
NOVEMBER														
Austria	4			2	2	1	0	1					1	1
Belgium	3			2	1	1	1							
Croatia	3			2	2						1	1		
Czech Republic	2					2	2							
Denmark	16			7	7	6	3	3			3	3		
Finland	1			1	1									
France	16			8	8	4	3	1			2	2	2	2
Germany	8			5	5	3	0	3						
Greece	1					1	0							
Iceland	3					2	0	2			1	1		
Ireland	49			18	12	22	7	5	2	0	7	6		
Italy	7			2	2	3	1	2			2	2		
Latvia	10			2	2	3	3				5	5		
Lithuania	2			2	2									
Netherlands	3			2	2	1	1							
Norway	22			6	5	9	3	4			4	4	3	1
Poland	1	1	0											
Portugal	102	1	0	13	11	3	0		26	0	59	20		
Slovenia	1			1	1									
Spain	6			2	2	2	2		1	0	1	1		
Sweden	8			5	5	1	0	1			2	2		
United Kingdom	62			9	4	52	14	2			1	0		
DECEMBER														
Austria	18			5	5	9	7	2			4	4		
Belgium	21			5	3	11	in process				5	in process		
Bulgaria	2			1	0	1	1							
Croatia	6			4	1	1	0	1			1	0		
Cyprus	2					1	0		1	0				
Czech Republic	2					2	1	1						
Denmark	1			1	in process									
Estonia	1			1	1									
Finland	1			1	1									
France	39			14	14	9	in process				16	in process		
Germany	13			6	6	6	4	2			1	1		
Greece	6			4	0	2	0							
Iceland	5			2	2	2	0	1			1	1		
Italy	12			2	2	6	2	4			4	4		
Latvia	1					1	0	1						
Lithuania	20	1	0	6	6	12	9	3			1	1		
Netherlands	10			1	1	9	7	2						
Norway	15			8	5	1	0				1	0	5	2
Poland	5	2	0	1	0	2	1	0						
Portugal	20			2	2	3	2	1			15	15		
Romania	8										8	8		
Slovenia	9			5	5	3	3				1	1		
Spain	30			12	12	6	0	6			12	12		
United Kingdom	18			4	in process	11	in process				3	in process		

Table 2b. Summary of clinical samples and virus isolates*, with collection dates from 1 September 2019, contained in packages received from EU/EEA Member States since week 40/2019: January to April 2020.

MONTH / Country	TOTAL RECEIVED Seasonal viruses	A Number received	A Number propagated[1]	H1N1pdm09 Number received	H1N1pdm09 Number propagated[1]	H3N2 Number received	H3N2 Number propagated[2]		B Number received	B Number propagated[1]	B Victoria lineage Number received	B Victoria lineage Number propagated[1]	B Yamagata lineage Number received	B Yamagata lineage Number propagated[1]
2020														
JANUARY														
Austria	2	1	0						1	0				
Belgium	52			29	in process	18	in process				5	in process		
Bulgaria	28			14	in process	12	in process				2	0		
Cyprus	27			4	0	22	in process				1	in process		
Czech Republic	5			2	2	3	3							
Denmark	10			4	in process	5	in process				1	1		
Estonia	17			8	in process	4	in process				5	in process		
Finland	4			2	2	2	2							
France	8					4	in process				4	in process		
Germany	24			6	6	9	8	1			8	8	1	1
Greece	45			22	8	20	5	3	2	0	1	1		
Italy	3			1	1	1	1				1	1		
Lithuania	2					2	1	1						
Norway	22			1	0	14	in process				6	in process	1	in process
Poland	9	1	0	4	in process	2	in process		2	in process				
Portugal	7			1	in process	1	in process				5	in process		
Romania	15			4	3	7	1		1	0	3	3		
Slovakia	1					1	in process							
Slovenia	7			4	in process	2	in process				1	1		
Spain	18			13	12				1	0	4	4		
United Kingdom	38			18	in process	11	in process		3	0	6	in process		
FEBRUARY														
Austria	2	1	0						1	0				
Belgium	50			28	28	17	17				5	5		
Bulgaria	26			5	5	12	9	1			9	9		
Cyprus	38			6	in process	21	in process				11	in process		
Denmark	9					6	in process				3	3		
Estonia	12	1	in process	5	in process	1	in process		3	in process	2	in process		
Finland	8			5	5	1	1				2	2		
France	32			14	in process	7	in process				9	in process	2	in process
Germany	25			13	13	7	5	2			5	5		
Iceland	10			4	4	2	2				4	4		
Ireland	12			1	in process				4	in process	7	in process		
Italy	25			7	in process	12	12				6	in process		
Norway	13			4	in process	5	in process				3	in process	1	in process
Poland	22			10	in process	9	in process		3	in process				
Portugal	32			29	in process	2	in process				1	in process		
Slovakia	8			2	2	4	4				2	2		
Slovenia	15			3	3	9	in process				3	3		
Sweden	18			8	8	5	3	2			5	5		
United Kingdom	14			1	1	2	2		7	in process	4	4		
MARCH														
Belgium	6			4	4						2	2		
Bulgaria	9			1	1	1	1				7	7		
Cyprus	9			1	in process	1	in process				7	in process		
Estonia	5	4	in process						1	in process				
Finland	4					2	2				2	2		
France	19			8	in process	4	in process				7	in process		
Germany	5			2	2	2	2				1	1		
Iceland	13			4	4	6	6				3	3		
Ireland	14			3	in process	1	in process		6	in process	4	in process		
Norway	37			5	in process	18	in process				14	in process		
Poland	4			4	in process									
Portugal	4			3	in process	1	in process							
Slovenia	8					2	2				6	6		
United Kingdom	19								17	in process	2	in process		
APRIL														
Iceland	1										1	1		
Norway	1										1	in process		
27 Countries	1608	14	0	523	282	597	199	92	85	0	371	192	18	7
		0.87%		32.5%		37.1%			5.3%		23.1%		1.1%	
		70.5%							29.5%					

*** Note: Where clinical sample and a virus isolate from the same patient were received, this is counted as one in the Total Received and following columns.**

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

Includes clinical samples in lysis-mix from Northern Ireland and Scotland and RNA extracts from Greece and Portugal for which genetic characterisation only can be performed. In addition, some clinical samples from Bulgaria, Estonia, Greece, Ireland, Poland and Portugal were not cultured as either sequencing from the clinical sample failed or sequences generated were identical to those from other clinical samples.

Cells with an orange background indicate samples that were sequenced only (due either to restricted characterisation conducted during COVID-19 lockdown or the samples having collection dates before 2020-01-31 characterisation of which will not be used to inform the WHO VCM in September 2020).

As of 2020-07-02

Influenza A(H1N1)pdm09 virus analyses

The first A(H1N1)pdm09 HA phylogeny is repeated from the May 2020 report and was generated based on sequences deposited in GISAID for recently circulating viruses, with collection dates from 1 February 2020, submitted to GISAID in May 2020 (Figure 1a). The second is again based on viruses with collection dates from 1 February 2020, but with sequences deposited in GISAID during June 2020; a total of 341 sequences had been deposited, 338 subgroup 6B.1A5A and three 6B.1A5B, so a representative phylogeny is shown including HA gene sequences of 87 subgroup 6B.1A5A and two subgroup 6B.1A5B viruses (Figure 1b). All recently circulating viruses fell into clade 6B.1A, defined by the amino acid substitutions **S74R**, **S84N**, **S162N** (introducing a potential N-linked glycosylation site), **S164T** (which alters the glycosylation motif at residues 162 to 164), **I216T** and **I295V** in **HA1**. Within clade 6B.1A, clusters of viruses (genetic groups) encoding a range of **HA** amino acid substitutions have emerged, with most recently circulating viruses carrying the substitution **S183P** in **HA1**, although this is not retained in all genetic groups. Figures 1a and 1b are annotated with **HA1 S183P** substitution groups assigned for the February 2019 WHO Vaccine Consultation Meeting (6B.1A/183P-1 to -7, abbreviated to 6B.1A1 to 6B.1A7); the recommended vaccine viruses for the northern hemisphere 2019–2020 and 2020–2021 influenza seasons are shown in red [1, 3]. The seven subclades are defined by the following HA amino acid substitutions:

1. Subclade **6B.1A1** viruses, represented by the current vaccine virus **A/Brisbane/02/2018**, carry an HA gene mutation encoding **HA1 S183P** amino acid substitution;
2. Subclade **6B.1A2** viruses, represented by **A/Denmark/2728/2019**, carry HA gene mutations encoding **HA1 S183P** and **L233I** with **HA2 V193A** amino acid substitutions - a subgroup within this subclade has emerged with additional **HA1** amino acid substitutions of **N129D, K130N, P137S, N156K** and **K211R** (e.g. **A/Hong Kong/110/2019**);
3. Subclade **6B.1A3** viruses, represented by **A/Norway/3737/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions;
4. Subclade **6B.1A4** represented by **A/Hungary/20/2018** carries HA gene mutations encoding **HA1 N129D, A144E** and **S183P** amino acid substitutions;
5. Subclade **6B.1A5** viruses carry HA gene mutations encoding **HA1 S183P** and **N260D** amino acid substitutions and splits into two subgroups designated **6B.1A5A** represented by **A/Norway/3433/2018** with additional **HA1** amino acid substitutions of **N129D** and **T185A**, and **6B.1A5B** represented by **A/Switzerland/3330/2017** with additional amino acid substitutions of **HA1 E235D** and **HA2 V193A**;
6. Subclade **6B.1A6** viruses, represented by **A/Ireland/84630/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions, like subclade **6B.1A3** viruses, but fall within a separate phylogenetic branch which is closer to subclade **6B.1A5** viruses;
7. Subclade **6B.1A7** viruses, represented by **A/Slovenia/1489/2019**, carry HA gene mutations encoding **HA1 K302T** and **HA2 I77M, N169S** and **E179D** amino acid substitutions sometimes with additional **HA1** substitutions of **E68D, S121N** and **L161I** (e.g. **A/Moscow/193/2019**). Note: a subgroup of this subclade has emerged with **P183S** (reversion)**, T185I, I240V** and **I286L** substitutions in **HA1** (e.g. **A/Estonia/120012/2019**).

The majority of recently circulating viruses have fallen in subgroup **6B.1A5A**, which contains a number of virus clusters, three of which have been detected in significant numbers defined by: (i) **HA1 D187A** and **Q189E** substitutions, (ii) **HA2 V193A** substitution and (iii) **HA1 N156K** substitution with the great majority in this cluster also having **HA1 K130N**, **L161V**, **V250A** and **HA2 E179D** substitutions. Relatively few viruses in subgroup **6B.1A5B** (with **HA1 K130N**, **K160M**, **T216K**, **E235D**, **H296N** and **HA2 V193A** substitutions) have also been detected. However, as indicated in previous reports, based on sequences deposited in GISAID for viruses detected from 1 February 2020 onwards, the vast majority fell in subgroup 6B.1A5A, with an approximately equal split between two of the genetic clusters defined above, (i) and (iii), with a minority falling in subgroup **6B.1A5B** (Figures 1a and 1b). This pattern was seen for viruses detected in the US and EU/EEA countries. Both phylogenies are made up largely with sequences from viruses detected in February and March, and have very similar profiles. The three viruses with collection dates in April all fall in genetic clusters (iii) (Figure 1a) and carry **HA1 N156K** substitution which is known to have significant antigenic effect based on HI assays conducted with post-infection ferret antisera raised against A(H1N1)pdm09 vaccine viruses.

The great majority of viruses in the various subgroups characterised to date, with the exception of those in genetic cluster (iii), have remained antigenically similar to the northern hemisphere 2019–2020 vaccine virus, A/Brisbane/02/2018, as assessed with post-infection ferret antisera and shown in earlier characterisation reports; this is also the case for the relatively small number of viruses tested with antisera raised against A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like viruses (with **HA1 D187A** and **Q189E** amino acid substitutions) that were recommended for use in the northern hemisphere 2020–2021 influenza season [3].

Tables 3-1 to 3-3 show the results of haemagglutination inhibition (HI) assays of A(H1N1)pdm09 viruses performed, with a panel of post-infection ferret antisera, since the May 2020 report. The 99 test viruses are sorted by date of collection and genetic group/subgroup, where known at the time of writing this report; 90 were subgroup **6B.1A5A** viruses, two were subgroup **6B.1A5B** viruses and sequencing is in progress (pending) for seven. Table 3-4 shows a summary of the results.

The panel of post-infection ferret antisera was raised against 10 individual viruses, three of which were egg-propagated viruses representing recently recommended vaccine viruses. Antisera raised against nine of the viruses, all but that raised against A/Denmark/3280/2019, showed similar HI reactivity recognising 64 to 74 (65-75%) test viruses at titres

within twofold of respective homologous titres and 72 to 74 (73-75%) at titres within fourfold (Table 3-4). A/Denmark/3280/2019 is representative of a cluster of viruses carrying HA1 amino acid substitutions N156K, K130N, L161I and V250A and antiserum raised against it recognised only 22 (22%) test viruses at titres within fourfold of the homologous titre; all 22 viruses carried the N156K substitution with additional HA1 amino acid substitutions (Tables 3-1 to 3-4). These 22 viruses showed poor reactivity with antisera raised against all three vaccine viruses and of five additional viruses showing low reactivity; sequence is pending for two, A/Haute Normandie/1230/2020 has HA1 V47A, E112D and R205G substitutions (Table 3-1), while B/Belgium/G0204/2020 and B/Belgium/G0290/2020 have HA1 R74K, P137S and S157L substitutions (Table 3-2).

Figure 1a. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, May 2020)

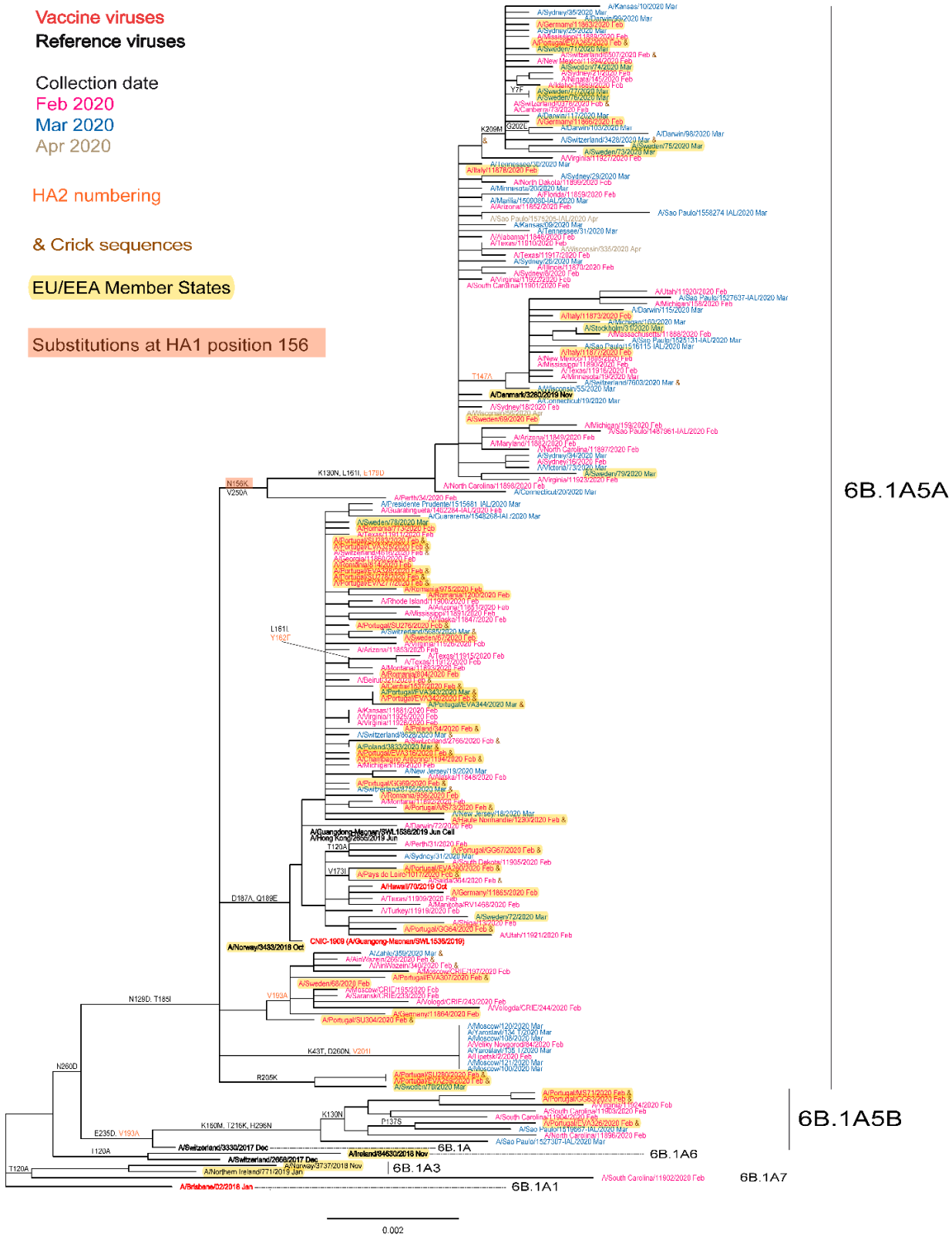


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, June 2020)

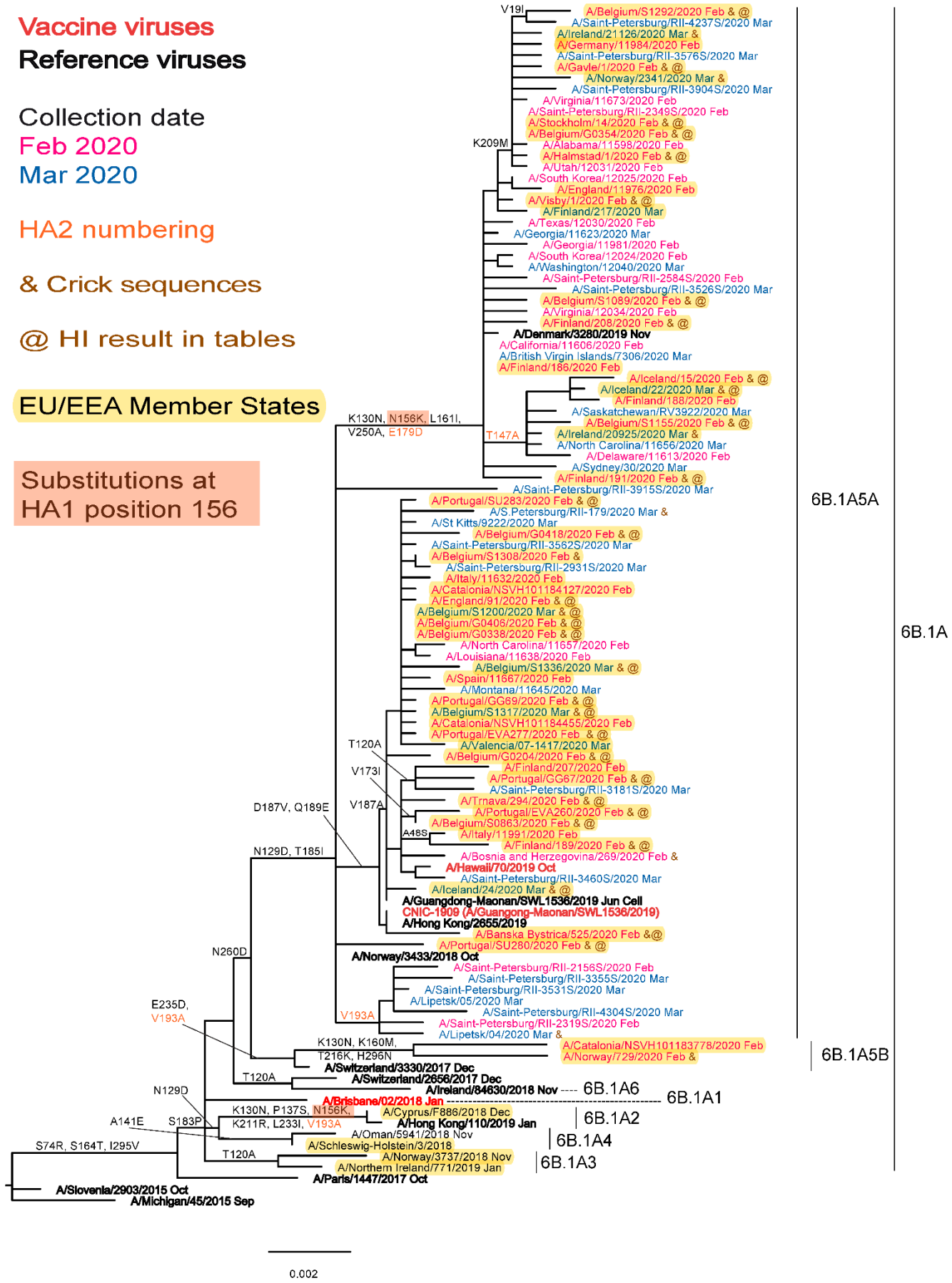


Table 3-1. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
								Post-infection fereret antisera						
								NEW			NEW			
	Passage history			A/Mich 45/15 Egg	A/Paris 1447/17 MDCK	A/Bris 02/18 Egg	A/Norway 3433/18 MDCK	A/Denmark 3280/19 MDCK	A/Ire 87733/19 Egg	A/G-M SWL1536/19 Egg	A/G-M SWL1538/19 MDCK	A/Swit 3330/17 Egg	A/Ire 84630/18 MDCK
	Ferret number			F31/16[*1]	F03/18[*2]	F09/19[*1]	F04/19[*1]	F08/20[*1]	F18/20[*1]	F12/20[*1] St Jude's	F09/20[*1] St Jude's	F23/18[*1]	F08/19[*1]
	Genetic group			6B.1	6B.1A	6B.1A1	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5B	6B.1A6
REFERENCE VIRUSES													
A/Michigan/45/2015	6B.1	2015-09-07	E3/E3	640	1280	640	1280	40	640	640	1280	640	640
A/Paris/1447/2017	6B.1A	2017-10-20	MDCK1/MDCK3	1280	2560	1280	2560	<	1280	1280	2560	1280	2560
A/Brisbane/02/2018	6B.1A1	2018-01-04	E3/E1	1280	2560	1280	2560	40	1280	1280	1280	1280	1280
A/Norway/3433/2018	6B.1A5A	2018-10-30	MDCK3	320	640	320	1280	<	320	640	1280	320	640
A/Denmark/3280/2019	6B.1A5A N156K	2019-11-10	MDCK4/MDCK3	80	80	80	80	1280	80	40	320	40	40
A/Ireland/87733/2019	6B.1A5A D187A, Q189E	2019-11-03	E3	640	1280	640	2560	40	1280	1280	2560	640	1280
A/Guangong-Maonan/SWL1536/2019	6B.1A5A D187A, Q189E	2019-06-17	E3/E1	640	1280	640	2560	<	640	1280	2560	640	640
A/Guangong-Maonan/SWL1536/2019	6B.1A5A D187A, Q189E	2019-06-17	C2/MDCK1	640	1280	640	2560	<	640	1280	2560	640	1280
A/Switzerland/3330/2017	6B.1A5B clone 35	2017-12-20	E6/E2	640	1280	640	2560	<	320	640	1280	640	640
A/Ireland/84630/2018	6B.1A6	2018-11-28	MDCK1/MDCK3	640	1280	640	1280	<	640	1280	1280	640	1280
TEST VIRUSES													
A/Bulgaria/886/2020	6B.1A5A	2020-02-03	SIAT2/MDCK1	640	1280	640	1280	<	640	1280	1280	640	640
A/Slovenia/973/2020	6B.1A5A	2020-02-03	MDCKx/MDCK1	640	1280	320	1280	<	320	640	1280	320	320
A/Portugal/GG64/2020	6B.1A5A	2020-02-03	MDCK2/MDCK1	640	1280	640	1280	<	640	1280	2560	320	640
A/Portugal/SU283/2020	6B.1A5A	2020-02-04	MDCK3/MDCK1	640	1280	640	1280	<	640	1280	1280	320	640
A/Bulgaria/885/2020	6B.1A5A	2020-02-05	SIAT2/MDCK1	640	1280	640	1280	<	640	640	1280	320	320
A/Bulgaria/918/2020	6B.1A5A	2020-02-06	SIAT2/MDCK1	1280	2560	640	2560	<	1280	2560	2560	640	1280
A/Pays de Loire/1017/2020	6B.1A5A	2020-02-07	MDCK1/MDCK1	640	1280	640	2560	<	640	1280	1280	640	640
A/Portugal/EVA260/2020	6B.1A5A	2020-02-08	MDCK3/MDCK1	1280	2560	1280	2560	<	640	1280	1280	640	1280
A/Portugal/EVA259/2020	6B.1A5A	2020-02-08	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	640
A/Portugal/SU280/2020	6B.1A5A	2020-02-09	MDCK2/MDCK1	640	1280	640	1280	<	320	640	1280	320	640
A/Portugal/GG67/2020	6B.1A5A	2020-02-10	MDCK2/MDCK1	640	1280	640	1280	<	640	640	1280	320	640
A/Slovenia/1261/2020	6B.1A5A N156K, V24I, I116V, K130N, L161I, DQ, K209M, V250A	2020-02-11	SIATx/MDCK1	40	<	40	80	640	40	40	80	40	40	<
A/Sachsen-Anhalt/20/2020	6B.1A5A	2020-02-13	C1/MDCK1	1280	1280	1280	2560	<	640	2560	2560	2560	640	1280
A/Portugal/EVA277/2020	6B.1A5A	2020-02-13	MDCK3/MDCK1	640	1280	640	2560	<	640	1280	1280	1280	640	640
A/Portugal/GG69/2020	6B.1A5A	2020-02-13	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	2560	1280	640	1280
A/Bulgaria/988/2020	6B.1A5A	2020-02-14	SIAT2/MDCK1	640	640	320	1280	<	640	640	1280	320	320	320
A/Niedersachsen/52/2020	6B.1A5A N156K, K130N, L161I, DQ, K209M, V250A	2020-02-14	C1/MDCK1	40	<	<	80	320	<	40	80	40	<	<
A/Saarland/6/2020	6B.1A5A N156K, K130N, L161I, DQ, K209M, V250A	2020-02-17	C1/MDCK1	40	40	40	160	640	40	40	160	80	40	40
A/Haute Normandie/1230/2020	6B.1A5A V47A, E112D, R205G	2020-02-18	MDCK1/MDCK1	80	<	40	160	640	40	80	160	80	40	40
A/Portugal/EVA316/2020	6B.1A5A	2020-02-18	MDCK1/MDCK1	640	1280	640	2560	<	640	1280	2560	1280	640	640
A/Bulgaria/1030/2020	6B.1A5A	2020-02-19	SIAT2/MDCK1	640	1280	640	2560	40	640	1280	2560	2560	640	1280
A/Sachsen/49/2020	6B.1A5A	2020-02-20	C1/MDCK1	1280	1280	640	2560	<	640	1280	2560	1280	640	1280
A/Slovenia/1564/2020	6B.1A5A	2020-02-24	SIATx/MDCK1	640	1280	640	1280	40	640	1280	2560	1280	320	640
A/Brandenburg/8/2020	6B.1A5A N156K, K130N, L161I, DQ, V250A	2020-02-24	C1/MDCK1	40	40	40	80	640	40	40	80	40	40	<
A/Mecklenburg-Vorpommern/4/2020	6B.1A5A N156K, K130N, P137S, L161I, DQ, V250A	2020-02-24	C1/MDCK1	40	<	40	80	640	40	40	80	40	40	40
A/Portugal/EVA311/2020	6B.1A5A	2020-02-24	MDCK2/MDCK1	640	1280	640	1280	<	640	1280	1280	1280	320	640
A/Baden-Wurttemberg/116/2020	6B.1A5A	2020-02-28	C1/MDCK1	1280	1280	640	2560	40	640	1280	2560	1280	640	640
A/Schleswig-Holstein/8/2020	6B.1A5A	2020-02-28	C1/MDCK1	640	1280	640	1280	40	640	1280	1280	640	320	640
A/Hessen/32/2020	6B.1A5A	2020-03-02	C1/MDCK1	640	1280	640	1280	40	640	1280	1280	1280	320	640
A/Thuringen/44/2020	6B.1A5A	2020-03-02	C1/MDCK1	640	1280	640	1280	40	640	1280	1280	640	640	640
A/Bulgaria/1243/2020	6B.1A5A	2020-03-04	SIAT2/MDCK1	640	1280	640	1280	<	640	1280	2560	1280	640	640
A/Portugal/EVA239/2020	6B.1A5B	2020-02-02	MDCK1/MDCK1	1280	2560	1280	1280	80	640	1280	1280	1280	640	1280

	Vaccine	Vaccine
	NH 2018-19	NH 2019-20
	SH 2019	SH 2020

	Vaccine	Vaccine
	NH 2020-21	NH 2020-21

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

1 < = <40; 2 < = <80; ND =Not Done

Sequences in phylogenetic trees

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

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection fereret antisera										
					A/Mich 45/15 Egg	A/Paris 1447/17 MDCK	A/Bris 02/18 Egg	A/Norway 3433/18 MDCK	A/Denmark 3280/19 MDCK	A/Ire 87733/19 Egg	A/G-M SWL1536/19 Egg	A/G-M SWL1536/19 MDCK	A/Swit 3330/17 Egg	A/Ire 84630/18 MDCK
					F31/16[-1]	F03/18[-2]	F09/19[+1]	F04/19[+1]	F08/20[+1]	St Jude's F18/20	F12/20[+1]	F09/20[+1]	F23/18[-1]	F08/19[+1]
					6B.1	6B.1A	6B.1A1	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5B	6B.1A6
REFERENCE VIRUSES														
A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	640	1280	640	1280	40	640	640	1280	640	1280
A/Paris/1447/2017		6B.1A	2017-10-20	MDCK1/MDCK3	1280	2560	1280	2560	<	1280	1280	2560	1280	2560
A/Brisbane/02/2018		6B.1A1	2018-01-04	E3/E1	1280	2560	1280	2560	40	1280	1280	1280	1280	1280
A/Norway/3433/2018		6B.1A5A	2018-10-30	MDCK3	320	640	320	1280	<	640	640	640	320	640
A/Denmark/3280/2019	N156K	6B.1A5A	2019-11-10	MDCK4/MDCK3	80	80	80	80	640	80	40	160	40	40
A/Ireland/87733/2019		6B.1A5A	2019-11-03	E3	1280	1280	640	2560	40	1280	1280	1280	1280	1280
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	2019-06-17	E3/E1	640	640	640	2560	<	640	1280	1280	640	640
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	2019-06-17	C2/MDCK1	640	1280	1280	2560	<	640	1280	1280	640	1280
A/Switzerland/3330/2017	clone 35	6B.1A5B	2017-12-20	E6/E2	640	1280	1280	2560	<	320	640	640	640	640
A/Ireland/84630/2018		6B.1A6	2018-11-28	MDCK1/MDCK3	640	1280	640	1280	<	640	1280	640	1280	1280
TEST VIRUSES														
A/Centre/675/2020		6B.1A5A	2020-02-03	MDCK1/MDCK2	640	1280	640	2560	<	640	1280	1280	640	1280
A/England/91/2020		6B.1A5A	2020-02-06	SIAT1/MDCK1	1280	1280	1280	2560	<	1280	1280	1280	1280	1280
A/Belgium/S1052/2020		6B.1A5A	2020-02-04	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	640
A/Belgium/S0547/2020		6B.1A5A	2020-02-04	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	640
A/Belgium/S0546/2020		6B.1A5A	2020-02-04	MDCK1/MDCK1	640	1280	640	1280	<	320	1280	1280	640	640
A/Belgium/S0535/2020		6B.1A5A	2020-02-04	MDCK1/MDCK1	640	1280	640	1280	<	320	1280	1280	320	640
A/Belgium/G0204/2020	S157L, R74K, P137S	6B.1A5A	2020-02-04	MDCK1/MDCK1	160	320	160	640	<	80	160	640	80	80
A/Belgium/S1046/2020		6B.1A5A	2020-02-07	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	640
A/Belgium/S0707/2020		6B.1A5A	2020-02-07	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	2560	640	640
A/Belgium/S1271/2020		6B.1A5A	2020-02-09	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	640
A/Banska Bystrica/525/2020		6B.1A5A	2020-02-10	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	640	1280
A/Belgium/G0255/2020		6B.1A5A	2020-02-10	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	640	640
A/Belgium/G0237/2020		6B.1A5A	2020-02-10	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	640	640
A/Belgium/S0960/2020		6B.1A5A	2020-02-11	MDCK1/MDCK1	640	1280	640	1280	<	640	640	640	640	640
A/Belgium/G0290/2020	S157L, R74K, P137S N156K, I9N, V19I, K130N, L161I, DQ, K209N, V250A	6B.1A5A	2020-02-11	MDCK1/MDCK1	320	320	160	640	<	160	320	640	160	80
A/Belgium/S1292/2020		6B.1A5A	2020-02-13	MDCK1/MDCK1	<	<	<	40	320	<	<	<	<	<
A/Belgium/G0311/2020		6B.1A5A	2020-02-14	MDCK1/MDCK1	640	1280	320	1280	<	320	640	640	320	640
A/Belgium/S0993/2020		6B.1A5A	2020-02-16	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	320	1280
A/Belgium/S0863/2020		6B.1A5A	2020-02-16	MDCK1/MDCK1	640	1280	640	1280	<	320	640	1280	320	640
A/Trnava/294/2020		6B.1A5A	2020-02-17	MDCKx/MDCK1	1280	1280	640	1280	40	640	1280	1280	640	1280
A/Belgium/G0354/2020	N156K, K130N, L161I, DQ, K209M, V250A	6B.1A5A	2020-02-17	MDCK1/MDCK1	<	<	<	40	320	<	<	<	<	<
A/Belgium/G0338/2020		6B.1A5A	2020-02-18	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	640	640
A/Belgium/S1115/2020		6B.1A5A	2020-02-19	MDCK1/MDCK1	1280	1280	1280	2560	<	640	2560	1280	640	1280
A/Belgium/G0353/2020		6B.1A5A	2020-02-19	MDCK1/MDCK1	640	1280	640	1280	<	640	640	1280	640	640
A/Belgium/G0350/2020	N156K, K130N, P137S, L161I, T184A, I185F, DQ, V250A	6B.1A5A	2020-02-19	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	1280
A/Iceland/04/2020		6B.1A5A	2020-02-20	MDCK1/MDCK1	40	<	<	80	640	40	40	40	40	<
A/Belgium/S1146/2020	N156K, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-23	MDCK1/MDCK1	640	640	640	1280	<	320	1280	1280	320	640
A/Iceland/11/2020	N156K, K130N, K154X, L161I, T184A, I185F, DQ, V250A	6B.1A5A	2020-02-24	MDCK1/MDCK1	80	80	80	160	640	80	80	80	80	40
A/Iceland/10/2020		6B.1A5A	2020-02-24	MDCK1/MDCK1	<	<	40	40	640	<	40	<	<	<
A/Belgium/G0406/2020		6B.1A5A	2020-02-24	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	1280
A/Belgium/S1089/2020	N156K, V47I, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-25	MDCK1/MDCK1	40	<	40	80	640	40	40	1280	40	<
A/Belgium/S1308/2020		6B.1A5A	2020-02-26	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	1280
A/Belgium/S1155/2020	N156K, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-26	MDCK1/MDCK1	80	80	80	160	640	80	80	80	80	40
A/Belgium/H0024/2020		6B.1A5A	2020-02-26	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	1280
A/Belgium/G0418/2020		6B.1A5A	2020-02-26	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	1280
A/Iceland/15/2020	N156K, K130N, L161I, T184A, I185F, DQ, V250A	6B.1A5A	2020-02-27	MDCK1/MDCK1	40	<	40	80	640	40	40	40	40	<
A/Belgium/S1200/2020		6B.1A5A	2020-03-01	MDCK1/MDCK1	640	640	640	1280	<	320	640	640	320	640
A/Belgium/S1317/2020		6B.1A5A	2020-03-03	MDCK1/MDCK1	1280	1280	640	1280	<	640	1280	1280	640	640
A/Belgium/S1336/2020		6B.1A5A	2020-03-05	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	320	640
A/Belgium/G0467/2020		6B.1A5A	2020-03-05	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	320	640
A/Iceland/22/2020	N156K, K130N, P137S, L161K, DQ, V250A	6B.1A5A	2020-03-07	MDCK1/MDCK1	<	<	<	80	160	<	40	40	<	<
A/Iceland/24/2020		6B.1A5A	2020-03-08	MDCK1/MDCK1	640	640	320	640	<	320	640	640	320	640
A/Iceland/34/2020		6B.1A5A	2020-03-22	MDCK1/MDCK1	640	640	320	1280	<	320	640	640	320	640
A/Iceland/36/2020		6B.1A5A	2020-03-25	MDCK1/MDCK1	640	1280	640	1280	<	640	1280	1280	160	640

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

1 <= <40; 2 <= <80; ND =Not Done

Sequences in phylogenetic trees

Vaccine	Vaccine
NH 2018-19	NH 2019-20
SH 2019	SH 2020

Vaccine
NH 2020-21

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

Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection fereret antisera									
					A/Mich 45/15	A/Paris 1447/17	A/Bris 02/18	A/Norway 3433/18	A/Denmark 3280/19	A/Ire 87733/19	A/G-M SWL1536/19	A/G-M SWL1536/19	A/Swit 3330/17	A/Ire 84630/18
		Passage history			Egg	MDCK	Egg	MDCK	MDCK	Egg	Egg	MDCK	Egg	MDCK
		Ferret number			F31/16*1	F03/18*2	F09/19*1	F04/19*1	F08/20*1	St Jude's F18/20	F12/20*1	F09/20*1	F23/18*1	F08/19*1
		Genetic group			6B.1	6B.1A	6B.1A1	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5B	6B.1A6
REFERENCE VIRUSES														
A/Michigan/45/2015		6B.1	2015-09-07	E3/E3	640	1280	640	1280	40	640	640	1280	640	1280
A/Paris/1447/2017		6B.1A	2017-10-20	MDCK1/MDCK3	1280	2560	1280	2560	<	1280	1280	2560	1280	2560
A/Brisbane/02/2018		6B.1A1	2018-01-04	E3/E1	1280	2560	1280	2560	40	1280	1280	1280	1280	1280
A/Norway/3433/2018		6B.1A5A	2018-10-30	MDCK3	320	640	640	1280	<	640	640	640	320	640
A/Denmark/3280/2019	N156K	6B.1A5A	2019-11-10	MDCK4/MDCK3	80	80	80	160	1280	80	80	160	80	40
A/Ireland/87733/2019		6B.1A5A	2019-11-03	E4	1280	1280	640	2560	40	1280	1280	1280	1280	1280
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	2019-06-17	E3/E1	640	640	640	2560	<	640	1280	1280	640	640
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	2019-06-17	C2/MDCK1	640	1280	1280	2560	<	640	1280	1280	640	1280
A/Switzerland/3330/2017	clone 35	6B.1A5B	2017-12-20	E6/E2	640	1280	1280	2560	<	320	640	640	640	640
A/Ireland/84630/2018		6B.1A6	2018-11-28	MDCK1/MDCK3	640	1280	640	1280	<	640	1280	1280	1280	1280
TEST VIRUSES														
A/Finland/173/2020		6B.1A5A	2020-01-25	MDCK1/MDCK1	320	1280	320	1280	<	320	640	320	320	320
A/Finland/177/2020		6B.1A5A	2020-01-29	MDCK1/MDCK1	640	1280	640	2560	<	640	1280	1280	640	640
A/Stockholm/14/2020	N156K, K130N, L161I, DQ, K209M, V250A	6B.1A5A	2020-02-03	SIAT0/MDCK1	80	<	40	160	640	40	40	80	40	<
A/Lund/3/2020		6B.1A5A	2020-02-04	SIAT0/MDCK1	640	1280	640	2560	40	640	1280	1280	640	640
A/Linkoping/3/2020	N156K, K130N, L161I, DQ, K209M, V250A	6B.1A5A	2020-02-08	SIAT0/MDCK1	40	<	<	160	640	<	40	40	40	<
A/Milano/47/2020		pending	2020-02-08	MDCK2/MDCK1	1280	2560	1280	2560	<	640	1280	1280	640	1280
A/Friuli Venezia Giulia/237/2020		pending	2020-02-08	MDCK2/MDCK1	640	1280	640	1280	<	640	1280	1280	640	640
A/Gavle/1/2020	N156K, K130N, L161I, I179V, DQ, K209M, V250A	6B.1A5A	2020-02-10	SIAT0/MDCK1	<	<	<	40	160	<	<	<	<	<
A/Stockholm/29/2020		6B.1A5A	2020-02-11	SIAT0/MDCK1	640	640	320	1280	<	320	1280	640	320	640
A/Finland/189/2020		6B.1A5A	2020-02-11	MDCK1/MDCK1	1280	1280	640	2560	<	640	1280	1280	640	1280
A/Milano/59/2020		pending	2020-02-11	MDCK3/MDCK1	1280	2560	1280	2560	<	640	1280	1280	640	1280
A/Halmstad/1/2020	N156K, K130N, L161I, DQ, K209M, V250A	6B.1A5A	2020-02-13	SIAT0/MDCK1	40	<	40	160	640	40	40	40	40	<
A/Lund/4/2020		6B.1A5A	2020-02-17	SIAT0/MDCK1	640	640	640	2560	40	640	1280	1280	640	640
A/Finland/191/2020	N156K, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-17	MDCK1/MDCK1	80	80	40	160	640	40	80	80	40	<
A/Milano/79/2020		pending	2020-02-17	MDCK3/MDCK1	1280	2560	1280	2560	<	640	1280	1280	640	1280
A/Finland/208/2020	N156K, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-18	MDCK1/MDCK1	40	<	40	80	320	40	40	40	40	<
A/Parma/27/2020		pending	2020-02-18	MDCK2/MDCK1	<	<	<	40	160	<	<	<	<	<
A/Finland/206/2020		6B.1A5A	2020-02-19	MDCK1/MDCK1	640	640	640	2560	<	640	1280	1280	640	640
A/Milano/77/2020		pending	2020-02-19	MDCK3/MDCK1	640	1280	640	2560	<	640	1280	1280	640	1280
A/Parma/32/2020		pending	2020-02-20	MDCK2/MDCK1	<	<	<	40	160	<	<	<	<	<
A/Visby/1/2020	N156K, F117L, K130N, L161I, DQ, V250A	6B.1A5A	2020-02-22	SIAT0/MDCK2	<	<	<	80	320	<	40	40	40	<
A/Finland/212/2020	N156K, K130N, L161I, DQ, K209M, V250A	6B.1A5A	2020-02-26	MDCK1/MDCK1	40	<	<	160	640	<	40	40	40	<
A/Finland/151/2019		6B.1A5B	2019-12-12	MDCK1/MDCK1	640	2560	640	1280	80	640	1280	1280	640	1280
					Vaccine		Vaccine				Vaccine			
					NH 2018-19		NH 2019-20				NH 2020-21			
					SH 2019		SH 2020							

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

1 < = <40; 2 < = <80; ND =Not Done

Sequences in phylogenetic trees

Table 3-4. Antigenic analysis of A(H1N1)pdm09 viruses by HI – Summary

Viruses	Other information		Haemagglutination inhibition titre: Post-infection fereret antisera									
		Passage history	A/Mich 45/15 Egg	A/Paris 1447/17 MDCK	A/Bris 02/18 Egg	A/Norway 3433/18 MDCK	A/Denmark 3280/19 MDCK	A/Ire 87733/19 Egg	A/G-M SWL1536/19 Egg	A/G-M SWL1536/19 MDCK	A/Swit 3330/17 Egg	A/Ire 84630/18 MDCK
		Ferret number	F31/16[*1]	F03/18[*2]	F09/19[*1]	F04/19[*1]	F08/20[*1]	St Jude's F18/20	F12/20[*1]	F09/20[*1]	F23/18[*1]	F08/19[*1]
		Genetic group	6B.1	6B.1A	6B.1A1	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5A	6B.1A5B	6B.1A6
REFERENCE VIRUSES												
A/Michigan/45/2015		6B.1	640	1280	640	1280	40	640	640	1280	640	1280
A/Paris/1447/2017		6B.1A	1280	2560	1280	2560	<	1280	1280	2560	1280	2560
A/Brisbane/02/2018		6B.1A1	1280	2560	1280	2560	40	1280	1280	1280	1280	1280
A/Norway/3433/2018		6B.1A5A	320	640	640	1280	<	640	640	640	320	640
A/Denmark/3280/2019	N156K	6B.1A5A	80	80	80	160	1280	80	80	160	80	40
A/Ireland/87733/2019		6B.1A5A	1280	1280	640	2560	40	1280	1280	1280	1280	1280
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	640	640	640	2560	<	640	1280	1280	640	640
A/Guangong-Maonan/SWL1536/2019	D187A, Q189E	6B.1A5A	640	1280	1280	2560	<	640	1280	1280	640	1280
A/Switzerland/3330/2017	clone 35	6B.1A5B	640	1280	1280	2560	<	320	640	640	640	640
A/Ireland/84630/2018		6B.1A6	640	1280	640	1280	<	640	1280	1280	1280	1280
TEST VIRUSES												
		Fold reduction in HI titre										
Number of viruses tested	99	≤2	73	64	65	74	18	62	72	73	71	68
		%	*73.7*	*64.6*	*65.7*	*74.7*	*18.2*	*62.6*	*72.7*	*73.7*	*71.7*	*68.7*
		4	1	8	7	0	4	10	1	1	2	4
		%	*1.0*	*8.1*	*7.0*		*4.0*	*10.1*	*1.0*	*1.0*	*2.0*	*4.0*
		≥8	25	27	27	25	77	27	26	25	26	27
		%	*25.3*	*27.3*	*27.3*	*25.3*	*77.8*	*27.3*	*26.3*	*25.3*	*26.3*	*27.3*
			Vaccine NH 2018-19 SH 2019		Vaccine NH 2019-20 SH 2020				Vaccine NH 2020-21			

Reference virus results are taken from an individual table as an example. Summaries for each antiserum are based on fold-reductions observed on the days that HI assays were performed

Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the May 2020 report and was generated based on sequences deposited in GISAID for recently circulating viruses, with collection dates from 1 February 2020, submitted to GISAID in May 2020 (Figure 2a). The second is again based on viruses with collection dates from 1 February 2020, but with sequences deposited in GISAID during June 2020; a total of 155 sequences (Figure 2b).

Viruses in clade 3C.2a have been dominant since the 2014–15 influenza season, and subgroup 3C.2a1b viruses predominated over the course of the 2018–19 season, but the HA gene sequences of viruses in both clades 3C.2a and 3C.3a continue to diverge. Notably, clade 3C.3a viruses have evolved to carry **HA1** amino acid substitutions of **L3I, S91N**, **N144K** (loss of a N-linked glycosylation motif at residues 144-146), **F193S** and **K326R**, and **D160N** in **HA2**, compared with cell culture-propagated A/Stockholm/6/2014, and levels of detection since January 2019 had increased in a number of WHO European Region countries and North America. Greater variation has been observed among clade 3C.2a viruses, resulting in the designation of new subclades/subgroups. Amino acid substitutions that define these subclades/subgroups are:

- 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** (a former vaccine virus)
- Subgroup **3C.2a1a**: Those in subclade **3C.2a1** plus **T135K** in **HA1**, resulting in the loss of a potential glycosylation site, and **G150E** in **HA2**, e.g. **A/Greece/4/2017**
- Subgroup **3C.2a1b**: Those in subclade **3C.2a1** plus **E62G, R142G** and **H311Q** in **HA1**, often with additional amino acid substitutions – notably **HA1 T131K** and **HA2 V200I**, the **3C.2a1b+T131K** cluster (e.g. **A/South Australia/34/2019**) or **HA1 T135K** (resulting in the loss of a potential glycosylation site) commonly with **T128A** (resulting in the loss of a potential glycosylation site), the **3C.2a1b+T135K-A** cluster (e.g. **A/La Rioja/2202/2018**) or a recently emerged, antigenically distinct group with **HA1 T135K, T128A, S137F, A138S** and **F193S**, the **3C.2a1b+T135K-B** cluster (e.g. **A/Hong Kong/2675/2019**)
- Clade **3C.3a**: represented by **A/Switzerland/9715293/2013** (see above), but recently a resurgence of clade **3C.3a** viruses, carrying additional substitutions of **S91N, N144K** (resulting in the loss of a potential glycosylation site)**, and F193S** in **HA1** and **D160N** in **HA2**, e.g. **A/England/538/2018** and **A/Kansas/14/2017**, the A(H3N2) vaccine virus for the 2019–20 influenza season.

The HA phylogeny generated for the May report, based on sequences recently deposited in GISAID, showed viruses in the **3C.2a1b** subgroup to have circulated in the greatest numbers, with approximately equal distribution between the **3C.2a1b+T131K**, **3C.2a1b+T135K-A** and **3C.2a1b+T135K-B** clusters, and just two from April falling in the **3C.2a1b+T131K** cluster (Figure 2a). The significant geographic spread of viruses in the antigenically distinct **3C.2a1b+T135K-B** cluster, influenced the selection of an A/Hong Kong/2671/2019-like virus as the A(H3N2) component of vaccines for the 2020–2021 northern hemisphere influenza season [3]. The geographic distribution of clade **3C.3a** viruses was more restricted with the great majority of recently detected viruses being reported from the European Region (Figure 2a). The updated phylogeny, for sequences deposited in June is again made up of sequences from viruses detected in North America and Europe during February and March (Figure 2b). The two phylogenies are very similar but for the dominance of clade **3C.3a** viruses in EU/EEA countries being more pronounced in Figure 2b.

The locations of A/Kansas/14/2017 (**3C.3a**), the A(H3N2) virus recommended for inclusion in vaccines for the northern hemisphere 2019–20 influenza season [1], and A/South Australia/34/2019 (**3C.2a1b+T131K**), the A(H3N2) virus recommended for inclusion in vaccines for the southern hemisphere 2020 influenza season [2], are indicated in Figures 2a and 2b in red. The location on the A/Hong Kong/2671/2019 (**3C.2a1b+T135K-B**) virus and its cell culture-equivalent A/Hong Kong/45/2019, recently recommended for egg- and cell culture-generated vaccines to be used in the 2020–2021 northern hemisphere season [3], are also indicated.

As described in many previous reports[2], 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 report[3], this is a particular problem for most viruses that fall in genetic clade **3C.2a**.

Since the May 2020 characterisation report of the viruses recovered, based on positive neuraminidase activity, 68 retained sufficient HA activity to allow antigenic analysis by HI (Tables 4-1 to 4-3). Test viruses are sorted by date of collection and genetic group/subgroup where known at the time of writing this report; 52 were clade **3C.2a** viruses, 15 were subgroup **3C.2a1b** viruses (four, five and six in clusters **T131K**, **T135K-A** and **T135K-B**, respectively) and one has not been sequenced. Results are summarised in Table 4-4.

Antisera raised against six individual clade **3C.2a** viruses reacted poorly with test viruses with 0-21% being recognised at titres within twofold and 0-78% at titres within fourfold of the respective homologous titres (Table 4-4). Antisera

[2] 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: 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:

https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/ERLI-Net%20report%20November%202014.pdf

raised against two previous egg-propagated vaccine viruses were responsible for the 0% recognition, A/South Australia/34/2019 (**3C.2a1b+T135K-B**), and 78% recognition, A/Singapore/INFIMH-16-0019/2016 (**3C.2a1b+T131K**). Antisera raised against two additional clade **3C.2a** viruses for which homologous titres were not available, A/Norway/3275/2018 (**3C.2a1b+T131K**) and A/La Rioja/2202/2018 (**3C.2a1b+T135K-A**), recognised only 5 (7%) and 4 (6%) test viruses respectively at titres of 160 or above. Antisera raised against two cell culture-propagated clade **3C.3a** viruses, A/England/538/2018 and A/Kansas/14/2017, both recognised 54 (79%) test viruses at titres within twofold, and 57 (84%) and 55 (81%) respectively at titres within fourfold, of homologous titres. Antiserum raised against the egg-propagated clade 3C.3a vaccine virus, NYMC X-327 (A/Kansas/14/2017), had a high homologous titre and only 7 (10%) and 30 (44%) test viruses were recognised at titres within twofold and fourfold of the homologous titre; however, the absolute titres with many of the test viruses matched those seen with antisera raised against cell culture-propagated A/England/538/2018 and A/Kansas/14/2017 which yielded significantly lower homologous titres.

Overall, the HI data show strong clade-specific recognition of test viruses by post-infection ferret antisera raised against cell culture-propagated reference viruses. Of the three egg-propagated viruses representing vaccine viruses and based test virus recognition fourfold reduced compared to homologous titres: A/Singapore/INFIMH-16-0019/2016 (**3C.2a1b+T131K**) has the consistently highest homologous titre (1280) and shows the worst recognition of test viruses with the great majority of titres being ≤40; NYMC X-327 (A/Kansas/14/2017, **3C.3a**) has high homologous titres (640-1280) and shows significant clade-specificity in recognition of test viruses; and A/Singapore/INFIMH-16-0019/2016 (**3C.2a1b+T131K**) has the lowest homologous titre (320) and shows the greatest cross-clade recognition with the majority of titres being ≥80.

Figure 2a. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, May 2020)

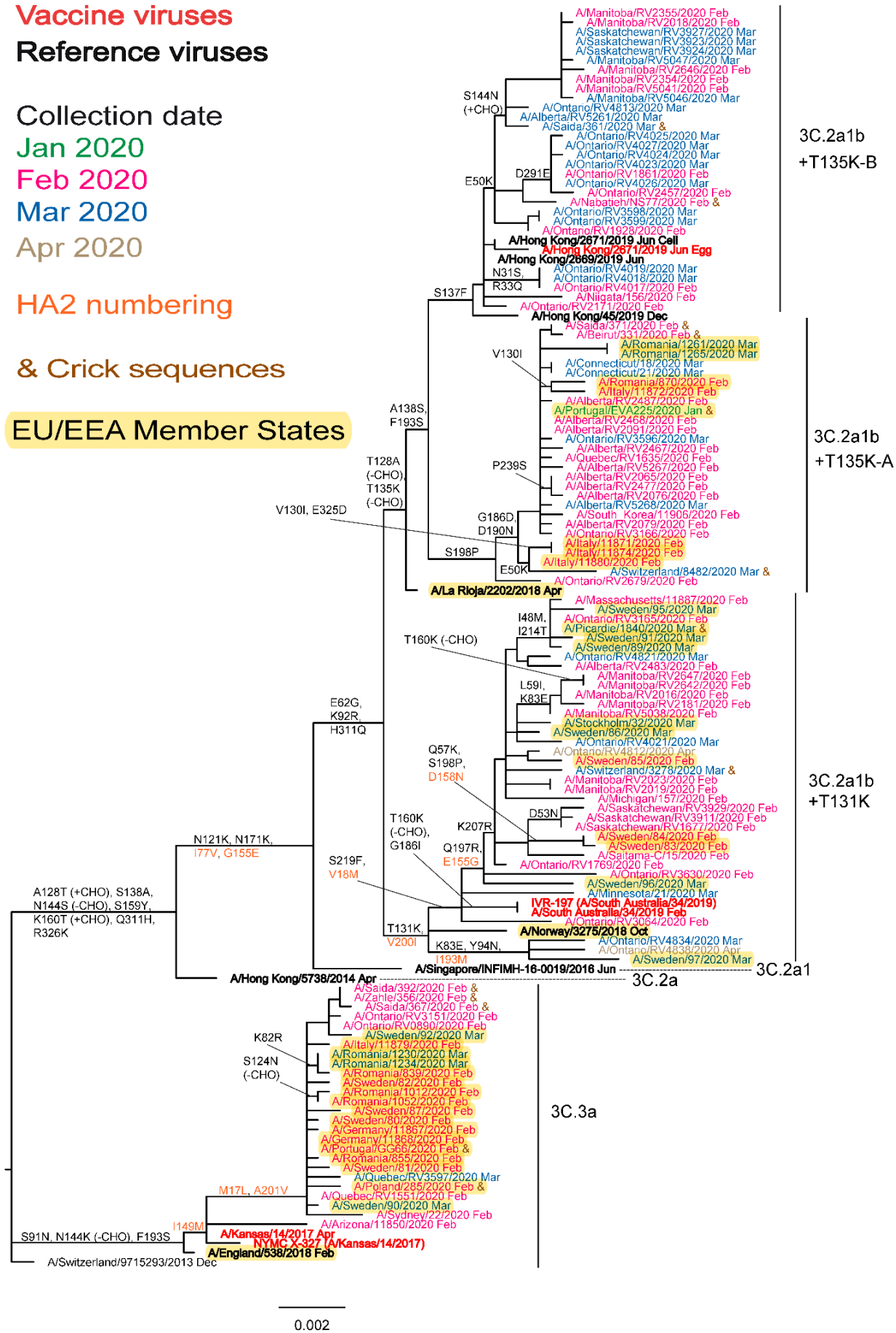


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, June 2020)

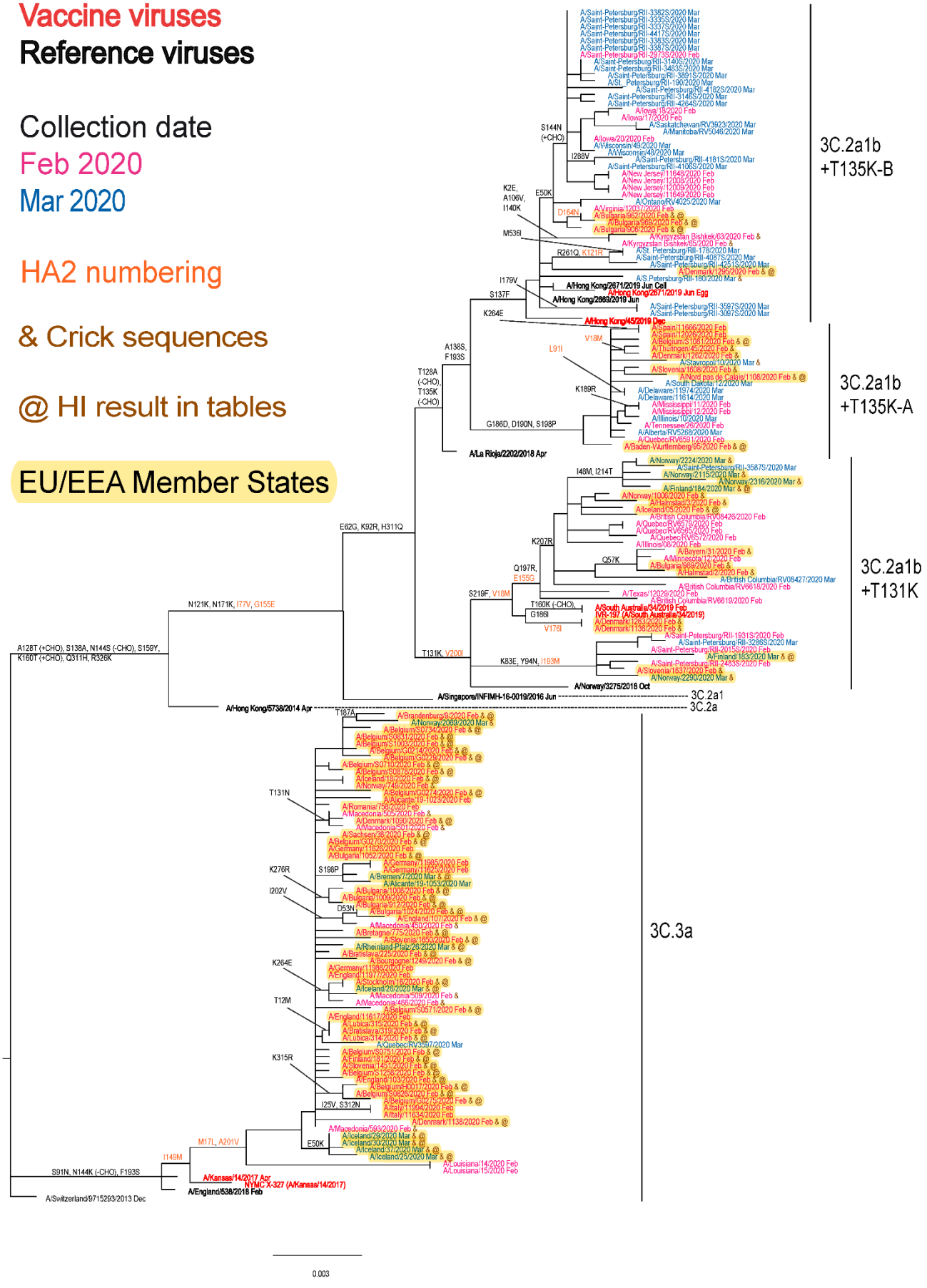


Table 4-1. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre											
				Post-infection ferret antisera											
	Passage history			A/HK 5738/14 MDCK St Jude's	A/Singapore 0019/16 Egg 10^{-4} F13/19	A/Norway 3275/18 SIAT F03/19	A/Sth Aus 34/19 Egg F45/19	A/La Rioja 2202/18 SIAT F26/18	A/HK 2671/19 Egg F44/19	A/HK 2671/19 Cell St Jude's F21/20	A/HK 2669/19 SIAT F04/20	A/Eng 538/18 SIAT F31/18	NYMC X-327 A/Kansas/14 Egg F16/19	A/Kansas 14/17 SIAT F17/19
	Ferret number														
	Genetic group			3C.2a	3C.2a1	3C.2a1b+T131K	3C.2a1b+T131K	3C.2a1b+T135K-A	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.3a	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES															
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK3/SIAT2	320	320	320	160	160	<	160	80	320	160	160	
A/Singapore/INFIMH-16-0019/2016	3C.2a1	2016-04-14	E5/E2	80	320	40	40	160	40	40	40	40	<	<	
A/South Australia/34/2019	3C.2a1b+T131K	2019-02-06	E6/E1	160	320	640	1280	80	160	40	<	80	40	40	
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	E8/E2	40	160	<	80	40	640	160	160	160	640	80	
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	CK1/SIAT4	160	320	320	320	320	320	640	640	320	80	160	
A/Hong Kong/2669/2019	3C.2a1b+T135K-B	2019-06-18	MDCK1/SIAT5	160	320	320	160	160	160	640	320	160	80	160	
A/England/538/2018	3C.3a	2018-02-26	MDCK1/SIAT4	80	80	<	40	40	80	160	<	640	160	640	
NYMC X-327 (A/Kansas/14/17)	3C.3a	2017-12-14	Ex/E1	<	40	<	<	<	160	80	<	320	640	320	
A/Kansas/14/2017	3C.3a	2017-12-14	SIAT3/SIAT2	40	80	<	40	40	40	160	<	640	160	320	
TEST VIRUSES															
A/Baden-Wurttemberg/95/2020	3C.2a1b+T135K-A	2020-02-10	C1/SIAT1	40	160	40	<	80	40	160	160	160	<	<	
A/Nord pas de Calais/1108/2020	3C.2a1b+T135K-A	2020-02-10	MDCK2/SIAT1	40	80	80	40	80	80	160	80	320	160	160	
A/Bulgaria/1772/2020	3C.2a1b+T135K-A	2020-03-09	SIAT2/SIAT1	80	320	160	80	160	320	320	160	320	160	160	
A/Bulgaria/906/2020	3C.2a1b+T135K-B	2020-02-04	SIAT2/SIAT1	40	80	40	<	80	40	320	160	40	<	<	
A/Bulgaria/962/2020	3C.2a1b+T135K-B	2020-02-11	SIAT2/SIAT1	80	160	80	<	160	80	320	160	80	<	40	
A/Bulgaria/969/2020	3C.2a1b+T135K-B	2020-02-12	SIAT2/SIAT1	40	40	80	<	40	80	320	160	40	<	<	
A/Bretagne/775/2020	3C.3a	2020-02-03	MDCK2/SIAT1	40	80	<	40	80	<	80	40	640	320	640	
A/England/103/2020	3C.3a	2020-02-05	SIAT1/SIAT1	40	80	<	<	40	40	80	<	640	160	320	
A/Bulgaria/912/2020	3C.3a	2020-02-06	SIAT2/SIAT1	80	80	<	<	80	<	80	<	640	320	320	
A/Bratislava/225/2020	3C.3a	2020-02-06	MDCKx/SIAT1	<	80	<	<	40	40	80	<	640	320	320	
A/Bulgaria/927/2020	3C.3a	2020-02-07	SIAT2/SIAT1	80	160	80	80	80	80	160	<	640	320	640	
A/England/107/2020	3C.3a	2020-02-11	SIAT1/SIAT1	<	40	<	<	<	<	80	<	640	160	320	
A/Sachsen/38/2020	3C.3a	2020-02-14	C1/SIAT1	80	80	<	<	40	<	160	<	640	320	320	
A/Bulgaria/1024/2020	3C.3a	2020-02-16	SIAT2/SIAT1	<	<	40	80	40	40	80	<	640	160	320	
A/Bulgaria/1009/2020	3C.3a	2020-02-17	SIAT2/SIAT1	<	80	<	<	<	<	40	<	640	160	320	
A/Bulgaria/1008/2020	3C.3a	2020-02-17	SIAT2/SIAT1	80	80	<	40	40	40	80	<	640	320	320	
A/Bourgogne/1249/2020	3C.3a	2020-02-17	MDCK1/SIAT1	40	80	<	40	40	<	80	<	640	160	320	
A/Lubica/314/2020	3C.3a	2020-02-17	MDCK1/SIAT1	<	80	<	<	<	40	80	<	640	320	320	
A/Lubica/315/2020	3C.3a	2020-02-18	MDCK1/SIAT1	<	80	<	<	<	40	80	<	320	160	160	
A/Bulgaria/1052/2020	3C.3a	2020-02-21	SIAT2/SIAT1	80	160	<	40	40	<	160	<	640	160	320	
A/Bratislava/319/2020	3C.3a	2020-02-21	MDCK1/SIAT1	40	80	<	<	40	40	80	<	640	160	320	
A/Brandenburg/9/2020	3C.3a	2020-02-23	C1/SIAT1	80	80	<	<	40	<	80	<	640	160	320	
A/Rheinland-Pfalz/26/2020	3C.3a	2020-03-02	C1/SIAT1	80	80	<	40	40	<	80	<	640	160	320	
A/Bremen/7/2020	3C.3a	2020-03-02	C1/SIAT1	40	80	<	<	40	<	80	<	640	160	320	
					Vaccine NH 2018-19		Vaccine SH 2020		Vaccine NH 2020-21				Vaccine NH 2019-20		

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

Sequences in phylogenetic trees

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/HK 5738/14	A/Singapore 0019/16	A/Norway 3275/18	A/Sth Aus 34/19	A/La Rioja 2202/18	A/HK 2671/19	A/HK 2671/19	A/Eng 538/18	NYMC X-327 A/Kansas/14	A/Kansas 14/17
		Passage history			MDCK	Egg 10^{-4}	SIAT	Egg	SIAT	Egg	Cell	SIAT	Egg	SIAT
		Ferret number			St Jude's F60/17 *1	F13/19 *1	F03/19 *1	F45/19 *1	F26/18 *1	F44/19 *1	St Jude's F21/20 *1	F31/18 *1	F16/19 *1	F17/19 *1
		Genetic group			3C.2a	3C.2a1	3C.2a1b+T131K	3C.2a1b+T131K	3C.2a1b+T135K-A	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES														
A/Hong Kong/5738/2014		3C.2a	2014-04-30	MDCK1/MDCK3/SIAT2	320	320	320	160	160	<	160	320	160	160
A/Singapore/INFIMH-16-0019/2016		3C.2a1	2016-04-14	E5/E2	80	320	40	40	160	40	40	40	40	<
A/South Australia/34/2019		3C.2a1b+T131K	2019-02-06	E6/E1	160	320	640	1280	80	320	40	80	40	40
A/Hong Kong/2671/2019		3C.2a1b+T135K-B	2019-06-17	E8/E2	40	80	<	80	40	640	160	160	640	80
A/Hong Kong/2671/2019		3C.2a1b+T135K-B	2019-06-17	CK1/SIAT4	160	320	320	160	320	320	640	320	160	160
A/England/538/2018		3C.3a	2018-02-26	MDCK1/SIAT4	40	80	<	40	40	80	160	640	320	640
NYMC X-327 (A/Kansas/14/17)		3C.3a	2017-12-14	Ex/E1	40	40	<	80	<	160	80	320	1280	320
A/Kansas/14/2017		3C.3a	2017-12-14	SIAT3/SIAT2	40	80	<	40	40	40	160	640	320	320
TEST VIRUSES														
A/Iceland/05/2020		3C.2a1b+T131K	2020-02-21	MDCK1/SIAT2	80	160	160	160	160	40	80	80	80	80
A/Slovenia/1752/2020		3C.2a1b+T131K	2020-03-05	MDCKx/SIAT1	40	160	160	160	40	<	40	<	40	<
A/Belgium/S1081/2020		3C.2a1b+T135K-A	2020-02-24	SIAT1/SIAT1	40	160	40	40	80	160	160	160	80	40
A/Stockholm/13/2020		3C.2a1b+T135K-B	2020-02-02	SIAT0/SIAT1	40	80	80	40	80	80	320	40	<	40
A/Belgium/S0751/2020		3C.3a	2020-02-01	SIAT1/SIAT2	<	40	<	40	<	40	40	640	160	320
A/Slovenia/944/2020		3C.3a	2020-02-03	MDCKx/SIAT1	40	80	<	40	<	40	<	640	160	320
A/Belgium/S0571/2020		3C.3a	2020-02-03	SIAT1/SIAT1	40	80	<	40	<	40	40	640	160	320
A/Belgium/G0229/2020		3C.3a	2020-02-03	SIAT1/SIAT1	<	<	<	40	<	<	<	320	80	160
A/Stockholm/16/2020		3C.3a	2020-02-04	SIAT0/SIAT1	40	80	<	80	<	40	80	640	320	640
A/Belgium/G0214/2020		3C.3a	2020-02-05	SIAT1/SIAT2	<	40	<	40	<	40	40	320	160	320
A/Belgium/S1258/2020		3C.3a	2020-02-07	SIAT1/SIAT1	80	80	<	80	<	40	80	640	320	320
A/Belgium/S0710/2020		3C.3a	2020-02-08	SIAT1/SIAT1	40	160	<	80	<	40	80	320	320	320
A/Belgium/H0017/2020		3C.3a	2020-02-09	SIAT1/SIAT1	<	40	<	80	<	40	40	320	160	320
A/Belgium/S0734/2020		3C.3a	2020-02-10	SIAT1/SIAT1	40	80	<	80	<	40	80	640	320	320
A/Belgium/G0275/2020		3C.3a	2020-02-10	SIAT1/SIAT1	40	80	<	40	<	40	40	640	160	320
A/Belgium/G0274/2020		3C.3a	2020-02-10	SIAT1/SIAT1	40	80	<	80	<	40	40	320	160	320
A/Belgium/G0270/2020		3C.3a	2020-02-10	SIAT1/SIAT1	<	40	<	40	<	<	40	320	160	320
A/Belgium/S0900/2020		3C.3a	2020-02-11	SIAT1/SIAT1	<	40	<	40	<	<	40	320	160	160
A/Belgium/S0831/2020		3C.3a	2020-02-11	SIAT1/SIAT1	<	80	<	40	<	40	40	320	160	320
A/Slovenia/1270/2020		3C.3a	2020-02-12	SIATx/SIAT1	40	80	40	80	40	40	80	640	320	320
A/Belgium/S0826/2020		3C.3a	2020-02-12	SIAT1/SIAT1	40	80	<	40	<	40	40	320	160	320
A/Belgium/S0878/2020		3C.3a	2020-02-14	SIAT1/SIAT2	<	40	<	40	<	40	40	320	160	320
A/Stockholm/30/2020		3C.3a	2020-02-17	SIAT0/SIAT1	<	40	<	40	<	40	40	640	160	320
A/Slovenia/1451/2020		3C.3a	2020-02-18	SIATx/SIAT1	40	80	<	40	<	40	80	640	160	320
A/Slovenia/1465/2020		3C.3a	2020-02-19	SIATx/SIAT1	<	40	<	40	<	40	40	640	160	320
A/Belgium/S1003/2020		3C.3a	2020-02-19	SIAT1/SIAT1	40	40	<	40	<	40	40	320	160	320
A/Slovenia/1506/2020		3C.3a	2020-02-21	SIATx/SIAT1	40	80	<	40	<	40	40	640	160	320
A/Slovenia/1650/2020		3C.3a	2020-02-27	SIATx/SIAT1	40	80	80	80	<	40	40	640	160	320
A/Iceland/18/2020		3C.3a	2020-02-29	MDCK1/SIAT1	40	80	<	40	<	40	80	640	320	320
A/Slovenia/1708/2020		3C.3a	2020-03-02	SIATx/SIAT1	40	80	<	80	<	40	80	640	320	320
A/Iceland/25/2020		3C.3a	2020-03-12	MDCK1/SIAT1	40	80	<	40	<	40	80	640	160	320
A/Iceland/30/2020		3C.3a	2020-03-13	MDCK1/SIAT1	<	80	<	40	<	<	40	320	160	320
A/Iceland/26/2020		3C.3a	2020-03-13	MDCK1/SIAT1	80	160	40	80	40	80	80	640	320	640
A/Iceland/29/2020		3C.3a	2020-03-16	MDCK1/SIAT1	<	80	<	40	<	40	40	320	160	320
A/Iceland/37/2020		3C.3a	2020-03-26	MDCK1/SIAT1	40	80	<	80	<	40	80	640	160	320
A/Iceland/23/2020		No seq	2020-03-09	MDCK1/SIAT1	<	80	40	40	40	160	320	80	<	40
Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used) 1 < = <40						Vaccine NH 2018-19		Vaccine SH 2020		Vaccine NH 2020-21			Vaccine NH 2019-20	
Sequences in phylogenetic trees														

Table 4-3. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
				Post-infection ferret antisera										
	Passage history			A/HK	A/Singapore	A/Norway	A/Sth Aus	A/La Rioja	A/HK	A/HK	A/Eng	NYMC X-327	A/Kansas
				5738/14	0019/16	3275/18	34/19	2202/18	2671/19	2671/19	538/18	A/Kansas/14	14/17
				MDCK	Egg 10^{-4}	SIAT	Egg	SIAT	Egg	Cell	SIAT	Egg	SIAT
				St Judes						St Judes			
	Ferret number			F60/17 $^{-1}$	F13/19 $^{-1}$	F03/19 $^{-1}$	F45/19 $^{-1}$	F26/18 $^{-1}$	F44/19 $^{-1}$	F21/20 $^{-1}$	F31/18 $^{-1}$	F16/19 $^{-1}$	F17/19 $^{-1}$
	Genetic group			3C.2a	3C.2a1	3C.2a1b+T131K	3C.2a1b+T131K	3C.2a1b+T135K-A	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES													
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK3/SIAT2	320	320	320	160	160	40	160	320	160	160
A/Singapore/INFIMH-16-0019/2016	3C.2a1	2016-04-14	E5/E2	160	320	40	40	160	80	40	40	40	<
A/South Australia/34/2019	3C.2a1b+T131K	2019-02-06	E6/E1	320	320	1280	1280	80	320	40	80	40	40
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	E8/E2	40	160	<	80	40	640	160	160	640	80
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	CK1/SIAT4	160	320	320	160	160	320	640	160	160	160
A/England/538/2018	3C.3a	2018-02-26	MDCK1/SIAT4	40	80	<	40	<	40	80	320	160	320
NYMC X-327 (A/Kansas/14/17)	3C.3a	2017-12-14	Ex/E1	80	80	<	80	40	320	160	320	1280	640
A/Kansas/14/2017	3C.3a	2017-12-14	SIAT3/SIAT2	40	80	<	40	40	40	80	320	160	320
TEST VIRUSES													
A/Finland/183/2020	3C.2a1b+T131K	2020-03-02	SIAT1/SIAT1	40	40	40	40	80	<	40	40	<	<
A/Finland/184/2020	3C.2a1b+T131K	2020-03-16	SIAT1/SIAT1	40	40	40	40	80	<	40	40	<	<
A/Finland/171/2020	3C.2a1b+T135K-A	2020-01-02	SIAT1/SIAT1	80	80	160	80	160	80	320	80	80	40
A/Denmark/1262/2020	3C.2a1b+T135K-A	2020-02-17	SIAT3/SIAT1	1280	640	640	640	640	320	1280	640	320	320
A/Finland/180/2020	3C.2a1b+T135K-B	2020-01-14	SIAT1/SIAT1	40	80	80	<	80	80	320	40	<	<
A/Denmark/1295/2020	3C.2a1b+T135K-B	2020-02-18	SIAT3/SIAT1	80	160	160	40	80	160	640	40	40	40
A/Finland/181/2020	3C.3a	2020-02-01	SIAT1/SIAT1	40	80	40	40	<	40	40	320	160	320
A/Denmark/1090/2020	3C.3a	2020-02-13	SIAT2/SIAT1	160	160	80	80	80	80	80	640	320	320
A/Denmark/1138/2020	3C.3a	2020-02-18	SIAT2/SIAT1	80	80	40	80	40	80	80	640	320	320
					Vaccine NH 2018-19		Vaccine SH 2020		Vaccine NH 2020-21			Vaccine NH 2019-20	

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

Sequences in phylogenetic trees

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

Viruses	Other information	Haemagglutination inhibition titre											
		Post-infection ferret antisera											
		A/HK	A/Singapore	A/Norway	A/Sth Aus	A/La Rioja	A/HK	A/HK	A/HK	A/Eng	NYMC X-327	A/Kansas
		5738/14	0019/16	3275/18	34/19	2202/18	2671/19	2671/19	2669/19	538/18	A/Kansas/14	14/17
	Passage history	MDCK	Egg 10^{-4}	SIAT	Egg	SIAT	Egg	Cell	SIAT	SIAT	Egg	SIAT
	Ferret number	St Jude's F60/17^{-1}	F13/19^{-1}	F03/19^{-1}	F45/19^{-1}	F26/18^{-1}	F44/19^{-1}	St Jude's F21/20^{-1}	F04/20^{-1}	F31/18^{-1}	F16/19^{-1}	F17/19^{-1}
	Genetic group	3C.2a	3C.2a1	3C.2a1b+T131K	3C.2a1b+T131K	3C.2a1b+T135K-A	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES												
A/Hong Kong/5738/2014	3C.2a	320	320	320	160	160	<	160	80	320	160	160
A/Singapore/INFIMH-16-0019/2016	3C.2a1	80	320	40	40	160	40	40	40	40	<	<
A/South Australia/34/2019	3C.2a1b+T131K	160	320	640	1280	80	160	40	<	80	40	40
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	40	160	<	80	40	640	160	160	160	640	80
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	160	320	320	320	320	320	640	640	320	80	160
A/Hong Kong/2669/2019	3C.2a1b+T135K-B	160	320	320	160	160	160	640	320	160	80	160
A/England/538/2018	3C.3a	80	80	<	40	40	80	160	<	640	160	640
NYMC X-327 (A/Kansas/14/17)	3C.3a	<	40	<	<	<	160	80	<	320	640	320
A/Kansas/14/2017	3C.3a	40	80	<	40	40	40	160	<	640	160	320
TEST VIRUSES												
Number of viruses tested		68	68	68*	68	68*	68	68	24	68	68	68
Fold reduction in HI titre												
2		1	12	5	0	4	1	9	5	54	7	54
%		*1.4*	*17.6*	*7.4*		*5.9*	*1.4*	*13.2*	*20.8*	*79.4*	*10.3*	*79.4*
4		15	41		0		3	6	1	3	23	1
%		*22.1*	*60.3*				*4.5*	*8.9*	*4.2*	*4.5*	*33.8*	*1.4*
8		52	15		68		64	53	18	11	38	13
%		*76.5*	*22.1*		*100.0*		*94.1*	*77.9*	*75.0*	*16.1*	*55.9*	*19.2*
		Vaccine NH 2018-19			Vaccine SH 2020		Vaccine NH 2020-21				Vaccine NH 2019-20	

* Homologous HI titres not available - only results for viruses yielding HI titres of ≥160 with the respective antisera are shown

Reference virus results are taken from individual tables as examples. Summaries for each antiserum are based on fold-reductions observed on the days that HI assays were performed.

Influenza B virus analyses

A total of 474 influenza type B viruses with collection dates after 31 August 2019 have been received at the WIC (Table 2). Of these, 389 were sent with pre-assignment to a lineage: 371 B/Victoria and 18 B/Yamagata.

Influenza B/Victoria-lineage

All recently circulating B/Victoria-lineage viruses have fallen in genetic **clade 1A**, represented by **B/Brisbane/60/2008** a former vaccine virus, but with additional **HA1** amino acid substitutions of **I117V, N129D** and **V146I** (e.g. **B/Ireland/3154/2016**). Viruses retaining full-length HAs have remained antigenically similar to B/Brisbane/60/2008. However, three genetic groups (described below with amino acid substitutions/deletions relative to B/Brisbane/60/2008 indicated) containing deletions of HA gene codons have emerged and the viruses in these groups are antigenically distinct from B/Brisbane/60/2008 and each other (as noted in the September 2018 characterisation report[4] and earlier ones), such that four antigenically distinguishable groups had been circulating:

- A group with double deletion of **HA1** residues **162** and **163** (**subclade △162-163** or **1A(△2)**) with amino acid substitutions of **D129G** and **I180V**, and **HA2 R151K** that spread worldwide and is represented by the current vaccine virus, **B/Colorado/06/2017**;
- A group with triple deletion of **HA1** residues **162** to **164** (**subclade △162-164A** or **1A(△3)A**), first detected in Asia, with amino acid substitutions of **I180T** and **K209N** that showed limited spread worldwide and is represented by **B/Hong Kong/269/2017**;
- A group with triple deletion of **HA1** residues **162** to **164** (**subclade △162-164B** or **1A(△3)B**), first detected in Africa, with amino acid substitution **K136E** often with **G133R** that showed geographic spread in recent months and is represented by the recently recommended vaccine virus **B/Washington/02/2019**.

The HA phylogeny generated for the May report showed continued dominance of **subclade 1A(△3)B** viruses, with the great majority having **HA1 K136E,** often with **G133R** substitution, and a number of virus clusters had emerged defined by specific amino acid substitutions, e.g. **HA1 N126K** or **E128K** or **D129N** or **N150K** with **G184E** or **N233K** (loss of a glycosylation site), and relatively few **subclade 1A(△2)** viruses had been detected (Figure 3a). The updated phylogeny for sequences deposited in GISAID during June is again largely made up of viruses detected in the USA and Europe (with a large number from the Russian Federation) during February and March with just one from April, a virus detected in Iceland (Figure 3b); the phylogeny profile is very similar to that of Figure 3a.

Following the spread of **1A(Δ2)** viruses a representative, B/Colorado/06/2017, was recommended for use in trivalent influenza vaccines for the 2019–20 northern hemisphere season [1]. Recent predominance of **1A(Δ3)B** viruses led to recommendation of a representative (B/Washington/02/2019) for use in trivalent influenza vaccines for the 2020 southern hemisphere and northern hemisphere 2020–2021 seasons [2, 3].

Of the B/Victoria-lineage viruses from EU/EEA countries received, 69 were assessed by HI assay since the May 2020 report (Tables 5-1 to 5-2). Test viruses are sorted by date of collection and genetic group/subgroup where known at the time of writing this report; 63 were subclade **1A(Δ3)B** viruses, one was a subclade **1A(Δ2)** virus and five have not been sequenced. Results are summarised in Table 5-3.

Poor test virus reactivity with ferret antisera raised against viruses in **clade 1A** (n=4) was observed. Antisera raised against three **subclade 1A(△2)** viruses recognised only 3 to 5 (4% to 7%) test viruses at titres within fourfold of their respective homologous titres, indicative of limited cross-reactivity with **subclade 1A(△3)B** viruses. Antisera raised against two **subclade 1A(△3)B** viruses recognised 71% and 78% of test viruses at titres within fourfold of their respective homologous titres. Overall, two patterns of reactivity were observed reflecting the subclade of the test virus with the **subclade 1A(△2)** virus reacting well with antisera raised against tissue culture-propagated B/Norway/2409/2017 and egg- and tissue culture-propagated B/Colorado/06/2017, while **subclade 1A(△3)B** viruses reacted well with antisera raised against egg- and tissue culture-cultivars of B/Washington/02/2019. Approximately 25% of the **subclade 1A(△3)B** test viruses showed eightfold or greater reductions in titre with antisera raised against B/Washington/02/2019 compared to homologous titres: those viruses with sequences available at the time of writing this report, carried unusual amino acid substitutions in HA1 (e.g. N126K/G or E128K or E136K or N150K or T155A or N233K (resulting in loss of a glycosylation sequon), sometimes with additional substitutions) (Tables 5-1 and 5-2, Figures 3a/b).

Influenza B/Yamagata-lineage

No B/Yamagata-lineage viruses have been characterised genetically at the WIC since the May report. The HA phylogeny, for viruses with collection dates from 1 January 2020, has been updated from the May report to contain nine sequences submitted to GISAID in June, one each from Australia and Spain, and three each from the Russian Federation and Trinidad for viruses detected in February and March (Figure 4). As for other recently detected B/Yamagata-lineage viruses, the HA genes fall in genetic **clade 3**, the B/Wisconsin/1/2010–B/Phuket/3073/2013 clade, within a subgroup defined by **HA1 L172Q** and **M251V** amino acid substitutions compared to B/Phuket/3073/2013. Some sub-clustering of sequences from recently collected viruses, defined by specific amino acid substitutions (e.g. **HA1 N164K, K211R,**

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

D229N or **D232N** [introducing a potential N-linked glycosylation site] sometimes with **R48K**), has occurred. Four of the sequences recently deposited in GISAID, from Australia and Trinidad, encode the **D232N** substitution, while the three from the Russian Federation are all characterised by **HA1** amino acid substitutions **S120T** and **D229N**. As noted in previous characterisation reports for 2018, none of these amino acid substitutions have any obvious antigenic effects based on 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 2019–2020 and 2020–2021 [1, 3] northern hemisphere and the 2020 [2] southern hemisphere seasons.

Figure 3a. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, May 2020)

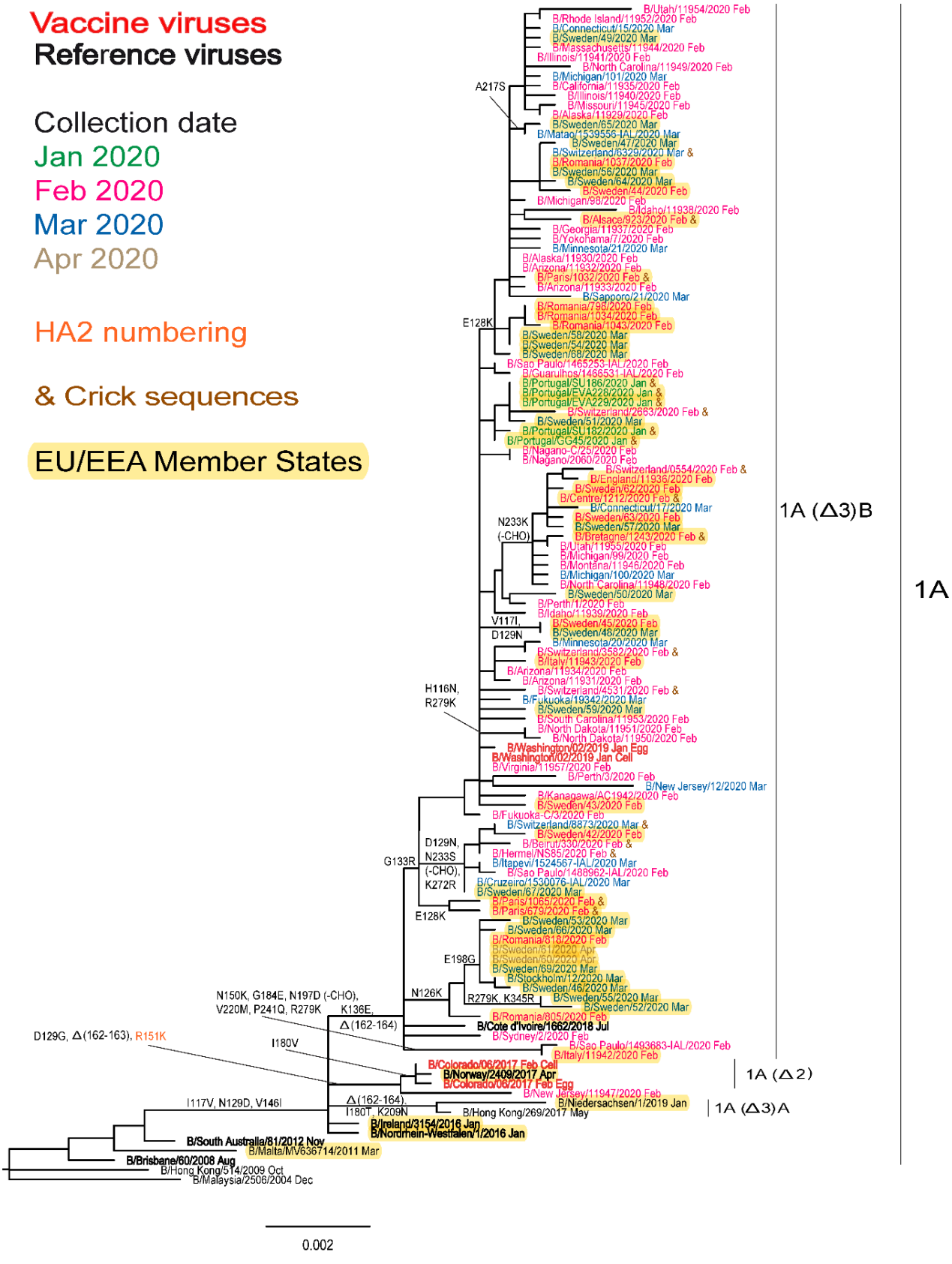


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, June 2020)

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

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
				Post-infection ferret antisera										
	Passage history			B/Bris 60/08 Egg	B/Bris 60/08 Egg	B/Sth Aus 81/12 Egg	B/Ireland 3154/16 MDCK	B/Nord-West 1/16 MDCK	B/Norway 2409/17 MDCK	B/Colorado 06/17 MDCK	B/Colorado 06/17 Egg	B/Wash'ton 02/19 MDCK	B/Wash'ton 02/19 Egg
	Ferret number			Sh 539, 540, 543, 544, 570, 571, 574[1,3]	F44/17[*2]	F25/16[*2]	F15/16[*2]	F38/17[*2]	F40/17[*2]	F21/18[*2]	F11/18[*2]	F37/19[*2]	F38/19[*2]
	Genetic group			1A	1A	1A	1A	1A	1A(Δ2)	1A(Δ2)	1A(Δ2)	1A(Δ3)	1A(Δ3)
REFERENCE VIRUSES													
B/Brisbane/60/2008	1A	2008-08-04	E4/E4	1280	640	640	40	80	<	10	160	<	160
B/South Australia/81/2012	1A	2012-11-28	E4/E2	2560	1280	1280	160	160	<	20	160	<	320
B/Ireland/3154/2016	1A	2016-01-14	MDCK1/MDCK4	1280	80	40	80	160	<	<	<	<	<
B/Nordrhein-Westfalen/1/2016	1A	2016-01-04	C2/MDCK2	1280	80	20	80	160	<	<	<	<	<
B/Norway/2409/2017	1A(Δ2)	2017-04-27	MDCK1/MDCK3	80	<	<	<	<	40	80	160	<	<
B/Colorado/06/2017	1A(Δ2)	2017-02-05	MDCK1/MDCK2	80	<	<	<	<	80	40	160	<	<
B/Washington/02/2019	1A(Δ2)	2017-02-05	E5/E2	640	160	40	<	<	40	40	320	<	160
B/Washington/02/2019	1A(Δ3) B	2019-01-19	C2/MDCK3	640	160	40	<	<	<	10	160	40	640
B/Washington/02/2019	1A(Δ3) B	2019-01-19	E3/E2	640	160	80	<	<	<	10	160	40	640
TEST VIRUSES													
B/Slovenia/912/2020	1A(Δ3) B	2020-02-02	SIATx/MDCK1	320	40	<	<	<	<	<	40	80	320
B/Belgium/S1068/2020	1A(Δ3) B	2020-02-08	MDCK1/MDCK1	160	<	<	<	<	<	<	<	<	160
B/Belgium/S1274/2020	1A(Δ3) B	2020-02-10	MDCK1/MDCK1	160	<	<	<	<	<	<	<	<	160
B/Belgium/S1071/2020	1A(Δ3) B	2020-02-12	MDCK1/MDCK1	160	10	<	<	<	<	<	<	<	160
B/Bulgaria/979/2020	1A(Δ3) B	2020-02-13	SIAT2/MDCK1	<	<	<	<	<	<	<	<	<	<
B/Belgium/G0316/2020	1A(Δ3) B	2020-02-13	MDCK1/MDCK1	320	40	<	<	<	<	<	20	<	320
B/Belgium/S1053/2020	1A(Δ3) B	2020-02-16	MDCK1/MDCK1	160	20	<	<	<	<	<	<	<	<
B/Bulgaria/1004/2020	1A(Δ3) B	2020-02-17	SIAT2/MDCK1	80	20	10	<	<	<	<	20	80	320
B/Iceland/03/2020	1A(Δ3) B	2020-02-19	MDCK1/MDCK1	80	10	<	<	<	<	<	<	<	160
B/Bulgaria/1054/2020	1A(Δ3) B	2020-02-21	SIAT2/MDCK1	<	<	10	<	<	<	<	<	320	<
B/Slovenia/1584/2020	1A(Δ3) B	2020-02-24	SIATx/MDCK1	160	40	<	<	<	<	<	<	40	<
B/Bulgaria/1084/2020	1A(Δ3) B	2020-02-25	SIAT2/MDCK1	80	20	20	<	<	<	<	20	40	320
B/Iceland/17/2020	1A(Δ3) B	2020-02-26	MDCK1/MDCK1	40	<	<	<	<	<	<	<	<	160
B/Iceland/12/2020	1A(Δ3) B	2020-02-26	MDCK1/MDCK1	80	<	<	<	<	<	<	<	<	160
B/Bulgaria/1114/2020	1A(Δ3) B	2020-02-27	SIAT2/MDCK1	80	20	40	<	<	<	<	20	80	320
B/Slovenia/1611/2020	1A(Δ3) B	2020-02-27	MDCKx/MDCK1	160	20	<	<	<	<	<	<	20	<
B/Iceland/16/2020	1A(Δ3) B	2020-02-27	MDCK1/MDCK1	40	<	<	<	<	<	<	<	<	160
B/Slovenia/1694/2020	1A(Δ3) B	2020-03-02	MDCKx/MDCK1	160	10	<	<	<	<	<	<	80	320
B/Slovenia/1692/2020	1A(Δ3) B	2020-03-02	SIATx/MDCK1	80	20	<	<	<	<	<	<	40	320
B/Iceland/21/2020	1A(Δ3) B	2020-03-02	MDCK1/MDCK1	80	20	<	<	<	<	<	<	<	320
B/Bulgaria/1251/2020	1A(Δ3) B	2020-03-04	SIAT2/MDCK1	160	20	10	<	<	<	<	<	80	320
B/Slovenia/1745/2020	1A(Δ3) B	2020-03-04	MDCKx/MDCK1	160	20	<	<	<	<	<	<	80	320
B/Slovenia/1743/2020	1A(Δ3) B	2020-03-04	MDCKx/MDCK1	80	20	<	<	<	<	<	<	40	160
B/Belgium/S1228/2020	1A(Δ3) B	2020-03-04	MDCK1/MDCK1	160	20	<	<	<	<	<	<	<	160
B/Belgium/G0472/2020	1A(Δ3) B	2020-03-04	MDCK1/MDCK1	320	20	<	<	<	<	<	<	<	<
B/Bulgaria/1170/2020	1A(Δ3) B	2020-03-05	SIAT2/MDCK1	160	40	20	<	<	<	10	40	80	640
B/Bulgaria/1308/2020	1A(Δ3) B	2020-03-05	SIAT2/MDCK1	320	80	20	<	<	<	<	40	80	320
B/Bulgaria/1279/2020	1A(Δ3) B	2020-03-05	SIAT2/MDCK1	160	20	20	<	<	<	<	40	80	320
B/Bulgaria/1774/2020	1A(Δ3) B	2020-03-09	SIAT2/MDCK1	160	80	20	<	<	<	10	80	80	640
B/Bulgaria/1722/2020	1A(Δ3) B	2020-03-09	SIAT2/MDCK1	160	40	20	<	<	<	10	40	80	640
B/Slovenia/1906/2020	1A(Δ3) B	2020-03-11	MDCKx/MDCK1	160	20	<	<	<	<	<	40	80	320
B/Slovenia/2232/2020	1A(Δ3) B	2020-03-17	MDCKx/MDCK1	320	40	<	<	<	<	<	20	80	320
B/Iceland/31/2020	1A(Δ3) B	2020-03-17	MDCK1/MDCK1	80	<	<	<	<	<	<	<	<	160
B/Iceland/38/2020	1A(Δ3) B	2020-04-20	MDCK1/MDCK1	80	<	<	<	<	<	<	<	40	160
B/Bulgaria/1006/2020	No seq	2020-02-17	SIAT2/MDCK1	<	<	<	40	<	40	<	<	<	40
B/Bulgaria/1051/2020	No seq	2020-02-21	SIAT2/MDCK1	<	<	<	<	<	<	<	<	320	<
B/Bulgaria/1165/2020	No seq	2020-02-28	SIAT2/MDCK1	<	<	<	<	<	<	<	<	<	<
B/Bulgaria/1169/2020	No seq	2020-02-29	SIAT2/MDCK1	<	<	<	<	<	<	<	<	<	<
B/Bulgaria/1257/2020	No seq	2020-03-04	SIAT2/MDCK1	160	20	<	<	<	<	<	<	640	<
B/Iceland/32/2020	No seq	2020-03-19	MDCK1/MDCK1	640	80	40	<	<	<	<	80	<	80

Vaccine SH 2019 NH 2019-20

Vaccine SH 2020 NH 2020-21

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used):
[1] < = <40; [2] < = <10; [3] hyperimmune sheep serum; [4] < = <20; ND = Not Done

Sequences in phylogenetic trees

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

Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre									
						Post-infection ferret antisera								
					B/Bris 60/08	B/Bris 60/08	B/Sth Aus 81/12	B/Ireland 3154/16	B/Nord-West 1/16	B/Norway 2409/17	B/Colorado 06/17	B/Colorado 06/17	B/Wash'ton 02/19	B/Wash'ton 02/19
		Passage history			Egg	Egg	Egg	MDCK	MDCK	MDCK	MDCK	Egg	MDCK	Egg
		Ferret number			Sh 539, 540, 543, 544, 570, 571, 574[*1,3]	F44/17[*2]	F25/16[*2]	F15/16[*2]	F38/17[*2]	F40/17[*2]	F21/18[*2]	F11/18[*2]	F37/19[*2]	F38/19[*2]
		Genetic group			1A	1A	1A	1A	1A	1A(Δ2)	1A(Δ2)	1A(Δ2)	1A(Δ3)	1A(Δ3)
REFERENCE VIRUSES														
B/Brisbane/60/2008		1A	2008-08-04	E4/E4	1280	640	640	40	80	<	10	160	<	160
B/South Australia/81/2012		1A	2012-11-28	E4/E2	2560	1280	1280	80	160	<	20	160	<	320
B/Ireland/3154/2016		1A	2016-01-14	MDCK1/MDCK4	1280	80	40	80	160	<	<	<	<	<
B/Nordrhein-Westfalen/1/2016		1A	2016-01-04	C2/MDCK2	1280	80	20	160	160	<	<	<	<	<
B/Norway/2409/2017		1A(Δ2)	2017-04-27	MDCK1/MDCK3	80	<	<	<	<	80	80	160	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	MDCK1/MDCK2	80	<	<	<	<	80	80	160	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	E5/E2	1280	160	40	<	<	40	40	320	<	160
B/Washington/02/2019		1A(Δ3)B	2019-01-19	C2/MDCK3	640	80	40	<	<	<	10	160	20	320
B/Washington/02/2019		1A(Δ3)B	2019-01-19	E3/E2	640	80	80	<	<	<	20	160	40	320
TEST VIRUSES														
B/Finland/203/2020		1A(Δ2)	2020-03-12	MDCK1/MDCK1	80	10	<	<	<	40	80	160	<	<
B/Denmark/20/2020	N126K, R133G, E195G	1A(Δ3)B	2020-01-22	SIAT2/MDCK1	160	40	10	<	<	<	<	10	<	40
B/Paris/679/2020		1A(Δ3)B	2020-02-03	MDCK1/MDCK1	160	20	<	<	<	<	10	20	80	320
B/Bratislava/202/2020		1A(Δ3)B	2020-02-03	MDCK1/MDCK1	640	80	<	<	<	10	10	40	80	320
B/Linkoping/1/2020		1A(Δ3)B	2020-02-03	SIAT0/MDCK1	160	40	<	<	<	<	<	40	40	320
B/Stockholm/7/2020		1A(Δ3)B	2020-02-05	SIAT0/MDCK1	320	40	<	<	<	<	10	40	80	320
B/Denmark/46/2020	N126K, R133G, E195G	1A(Δ3)B	2020-02-05	SIAT2/MDCK1	160	40	<	<	<	10	<	40	10	40
B/Alsace/923/2020		1A(Δ3)B	2020-02-06	MDCK1/MDCK1	160	20	<	<	<	<	10	20	80	320
B/England/127/2020		1A(Δ3)B	2020-02-06	MDCK1/MDCK1	640	40	<	<	<	<	10	40	80	320
B/Eskilstuna/2/2020		1A(Δ3)B	2020-02-07	SIAT0/MDCK1	160	40	<	<	<	<	<	40	40	320
B/Denmark/65/2020		1A(Δ3)B	2020-02-09	SIAT2/MDCK1	160	20	<	<	<	<	<	20	40	320
B/Paris/1032/2020		1A(Δ3)B	2020-02-10	MDCK1/MDCK1	320	40	20	<	<	20	20	80	80	320
B/England/95/2020		1A(Δ3)B	2020-02-10	SIAT1/MDCK1	320	20	<	<	<	10	10	40	40	320
B/Paris/1065/2020		1A(Δ3)B	2020-02-11	MDCK1/MDCK1	160	20	<	<	<	10	10	20	80	320
B/Eskilstuna/3/2020	N126K, R133G, T179A, R276K,	1A(Δ3)B	2020-02-12	SIAT0/MDCK1	80	20	<	<	<	<	10	20	10	40
B/Finland/195/2020		1A(Δ3)B	2020-02-12	MDCK1/MDCK1	160	20	<	<	<	<	<	40	40	320
B/Bremen/1/2020	D129N, N230S, K269R	1A(Δ3)B	2020-02-17	C2/MDCK1	80	40	10	<	<	<	10	40	20	80
B/Berlin/12/2020		1A(Δ3)B	2020-02-18	C1/MDCK1	320	40	20	<	<	10	10	40	40	320
B/Saarland/1/2020		1A(Δ3)B	2020-02-18	C1/MDCK1	320	40	20	<	<	10	10	40	80	320
B/Bretagne/1243/2020		1A(Δ3)B	2020-02-18	MDCK1/MDCK1	160	40	20	<	<	<	10	40	40	320
B/Centre/1212/2020		1A(Δ3)B	2020-02-18	MDCK1/MDCK1	160	40	20	<	<	<	10	40	40	320
B/England/130/2020		1A(Δ3)B	2020-02-18	SIAT1/MDCK1	80	10	<	<	<	<	10	20	80	320
B/Denmark/77/2020		1A(Δ3)B	2020-02-18	SIAT2/MDCK1	320	40	<	<	<	<	<	40	80	320
B/Finland/197/2020		1A(Δ3)B	2020-02-20	MDCK1/MDCK1	80	20	<	<	<	10	<	40	40	320
B/England/134/2020		1A(Δ3)B	2020-02-24	SIAT1/MDCK1	320	40	20	<	<	<	10	40	80	320
B/Nordrhein-Westfalen/6/2020	D129N, N230S, K269R	1A(Δ3)B	2020-02-25	C1/MDCK1	160	20	20	<	<	<	10	80	40	80
B/Bratislava/439/2020		1A(Δ3)B	2020-02-27	MDCK1/MDCK1	320	40	<	<	<	<	<	20	80	320
B/Stockholm/10/2020	D129N, N230S, K269R	1A(Δ3)B	2020-02-29	SIAT0/MDCK1	160	40	<	<	<	<	10	40	20	80
B/Mecklenburg-Vorpommern/1/2020	N126K, R133G, E195G	1A(Δ3)B	2020-03-02	C1/MDCK1	160	40	20	<	<	<	<	40	10	40
B/England/116/2020	D129N, R133G	1A(Δ3)B	2020-03-06	SIAT1/MDCK1	160	20	<	<	<	<	<	20	20	40
B/Finland/202/2020		1A(Δ3)B	2020-03-06	MDCK1/MDCK1	160	40	<	<	<	10	<	40	40	320
B/Bayern/6/2020		No seq	2020-02-26	C1/MDCK1	160	20	10	<	<	<	10	80	40	640
												Vaccine SH 2019 NH 2019-20		Vaccine SH 2020 NH 2020-21

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used):
[1] < = <40; [2] < = <10; [3] hyperimmune sheep serum; [4] < = <20; ND = Not Done

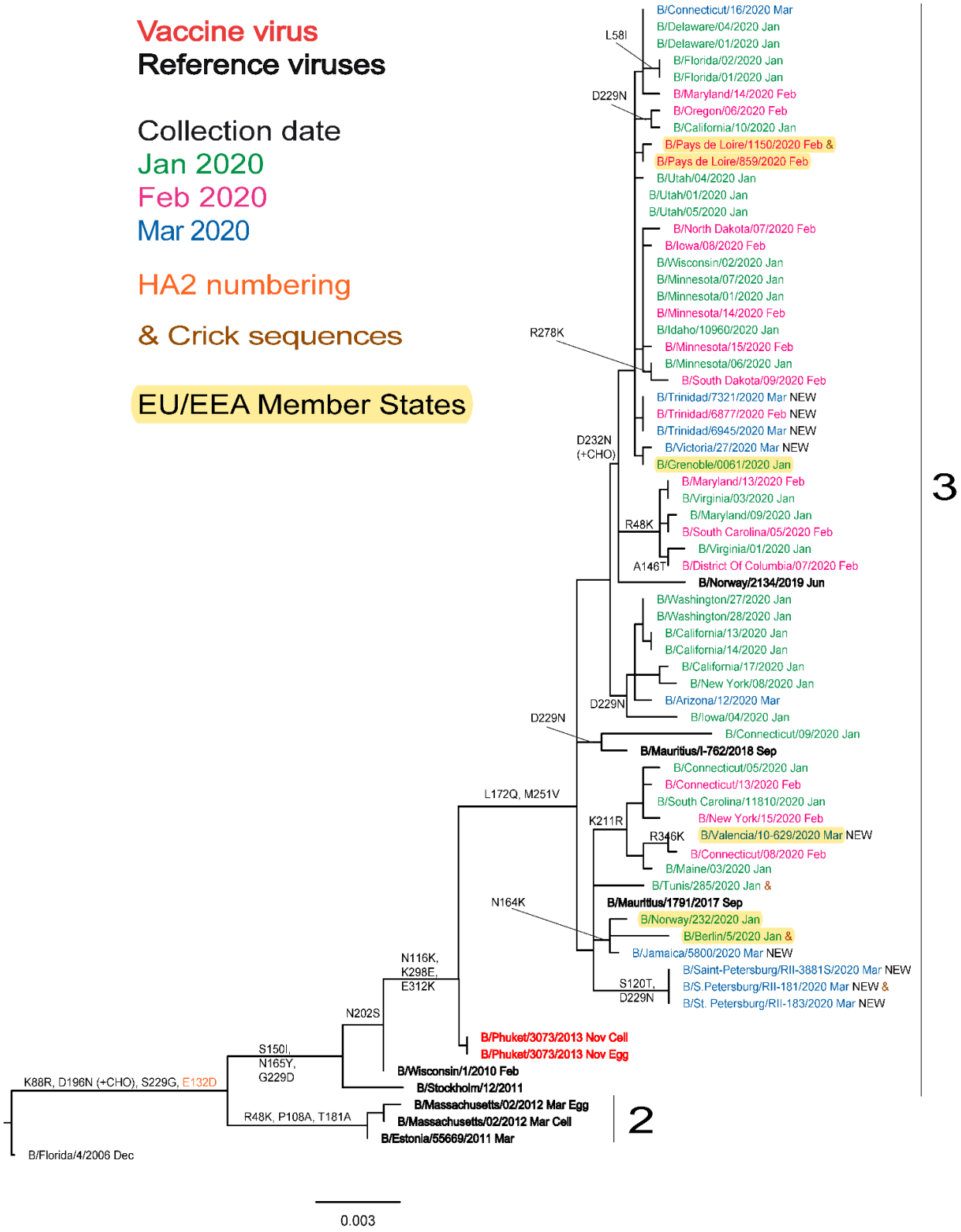
Sequences in phylogenetic trees

Table 5-3. Antigenic analysis of influenza B/Victoria-lineage viruses by HI - Summary

Viruses	Other information	Post-infection ferret antisera									
		B/Bris 60/08	B/Sth Aus 81/12	B/Ireland 3154/16	B/Nord-West 1/16	B/Norway 2409/17	B/Colorado 06/17	B/Colorado 06/17	B/Wash'ton 02/19	B/Wash'ton 02/19
	Passage history	Egg	Egg	MDCK	MDCK	MDCK	MDCK	Egg	MDCK	Egg
	Ferret number	F44/17 [-2]	F25/16 [-2]	F15/16 [-2]	F38/17 [-2]	F40/17 [-2]	F21/18 [-2]	F11/18 [-2]	F37/19 [-2]	F38/19 [-2]
	Genetic group	1A	1A	1A	1A	1A(Δ2)	1A(Δ2)	1A(Δ2)	1A(Δ3)	1A(Δ3)
REFERENCE VIRUSES										
B/Brisbane/60/2008	1A	640	640	40	80	<	10	160	<	160
B/South Australia/81/2012	1A	1280	1280	80	160	<	20	160	<	320
B/Ireland/3154/2016	1A	80	40	80	160	<	<	<	<	<
B/Nordrhein-Westfalen/1/2016	1A	80	20	160	160	<	<	<	<	<
B/Norway/2409/2017	1A(Δ2)	<	<	<	<	80	80	160	<	<
B/Colorado/06/2017	1A(Δ2)	<	<	<	<	80	80	160	<	<
B/Colorado/06/2017	1A(Δ2)	160	40	<	<	40	40	320	<	160
B/Washington/02/2019	1A(Δ3) B	80	40	<	<	<	10	160	20	320
B/Washington/02/2019	1A(Δ3) B	80	80	<	<	<	20	160	40	320
TEST VIRUSES										
	Fold reduction in									
Number of viruses tested	69	0	0	1	0	2	1	1	49	40
	%			*1.4*		*2.9*	*1.4*	*1.4*	*71.0*	*58.0*
	4	0	0	0	0	1	4	4	0	14
	%					*1.4*	*5.8*	*5.8*		*20.3*
	≥8	69	69	68	69	66	64	64	20	15
	%	*100.0*	*100.0*	*98.6*	*100.0*	*95.7*	*92.8*	*92.8*	*29.0*	*21.7*
							Vaccine SH 2019 NH 2019-20		Vaccine SH 2020 NH 2020-21	

Reference virus results are taken from an individual table as an example. Summaries for each antiserum are based on fold-reductions observed on the days that HI assays were performed

Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, June 2020)



Summaries of data submitted to TESSy

Genetic characterisation

For the 2019–20 season, as of week 20/2020, 2 752 viruses were characterised genetically and ascribed to a genetic clade (no additional characterisations were reported during weeks 21-25/2020):

- 982 were A(H1N1)pdm09 viruses, with 945 being subclade 6B.1A5 (904 subgroup 6B.1A5A represented by A/Norway/3433/2018 and 41 subgroup 6B.1A5B represented by A/Switzerland/3330/2018), 19 being subgroup 6B.1A7 represented by A/Slovenia/1489/2019, 11 being subgroup 6B.1A1 represented by A/Brisbane/02/2018 and seven attributed to a known group not listed in the 2019–20 reporting categories.
- 1 048 were A(H3N2) viruses, with 342 being subgroup 3C.2a1b+T131K represented by A/South Australia/34/2019, 560 being clade 3C.3a represented by A/Kansas/14/2017, 81 being subgroup 3C.2a1b+T135K-B represented by A/Hong Kong/2675/2019, 64 being subgroup 3C.2a1b+T135K-A represented by A/La Rioja/2202/2018 and one attributed to a known group not listed in the 2019–20 reporting categories.
- 26 were B/Yamagata-lineage clade 3 represented by the vaccine virus B/Phuket/3073/2013 with a further two attributed to a known group not listed in the 2019–20 reporting categories.
- 694 were B/Victoria-lineage viruses, with 630 being subclade 1A(Δ3)B represented by B/Washington/02/2019, 19 being subclade 1A(Δ2) represented by the vaccine virus B/Colorado/06/2017, five being subclade 1A(Δ3)A represented by B/Hong Kong/269/2017 and 40 attributed to a known group not listed in the 2019–20 reporting categories.

Antiviral susceptibility

Up to week 25/2020, a total of 1 993 viruses (825 A(H1N1)pdm09, 694 A(H3N2) and 474 type B) collected up to week 14/2020 of the 2019–20 season had been tested for susceptibility to neuraminidase inhibitors, oseltamivir and zanamivir. Three A(H1N1)pdm09 viruses carried amino acid substitution H275Y in NA, with one of them also having H295S substitution, both of which are indicative of highly reduced inhibition (HRI) by oseltamivir. A further two A(H1N1)pdm09 viruses showed reduced inhibition (RI) by oseltamivir in phenotypic assays, one of which also showed RI by zanamivir. One A(H3N2) virus showed HRI by oseltamivir with RI by zanamivir and carried NA R292K amino acid substitution. One B/Victoria-lineage virus showed HRI by oseltamivir and RI by zanamivir in phenotypic assays.

At the WIC this season, 690 viruses from EU/EEA countries have been assessed phenotypically against oseltamivir and zanamivir: 259 A(H1N1)pdm09, 238 A(H3N2), 186 B/Victoria-lineage and seven B/Yamagata-lineage. Two A(H1N1)pdm09 viruses (A/Denmark/3295/2019 and A/Denmark/3311/2019) showed HRI by zanamivir associated with NA Q136K amino acid substitution, one A(H3N2) virus (A/Limoges/2326/2019) showed RI by zanamivir associated with NA T148I substitution (resulting in the loss of a potential N-linked glycosylation motif) and one B/Victoria-lineage virus (B/Estonia/125782/2020) showed RI by zanamivir.

Influenza A(H7N9) virus

On 1 April 2013, World Health Organization (WHO) Global Alert and Response [4] 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 [5]. 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 [6]. WHO posted an analysis of information on A(H7N9) viruses on 10 February 2017 [7], and ECDC published a rapid risk assessment on the implications of A(H7N9) for public health on 3 July 2017 [8]. Current risk assessments are included in the WHO monthly summary and assessment of influenza at human-animal interface (accessed 6 July 2020). The assessment published on 16 July 2020 indicates that there have been no publicly available reports from animal health authorities in China or other countries on influenza A(H7N9) virus detections in animals in recent months [9]. The most recent human case was detected in mid-March 2019 [10]. 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 30 June 2020 and can be found on the ECDC website [11].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human–animal interface was published by WHO on 16 July 2020, while no new human cases were reported, according to reports received by the World Organisation for Animal Health (OIE), various influenza A(H5) subtypes continue to be detected in birds in Africa, Europe and Asia [9]. No new human cases of A(H5N1) infection have been detected since the case in Nepal in March 2019, the first human case of A(H5N1) infection reported to WHO since 2017; there have been, however, reports of A(H5N1) infection in domestic birds since February 2019 [12]. On 18 November 2016, ECDC published a rapid risk assessment related to outbreaks of highly pathogenic avian influenza H5N8 viruses in Europe [13]. As described above, the EU Reference Laboratory for

Avian Influenza, in collaboration with ECDC and the European Food Safety Authority, published on 30 June 2020 the latest overview of avian influenza, which can be found on the ECDC website [11].

Influenza A(H9N2) virus

Since the last update on 8 May 2020, two new laboratory-confirmed human cases of influenza A(H9N2) virus infections in China were reported, both in children with mild disease symptoms and exposure to poultry [9]: one in Shandong province (9 May 2020, disease onset 28 April) and one in Fujian province (13 May 2020, disease onset 4 May). Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa.

Other influenza zoonotic events

Since the last update on 8 May 2020, two additional zoonoses with swine viruses were reported [9]: one A(H1N2)v in Brazil in a 22 year-old female (22 June 2020, onset 12 April) and one A(H1N1)v (clade 1C.2.2) in Germany in a two year-old male (3 July 2020, onset 9 June). Both patients had swine exposure and recovered well.

WHO CC reports

A description of results generated by the London WHO CC at the WIC and used at the most recent WHO vaccine composition meeting (held in Geneva, Switzerland 24–28 February 2020), and previous ones, can be found at https://www.crick.ac.uk/partnerships/worldwide-influenza-centre/annual-and-interim-reports (accessed 7 July 2020).

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 highlighted in yellow. Sequences for most viruses from non-EU/EEA countries were recovered from the GISAID EpiFlu database. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from the GISAID 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.

References

1. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2019–2020 northern hemisphere influenza season. Wkly Epidemiol Rec. 2019 Mar 22;94(12):141-150. Available from: https://apps.who.int/iris/bitstream/handle/10665/311441/WER9412-141-150.pdf
2. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2020 southern hemisphere influenza season. Wkly Epidemiol Rec. 2019 Oct 18;94(42):473-496. Available from: https://apps.who.int/iris/bitstream/handle/10665/329390/WER9442-eng-fre.pdf
3. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2020- 2021 northern hemisphere influenza season. Wkly Epidemiol Rec. 2020 Mar 20;95(12):105-116. Available from: http://extranet.who.int/iris/restricted/bitstream/handle/10665/331503/WER9512-eng-fre.pdf
4. World Health Organization. Emergencies preparedness, response – Human infection with influenza A(H7N9) virus in China. 1 April 2013 [internet]. Geneva: WHO; 2013 [accessed 6 July 2020]. Available from: http://www.who.int/csr/don/2013_04_01/en/index.html
5. World Health Organization. Influenza – Avian influenza A(H7N9) virus [internet]. Geneva: WHO; 2017 [accessed 6 July 2020]. Available from: http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/
6. World Health Organization. Emergencies preparedness, response –Human infection with avian influenza A(H7N9) virus – China [internet]. Geneva: WHO; 2017 [accessed 6 July 2020]. Available from: http://www.who.int/csr/don/26-october-2017-ah7n9-china/en/
7. World Health Organization. Analysis of recent scientific information on avian influenza A(H7N9) virus. 10 February 2017 [internet]. Geneva: WHO, 2017 [accessed 6 July 2020]. Available from: http://www.who.int/influenza/human_animal_interface/avian_influenza/riskassessment_AH7N9_201702/en
8. European Centre for Disease Prevention and Control. Influenza A(H7N9) virus in China - implications for public health - 7th update, 3 July 2017. Stockholm: ECDC; 2017. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/2017-07-03-RRA-Disease-China_H7N9_0.pdf
9. World Health Organization. Influenza at the human-animal interface. Summary and assessment, 9 May to 10 July 2020 [internet]. Geneva: WHO; 2020. Available from: https://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_10_07_2020.pdf
10. World Health Organization. Influenza at the human–animal interface. Summary and assessment, 13 February to 9 April 2019 [internet]. Geneva: WHO; 2019. Available from: https://www.who.int/influenza/human_animal_interface/Influenza_Summary_IRA_HA_interface_09_04_2019.pdf
11. European Centre for Disease Prevention and Control, European Food Safety Authority, European Union Reference Laboratory for Avian influenza. Avian influenza overview February – May 2020. Parma and Stockholm: EFSA, ECDC; 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Avian-influenza-overview-February%E2%80%93May-2020.pdf
12. World Health Organization. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2020. Geneva: WHO; 2020. Available from: https://www.who.int/influenza/human_animal_interface/2020_10_07_tableH5N1.pdf
13. European Centre for Disease Prevention and Control. Outbreak of highly pathogenic avian influenza A(H5N8) in Europe – 18 November 2016. Stockholm: ECDC; 2016. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/risk-assessment-avian-influenza-H5N8-europe.pdf