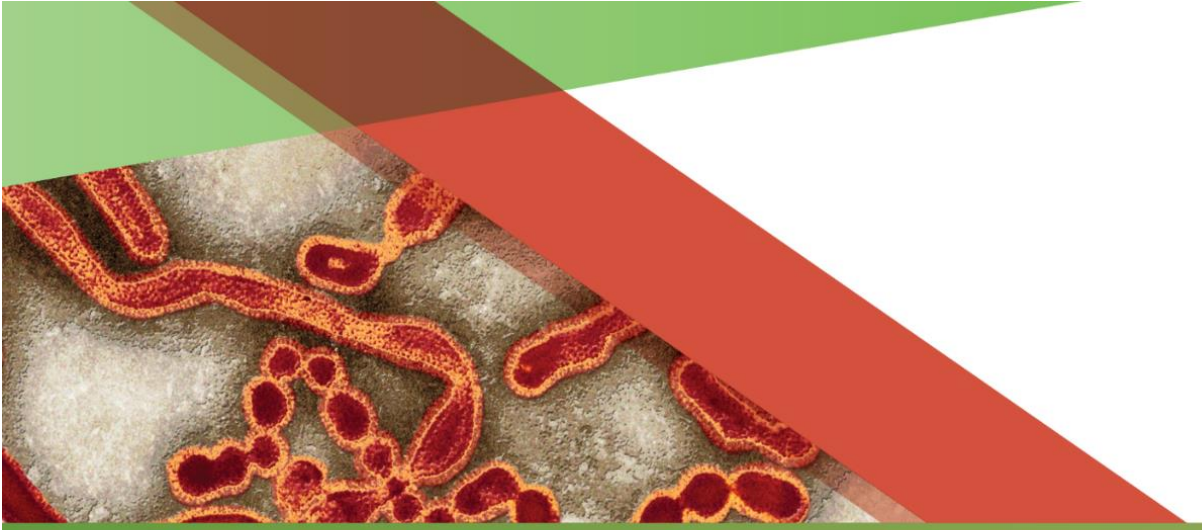

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, March 2022

Summary

This is the fifth report for the 2021-2022 influenza season. As of week 13/2022, 90 644 influenza detections across the WHO European Region were reported to the European Surveillance System (TESSy), a rise of over 87 000 since week 47/2021 with most being reported from week 49/2021 onwards. Of these 90 644 detections, 98% were type A viruses, with A(H3N2) (93%) dominating over A(H1N1)pdm09 (7%), and 2% type B of which only 32 were ascribed to a lineage, with all but one being B/Victoria. This represents a large increase (89 859, 115-fold) in detections compared to the 2020-2021 season, on the back of a large increase (1 548 266, 347%) in the number of samples tested. However, while there have been clear indications of an influenza epidemic in 2021-2022 with the epidemic threshold of 10% positivity within sentinel specimens having been crossed for 10 weeks as of week 13/2022 (unlike in 2020-2021), numbers of detections are significantly reduced compared to earlier seasons (e.g. 44% reduced compared to 2019-2020). The increased testing but reduced number of influenza detections is undoubtedly related to the emergence of SARS-CoV-2 and measures introduced to combat it.

Since the February 2022 characterisation report[1], one shipment from an EU/EEA country (Slovenia) was received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC). This report focuses on viruses with collection dates after 31 August 2021 for which HA gene sequences were made available in GISAID after 8 February 2022 to 4 April 2022, together with sequences generated and antigenic data determined at the WIC.

Globally, relatively few A(H1N1)pdm09 viruses have been detected in the course of the 2021-2022 season with 6B.1A.5a.1 and 6B.1A.5a.2 subgroups being most commonly detected, but with dominance of a particular subgroup varying between countries. The subgroups are antigenically different and 6B.1A.5a.1 viruses have been most numerous in Europe. An emergent genetic group within this subgroup showing antigenic drift, defined by HA1 P137S and G155E amino acid substitutions, has been detected. At the February 2022 WHO influenza vaccine composition meeting (VCM), the recommendation was to retain A/Victoria/2570/2019-like viruses (6B.1A.5a.2) as the vaccine component for the northern hemisphere 2022-2023 influenza season.

In Europe and across the world, A(H3N2) viruses have been dominant with the vast majority of recently detected viruses falling in subgroup 3C.2a1b.2a being 'Bangladesh-like' (3C.2a1b.2a.2). While small clusters of viruses showing

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, February 2022. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/en/publications-data/influenza-virus-characterisation-summary-europe-february-2022

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antigenic drift have emerged among the 'Bangladesh-like' viruses, the great majority of these viruses retained good recognition by post-infection ferret antisera raised against A/Darwin/9/2021-like and A/Darwin/6/2021-like (3C.2a1b.2a.2) viruses which were recommended for egg- and cell-based vaccines to be used in the 2022 southern hemisphere season. At the February 2022 WHO VCM, the recommendation was to change the A(H3N2) vaccine components for the northern hemisphere 2022-2023 influenza season to match those to be used in the 2022 southern hemisphere season.

In Europe and across the world, few B/Victoria-lineage viruses have been detected during the 2021-2022 influenza season. All have lost encoding of a three amino acid triplet (HA1 residues 162-164) placing them in subclade V.1A.3 represented by B/Washington/02/2019 the vaccine virus recommended for inclusion in influenza vaccines for the 2021-2022 northern hemisphere season. The great majority of HA sequences from recently detected viruses, in geographically dispersed countries, have fallen in the V1A.3a group defined by a series of HA1 amino acid substitutions including N150K, with most falling in the V1A.3a.2 subgroup with defining HA1 A127T, P144L and K203R amino acid substitutions. Viruses in subgroup V1A.3a.2 are not recognised well by post-infection ferret antisera raised against B/Washington/02/2019-like viruses and B/Austria/1359417/2021-like (V.1A.3a.2) viruses were recommended for use in the southern hemisphere 2022 and the northern hemisphere 2022-2023 influenza seasons.

No cases of infection with circulating B/Yamagata-lineage viruses have been confirmed since March of 2020. All HA gene sequences from the 77 viruses detected in 2020, inclusive of 12 from EU/EEA countries, belong to genetic clade Y3 and carry three HA1 amino acid substitutions (L172Q, D229N and M251V) compared to B/Phuket/3073/2013-like viruses which are still recommended for use in quadrivalent influenza vaccines. There is a need to share all B/Yamagata-lineage viruses detected recently for detailed characterisation to determine if there are any in circulation that are not related to Live Attenuated Influenza Vaccines.

Table 1 shows a summary of influenza virus detections in the WHO European Region reported to The European Surveillance System (TESSy) database during the 2021-2022 season (weeks 40/2021-13/2022), compared to the same period in the 2020-2021 season. There has been a vast increase in the number of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (1 548 266, 347%), even when compared with a more 'normal' season, 2019-2020 (1 436 993, 295%: results not shown), which led into the COVID-19 pandemic. With this increased testing there has been a rise in the number of influenza-positive samples (89 859, 115-fold), though there was a reduction compared to the same period in 2019-2020 (69 970, 44%: results not shown). These data probably relate to a number of factors: (i) significant numbers of samples taken from patients fulfilling ILI and/or ARI criteria being infected with other agents, possibly SARS-CoV-2, the virus responsible for the COVID-19 pandemic; (ii) restrictions on travel and social/work place gatherings, imposed to help curtail the spread of SARS-CoV-2, also impeding the spread of influenza viruses; (iii) increased use of personal protective equipment (e.g. face masks) and hygiene measures (e.g. hand-washing and surface disinfection), and; (iv) viral interference, with SARS-CoV-2 infection impeding infection by influenza viruses.

With these caveats, and being mindful of the low number of detections during of the 2020-2021 season, the ratio of type A to type B detections has increased compared to the 2020-2021 season (1:1 to 50.3:1), with a greater dominance of A(H3N2) over A(H1N1)pdm09 viruses. While the number of influenza B virus detections has increased from 393 to 1 766 (449%), only small numbers were ascribed to a lineage in both time periods (Table 1) and based on sequences available in GISAID, B/Yamagata lineage viruses with collection dates after March 2020 have not been characterised genetically. Currently, it appears that measures introduced relating to the COVID-19 pandemic are still having an effect, but there has been a clear indication of an influenza season in the Region during 2021-2022 with the rate of influenza positivity in sentinel samples above 10%, the epidemic threshold set for the Region, for 10 weeks (weeks 49/2021 to 1/2022 and weeks 9-13/2022) with A(H3N2) viruses dominating.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2021-22 season (weeks 40/2021-13/2022)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40/2021-13/2022			Totals*		Cumulative number of detections for weeks 40/2020-13/2021			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**4765**	**84113**	**88878**	**98.1**	**50.3:1**	**22**	**370**	**392**	**49.9**	**1:1**
A(H1N1)pdm09	284	1605	1889	7.5		13	29	42	47.2	
A(H3N2)	3488	19647	23135	92.5	12.2:1	6	41	47	52.8	1.1:1
A not subtyped	993	62861	63854			3	300	303		
Influenza B	**59**	**1707**	**1766**	**1.9**		**16**	**377**	**393**	**50.1**	
Victoria lineage	7	24	31	96.9	31:1	2	11	13	81.3	4.3:1
Yamagata lineage	0	1	1	3.1		0	3	3	9.7	
Lineage not ascribed	52	1682	1734			14	363	377		
Total detections (total tested)	**4 824 (46 304)**	**85 820 (>2 129 302)**	**90 644 (>2 175 606)**			**38 (32 556)**	**747 (>594 784)**	**785 (>627 340)**		

[a] Numbers taken from Flu News Europe to week 13/2022 and week 13/2021 reports for the two influenza seasons

* 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 Victoria:Yamagata lineages.

Genetic and antigenic characterisation data generated at the WIC for viruses with collection dates after 31 August 2020 until 31 January 2021, up to a report deadline of 15 February 2021, contributed to the WIC virus characterisation report that was presented at the WHO influenza vaccine composition meeting (VCM) in February 2021 when recommendations were made for the northern hemisphere 2021-2022 season [1]. Data generated on viruses with collection dates after 31 January 2021 until 31 August 2021 informed the September 2021 VCM when recommendations were made for the 2022 southern hemisphere season [2]. Data presented in the February report for viruses with collection dates after 31 August 2021 until 31 January 2022 contributed to the most recent VCM (21-24 February) where recommendations were made

for the 2022-2023 northern hemisphere influenza seasons [3]. For the 2022-2023 northern hemisphere season it was recommended to change the A(H3N2) and B/Victoria-lineage components of influenza vaccines to match those to be used in 2022 southern hemisphere vaccination campaigns.

Due to the relatively low number of influenza-positive specimens detected until recently, and thereby available for sharing with WIC, this and recent influenza characterisation reports have been mainly based on phylogenetic analyses of complete HA gene sequences submitted to the EpiFlu™ database of the Global Initiative on Sharing All Influenza Data (GISAID), inclusive of sequences generated at the WIC, with those from EU/EEA countries highlighted. A(H1N1)pdm09, A(H3N2) and B/Victoria-lineage HA gene phylogenies for viruses with collection dates after 31 August 2021, for representative non-WIC generated sequences available in GISAID, generated for the February report are presented (Figures 1a, 2a and 3a). Additional phylogenies for A(H1N1)pdm09 and B/Victoria-lineage viruses including all those sequences made available in GISAID and generated by WIC after 8 February 2022 are included (Figures 1b and 3b) together with an A(H3N2) representative tree for sequences from both sources (Figure 2b). Table 2 shows the numbers of HA sequences, derived from viruses with collection dates after 31 August 2021, available and used in the respective phylogenies generated for the March report.

Table 2. Summary of the numbers of HA gene sequences available and used in generating the phylogenies presented in this report

Virus subtype/lineage	Number of HA sequences from viruses collected in the 2021-2022 season			
	GISAID (non-WIC) total[1]	GISAID representatvive set[2]	WIC total[1]	WIC representative set[2]
A(H1N1)pdm09	127	127	39	39
A(H3N2)	1425	145	275	100
B/Victoria	49	49	25	25

[1] Number of individual virus sequences released/generated since the February 2022 report

[2] Number of sequences included in the representative phylogenies for the March 2022 report

Since week 40/2021, 32 shipments of specimens (virus isolates and/or clinical specimens) were received at the WIC from WHO Global Influenza Surveillance and Response System (GISRS) recognised National Influenza Centres (NICs) in a total of 17 EU/EEA countries and the UK (Table 3). Of the 485 samples received 468 (96.5%) were type A viruses and 17 (3.5%) were type B viruses. A single shipment, from Slovenia, was received since the February report.

A total of 39 viruses from EU/EEA countries, seven A(H1N1)pdm09 and 32 A(H3N2), have been characterised antigenically since the February report (Tables 3 and 4 respectively).

Table 3. Summary of seasonal influenza clinical samples and virus isolates* with collection dates after week 39/2021 contained in packages received from EU/EEA Member States

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]
2021														
September														
Belgium	1					1	1							
Croatia	3					3	2	0						
Denmark	5					5	5							
France	11			1	0	10	9	0						
Italy	1					1	1	0						
Netherlands	13					12	in process				1	1		
Spain	1					1	0	0						
Sweden	2			1	1	1	1							
UK (England)	2					2	2							
October														
Denmark	3			1	1	2	1	0						
Estonia	1					1	0	0						
France	12			9	8	3	3							
Germany	2					2	in process							
Ireland	1					1	1							
Italy	5			3	3	2	2							
Netherlands	36					36	in process							
Norway	7					7	7							
Portugal	3					2	0	0	1	0				
Spain	4					4	in process							
Sweden	2					2	2							
UK (England)	8					8	8							
UK (Scotland)	5										1		4	
November														
Belgium	2					2	2							
Croatia	1			1	1									
Estonia	1					1	0	0						
France	28			18	13	10	8	0						
Germany	5					4	in process				1	1		
Ireland	3					2	0	0			1	1		
Ireland	1					1								
Italy	5					5	5							
Netherlands	23					23	in process							
Norway	8					8	5	0						
Romania	1										1	1		
Spain	36			1	1	33	in process		1	0	1	0		
Slovenia	2					2	in process							
Sweden	5					5	5							
UK (Scotland)	2					2								
UK (N. Ireland)	3					2			1					
December														
Belgium	15	1	in process	7	in process	7	2	0						
Croatia	7			1	1	6	5	0						
Estonia	7					7	1	0						
France	1					1	1							
Germany	10					10	in process							
Hungary	2					2	2							
Ireland	4			1	0	3	3							
Ireland	1					1								
Latvia	5					5	5							
Netherlands	26			5	5	21	in process							
Norway	1					1	1							
Portugal	17	1	0			13	3	0	1	0	2	1		
Romania	5					5	5							
Slovenia	1					1	in process							
Spain	53					52	in process				1	0		
2022														
January														
Belgium	16			7	in process	9	in process							
Estonia	4					4	4							
Germany	3					3	3							
Hungary	2					2	2							
Ireland	2					2	0	0						
Ireland	2			1		1								
Latvia	1					1	1							
Norway	9			1	0	8	4	0						
Romania	4			1	1	3	3							
Slovenia	2					2	in process							
Spain	3					3	in process							
FEBRUARY														
Slovenia	12					12	in process							
Spain	10					10	in process							
MARCH														
Slovenia	2					2	in process							
Spain	4			2	in process	2	in process							
18 Countries	485	2	0	61	35	405	115	0	4	0	9	5	4	0
		0.41%		12.6%		83.5%			0.8%		1.9%		0.8%	
		96.5%							3.5%					

*** 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 (H3N2 only)
Samples provided in lysis buffer, so genetic characterisation only possible
Based on a 'sequence first' approach, some clinical samples were not cultured due to either failed sequence (associated with high (>30) Ct values in rtRTPCR) or multiples of viruses with identical sequence or SARS-CoV-2 co-positivity

As of 2022-04-06

Influenza A(H1N1)pdm09 virus analyses

All recently circulating viruses have fallen 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 VCM, updated for the September 2020 WHO VCM, and with a new nomenclature introduced at the time of the September 2021 WHO VCM (**6B.1A.1 to 6B.1A.7**). The recommended vaccine viruses for the northern hemisphere 2021-2022 and 2022-2023, and southern hemisphere 2022 (egg-based A/Victoria/5270/2019-like and cell-based A/Wisconsin/588/2019-like) influenza seasons are shown in red [1, 3, 2]. The seven subclades are defined by the following HA amino acid substitutions:

1. Subclade **6B.1A.1** viruses, represented by the 2019-2020 vaccine virus **A/Brisbane/02/2018**, carry an HA gene mutation encoding **HA1 S183P** amino acid substitution.
2. Subclade **6B.1A.2** viruses, represented by **A/Denmark/2728/2019**, carry HA gene mutations encoding **HA1 S183P** and **L233I** with **HA2 V193A** amino acid substitutions – a group 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.1A.3** viruses, represented by **A/Norway/3737/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions.
4. Subclade **6B.1A.4** represented by **A/Hungary/20/2018** carries HA gene mutations encoding **HA1 N129D, A144E** and **S183P** amino acid substitutions.
5. Subclade **6B.1A.5** viruses carry HA gene mutations encoding **HA1 S183P** and **N260D** amino acid substitutions and split into two groups designated **6B.1A.5a** represented by **A/Norway/3433/2018** with additional **HA1** amino acid substitutions of **N129D** and **T185A**, and **6B.1A.5b** represented by **A/Switzerland/3330/2017** with additional amino acid substitutions of **HA1 E235D** and **HA2 V193A**. Two subgroups within the **6B.1A.5a** group have been defined based on **HA1** amino acid substitutions of **D187V/A** and **Q189E** (**6B.1A.5a.1**) or **K130N**, **N156K**, **L161I** and **V250A** (**6B.1A.5a.2**).
6. Subclade **6B.1A.6** viruses, represented by **A/Ireland/84630/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions, like subclade **6B.1A.3** viruses, but fall within a separate phylogenetic branch which is closer to subclade **6B.1A.5** viruses.
7. Subclade **6B.1A.7** 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 group within this subclade has emerged with **P183S** (reversion)**, T185I, I240V** and **I286L** substitutions in **HA1** (e.g. **A/Estonia/120012/2019**).

Relatively few (132) A(H1N1)pdm09 HA sequences from viruses with collection dates after 31 August 2021 were available in GISAID for phylogenetic analysis at the time of preparing the February report. The representative set chosen for phylogenetic analysis showed an apparent dominance of subgroup **6B.1A.5a.2** viruses over **6B.1A.5a.1** viruses, but the **6B.1A.5a.2** set was dominated by viruses from Bangladesh with collection dates in September through November (Figure 1a). Viruses from EU/EEA countries fell within both subgroups but with a preponderance of those from the Netherlands falling in the **6B.1A.5a.1** subgroup.

During March 2022, a total of 166 A(H1N1)pdm09 HA sequences from viruses with collection dates after 31 August 2021 became available from GISAID and WIC (Table 2). These were composed of: a set of **6B.1A.5a** viruses from Zambia with collection dates in 2021 together with one from Mozambique collected in January 2022; a large number of **6B.1A.5a.1** subgroup viruses detected in African countries (Cameroon, Cote d'Ivoire, Ghana, Niger and Togo), South America (Argentina) and the European Region (Belgium, Croatia, France, Kosova, the Netherlands and the UK (England)); and a smaller number of **6B.1A.5a.2** subgroup viruses detected in India, Oman, USA and the European Region (Albania, the Netherlands and the UK (England)) (Figure 1b). The large majority of HA gene sequences from viruses with collection dates in 2022 fell in the **6B.1A.5a.1** subgroup.

A set of seven A(H1N1)pdm09) viruses from Belgium, with collection dates in December 2021 and January 2022, characterised antigenically since the February report all fell in subgroup **6B.1A.5a.1** (Table 3). Generally, the seven test viruses were recognised well in HI by seven of the antisera but not those raised against the three subgroup **6B.1A.5a.2** viruses which included the current vaccine virus, A/Victoria/2570/2019 (IVR-215). The test virus A/Belgium/H0017/2022 was recognised less well by five of the antisera, notably those raised against the egg-propagated cultivar of A/Guangdong-Maonan/SWL1536/2019, and fell in a phylogenetic cluster of **6B.1A.5a.1** viruses with **HA1 P137S** and **G155E** substitutions (Table 3 and Figure 1b). At the recent WHO VCM, held in Geneva from 21-24 February 2022, A/Victoria/2570/2019-like viruses were recommended for use in the northern hemisphere 2022-2023 influenza season [3]. This decision was largely based on the fact that antisera induced by **6B.1A.5a.1** subgroup viruses, in ferrets and humans, gave poor recognition of **6B.1A.5a.2** subgroup viruses and most of the human population are unlikely to have been exposed to **6B.1A.5a.2** subgroup viruses given their low level circulation during the COVID-19 pandemic.

Figure 1a. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, February 2022)

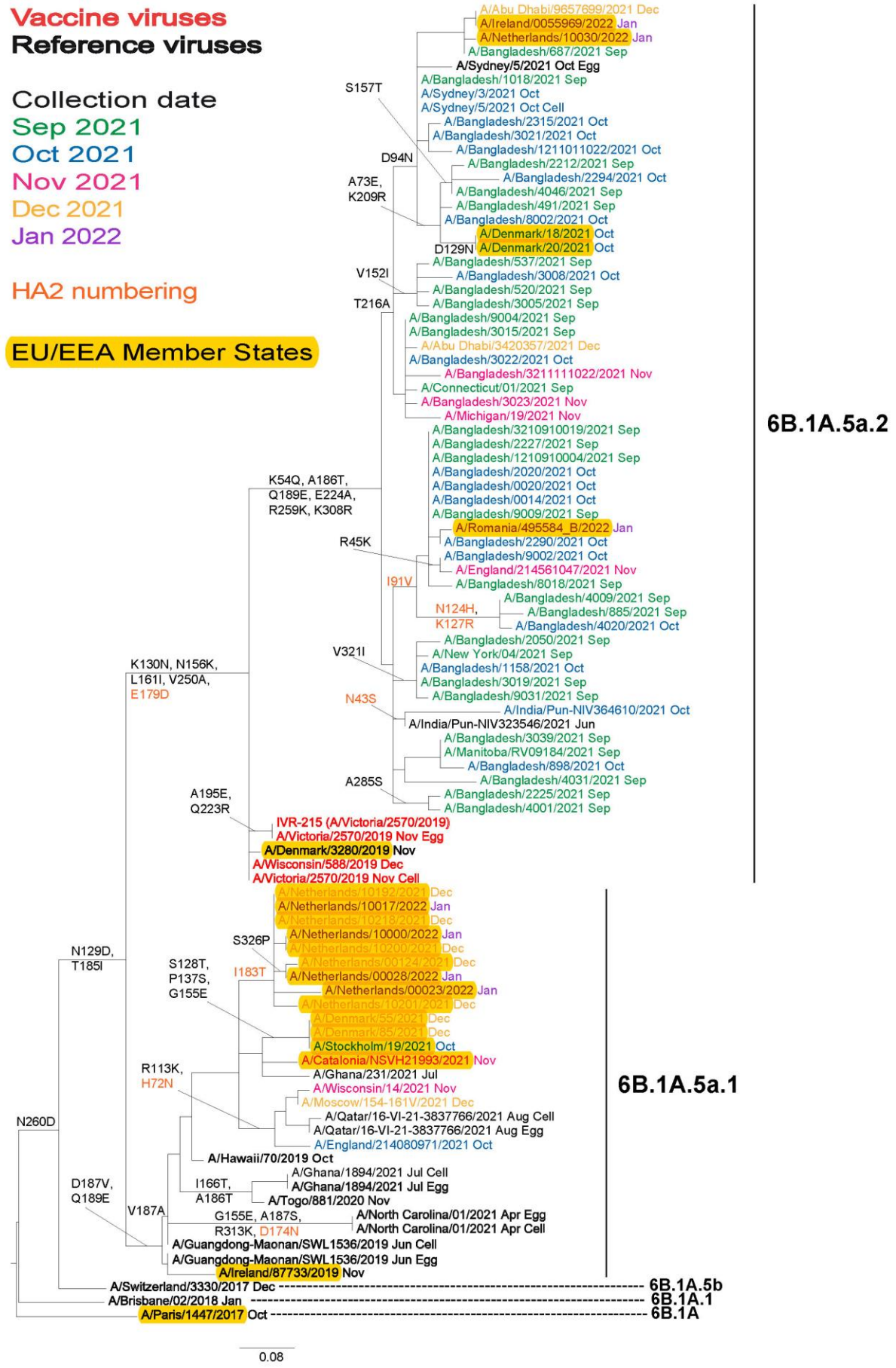


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAD/WIC, March 2022)

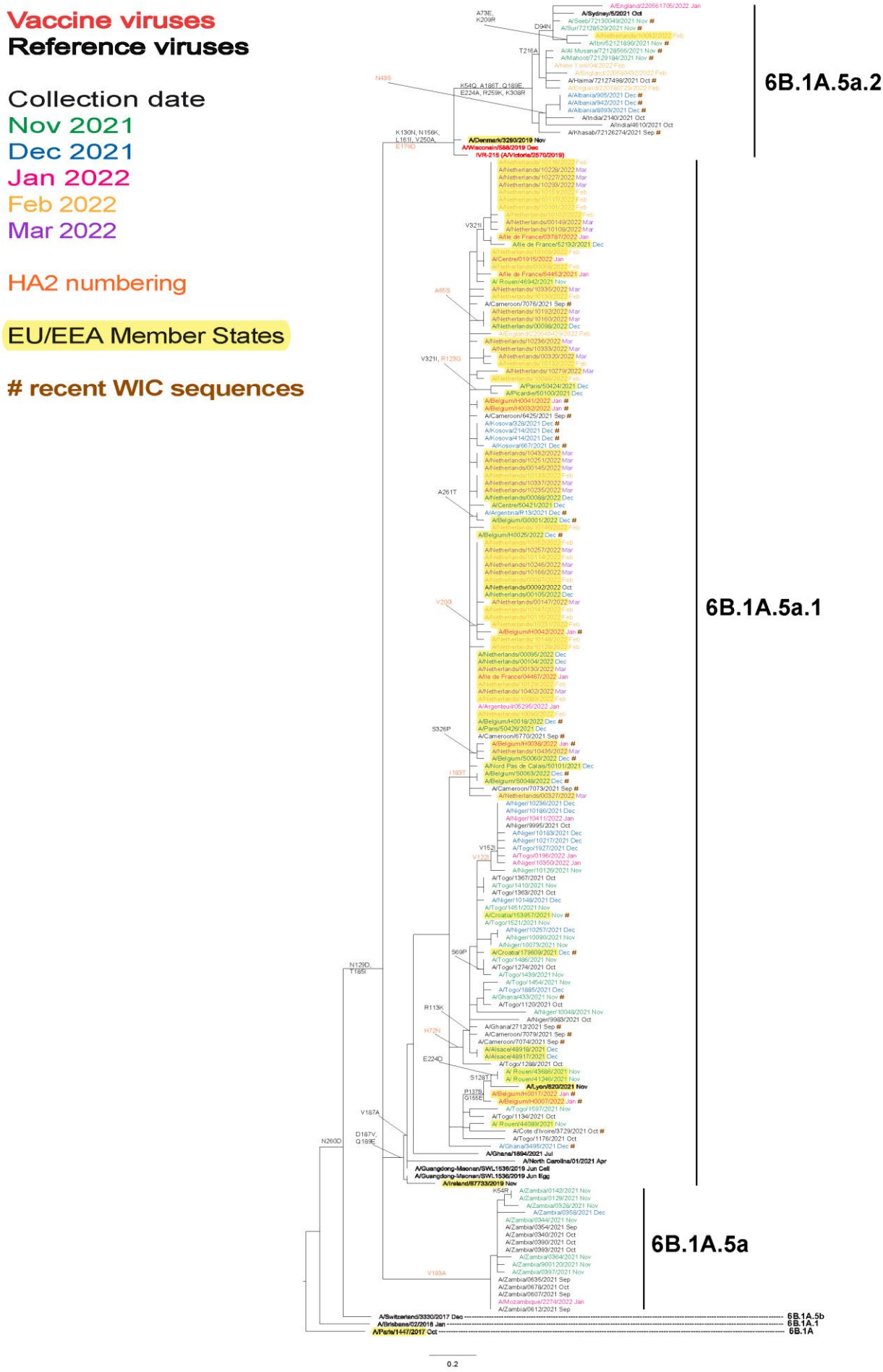


Table 3. Antigenic analysis of influenza A(H1N1)pdm09 viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
				Post-infection ferret antisera										
				A/Paris 1447/17 MDCK	A/Bris 02/18 Egg	A/Swit 3330/17 Egg	A/Ire 87733/19 Egg	A/G-M SWL1536/19 Egg	A/G-M SWL1536/19 MDCK	A/Ghana 1894/21 Egg	A/Denmark 3280/19 MDCK	IVR-215 A/Vic/2570/19 Egg	A/Sydney 5/21 Egg
Passage history							St Jude's						
Ferret number				F03/18*2	F09/19*1	F23/18*1	F18/20*1	F12/20*1	F09/20*1	F02/22*1	F08/20*1	F37/21*1	F04/22*1
Genetic group				6B.1A	6B.1A.1	6B.1A.5b	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.1	6B.1A.5a.2	6B.1A.5a.2	6B.1A.5a.2
REFERENCE VIRUSES													
A/Paris/1447/2017	6B.1A	2017-10-20	MDCK1/MDCK3	1280	1280	1280	1280	2560	2560	1280	40	80	40
A/Brisbane/02/2018	6B.1A.1	2018-01-04	E3/E2	1280	1280	1280	640	2560	1280	1280	40	160	80
A/Switzerland/3330/2017	clone 35 6B.1A.5b	2017-12-20	E6/E2	1280	640	1280	640	1280	1280	640	40	80	40
A/Ireland/87733/2019	6B.1A.5a.1	2019-11-03	E4	1280	640	640	1280	2560	2560	1280	40	80	80
A/Guangdong-Maonan/SWL1536/2019	6B.1A.5a.1	2019-06-17	E3/E2	640	640	320	640	1280	1280	640	40	80	40
A/Guangdong-Maonan/SWL1536/2019	6B.1A.5a.1	2019-06-17	C2/MDCK1	1280	640	640	1280	2560	2560	1280	80	160	80
A/Ghana/1894/2021	6B.1A.5a.1	2021-07-21	E3	1280	640	640	640	1280	1280	640	40	80	40
A/Denmark/3280/2019	6B.1A.5a.2	2019-11-10	MDCK4/MDCK6	160	80	80	160	160	160	80	2560	>5120	2560
IVR-215 (A/Victoria/2570/2019)	6B.1A.5a.2	2018-11-22	E4/D7/E2	80	40	40	80	160	160	80	1280	2560	1280
A/Sydney/5/2021	6B.1A.5a.2	2021-10-16	E3/E1	80	40	80	40	80	80	80	1280	2560	2560
TEST VIRUSES													
A/Belgium/H0025/2022	6B.1A.5a.1	2021-12-10	MDCK1/MDCK1	640	320	320	640	1280	1280	640	<	40	40
A/Belgium/H0018/2022	6B.1A.5a.1	2021-12-20	MDCK1/MDCK1	1280	320	320	640	1280	1280	640	40	40	40
A/Belgium/S0060/2022	6B.1A.5a.1	2021-12-26	MDCK1/MDCK1	640	320	320	320	1280	1280	1280	<	80	40
A/Belgium/S0048/2022	6B.1A.5a.1	2021-12-26	MDCK1/MDCK1	1280	320	320	640	1280	1280	640	40	80	40
A/Belgium/H0017/2022	6B.1A.5a.1	2022-01-05	MDCK1/MDCK1	320	160	160	160	320	1280	640	40	80	80
A/Belgium/H0032/2022	6B.1A.5a.1	2022-01-18	MDCK1/MDCK1	640	320	320	320	640	1280	640	<	80	40
A/Belgium/H0041/2022	6B.1A.5a.1	2022-01-19	MDCK1/MDCK1	640	320	320	640	1280	1280	640	<	80	80
				Vaccine NH 2019-20 SH 2020				Vaccine NH 2020-21				Vaccine SH 2021 NH 2021-22 SH 2022 NH 2022-23	

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40; 2 < = <80; ND = Not Done

Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny was generated using a representative set of non-WIC generated HA gene sequences released in GISAID, as of 8 February 2022, for viruses with collection dates in the 2021-2022 influenza season (Figure 2a). The second phylogeny is based on representative A(H3N2) HA sequences made available in GISAID and generated at the WIC since 8 February 2022 for viruses with collection dates in the 2021-2022 influenza season (Figure 2b).

Viruses in clade **3C.2a** have been dominant since the 2014-15 influenza season with group **3C.2a1b** viruses predominating over the course of the 2019-2020 season in most WHO-defined regions of the world but for the European Region, where there was equivalence of clade **3C.3a** viruses. The HA gene sequences of viruses in both clades **3C.2a** and **3C.3a** continue to diverge. Notably, clade **3C.3a.1** 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. Greater variation has been observed among clade **3C.2a** viruses, resulting in the designation of new subclades/groups/subgroups. Amino acid substitutions that define these subclades/groups/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).
- Group **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.**
- Group **3C.2a1b**: Those in subclade **3C.2a1** plus **E62G, R142G** and **H311Q** in **HA1**, often with additional amino acid substitutions – notably **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.1** subgroup (e.g. **A/La Rioja/2202/2018**) or **HA1 T131K** and **HA2 V200I**, the **3C.2a1b.2** subgroup (e.g. **A/South Australia/34/2019**). Distinct clusters of viruses within both these subgroups have emerged defined by specific **HA1** and/or **HA2** amino acid substitutions: **3C.2a1b.1a** with additional amino acid substitutions of **HA1 A138S**, **F193S** and **S198P**, many also with **G186D** and **D190N** (e.g. **A/Denmark/3284/2019**); **3C.2a1b.1b** with additional amino acid substitutions of **HA1 S137F, A138S** and **F193S** (e.g. **A/Hong Kong/2671/2019**); **3C.2a1b.2a** with additional amino acid substitutions of **HA1 K83E** and **Y94N** with **HA2 I193M** (e.g. **A/Slovenia/1637/2020**); **3C.2a1b.2b** with **HA2 V18M** substitution, often with additional **HA1** substitutions (e.g. **A/Bretagne/1323/2020**).
- Clade **3C.3a**: represented by a former vaccine virus, **A/Switzerland/9715293/2013**, with recently circulating clade **3C.3a.1** 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 2019-2020 northern hemisphere influenza season.

The significant geographic spread of viruses in the antigenically distinct **3C.2a1b.1b** cluster influenced the selection of an A/Hong Kong/2671/2019-like or an A/Hong Kong/45/2019-like virus as the A(H3N2) component of vaccines for the 2020-2021 northern hemisphere and 2021 southern hemisphere influenza seasons.

Figure 2a indicates a single **3C.3a.1** virus and small numbers of **3C.2a1b.1b** and **3C.2a1b.1a** (notably in Africa) viruses detected and characterised during the 2021-2022 influenza season. The great majority of viruses with collection dates after 31 August 2021 were 'Bangladesh-like' (**3C.2a1b.2a.2** with **HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N** and **Y195F**). The latter viruses were split into four subgroups defined by specific **HA1** amino acid substitutions: (i) **S205F** and **A212T**; (ii) **H56Y** and **S270T**; (iii) **D53N**, commonly with **N96S** and **I192F**; (iv) **D53G** often with **I25V**, **R201K** and **S219Y** or **D104G** and **K276R**. Subgroups (iii) and (iv) also share **HA1 H156S** amino acid substitution.

The second phylogeny, based on a representative set of sequences derived from those released in GISAID and those generated by WIC after 8 February 2022 (Table 2) for samples shared by many countries, shows a very similar profile with the vast majority being derived from 'Bangladesh-like' (**3C.2a1b.2a.2**) viruses (Figure 2b). Just two viruses from Timor-Leste collected in January 2022 were 'Cambodia-like' (**3C.2a1b.2a.1** with **HA1** substitutions of **K171N**, **G186S** and **S198P**). Similarly, small numbers of **3C.2a1b.1a** viruses were detected by countries in WHO African (Cote d'Ivoire, Ghana, Mozambique, Niger, South Africa, Togo and Zambia) and European (Belgium, France, Norway and Spain) Regions and **3C.2a1b.1b** viruses were received from African (Kenya) and European (France) Regions. In both phylogenies, sequences derived from samples collected in EU/EEA countries are dispersed throughout the trees with the 'Bangladesh-like' (**3C.2a1b.2a.2**) viruses falling into multiple virus clusters defined by specific amino acid substitutions (Figures 2a and 2b).

'Bangladesh-like' **3C.2a1b.2a.2** viruses, A/Darwin/9/2021 and A/Darwin/6/2021 for egg- and cell-based vaccines respectively, were recently recommended for use in the southern hemisphere 2022 and northern hemisphere 2022-2023 influenza seasons [2, 3].

The locations of HA sequences for egg- and cell culture-propagated cultivars of A/Cambodia/e0826360/2020 (**3C.2a1b.2a.1**) recommended for use in northern hemisphere 2021-2022 vaccines [1], are indicated on the phylogenies, as are egg- and cell-culture based vaccines to be used in the 2022 southern hemisphere and northern hemisphere seasons, A/Darwin/9/2021 and A/Darwin/6/2021 (**3C.2a1b.2a.2**) respectively [2, 3] (Figures 2a and 2b).

As described in many previous reports[2], influenza A(H3N2) viruses had been 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 was a significant problem for most viruses that fell in genetic clade **3C.2a**, although there was some alleviation of this during 2019-2020 with continuation into the 2020-2021 influenza season. This issue is now much alleviated for 'Bangladesh-like' **3C.2a1b.2a.2** viruses which agglutinate guinea pig RBCs well, allowing HI assays to be performed. No A(H3N2) viruses recovered failed to agglutinate guinea pig RBCs at the time of writing this report (Table 3).

While the number of detections of seasonal influenza viruses was low from April 2020 to July 2021, compared to previous years, the WHO Collaborating Centres for Influenza have shown viruses in these emerged virus clusters to be antigenically distinguishable from one another and other A(H3N2) virus subgroups.

Of the 32 A(H3N2) viruses characterised antigenically since the February report, 29 were 'Bangladesh-like' **3C.2a1b.2a.2** viruses and three were **3C.2a1b.1a** viruses (Tables 4-1 to 4-3). Of the three **3C.2a1b.1a** viruses, one, two and two were recognised well, within fourfold of the respective homologous titres, by antisera raised against cell culture-propagated viruses A/Denmark/3264/2019 (1a), A/Hong Kong/2671/2019 (1b) and A/Cambodia/925256/2020 (2a.1), respectively. The 29 'Bangladesh-like' **3C.2a1b.2a.2** test viruses were recognised well only by post-infection ferret antisera raised against viruses with 3C.2a1b.2a.2 HAs. Antisera raised against cell culture-propagated A/Bangladesh/4005/2020 and A/Stockholm/5/2021 recognised 23 (79%) and 29 (100%) of the test viruses at titres within fourfold of the homologous titres, respectively. Similarly, the antiserum raised against cell culture-propagated A/England/214191723/2021 recognised 20/20 (100%) test viruses at titres within fourfold of the homologous titres. The antiserum raised against egg-propagated A/Darwin/9/2021 recognised 15 (52%) of the test viruses at titres within fourfold of the homologous titres.

Results of HI assays with panels of post-infection ferret antisera raised against A(H3N2) vaccine and reference viruses for viruses detected in EU/EEA countries can be seen in previous influenza characterisation reports on ECDC's website. Overall, these data show strong clade/subclade-specific recognition of test viruses by post-infection ferret antisera raised against cell culture-propagated reference viruses, with limited cross-clade/subclade recognition and further reductions in recognition of cell culture-propagated recently circulating viruses by antisera raised against A(H3N2) egg-propagated vaccine viruses.

[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

Figure 2a. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, February 2022)

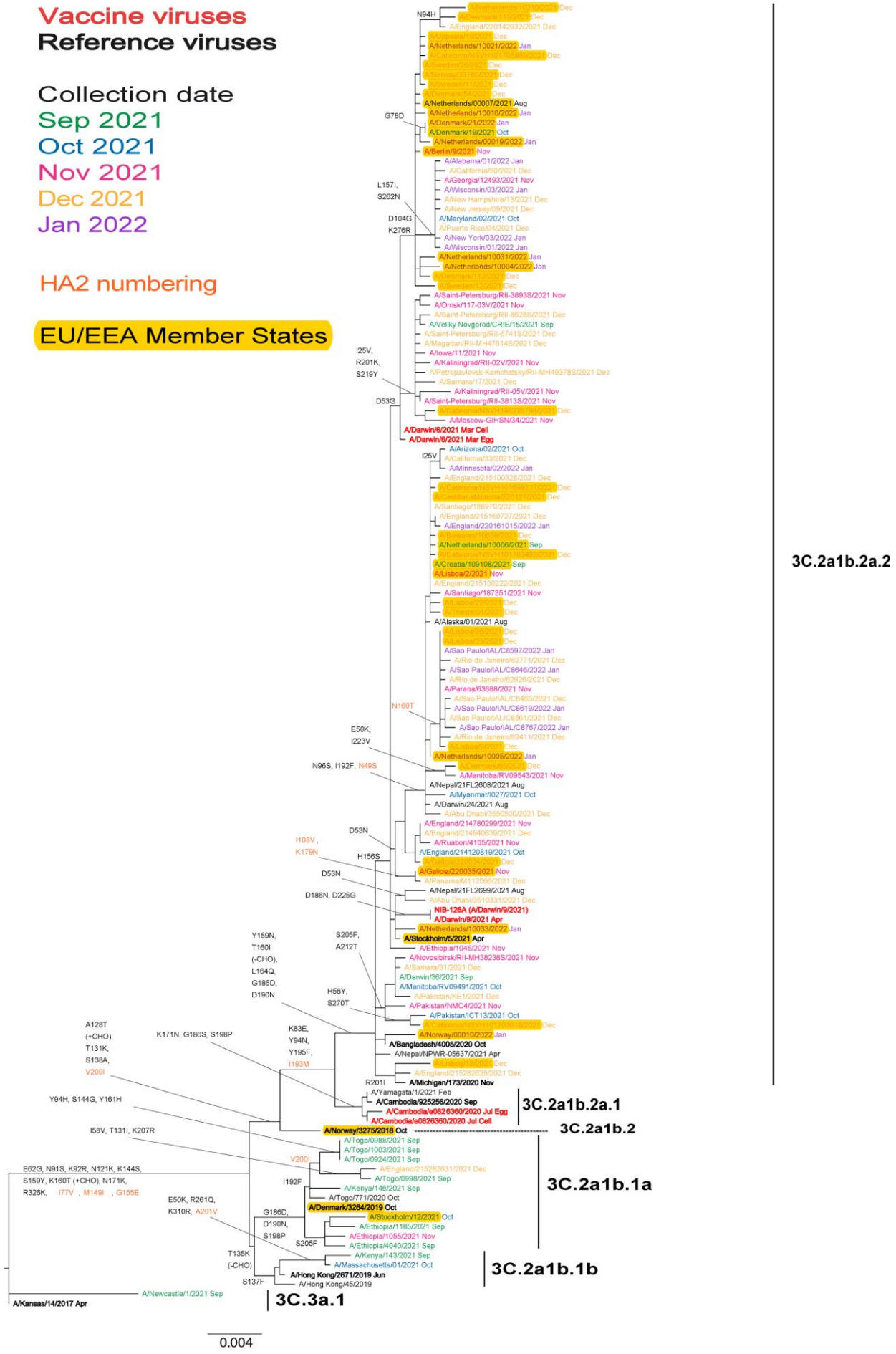


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID/WIC, March 2022)

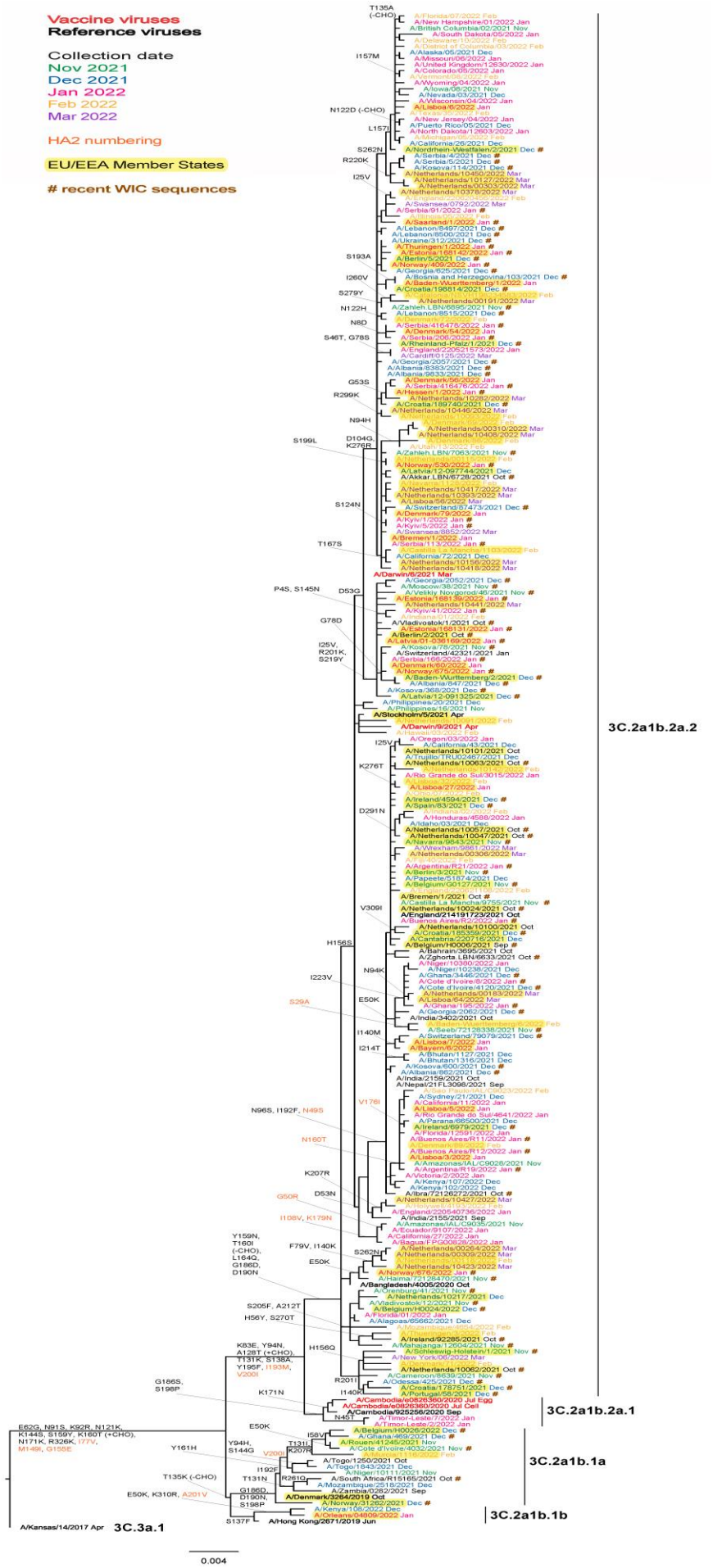


Table 4-1. Antigenic analysis of influenza A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre								
				Post-infection ferret antisera								
				A/Denmark	A/HK	A/Camb	A/Camb	A/Bang	A/Darwin	A/Stock	A/Kansas
	Passage history			3264/19	2671/19	e0826360/20	925256/20	4005/20	9/21	5/21	14/17
				SIAT	Cell	Egg	SIAT	SIAT	Egg	SIAT	SIAT
	Ferret number			F19/20^{*1}	St Judes F21/20^{*1}	F10/21^{*1}	F03/21^{*1}	F07/21^{*1}	F38/21^{*1}	F35/21^{*1}	F17/19^{*1}
	Genetic group			3C.2a1b.1a	3C.2a1b.1b	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.3a.1
REFERENCE VIRUSES											
A/Denmark/3264/2019	3C.2a1b.1a	2019-10-25	SIAT3/SIAT4	320	320	160	640	320	320	160	160
A/Hong Kong/2671/2019	3C.2a1b.1b	2019-06-17	MDCK1/SIAT4	160	320	160	640	320	320	160	160
A/Cambodia/e0826360/2020	3C.2a1b.2a.1	2020-07-16	E5/E2	160	40	1280	160	160	640	320	80
A/Cambodia/925256/2020	3C.2a1b.2a.1	2020-09-25	SIAT5	160	160	320	1280	320	640	320	160
A/Bangladesh/4005/2020	3C.2a1b.2a.2	2020-10-04	SIAT3	320	80	320	160	640	1280	640	320
A/Darwin/9/2021	3C.2a1b.2a.2	2021-04-17	E3/E2	160	<	320	160	320	2560	640	80
A/Stockholm/5/2021	3C.2a1b.2a.2	2021-04-16	S0/S3	80	<	160	80	320	2560	640	80
A/Kansas/14/2017	3C.3a.1	2017-12-14	SIAT3/SIAT2	40	<	80	80	80	160	80	320
TEST VIRUSES											
A/Norway/31262/2021	3C.2a1b.1a	2021-12-02	SIAT2	160	80	80	320	80	80	40	40
A/Ireland/45934/2021	3C.2a1b.2a.2	2021-12-12	SIAT2	40	<	40	40	160	640	320	<
A/Latvia/12-045953/2021	3C.2a1b.2a.2	2021-12-14	P2/SIAT1	40	<	80	40	160	640	320	<
A/Latvia/12-091326/2021	3C.2a1b.2a.2	2021-12-24	P2/SIAT1	160	<	160	80	320	1280	640	40
A/Latvia/12-091325/2021	3C.2a1b.2a.2	2021-12-24	P2/SIAT1	320	40	640	160	640	2560	1280	160
A/Latvia/12-091317/2021	3C.2a1b.2a.2	2021-12-26	P2/SIAT1	160	<	160	40	320	1280	320	40
A/Latvia/12-097744/2021	3C.2a1b.2a.2	2021-12-28	P2/SIAT1	40	<	40	40	80	640	160	<
A/Latvia/01-036169/2022	3C.2a1b.2a.2	2022-01-08	P2/SIAT1	160	<	320	160	320	1280	640	80
A/Norway/675/2022	3C.2a1b.2a.2	2022-01-11	SIAT2	160	<	320	80	320	1280	640	80
A/Latvia/01-089648/2022	3C.2a1b.2a.2		P2/SIAT1	40	<	40	40	160	640	320	<
						Vaccine NH 2021-22			Vaccine SH 2022 NH 2022-23		

Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40, ND = Not Done

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

Viruses	Other information		Collection date	Passage history	Haemagglutination inhibition titre								
					Post-infection ferret antisera								
					A/Denmark 3264/19	A/HK 2671/19	A/Camb e0826360/20	A/Camb 925256/20	A/Bang 4005/20	A/Stock 5/21	A/Eng 214191723/21	A/Darwin 9/21	A/Kansas 14/17
		Passage history			SIAT	Cell	Egg	SIAT	SIAT	SIAT	SIAT	Egg	SIAT
		Ferret number			F19/20*1	St Judes F21/20*1	F10/21*1	F03/21*1	F07/21*1	F35/21*1	F07/22*1	F38/21*1	F17/19*1
		Genetic group			3C.2a1b.1a	3C.2a1b.1b	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.3a.1
REFERENCE VIRUSES													
A/Denmark/3264/2019		3C.2a1b.1a	2019-10-25	SIAT3/SIAT4	640	640	160	640	160	320	40	320	80
A/Hong Kong/2671/2019		3C.2a1b.1b	2019-06-17	MDCK1/SIAT4	320	640	160	640	320	160	40	320	160
A/Cambodia/e0826360/2020		3C.2a1b.2a.1	2020-07-16	E5/E2	320	40	2560	320	320	320	640	1280	160
A/Cambodia/925256/2020		3C.2a1b.2a.1	2020-09-25	SIAT5	160	160	160	640	160	160	40	320	160
A/Bangladesh/4005/2020		3C.2a1b.2a.2	2020-10-04	SIAT3	320	80	320	320	640	640	640	2560	320
A/Stockholm/5/2021		3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	160	40	80	160	320	640	640	2560	80
A/England/214191723/2021		3C.2a1b.2a.2	2021-10-12	MDCK1/SIAT2	40	<	40	80	160	320	640	1280	<
A/Darwin/9/2021		3C.2a1b.2a.2	2021-04-17	E3/E2	320	40	320	160	320	640	640	5120	80
A/Kansas/14/2017		3C.3a.1	2017-12-14	SIAT3/SIAT2	80	40	80	80	80	160	80	160	640
TEST VIRUSES													
A/Belgium/H0026/2022		3C.2a1b.1a	2021-12-10	SIAT1/SIAT1	40	40	40	160	80	40	<	80	<
A/Andalucia/9561/2021		3C.2a1b.2a.2	2021-10-08	SIAT1/SIAT1	320	40	320	160	640	320	640	1280	160
A/Norway/23748/2021		3C.2a1b.2a.2	2021-10-14	SIAT1	160	<	320	80	320	320	320	640	80
A/Norway/23662/2021		3C.2a1b.2a.2	2021-10-14	SIAT1	160	<	320	80	320	320	320	640	80
A/Norway/24121/2021		3C.2a1b.2a.2	2021-10-15	SIAT1	160	<	320	80	320	320	320	640	80
A/Norway/24931/2021		3C.2a1b.2a.2	2021-10-24	SIAT1	40	<	<	40	80	160	320	320	<
A/Andalucia/9929/2021		3C.2a1b.2a.2	2021-11-11	SIAT1/SIAT1	40	<	80	80	160	320	640	640	<
A/Castilla La Mancha/9755/2021		3C.2a1b.2a.2	2021-11-16	SIAT1	40	<	40	40	80	160	320	640	<
A/Andalucia/9928/2021		3C.2a1b.2a.2	2021-11-23	SIAT1/SIAT1	40	<	40	40	160	320	640	640	<
A/Navarra/9875/2021		3C.2a1b.2a.2	2021-11-24	SIAT1	40	<	40	40	80	320	640	640	<
A/Navarra/9843/2021		3C.2a1b.2a.2	2021-11-24	SIAT1	80	<	80	80	160	640	640	2560	40
A/Norway/29512/2021		3C.2a1b.2a.2	2021-11-25	SIAT1	80	<	40	80	160	320	640	1280	<
A/Spain/58/2021		3C.2a1b.2a.2	2021-12-14	SIAT1	40	<	40	40	160	160	640	640	<
A/Belgium/H0024/2022		3C.2a1b.2a.2	2021-12-14	SIAT1/SIAT1	160	<	320	80	320	320	160	640	80
A/Portugal/58/2021		3C.2a1b.2a.2	2021-12-15	SIAT1	40	<	40	40	160	160	640	640	<
A/Portugal/57/2021		3C.2a1b.2a.2	2021-12-15	SIAT1	160	40	320	80	320	640	640	1280	80
A/Spain/90/2021		3C.2a1b.2a.2	2021-12-21	SIAT1	80	<	40	40	160	160	160	640	<
A/Spain/88/2021		3C.2a1b.2a.2	2021-12-22	SIAT1	40	<	40	40	80	160	320	640	<
							Vaccine NH 2021-22					Vaccine SH 2022 NH 2022-23	

*Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40, ND = Not Done

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

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre									
				Post-infection ferret antisera									
				A/Denmark	A/HK	A/Camb	A/Camb	A/Bang	A/Stock	A/Eng	A/Darwin	A/Kansas
	Passage history			3264/19	2671/19	e0826360/20	925256/20	4005/20	5/21	21419172 3/21	9/21	14/17
				SIAT	Cell	Egg	SIAT	SIAT	SIAT	SIAT	Egg	SIAT
	Ferret number			F19/20[-1]	St Judes F21/20[-1]	F10/21[-1]	F03/21[-1]	F07/21[-1]	F35/21[-1]	F07/22[-1]	F38/21[-1]	F17/19[-1]
	Genetic group			3C.2a1b.1a	3C.2a1b.1b	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.3a.1
REFERENCE VIRUSES												
A/Denmark/3264/2019	3C.2a1b.1a	2019-10-25	SIAT3/SIAT4	320	320	160	640	160	160	40	320	80
A/Hong Kong/2671/2019	3C.2a1b.1b	2019-06-17	MDCK1/SIAT4	320	320	160	640	160	160	40	320	160
A/Cambodia/e0826360/2020	3C.2a1b.2a.1	2020-07-16	E5/E2	160	<	1280	320	320	320	320	640	160
A/Cambodia/925256/2020	3C.2a1b.2a.1	2020-09-25	SIAT6	160	160	160	640	160	160	<	320	160
A/Bangladesh/4005/2020	3C.2a1b.2a.2	2020-10-04	SIAT3	160	160	320	320	640	640	640	1280	320
A/Stockholm/5/2021	3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	160	<	160	80	320	640	320	1280	80
A/England/214191723/2021	3C.2a1b.2a.2	2021-10-12	MDCK1/SIAT2	80	<	80	80	160	320	640	1280	40
A/Darwin/9/2021	3C.2a1b.2a.2	2021-04-17	E3/E2	160	40	640	80	320	640	640	2560	80
A/Kansas/14/2017	3C.3a.1	2017-12-14	SIAT3/SIAT2	40	40	80	80	40	80	80	160	640
TEST VIRUSES												
A/Rouen/41245/2021	3C.2a1b.1a	2021-11-15	SIAT1	40	80	<	80	40	<	<	40	40
A/Estonia/167744/2021	3C.2a1b.2a.2	2021-12-08	SIAT1	160	<	320	80	160	640	640	1280	80
A/Belgium/H0013/2022	3C.2a1b.2a.2	2022-01-17	SIAT1	<	<	80	40	160	320	640	640	40
A/Belgium/H0008/2022	3C.2a1b.2a.2	2022-01-17	SIAT1	80	<	80	40	80	160	160	320	40

Vaccine NH 2021-22

Vaccine SH 2022 NH 2022-23

*Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40, ND = Not Done

Influenza B virus analyses

Influenza B/Victoria-lineage

All recently circulating B/Victoria-lineage viruses have fallen in genetic clade **V1A**, represented by **B/Brisbane/60/2008**, a former vaccine virus, but with additional **HA1** amino acid substitutions of **I117V** and **N129D** (e.g. **B/Ireland/3154/2016**). Viruses retaining full-length HAs had 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. 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 **V1A.1**) with amino acid substitutions of **D129G** and **I180V**, and **HA2 R151K** that spread worldwide and is represented by a previous vaccine virus, **B/Colorado/06/2017**.
- A group with triple deletion of **HA1** residues **162** to **164** (subclade **V1A.2**) first detected in Asia, with amino acid substitutions of **I180T** and **K209N** that showed limited geographic spread (with no detections having been made recently), represented by **B/Hong Kong/269/2017**.
- A group with triple deletion of **HA1** residues **162** to **164** (subclade **V1A.3**) first detected in Africa, with amino acid substitution **K136E** often with **G133R** that showed geographic spread and became dominant, represented by **B/Washington/02/2019** the vaccine virus recommended after the WHO VCM in February 2021 [1].

The phylogeny generated for the February report for non-WIC HA sequences available in GISAID shows all recent viruses to fall in the **V1A.3** subclade with small numbers of viruses being related to **B/Washington/02/2019** but with viruses from the Americas having additional **HA1** substitutions of **T73I** and **N233K** (resulting in loss of a glycosylation site) and those from Kenya having **HA1 K75E**, **E128K**, **T155A** and **G230N** substitutions (Figure 3a). The great majority of viruses fall in the **V1A.3a** group characterised by **HA1 N150K**, **G184E**, **N197D** (resulting in loss of a glycosylation site) and **R279K**, with this group splitting into two subgroups designated **V1A.3a.1** (characterised by **HA1 V220M** and **P241Q** substitutions, detected predominantly in China) and **V1A.3a.2** (characterised by **HA1 A127T**, **P144L** and **K203R**, often with additional substitutions, which has spread worldwide and is represented by the **B/Austria/1359417/2021** vaccine virus).

The second phylogeny, based on sequences becoming available in GISAID and generated at the WIC during March 2022, shows a very similar profile but with subgroup **V1A.3a.2** viruses dominating recently. However, clusters of **V1A.3 B/Washington/02/2019**-like viruses were detected in different countries earlier in the season: from Madagascar, characterised by **HA1 E128K** and **T170I** substitutions; from Honduras, characterised by **HA1 T73I** and **N233K** (resulting in loss of a glycosylation site) substitutions; and from Kenya, characterised by **HA1 K75E**, **E128K**, **T155A** and **G230N** substitutions (Figure 3b).

Relatively few B/Victoria-lineage viruses have been detected in the WHO European Region (Table 1), but the WHO Collaborating Centres for Influenza have shown the **V.1A.3a** group viruses with additional HA1 substitutions to be antigenically distinct from one another. No B/Victoria-lineage viruses from EU/EEA countries were characterised at the WIC since the February report. Those characterised earlier in the 2021-2022 season were subgroup **V1A.3a.2** viruses which were recognised poorly by post-infection ferret antiserum raised against **B/Washington/02/2019**, the current vaccine virus, but well (with HI titres of at least 320 with the antiserum raised against the egg-propagated variant with **HA1 G141R** substitution) by antisera raised against **B/Austria/1359417/2021**, the recommended vaccine virus for southern hemisphere 2022 and northern hemisphere 2022-2023 influenza seasons [2, 3].

Influenza B/Yamagata-lineage

It is assumed that no B/Yamagata-lineage viruses have been detected after March 2020 as no sequences for such viruses with collection dates after this had been released is GISAID as of 4 April 2022. Figure 4 is repeated from the September 2021 report. All sequences fell in genetic clade **3** (**Y3**), 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 which is recommended for inclusion in quadrivalent vaccines for the 2020-2021 and 2021-2022 northern hemisphere, 2021 and 2022 southern hemisphere seasons [1, 2, 3, 4]. Some sub-clustering of sequences, defined by specific amino acid substitutions (e.g. **HA1 N164K, K211R, D229N** or **D232N** [introducing a potential N-linked glycosylation site] sometimes with **R48K**), has occurred. As noted in previous characterisation reports, 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. Of the four samples recently shared with WIC by UK (Scotland: Table 3) only one yielded good sequence which showed it to be associated with Live Attenuated Influenza Vaccine (LAIV).

A concerted effort by all NICs of GISRS is required to identify B/Yamagata-lineage viruses for detailed characterisation to determine if there are any in circulation that are non-LAIV-related.

[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

Figure 3a. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, February 2022)

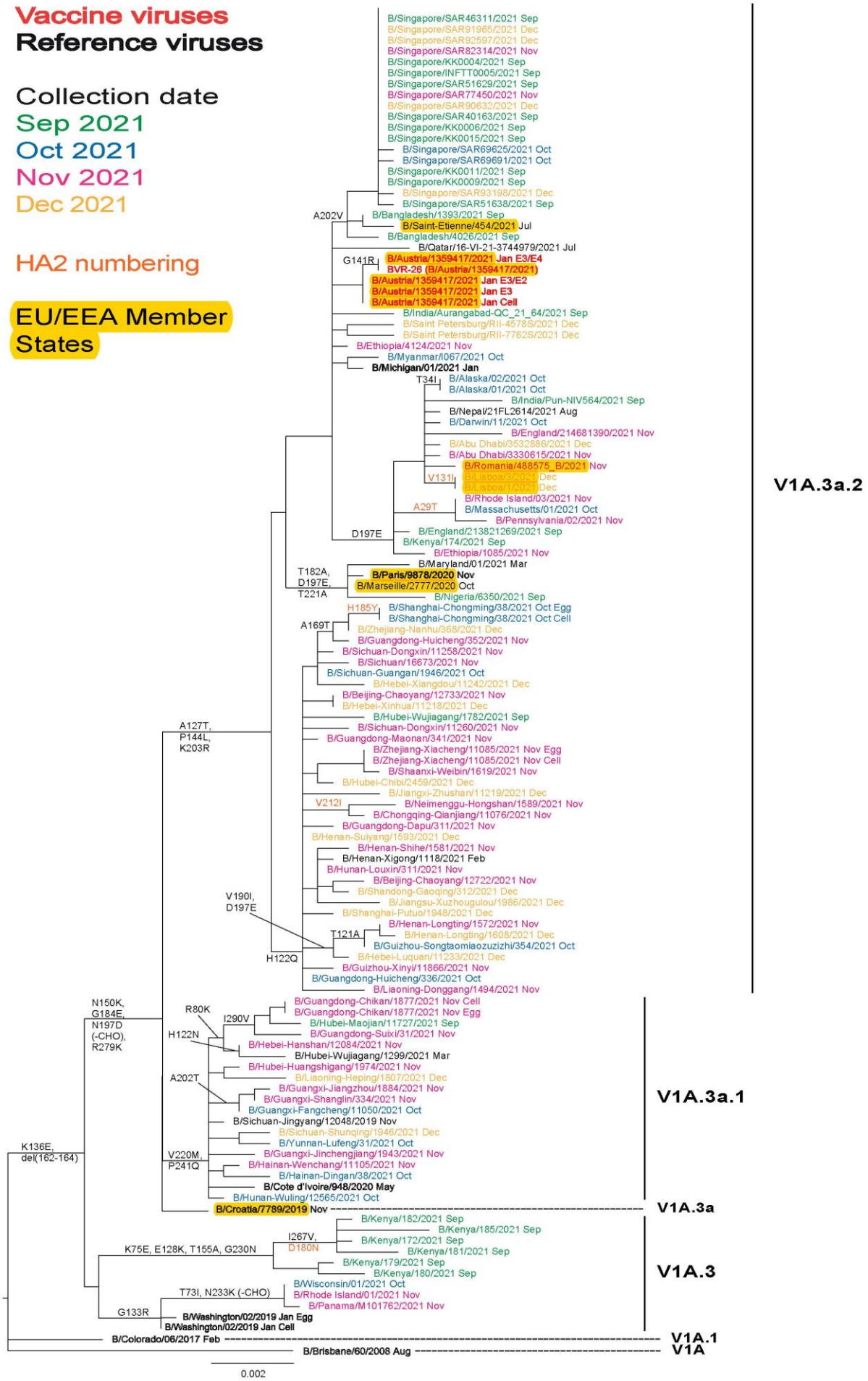


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID/WIC, March 2022)

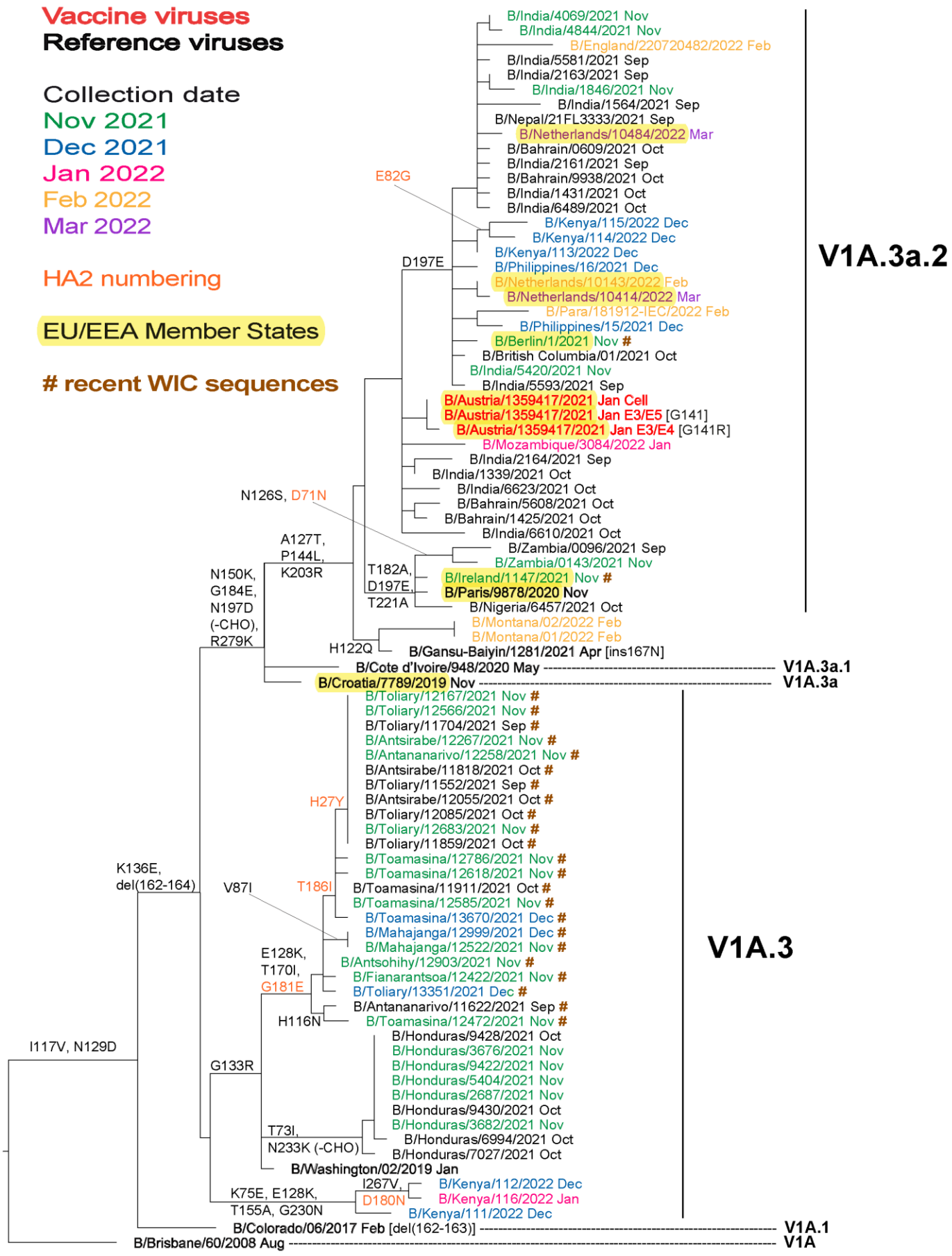
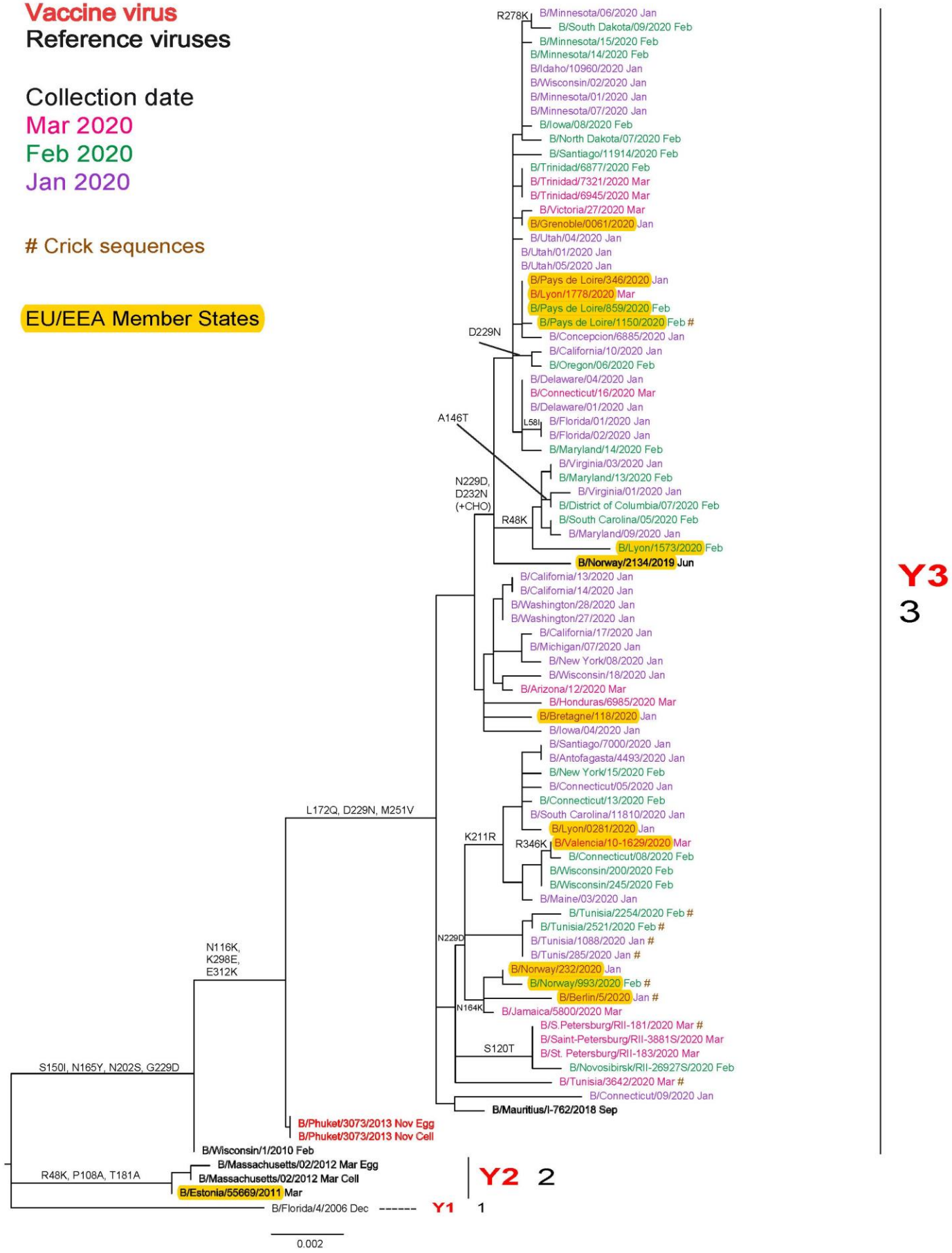


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, September 2021)



Summary of data submitted to the European Surveillance System

Genetic characterisation

1 894 viruses detected over the course of the 2021-2022 season (weeks 40/2021-13/2022) were genetically characterised:

- Of 135 A(H1N1)pdm09 viruses, 117 belonged to clade 6B.1A.5a.1 (represented by A/Guangdong-Maonan/SWL1536/2019) and 11 belonged to clade 6B.1A.5a.1 (represented by A/Victoria/2570/2019). Seven were not attributed to a clade.
- Of 1 741 A(H3N2) viruses, 1 731 belonged to the 'Bangladesh-like' clade (3C.2a1b.2a.2) represented by A/Bangladesh/4005/2020, one to the 'Cambodia-like' clade (3C.2a1b.2a.1) and nine were attributed to clade 3C.2a1b.1a (represented by A/Denmark/3264/2019).
- Eleven B/Victoria-lineage viruses, two belonging to clade V1A.3 (represented by B/Washington/02/2019) and eight to clade V1A.3a.2 (represented by B/Austria/1359417/2021). One was not attributed to a clade.
- Seven viruses were reported as B/Yamagata-lineage with four being B/Phuket/3073/2013-like. However, the possibility that these seven viruses were derived from live attenuated influenza vaccine (LAIV) could not be excluded.

Antiviral susceptibility

Up to week 13/2022, 1 669 viruses were assessed for susceptibility to neuraminidase inhibitors (NAIs): 1 173 A(H3), 116 A(H1)pdm09 and three B virus were assessed genotypically, and 353 A(H3), 11 A(H1)pdm09 and 13 B viruses were assessed phenotypically. Susceptibility to the PA inhibitor baloxavir marboxil was assessed genotypically for 1 001 viruses: 898 A(H3), 98 A(H1)pdm09 and three B viruses. Phenotypically no viruses with reduced susceptibility were identified and genotypically two A(H3) viruses showed PA amino acid substitutions potentially associated with reduced susceptibility to baloxavir marboxil.

At the WIC, 206 influenza viruses detected within EU/EEA countries during the 2021-2022 season have been assessed phenotypically against oseltamivir and zanamivir: 49 A(H1)pdm09, 152 A(H3) and five B/Victoria-lineage. All viruses showed normal inhibition (NI) by both NAIs and their PA gene sequences had no markers associated with reduced susceptibility to baloxavir marboxil.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response System [4] reported that the China Health and Family Planning Commission had notified WHO of three cases of human infection with influenza A(H7N9). 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, although few human cases were reported during the 2017-18 season [5]. WHO posted an analysis of information on A(H7N9) viruses on 10 February 2017 [6], and ECDC published a rapid risk assessment on the implications of A(H7N9) for public health on 3 July 2017 [7]. Current risk assessments can be found on WHO's website https://www.who.int/teams/global-influenza-programme/avian-influenza/monthly-risk-assessment-summary (accessed 11 April 2022). The assessment published on 1 March 2022 indicated that there had 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 [8]. The H7N9 situation update published by the Food and Agricultural Organization of the United Nations on 3 February 2021 indicated there had been 14 detections in chickens in Shandong province, China during October 2020, but the report published on 2 February 2022 indicated that there had been no additional detections since then [9], and an e-mailed notification on 6 April indicated that this was still the case. 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 approved on 30 March 2022 and can be found on ECDC's website [11].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 1 March 2022. Since the previous risk assessment on 21 January 2022, eight human cases of infection with avian influenza A(H5N6) viruses were reported from China [8]. The A(H5N6) cases had disease onset dates in November 2021 and January 2022 with all patients reporting exposure to poultry. At the time of report publication, three cases were fatal (a 12-year-old female and 46- and 75-year-old males) with the remaining five being critical. The most recent confirmed case of human infection with an A(H5N1) virus was reported by England and a full report into the investigation of this case has been published [12].

On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [13]. The latest collaborative report from ECDC and the European Food Safety Authority (EFSA), reported 2 653 highly pathogenic avian influenza (HPAI) A(H5) detections between 9 December 2021 and 15 March 2022, 1 030 in poultry, 1 489 in wild birds and 133 in domestic birds [11]. Detections occurred in 33 EU/EEA countries and the UK. Of the poultry detections, 609 were reported by France, 131 by Italy, 73 by Hungary and 53 by Poland. The majority of wild bird detections were reported by Germany (767), the Netherlands (293), Denmark (74) and the UK (118). Genetic analyses indicated that the circulating viruses belonged to clade 2.3.4.4b, with such viruses having been circulating in Europe since October 2020. Some of these viruses were also detected in wild mammal species in Finland, Ireland, the Netherlands and Slovenia, showing genetic markers of adaptation to replication in mammals. According to reports compiled by the Food and Agricultural Organization of the United Nations (FAO) as of 23 March 2022, various influenza A(H5Nx) subtypes continued to be detected in wild and/or domestic birds in Africa, Americas, Asia and Europe, and since 23 February 2022 a total of 966 HPAI (27 not subtyped, 110 H5Nx, 817 H5N1, 11 H5N2 and one H5N8) and no LPAI outbreaks had been reported, with mention of three A(H5N6) human infections in China [14].

Influenza A(H9N2) virus

Since the previous WHO update on 21 January 2022 six laboratory-confirmed human cases of influenza A(H9N2) virus infection, all but one in children, were reported by China with onset dates in October through January [8]. All cases reported mild severity with four having confirmed poultry exposure. There were neither epidemiological links nor clusters of cases and A(H9) viruses were detected in associated environmental samples. The FAO report of 23 March 2022 reported two cases of human infection in China and one from a child in Cambodia in 2021 [14]. Public Health England has published and updated risk assessment for avian influenza A(H9N2) [15]. Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa.

Other influenza zoonotic events

Since the previous WHO update on 21 January 2022, one A(H1N1)v and one A(H1N2)v zoonotic events with swine-related variant influenza A viruses were reported [8]. Both patients were adults who reported exposure to pigs and no onward transmissions were detected. The A(H1N1)v case was reported by Denmark and the patient was admitted to intensive care on 24 November 2021 and subsequently identified as being influenza-positive. The A(H1N2)v case was detected in California, USA and the patient was not hospitalised. The variant viruses were similar to those circulating in swine in their respective locations.

WHO Collaborating Centre reports

A description of results generated by the London WHO Collaborating Centre at the WIC and used at the September 2021 WHO VCM (held online: 13-23 September 2021 for seasonal influenza viruses), and previous ones, can be found at https://www.crick.ac.uk/partnerships/worldwide-influenza-centre/annual-and-interim-reports (accessed 8 April 2022). The report for the February 2022 VCM will be posted shortly.

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(s) of sample collection. Isolates from WHO NICs in EU/EEA countries are highlighted in yellow. Sequences for many 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.

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