Influenza virus characterization

Summary report, Europe, December 2022

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Acknowledgments

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Summary

The November 2022 characterization report[1], was the second report for the 2022-2023 influenza season. As of week 52/2022, 109 321 detections had been reported. Of these detections, 94% were type A viruses, with A(H3N2) and A(H1N1)pdm09 showing near equal proportions, 51% and 49% respectively, and 6% type B of which 707 were ascribed to a lineage, with all being B/Victoria. This represents a 5-fold increase in detections compared to the 2021-2022 season, despite only a modest increase (5%) in the number of samples tested. The epidemic threshold of 10% positivity within sentinel specimens was crossed in week 45/2022.

Eight shipments from countries within the WHO European Region were received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC) since the November report. This report focuses on viruses with collection dates after 31 August 2022 for which HA gene sequences were submitted to, and released in, the EpiFlu™ database of the Global Initiative on Sharing All Influenza Data (GISAID) in December 2022, together with sequences and antigenic data generated at the WIC.

Globally, the great majority of the A(H1N1)pdm09 viruses detected in the first 13 weeks of the 2022-2023 season have fallen in the HA 6B.1A.5a.2 subgroup. As a percentage of type A viruses detected in the WHO European Region there has been an increase to 49% from 4% in the same period in 2021. Clear antigenic discrimination of 6B.1A.5a.1 and 6B.1A.5a.2 viruses has been shown in many previous reports. While circulating 6B.1A.5a.2 viruses are well recognised by post-infection ferret antisera raised against A/Victoria/2570/2019-like viruses, being used in vaccines for the northern hemisphere 2022-2023 influenza season, they are recognised less well by post-vaccination sera from humans. Recently circulating 6B.1A.5a.2 viruses carry HA1 K54Q, A186T, Q189E, E224A, R259K and K308R amino acid substitutions compared to A/Victoria/2570/2019 so the recommendation was to change the vaccine component to an A/Sydney/5/2021-like virus (carrying these substitutions) for the southern hemisphere 2023 season. A(H1N1)pdm09 viruses continue to diversify and viruses with additional HA1 amino acid substitutions of P137S, K142R, D260E and T277A are of concern.

In Europe and across the world A(H3N2) viruses have been dominant with the great majority of recently detected viruses, as assessed from sequence deposition in GISAID's EpiFlu™ database, falling in the 'Bangladesh-like' (3C.2a1b.2a.2) subgroup, but with recent detections of 'Cambodia-like' 3C.2a1b.2a.1 viruses in China. While clusters of viruses showing genetic and associated 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 egg-propagated A/Darwin/9/2021 which has been recommended for egg-based vaccines to be used in the 2022 and 2023 southern hemisphere, and 2022-23 northern hemisphere seasons. Antisera raised against a range of cell culture- and egg-propagated 3C.2a1b.2a.2 viruses generally gave good recognition of the 57 3C.2a1b.2a.2 test viruses from France, Portugal and Spain analysed since the November report.

In Europe and across the world generally, few B/Victoria-lineage viruses have been detected during weeks 40-52/2022. The vast majority of viruses with collection dates after 31 August 2022 for which sequences have been deposited in GISAID's EpiFlu™ database have HA genes that fall in the V1A.3a.2 subgroup with defining HA1 A127T, P144L and K203R amino acid substitutions. B/Austria/1359417/2021-like (V1A.3a.2) viruses have been recommended for use in the southern hemisphere 2022 and 2023, and the northern hemisphere 2022-2023 influenza seasons and post-infection ferret antisera raised against such viruses react well with recently circulating V1A.3a.2 viruses. Sequence from a single V1A.3 B/Washington/02/2019-like virus, with a collection date after 31 August 2022, was deposited in EpiFlu™ during December 2022.

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 16 from the WHO European Region, belonged to genetic clade Y3 and had 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 need to share all B/Yamagata-lineage viruses detected recently for detailed characterization 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 2022-2023 season (weeks 40-52/2022), compared to the same period in the 2021-2022 season. There has been a slight increase in the number of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (8 616, 5%), notably from sentinel sources, but a significant rise in the number of influenza detections (86 075, ~5-fold). In the same period of 2020, during the earlier stages of the COVID-19 pandemic, just 198 250 specimens were tested (~5-fold less) and only 415 influenza detections were reported (results not shown). These data probably relate to a number of factors: (i) significant

[1]Influenza virus characterization: summary report, Europe November 2022. WHO Regional Office for Europe and European Centre for Disease Prevention and Control; Copenhagen and Stockholm; 2022 (https://apps.who.int/iris/handle/10665/365415, accessed 30 December 2022).

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) residual effects of measures introduced to help curtail the spread of SARS-CoV-2, and; (iii) with large swathes of the human population now carrying a significant level of immunity to SARS-CoV-2 following either infection and/or vaccination, influenza has been able to re-establish itself after nearly two years of low-level circulation.

With these caveats, the ratio of type A to type B detections decreased slightly in 2022-2023 compared to 2021-2022, but with a greatly reduced dominance of A(H3N2) over A(H1N1)pdm09 viruses in 2022-2023. While the number of influenza B virus detections has increased from 1 022 to 6 469 (>6-fold), the number of viruses ascribed to a lineage has increased from 1.3% to 10.9% with all being of the B/Victoria lineage (Table 1). This is supported by sequences available in GISAID with no B/Yamagata lineage viruses, with collection dates after March 2020, having been characterised genetically. Currently, it appears that measures introduced relating to the COVID-19 pandemic are still having an effect, with greater numbers of respiratory clinical specimens being tested for influenza, but the 2022-2023 season started early in week 45/2022 with detections in sentinel systems being above the 10% epidemic threshold since that week and detections having greatly surpassed those reported during the first 13 weeks of the previous season. This is also supported by the rate of influenza positivity in sentinel samples which showed a slight rise towards the end of the 2021-2022 season and continued to hover around 7% until week 44/2022, thereafter crossing the 10% epidemic threshold and rising week-on-week until week 51/2022 (37%) before decreasing to 34% in week 52/2022, possibly due to reduced sentinel surveillance consultations and/or timely reporting to TESSy during the festive period (Figure 1).

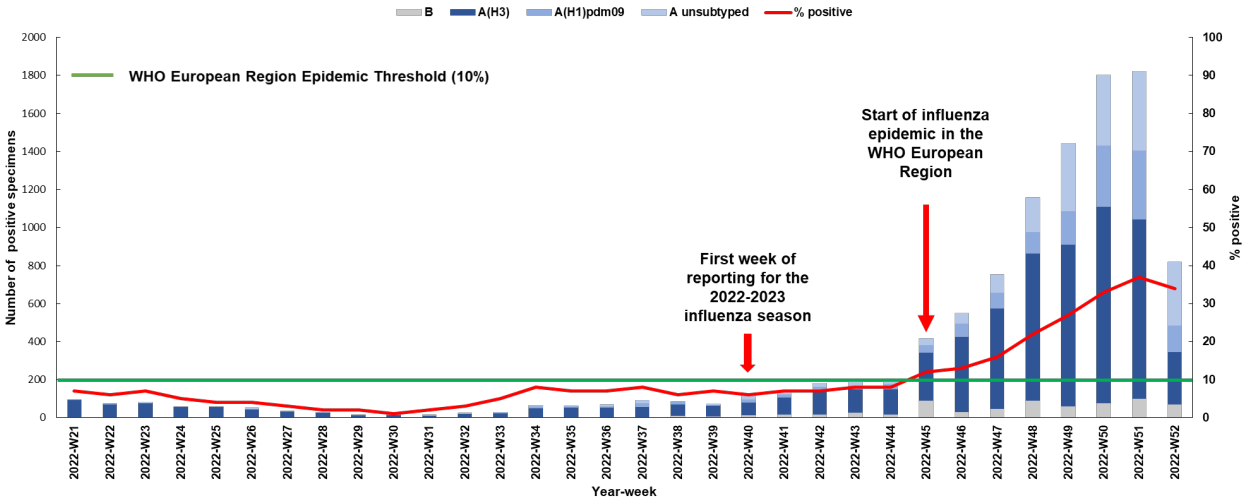
Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2022-2023 season (weeks 40-52/2022)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40-52/2022			Totals*		Cumulative number of detections for weeks 40-52/2021			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**8957**	**93895**	**102852**	**94.1**	**15.9:1**	**1116**	**21108**	**22224**	**95.6**	**21.7:1**
A(H1N1)pdm09	1410	17056	18466	48.9		46	274	320	3.6	
A(H3N2)	5582	13726	19308	51.1	1:1	679	7991	8670	96.4	27.1:1
A not subtyped	1965	63113	65078			391	12843	13234		
Influenza B	**646**	**5823**	**6469**	**5.9**		**19**	**1003**	**1022**	**4.4**	
Victoria lineage	200	507	707	100.0		5	7	12	92.3	12:1
Yamagata lineage	0	0	0			0	1	1	7.7	
Lineage not ascribed	446	5316	5762			14	995	1009		
Total detections (total tested)	**9 603 (47 319)**	**99 718 (>864 308)**	**109 321 (>911 627)**			**1 135 (26 648)**	**22 111 (>841 768)**	**23 246 (>868 416)**		

[a] Numbers taken from Flu News Europe week 52 reports for the two most recent 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.

Figure 1. Influenza positivity in sentinel-source specimens by week (2022-2023) – WHO European Region[a]



[a] Figure adapted from FluNewsEurope weeks 36-39/2022 and 52/2022 reports (https://flunewseurope.org/Archives)

Genetic and antigenic characterization data generated at the WIC for viruses with collection dates after 31 August 2022 until 31 January 2023 will inform the WHO influenza vaccine composition meeting (VCM) in February 2023 when recommendations will be made for the northern hemisphere 2023-2024 influenza season. Recommendations for the 2021-2022 northern [1] and 2022 southern [2] hemisphere seasons have been made and implemented. Data presented for viruses with collection dates after 31 August 2021 until 31 January 2022 contributed to the VCM for the northern hemisphere 2022-2023 season, where it was recommended to change the A(H3N2) and B/Victoria-lineage components of influenza vaccines to match those used in 2022 southern hemisphere vaccination campaigns [3]. At the last VCM (19-22 September), which focussed on data from viruses collected after 31 January 2022 until 31 August 2022, it was recommended to change the A(H1N1)pdm09 vaccine component for the 2023 southern hemisphere season [4].

This and recent influenza characterization reports (https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/influenza-virus-characterization) have been based mainly on phylogenetic analyses of complete HA gene

sequences submitted to GISAID's EpiFlu™ database, inclusive of sequences generated at the WIC. Here A(H1N1)pdm09, A(H3N2) and B/Victoria-lineage HA gene phylogenies prepared for the November report are shown (Figures 2a, 3a and 4a). Additional phylogenies (Figures 2b, 3b and 4b) are presented for HA sequences derived from viruses with collection and HA sequence submission dates from the days indicated in Table 2, with a sequence download date of 31 December 2022. The numbers of HA sequences, downloaded from GISAID, numbers remaining after de-duplication and the numbers used in the new representative phylogenies generated for this December report are shown.

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

Virus subtype/lineage	Global full length HA sequences available as of 2022-12-31				
	Virus collection date (from)	Sequence submission date (from)	Number Downloaded	Number de-duplicated and aligned	Number used in phylogenies*
A(H1N1)pdm09	2022-09-01	2022-12-01	1083	1003	134
A(H3N2)	2022-09-01	2022-12-01	1905	1770	126
B/Victoria	2022-09-01	2022-12-01	168	168	137
B/Yamagata	2022-01-01	2022-12-01	0	0	0

*** Inclusive of sequences generated recently at the WIC, but not including sequences from reference and vaccine viruses**

Nineteen shipments containing specimens (n = 569: virus isolates and/or clinical specimens) with collection dates after 31 August 2022 were received at the WIC (eight since the November report) from WHO Global Influenza Surveillance and Response System (GISRS) recognised National Influenza Centres (NICs) in four WHO European Region Member States (Table 3). Many of the samples contained in the recently received shipments were in the virus characterization process at the time of preparing this report.

A total of 80 viruses from the WHO European Region (17 A(H1N1)pdm09, 57 A(H3N2 and six B/Victoria-lineage) were characterised antigenically since the November report (Tables 4, 5 and 6 respectively). Of these, 14, 56 and three, respectively, have collection dates after 31 August 2022.

Table 3. Summary of seasonal influenza clinical samples and virus isolates* with collection dates after 2022-08-31 contained in packages received from WHO European Region Member States

MONTH	TOTAL RECEIVED	A		H1N1pdm09		H3N2			B		B Victoria lineage		B Yamagata lineage	
Country/area	Seasonal viruses	Number received	Number propagated[1]	Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]	Number received	Number propagated[1]
2022														
September														
France	19			3	in process	14	in process				2	in process		
Germany	6					5	5				1	1		
Netherlands	5			5	2									
Norway	14			12	1	1	in process				1	in process		
Portugal	2					2	2							
Spain	33			10	in process	23	in process							
October														
Denmark	11			4	in process	5	in process				2	in process		
France	28			9	in process	12	in process				7	in process		
Germany	18					18	18							
Ireland	13	2	in process	5	in process	6	in process							
Lithuania	4	1	in process			3	in process							
Netherlands	1			1	0									
Norway	35			18	in process	12	in process				5	in process		
Portugal	33			8	in process	25	25							
Spain	166	3	in process	15	in process	147	in process		1	in process				
UK (N. Ireland)	14			13		1								
November														
Denmark	3					2	in process				1	in process		
France	9					7	in process				2	in process		
Georgia	10	9	in process			1	in process							
Ireland	23	1	in process	12	in process	9	in process				1	in process		
Lithuania	29			13	in process	14	in process		2	in process				
Norway	13			9	in process	4	in process							
Portugal	4			1	in process	3	3							
Spain	89	21	in process	7	in process	60	in process				1	0		
Turkmenistan	1					1	in process							
December														
Turkmenistan	5					1	in process				4	in process		
TOTAL	569	37	0	142	3	362	53	0	3	0	25	1	0	0
12 Countries/areas		6.5%		25.0%		63.6%			0.5%		4.4%		0.0%	
		95.1%							4.9%					

*** 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 only genetic characterisation possible
Some samples not cultured because Ct value high (>30), failed sequence, identical sequence, mixed sequence or SARS-COV-2 positive

As of 2023-01-01

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 had emerged, with circulating viruses carrying the substitution **S183P** in **HA1**, although this was not retained in all genetic groups. Figures 2a and 2b 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] as are egg- and cell-based A/Sydney/5/2021, recently recommended for use in the southern hemisphere 2023 season [4]. HA amino acid substitutions defining the seven subclades have been defined in earlier reports. This report focuses on subclade **6B.1A.5** viruses which have circulated recently.

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**).

The phylogeny prepared for the November report focused on HA sequences derived from viruses with collection dates after 31 August 2022 for which sequences were submitted to GISAID in November 2022 (Figure 2a). Few **6B.1A.5a.1** subgroup viruses had been reported to GISAID, among them was a cluster of viruses from Kenya with amino acid substitutions of **HA1 R113K** and **HA2 H72N** compared to a previous vaccine virus, A/Guangdong-Maonan/SWL1536/2019. Recently detected viruses were in subgroup **6B.1A.5a.2**, all having **HA1 K54Q**, **A186T**, **Q189E**, **E224A**, **R259K** and **K308R** substitutions compared to the vaccine virus A/Victoria/2570/2019 [1, 2, 3], and virus clusters had emerged defined by amino acid substitutions: (i) **HA1 T216A** often with **D94N** , the cluster showing wide geographic distribution and containing the vaccine virus A/Sydney/5/2021 [4]; (ii) **HA1 A48P**, and; (iii) **HA1 K142R**, **D260E** and **HA2 I91V**, **N124H**, frequently with **HA1 P137S**, **T277A** and **HA2 E29D**. Cluster (iii) viruses dominated and the great majority of these had the additional **HA1 P137S**, **T277A** and **HA2 E29D** amino acid substitutions. Further diversification had occurred with virus clusters having emerged defined by amino acid substitutions of either **HA1 I185V** or **HA2 I91V** alone, while a subset of cluster (iii) viruses had **HA1 T216A** substitutions. Viruses detected in the WHO European Region were dispersed throughout the phylogeny.

The phylogeny prepared for this December report focused on HA sequences derived from viruses with collection dates after 31 August 2022 for which sequences were submitted to GISAID in December 2022 (Table 2 and Figure 2b). The two phylogenies have virtually identical structures with subgroup **6B.1A.5a.2** viruses dispersed throughout the same clusters and subsets. Of note, large numbers of viruses in the 'core' amino acid substitution category (having **HA1 K54Q**, **A186T**, **Q189E**, **E224A**, **R259K** and **K308R** substitutions compared to the vaccine virus A/Victoria/2570/2019 [1, 2, 3]) have been detected based on sequences submitted to GISAID. Sequences derived from a small number of **6B.1A.5a.1** subgroup viruses, detected in September through December, became available in December and included viruses from the Netherlands and Norway.

Since the November report, 17 A(H1N1)pdm09 viruses from the WHO European Region have been characterised antigenically (Tables 4-1 to 4-3). The panel of post-infection ferret antisera used in HI assays, four raised against subgroup **6B.1A.5a.1** viruses and five against **6B.1A.5a.2** viruses, gives clear discrimination of reference viruses in the two subgroups. This discrimination carried through to the 16 **6B.1A.5a.2** test viruses and the single **6B.1A.5a.1** test virus (A/Norway/31736/2022). All 16 **6B.1A.5a.2** test viruses were recognised well (within fourfold of the homologous titres, the great majority within twofold) by antisera raised against all **6B.1A.5a.2** reference viruses, which included the vaccine viruses A/Victoria/2570/2019 and A/Sydney/5/2021. Thirteen of the **6B.1A.5a.2** test viruses were known to be cluster (iii) viruses carrying the additional **HA1 P137S**, **T277A** and **HA2 E29D** amino acid substitutions.

Antisera induced by **6B.1A.5a.1** viruses in ferrets and humans yielded poor recognition of **6B.1A.5a.2** viruses and many humans were unlikely to have been exposed to **6B.1A.5a.2** subgroup viruses given their

low-level circulation during the COVID-19 pandemic. Hence, A/Victoria/2570/2019-like viruses were recommended for use in the northern hemisphere 2022-2023 influenza season [3]. The different clusters of **6B.1A.5a.2** viruses were not differentiated by post-infection ferret antisera but human serology indicated poor recognition of many **6B.1A.5a.2** viruses. For this reason, egg- and cell culture-propagated A/Sydney/5/2021-like viruses, carrying the **HA1 K54Q**, **A186T**, **Q189E**, **E224A**, **R259K** and **K308R** substitutions compared to A/Victoria/2570/2019, were recommended for vaccine formulations to be used in the 2023 southern hemisphere season [4].

Figure 2a. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID/WIC, Nov 2022)

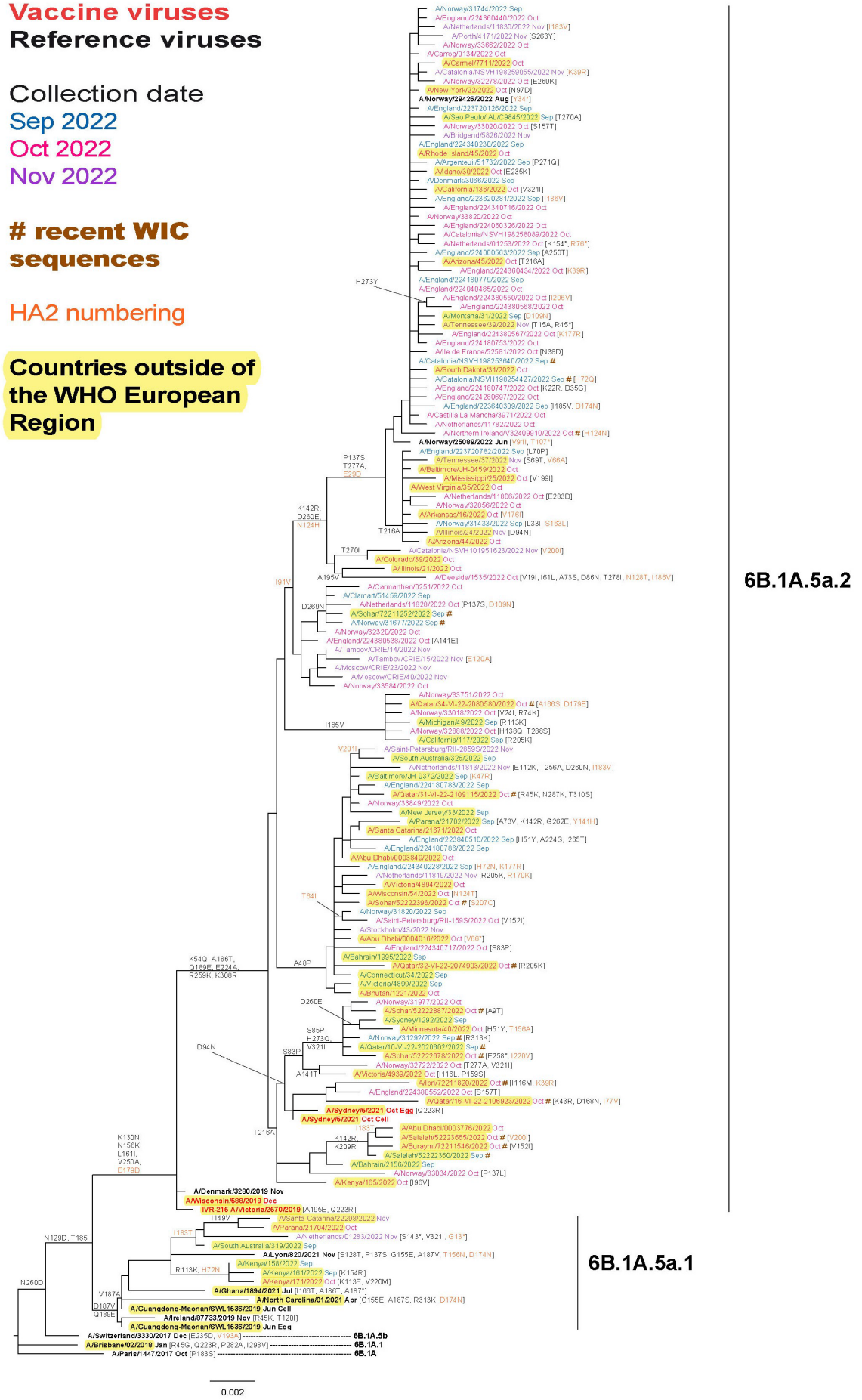


Figure 2b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID/WIC, Dec 2022)

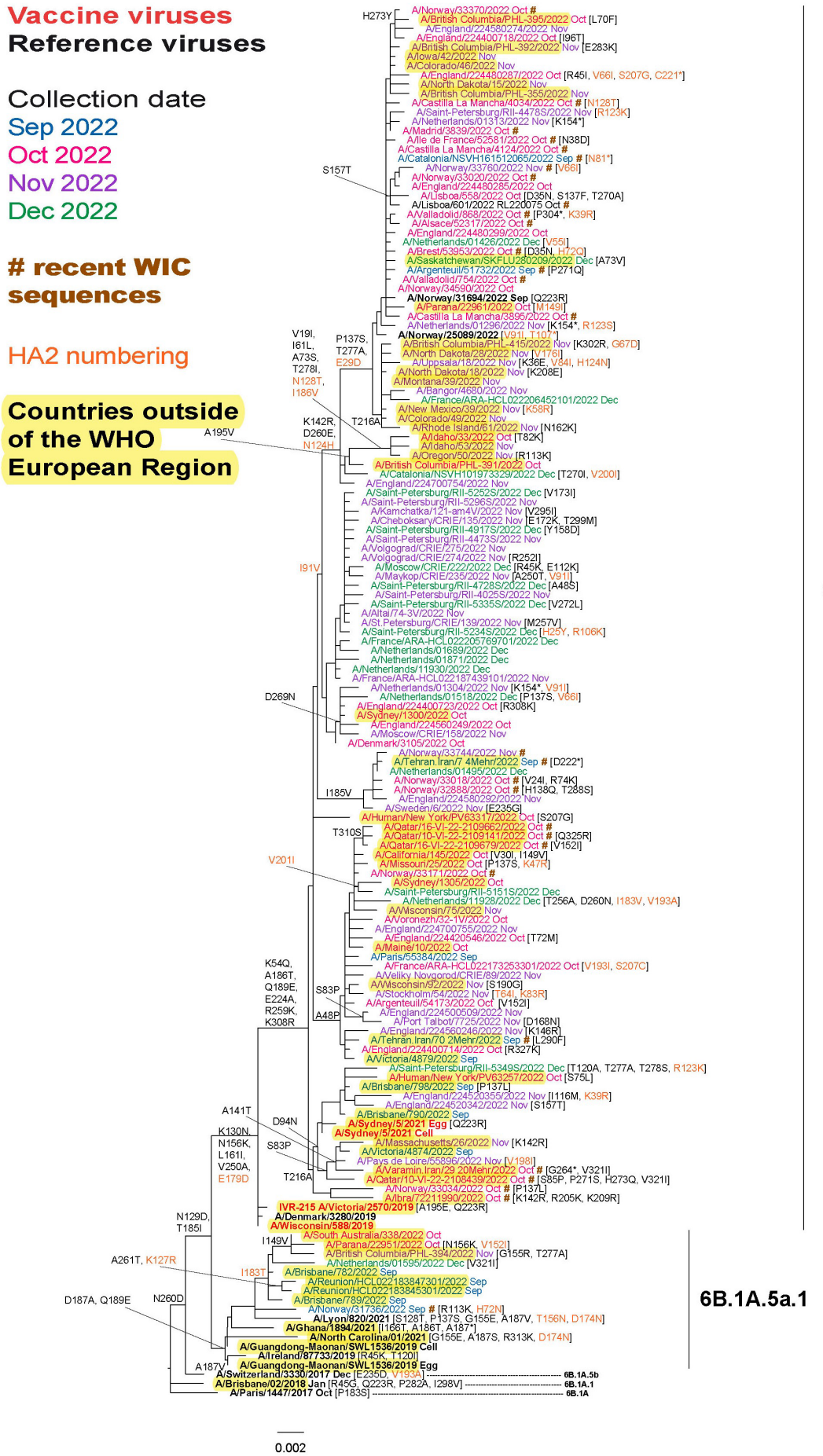


Table 4-1. Antigenic analysis of influenza A(H1N1)pdm09 viruses by HI

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection ferret antisera								
					A/G-M SWL1536/19 MDCK F09/20 6B.1A.5a.1	A/G-M SWL1536/19 Egg F12/20 6B.1A.5a.1	A/Ghana 1894/21 Egg F02/22 6B.1A.5a.1	A/Lyon 820/21 Egg F06/22 6B.1A.5a.1	A/Denmark 3280/19 MDCK F28/20 6B.1A.5a.2	IVR-215 A/Vic/2570/19 Egg F37/21 6B.1A.5a.2	A/Nor 25089/22 MDCK F38/22 6B.1A.5a.2	A/Sydney 5/21 Egg F04/22 6B.1A.5a.2
REFERENCE VIRUSES													
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	C2/MDCK1	1280	2560	1280	320	40	80	<40	40	
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	E3/E2	640	1280	640	160	40	40	<40	40	
A/Ghana/1894/2021		6B.1A.5a.1	2021-07-21	E2/E1	2560	2560	2560	320	80	160	<40	40	
A/Lyon/820/2021		6B.1A.5a.1	2021-11-16	E1/E2	320	160	160	320	40	40	<40	40	
A/Denmark/3280/2019	K130N, N156K, L161I, V250A	6B.1A.5a.2	2019-11-10	MDCK4/MDCK5	80	80	<40	40	2560	2560	1280	2560	
IVR-215 (A/Victoria/2570/2019)	K130N, N156K, L161I, V250A (A195E, Q223R)	6B.1A.5a.2	2018-11-22	E4/D7/E2	80	80	<40	40	640	1280	1280	640	
A/Norway/25089/2022	K54Q, A186T, Q189E, E224A, R259K, K308R, T216A	6B.1A.5a.2	2022-06-15	MDCK2	<40	40	<40	<40	2560	2560	2560	2560	
A/Sydney/5/2021	K54Q, A186T, Q189E, E224A, R259K, K308R, D94N, T216A (Q223R)	6B.1A.5a.2	2022-10-31	E3/E2	80	40	<40	40	1280	1280	640	1280	
TEST VIRUSES													
A/Netherlands/11719/2022	P137S, K142R	6B.1A.5a.2	2022-08-28	MDCK1	<40	<40	<40	<40	640	640	1280	640	
A/Catalonia/NSVH161512065/2022	P137S, K142R	6B.1A.5a.2	2022-09-14	MDCK1	<40	<40	<40	<40	640	640	1280	640	
						Vaccine NH 2020-21				Vaccine SH 2021 NH 2021-22 SH 2022 NH 2022-23		Vaccine SH 2023	

< relates to the lowest dilution of antiserum used

Table 4-2. Antigenic analysis of influenza A(H1N1)pdm09 viruses by HI

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre: Post-infection ferret antisera								
					A/G-M SWL1536/19 MDCK F09/20 6B.1A5a.1	A/G-M SWL1536/19 Egg F12/20 6B.1A.5a.1	A/Ghana 1894/21 Egg F02/22 6B.1A.5a.1	A/Lyon 820/21 Egg F06/22 6B.1A5a.1	A/Denmark 3280/19 MDCK F28/20 6B.1A.5a.2	IVR-215 A/Vic/2570/19 Egg F37/21 6B.1A.5a.2	A/Nor 25089/22 MDCK F38/22 6B.1A.5a.2	A/Sydney 5/21 Egg F04/22 6B.1A.5a.2
REFERENCE VIRUSES												
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	C2/MDCK1	2560	2560	1280	320	40	80	<40	40
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	E3/E2	1280	1280	640	160	<40	40	<40	<40
A/Ghana/1894/2021		6B.1A.5a.1	2021-07-21	E2/E1	2560	2560	1280	320	80	80	<40	40
A/Lyon/820/2021		6B.1A.5a.1	2021-11-16	E1/E2	640	320	160	640	40	80	40	40
A/Denmark/3280/2019	K130N, N156K, L161I, V250A	6B.1A.5a.2	2019-11-10	MDCK4/MDCK5	80	80	40	80	1280	1280	1280	1280
IVR-215 (A/Victoria/2570/2019)	K130N, N156K, L161I, V250A (A195E, Q223R)	6B.1A.5a.2	2018-11-22	E4/D7/E2	160	80	40	80	1280	1280	1280	1280
A/Norway/25089/2022	K54Q, A186T, Q189E, E224A, R259K, K308R, T216A	6B.1A.5a.2	2022-06-15	MDCK2	40	40	<40	<40	2560	1280	2560	1280
A/Sydney/5/2021	K54Q, A186T, Q189E, E224A, R259K, K308R, D94N, T216A	6B.1A.5a.2	2021-10-16	MDCK3/MDCK3	40	40	<40	40	2560	2560	1280	2560
A/Sydney/5/2021	K54Q, A186T, Q189E, E224A, R259K, K308R, D94N, T216A (Q223R)	6B.1A.5a.2	2022-10-31	E3/E2	80	40	<40	40	1280	640	640	1280
TEST VIRUSES												
A/Paris/54771/2022	P137S, K142R	6B.1A.5a.2	2022-07-27	MDCK1/MDCK1	<40	<40	<40	<40	640	640	640	640
A/Argenteuil/51732/2022	P137S, K142R	6B.1A.5a.2	2022-09-27	MDCK1/MDCK1	<40	<40	<40	<40	640	640	1280	640
A/Alsace/52317/2022	P137S, K142R	6B.1A.5a.2	2022-10-04	MDCK1/MDCK1	<40	40	<40	<40	1280	1280	1280	1280
A/Ile_de_France/52581/2022	P137S, K142R	6B.1A.5a.2	2022-10-10	MDCK1/MDCK1	40	40	<40	<40	640	1280	1280	640
A/Brest/53953/2022	P137S, K142R	6B.1A.5a.2	2022-10-13	MDCK2/MDCK1	<40	<40	<40	<40	640	640	1280	640
A/Argenteuil/54173/2022	P137S, K142R	6B.1A.5a.2	2022-10-15	MDCK1/MDCK1	40	40	<40	<40	640	640	640	640
					Vaccine NH 2020-21					Vaccine SH 2021 NH 2021-22 SH 2022 NH 2022-23		Vaccine SH 2023

< relates to the lowest dilution of antiserum used

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

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	A/G-M SWL1536/19 MDCK F09/20 6B.1A.5a.1	A/G-M SWL1536/19 Egg F12/20 6B.1A.5a.1	A/Ghana 1894/21 Egg F02/22 6B.1A.5a.1	A/Lyon 820/21 Egg F06/22 6B.1A.5a.1	A/Denmark 3280/19 MDCK F28/20 6B.1A.5a.2	IVR-215 A/Vic/2570/19 Egg F37/21 6B.1A.5a.2	NEW A/Nor 25089/22 MDCK F38/22 6B.1A.5a.2	NEW A/Sydney 5/21 MDCK F46/22 6B.1A.5a.2	NEW A/Sydney 5/21 MDCK F47/22 6B.1A.5a.2	NEW A/Sydney 5/21 Egg F04/22 6B.1A.5a.2
REFERENCE VIRUSES														
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	C2/MDCK1	1280	2560	1280	160	40	80	40	40	40	<40
A/Guangdong-Maonan/SWL1536/2019		6B.1A.5a.1	2019-06-17	E3/E2	1280	1280	320	160	40	40	<40	<40	40	<40
A/Ghana/1894/2021		6B.1A.5a.1	2021-07-21	E2/E1	2560	2560	1280	320	80	80	<40	40	80	40
A/Lyon/820/2021		6B.1A.5a.1	2021-11-16	E1/E2	320	160	160	320	40	40	40	40	<40	<40
A/Denmark/3280/2019	K130N, N156K, L161I, V250A	6B.1A.5a.2	2019-11-10	MDCK4/MDCK5	80	40	<40	40	1280	1280	1280	1280	1280	1280
IVR-215 (A/Victoria/2570/2019)	K130N, N156K, L161I, V250A (A195E, Q223R)	6B.1A.5a.2	2018-11-22	E4/D7/E2	160	40	40	40	640	1280	640	640	1280	640
A/Norway/25089/2022	K54Q, A186T, Q189E, E224A, R259K, K308R, T216A	6B.1A.5a.2	2022-06-15	MDCK2	40	40	<40	<40	1280	2560	2560	1280	2560	2560
A/Sydney/5/2021	K54Q, A186T, Q189E, E224A, R259K, K308R, D94N, T216A	6B.1A.5a.2	2021-10-16	MDCK3/MDCK3	40	40	<40	<40	2560	2560	2560	2560	5120	2560
A/Sydney/5/2021	K54Q, A186T, Q189E, E224A, R259K, K308R, D94N, T216A (Q223R)	6B.1A.5a.2	2022-10-31	E3/E2	80	40	<40	40	1280	1280	1280	1280	2560	2560
TEST VIRUSES														
A/Norway/31736/2022		6B.1A.5a.1	2022-09-16	MDCK1	2560	2560	1280	320	40	80	40	40	80	40
A/Norway/30508/2022		6B.1A.5a.2	2022-08-30	MDCK1	<40	<40	<40	<40	320	640	640	640	1280	1280
A/Madrid/3839/2022	P137S, K142R	6B.1A.5a.2	2022-10-03	MDCK1	40	<40	<40	<40	1280	2560	2560	1280	2560	2560
A/Castilla La Mancha/3971/2022	P137S, K142R	6B.1A.5a.2	2022-10-05	MDCK1	40	<40	<40	<40	1280	1280	2560	1280	1280	1280
A/Castilla La Mancha/3896/2022	P137S, K142R	6B.1A.5a.2	2022-10-06	MDCK1	40	<40	<40	<40	1280	2560	>5120	1280	2560	2560
A/Castilla La Mancha/3895/2022	P137S, K142R	6B.1A.5a.2	2022-10-06	MDCK1	40	<40	<40	<40	1280	1280	2560	1280	1280	1280
A/Castilla La Mancha/3900/2022	P137S, K142R	6B.1A.5a.2	2022-10-07	MDCK1	40	<40	<40	<40	1280	1280	1280	1280	1280	1280
A/Castilla La Mancha/4034/2022	P137S, K142R	6B.1A.5a.2	2022-10-15	MDCK1	40	<40	<40	<40	1280	1280	1280	1280	1280	640
A/Salamanca/1234/2022		seq pending	2022-11-08	MDCK2	40	<40	<40	<40	2560	2560	2560	2560	>5120	2560
						Vaccine NH 2020-21				Vaccine SH 2021 NH 2021-22 SH 2022 NH 2022-23			Vaccine SH 2023	

< relates to the lowest dilution of antiserum used

Influenza A(H3N2) virus analyses

A(H3N2) viruses with HA sequences 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. Since 2019-2020 group **3C.2a1b** viruses have dominated and **3C.3a** viruses have not been detected after the period February to August 2020.

Group **3C.2a1b** viruses contain HA amino acid substitutions found in subclade **3C.2a1** (those in clade **3C.2a** plus **N171K** in **HA1** and **I77V** and **G155E** in **HA2**, with most carrying **N121K** in **HA1**, e.g. **A/Singapore/INFIMH-16-0019/2016,** a former vaccine virus), plus **E62G, R142G** and **H311Q** in **HA1**, often with additional amino acid substitutions – notably either **HA1 T135K** commonly with **T128A** (both of which result in loss of potential glycosylation sites) yielding the **3C.2a1b.1** subgroup (e.g., **A/La Rioja/2202/2018**) or **HA1 T131K** and **HA2 V200I** producing 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)**.

The first phylogeny, based on HA sequences derived from viruses with collection dates after 31 August 2022 made available in GISAID and generated at the WIC during November 2022, was dominated by sequences derived from viruses detected in countries outside of the WHO European Region (Figure 3a). There was a lack of recently submitted sequences from 'Cambodia-like' **3C.2a1b.2a.1** viruses. All recently collected viruses 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 major subgroups defined by specific **HA1** amino acid substitutions: (i) **E50K** with a range of additional substitutions, e.g., **F79V**, **I140K**, **S262N** and **R33Q**; (ii) **D53N**, **N96S** (gain a glycosylation site) and **I192F**, many with additional substitutions defining specific virus clusters ; (iii) **D53G** commonly with **D104G** and **K276R**, and additional substitutions (e.g., **T167S**); (iv) **D53G**, **D104G**, **I140K**, **K276R** and **R299K**. Subgroups (ii), (iii) and (iv) also share **HA1 H156S** amino acid substitution. Sequences derived from samples collected in the WHO European Region were dispersed throughout the 'Bangladesh-like' (**3C.2a1b.2a.2**) portion of the phylogeny with viruses falling into multiple virus clusters defined by specific amino acid substitutions. Overall, subgroups (i) and (ii) were dominant being mainly populated by sequences derived from viruses detected in countries outside of the WHO European Region. Most of the new sequences falling in subgroup (iii) and all of those in subgroup (iv) were from viruses detected in the WHO European Region. Further diversification was observed in all four subgroups and a new subgroup had emerged in South Africa defined by HA1 amino acid substitutions **E83K**, **K121E**, **S205F**, **A212T** and **R261Q**.

The second phylogeny is based on HA sequences derived from viruses with collection dates after 31 August 2022 made available in GISAID and generated at the WIC during December 2022 (Table 2 and Figure 3b). The two phylogenies show similar profiles with the four subgroups identified above (i to iv) continuing to circulate in varying proportions. Virus diversification had continued, notably: within subgroup (i) the proportion of viruses with **R33Q** and **S262N** substitutions had increased within the WHO European Region; within subgroup (ii) a new cluster of viruses with **T135K** (resulting in loss of a glycosylation site) and **S145N** substitutions had emerged in the Americas, and; within the WHO European Region viruses in subgroup (iii) had emerged with **T135K** (resulting in loss of a glycosylation site) substitution. Further examples of the subgroup that had emerged in South Africa had not been released in GISAID. Sequences for 'Cambodia-like' **3C.2a1b.2a.1** viruses detected in China in September and October of 2022 had been released, all of which contained an additional **HA1 I48T** amino acid substitution.

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 in red on the phylogenies, as are egg- and cell-culture based 'Bangladesh-like' vaccines to be used in the 2022 and 2023 southern hemisphere and 2022-2023 northern hemisphere seasons, A/Darwin/9/2021 and A/Darwin/6/2021 (**3C.2a1b.2a.2**) respectively [2, 4, 3] (Figures 3a and 3b).

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.

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.

Results for 57 A(H3N2) 'Bangladesh-like' (**3C.2a1b.2a.2)** test viruses from France, Portugal and Spain, fully characterised antigenically since the November report, are shown in Tables 5-1 to 5-3. The test viruses fell within the four genetic subgroups identified above (i, n=45; ii, n = 4; iii, n = 2; iv, n = 6) and viruses in subgroups (i) and (ii) were generally recognised with twofold of the homologous titre by the antiserum raised against cell culture-propagated A/Thuringen/10/2022 (subgroup (i)), while viruses in subgroups (iii) and (iv) were recognised less well. The remaining reference **3C.2a1b.2a.2** viruses against which antisera were raised all contained **HA1 H156S** amino acid substitutions. Cell culture-propagated A/Stockholm/5/2021 and egg-propagated A/Darwin/9/2021 represent current vaccine-related viruses. The antiserum raised against A/Stockholm/5/2021 recognised all but one of the test viruses at titres within fourfold of the homologous titres (640), most within twofold, while that raised against A/Darwin/9/2021 (with slightly higher homologous titres in the range 640-1280) performed less well but yielded titres of at least 160 with all but two of the test viruses. Of the antisera raised against the subgroup (ii) reference viruses: that raised against cell culture-propagated A/Norway/24873/2021 (homologous titre 160) performed as well as that raised against A/Darwin/9/2021, while; that raised against egg-propagated A/Norway/24873/2021 (homologous titre 640) showed greater fold-drops against the test viruses but gave absolute titres comparable to those seen with the antiserum raised against cell culture-propagated A/Norway/24873/2021. Generally, antisera raised against subgroup (iii) (n = 1), subgroup (iv) (n = 1) and A/Poland/97/2022 viruses recognised those test viruses with **HA1 H156S** substitutions better than those that retained **H156**.

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 characterization 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 3a. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID/WIC, Nov 2022)

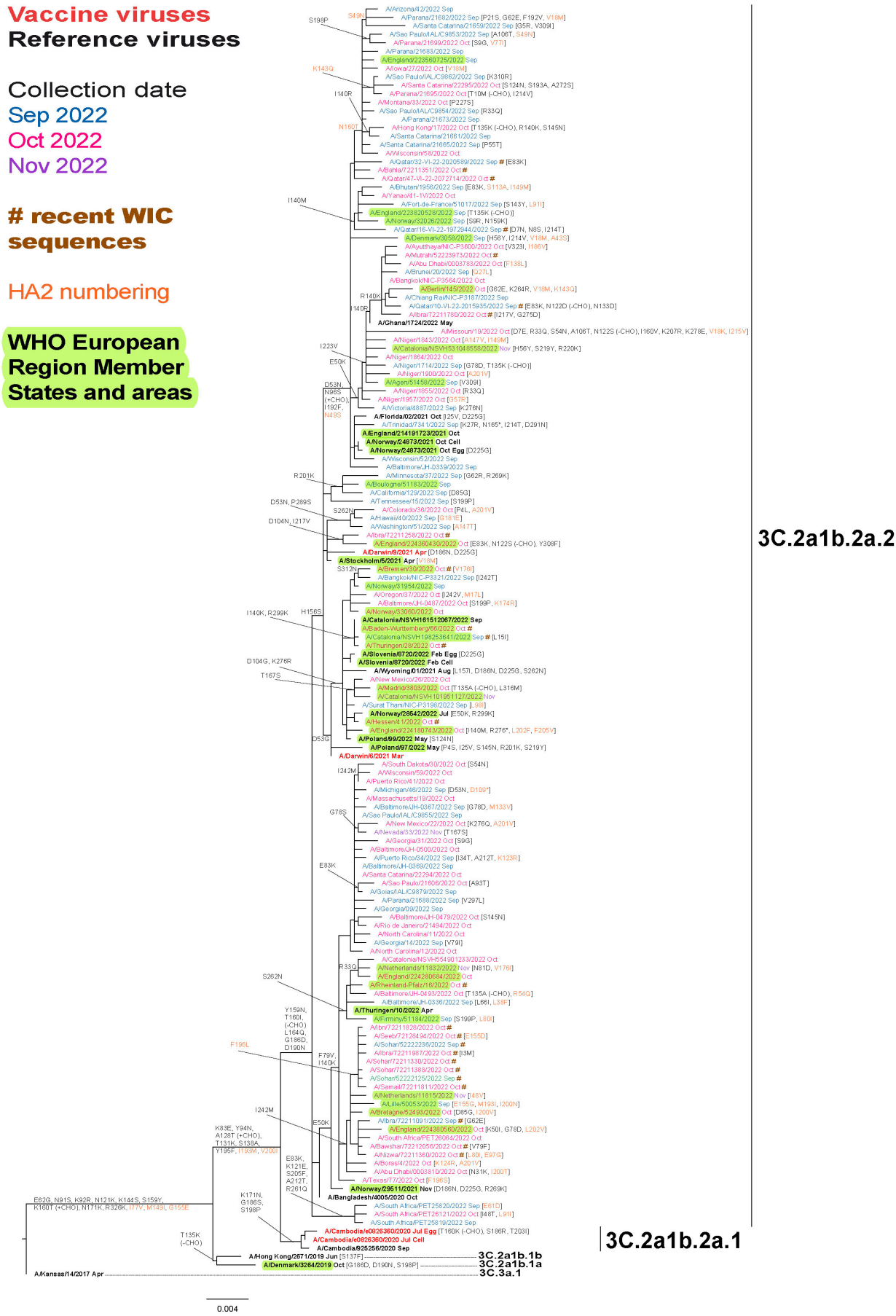


Figure 3b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID/WIC, Dec 2022)

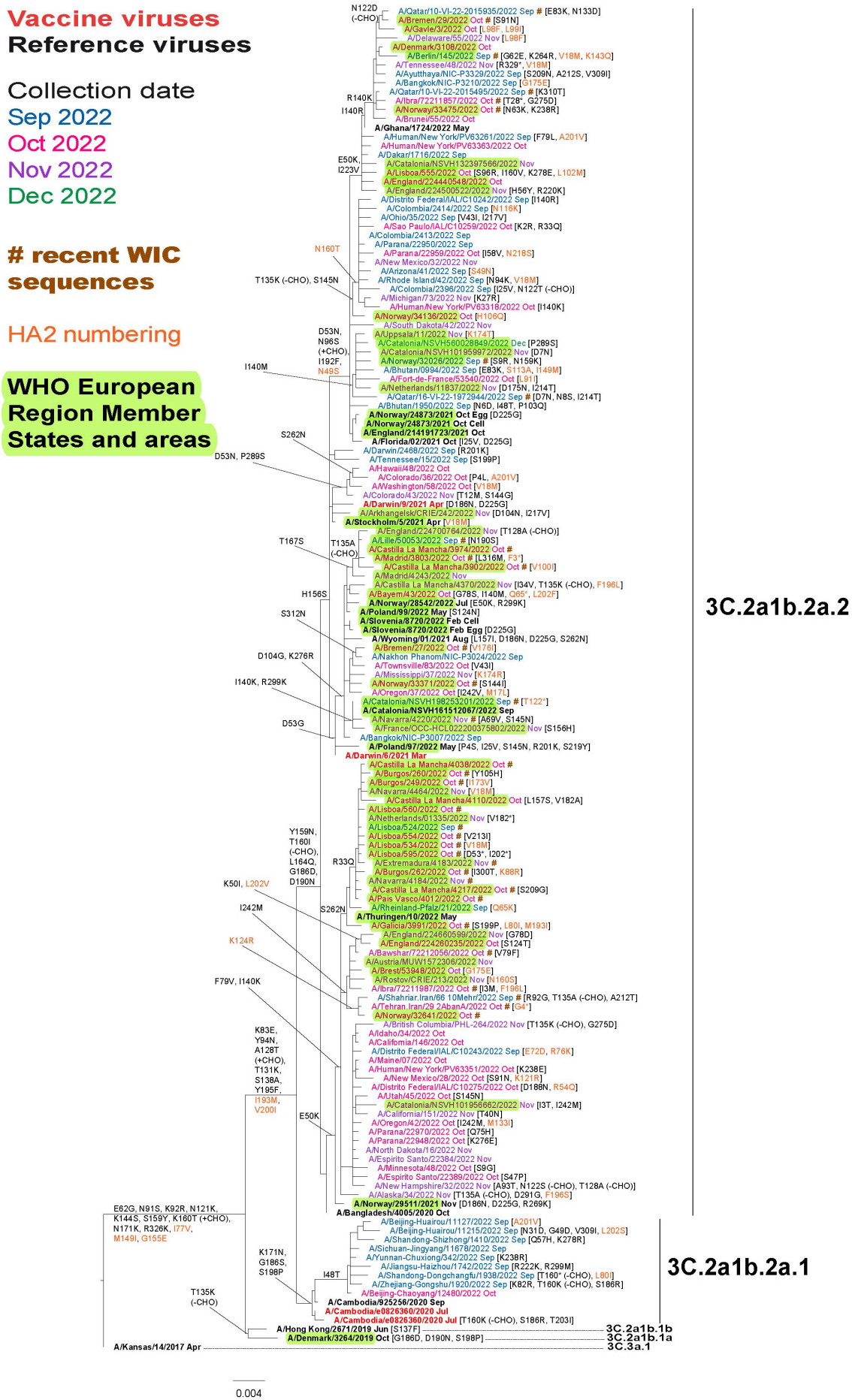


Table 5-1. Antigenic analysis of influenza A(H3N2) viruses by HI

Viruses	Other information	HA1 substitutions compared to A/Bangladesh/4005/2020	Passage history Ferret number Genetic group	Collection date	Passage history	Haemagglutination inhibition titre Post-infection ferret antisera A/Camb 925256/20 SIAT F03/21 3C.2a1b.2a.1	A/Camb e0826360/20 Egg F10/21 3C.2a1b.2a.1	A/Thuringen 10/22 SIAT F36/22 3C.2a1b.2a.2	A/Stock 5/21 SIAT F35/21 3C.2a1b.2a.2	A/Darwin 9/21 Egg F39/21 3C.2a1b.2a.2	A/Norway 24873/21 SIAT F10/22 3C.2a1b.2a.2	A/Norway 24873/21 Egg F11/22 3C.2a1b.2a.2	A/Poland 97/22 SIAT F39/22 3C.2a1b.2a.2	A/Slov 8720/2022 SIAT F24/22 3C.2a1b.2a.2	A/Catal NSVH161512067/22 SIAT F41/22 3C.2a1b.2a.2
REFERENCE VIRUSES															
A/Cambodia/925256/2020			3C.2a1b.2a.1	2020-09-25	S1AT5	320	160	<40	160	160	<40	<40	80	<40	<40
A/Cambodia/e0826360/2020			3C.2a1b.2a.1	2020-07-16	E5/E2	160	1280	80	160	320	40	80	80	80	<40
A/Thuringen/10/2022		E50K, F79Y, I140K, S262N subgroup (i)	3C.2a1b.2a.2	2022-04-01	P1/S1AT2	40	160	320	320	320	80	160	80	160	80
A/Stockholm/5/2021		H156S	3C.2a1b.2a.2	2021-04-16	S1AT0/S1AT3	80	160	80	640	640	80	160	320	320	160
A/Darwin/9/2021		H156S (D186N, D225G)	3C.2a1b.2a.2	2021-04-17	E3/E4	160	640	320	1280	1280	320	320	640	640	320
A/Norway/24873/2021		D53N, N96S(+cho), H156S, I192F subgroup (ii)	3C.2a1b.2a.2	2021-10-24	S1AT3	80	80	160	640	320	160	320	320	160	160
A/Norway/24873/2021		D53N, N96S(+cho), H156S, I192F (D225G) subgroup (ii)	3C.2a1b.2a.2	2021-10-24	E3/E1	80	320	320	1280	640	320	640	640	640	160
A/Poland/97/2022		P3S, I25V, D53G, S145N, H156S, R201K, S219Y	3C.2a1b.2a.2	2022-05-09	S1AT2	80	160	80	1280	640	160	320	640	320	640
A/Slovenia/8720/2022		D53Q, D104G, H156S, K276R subgroup (iii)	3C.2a1b.2a.2	2022-02-10	S1AT1/MDCK1/S1AT3	40	80	80	640	320	160	160	320	640	640
A/Catalonia/NSVH161512067/2022		D53Q, D104G, I140K, H156S, K276R, R299K subgroup (iv)	3C.2a1b.2a.2	2022-09-14	S1AT1/S1AT2	40	80	80	320	320	160	160	640	640	640
TEST VIRUSES															
	Subgroup (i)														
A/Lisboa/533/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-02	S1AT2/S1AT1	40	80	160	160	160	40	80	80	80	40
A/Lisboa/576/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-03	S1AT2/S1AT1	160	80	320	160	160	320	80	80	640	80
A/Lisboa/583/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-04	S1AT2/S1AT1	80	160	320	320	160	80	80	80	80	40
A/Lisboa/580/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-07	S1AT2/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/582/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-09	S1AT2/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/537/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-10	S1AT2/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/596/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-18	S1AT1/S1AT1	80	80	160	160	160	40	80	80	80	40
A/Lisboa/593/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-20	S1AT1/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/545/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-20	S1AT2/S1AT1	80	80	160	320	160	40	80	80	80	40
A/Lisboa/544/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-24	S1AT2/S1AT1	80	160	320	320	160	80	80	80	80	40
A/Lisboa/616/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-26	S1AT1/S1AT1	80	160	320	320	160	40	80	80	80	40
A/Lisboa/560/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-26	S1AT1/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/626/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-10-27	S1AT1/S1AT1	80	80	320	320	160	40	80	80	80	40
A/Lisboa/663/2022		R33Q, E50K, F79Y, I140K, S262N	3C.2a1b.2a.2	2022-11-02	S1AT1/S1AT1	40	80	160	160	160	40	80	40	80	40
A/Lisboa/584/2022		R33Q, E50K, F79Y, A93T, I140K, S262N	3C.2a1b.2a.2	2022-10-05	S1AT2/S1AT1	80	160	320	320	160	80	80	80	80	40
A/Lisboa/554/2022		R33Q, E50K, F79Y, I140K, V213I, S262N	3C.2a1b.2a.2	2022-10-24	S1AT1/S1AT1	80	160	320	320	160	80	80	80	80	40
A/Lisboa/595/2022		R33Q, E50K, D53X, F79Y, I140K, I202X, S262N	3C.2a1b.2a.2	2022-10-24	S1AT1/S1AT1	80	160	320	320	320	80	160	80	160	80
	Subgroup (iv)														
A/Catalonia/NSVH198253092/2022		D53Q, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-09-01	S1AT2	40	80	80	320	320	40	160	320	320	320
A/Lisboa/566/2022		D53Q, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-10-31	S1AT1/S1AT1	<40	40	40	160	160	40	80	160	160	160
							Vaccine NH 2021-22			Vaccine SH 2022 NH 2022-23 SH 2023					

< relates to the lowest dilution of antiserum used

* For 3C.2a1b.2a.2 viruses HA1 substitutions compared to A/Bangladesh/4005/2020 are shown as related to HA phylogenies (Figures 3a and 3b)

Table 5-2. Antigenic analysis of influenza A(H3N2) viruses by HI

Viruses	Other information HA1 substitutions compared to A/Bangladesh/4005/2020	Passage history Ferret number Genetic group	Collection date	Passage history	Haemagglutination inhibition titre Post-infection ferret antisera A/Camb 925256/20 SIAT F03/21 3C.2a1b.2a.1	A/Camb e0826360/20 Egg F10/21 3C.2a1b.2a.1	A/Thuringen 10/22 SIAT F36/22 3C.2a1b.2a.2	A/Stock 5/21 SIAT F35/21 3C.2a1b.2a.2	A/Darwin 9/21 Egg F39/21 3C.2a1b.2a.2	A/Norway 24873/21 SIAT F10/22 3C.2a1b.2a.2	A/Norway 24873/21 Egg F11/22 3C.2a1b.2a.2	A/Poland 97/22 SIAT F39/22 3C.2a1b.2a.2	A/Slov 8720/2022 SIAT F24/22 3C.2a1b.2a.2	A/Catal NSVH161512067/22 SIAT F41/22 3C.2a1b.2a.2
REFERENCE VIRUSES														
A/Cambodia/925256/2020		3C.2a1b.2a.1	2020-09-25	SIAT5	320	ND	ND	ND	ND	ND	ND	ND	ND	ND
A/Cambodia/e0826360/2020		3C.2a1b.2a.1	2020-07-16	E5/E2	80	640	40	160	320	40	80	40	80	<40
A/Thuringen/10/2022	E50K, F79V, I140K, S262N subgroup (i)	3C.2a1b.2a.2	2022-04-01	P1/SIAT2	80	160	320	640	320	80	80	80	160	40
A/Stockholm/5/2021	H156S	3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	80	80	80	640	640	80	160	320	320	80
A/Darwin/9/2021	H156S (D186N, D225G)	3C.2a1b.2a.2	2021-04-17	E3/E4	80	320	160	640	640	160	320	320	320	160
A/Norway/24873/2021	D53N, N96S(+cho), H156S, I192F subgroup (ii)	3C.2a1b.2a.2	2021-10-24	SIAT3	80	80	80	320	320	160	320	160	160	80
A/Norway/24873/2021	D53N, N96S(+cho), H156S, I192F (D225G) subgroup (ii)	3C.2a1b.2a.2	2021-10-24	E3/E1	40	320	160	640	640	160	640	320	640	80
A/Poland/97/2022	P3S, I25V, D53G, S145N, H156S, R201K, S219Y	3C.2a1b.2a.2	2022-05-09	SIAT2	40	160	80	1280	640	160	320	640	640	320
A/Slovenia/8720/2022	D53G, D104G, H156S, K276R subgroup (iii)	3C.2a1b.2a.2	2022-02-10	SIAT1/MDCK1/SIAT3	40	80	80	640	640	80	160	640	640	320
A/Catalonia/NSVH161512067/2022	D53G, D104G, I140K, H156S, K276R, R299K subgroup (iv)	3C.2a1b.2a.2	2022-09-14	SIAT1/SIAT2	40	80	80	320	320	80	160	320	320	320
TEST VIRUSES														
	Subgroup (i)													
A/Lisboa/532/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-09-17	SIAT2/SIAT1	40	80	160	320	160	40	80	80	80	40
A/Lisboa/524/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-09-25	SIAT2/SIAT2	40	80	160	320	160	40	80	80	80	40
A/Lisboa/534/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-02	SIAT2/SIAT1	40	80	320	160	160	40	80	80	80	40
A/Lisboa/578/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-04	SIAT2/SIAT1	80	80	320	320	320	80	80	80	80	40
A/Lisboa/577/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-04	SIAT2/SIAT1	40	80	160	160	160	40	80	80	80	40
A/Lisboa/535/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-04	SIAT2/SIAT1	40	80	320	320	320	80	80	80	80	40
A/Ile de France/52489/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-07	MDCK2/SIAT1	40	80	160	320	160	40	80	80	80	40
A/Lisboa/579/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-09	SIAT2/SIAT1	80	160	320	320	320	80	80	80	80	40
A/Ile de France/53172/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-11	MDCK1/SIAT1	40	80	320	320	160	40	80	80	80	40
A/Lisboa/572/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-11-04	SIAT1/SIAT1	80	160	320	320	320	80	80	80	80	40
A/Lisboa/570/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-11-04	SIAT1/SIAT1	80	160	320	320	160	40	80	80	80	40
A/Lourdes/53101/2022	R33Q, E50K, F79V, N81D, I140K, S262N	3C.2a1b.2a.2	2022-10-08	MDCK1/SIAT1	40	160	320	320	160	40	80	80	80	40
A/Lisboa/561/2022	R33Q, E50K, F79V, I140K, S144N(+cho), S262N	3C.2a1b.2a.2	2022-10-24	SIAT1/SIAT1	80	160	320	320	320	80	160	80	160	40
A/Lisboa/597/2022	R33Q, E50K, F79V, I140K, S262N, S279F	3C.2a1b.2a.2	2022-10-19	SIAT1/SIAT1	40	80	320	320	160	40	80	80	80	40
A/Lisboa/557/2022	R33Q, E50K, F79V, I140K, Q173H, S262N, S279F	3C.2a1b.2a.2	2022-10-20	SIAT1/SIAT1	40	80	320	320	320	40	80	80	80	40
	Subgroup (ii)													
A/Fort-De-France/53862/2022	D53N, N96S(+cho), I140M, H156S, I192F	3C.2a1b.2a.2	2022-10-18	MDCK1/SIAT1	80	80	160	640	320	160	320	160	160	80
A/Catalonia/NSVH198242704/2022	D53N, N96S(+cho), I140M, H156S, D188N, I192F	3C.2a1b.2a.2	2022-05-05	SIAT1/SIAT2	80	80	160	320	640	160	320	320	160	160
A/Ile de France/55137/2022	E50K, D53N, N96S(+cho), I140K, H156S, I192F, I223V	3C.2a1b.2a.2	2022-11-08	MDCK1/SIAT1	<40	<40	40	80	160	160	160	80	80	40
	Subgroup (iii)													
A/Lille/50053/2022	D53G, D104G, T135A(-cho), H156S, T167S, N190S, K276R	3C.2a1b.2a.2	2022-09-06	MDCK1/SIAT1	80	160	40	320	320	40	160	320	320	160
A/Madrid/3803/2022	D53G, D104G, T135A(-cho), H156S, T167S, K276R, L316M	3C.2a1b.2a.2	2022-10-05	SIAT2/SIAT2	40	80	40	320	320	40	80	320	320	640
	Subgroup (iv)													
A/Catalonia/NSVH560025968/2022	D53G, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-09-01	SIAT1	<40	40	80	160	160	40	80	160	320	160
A/Catalonia/NSVH198253201/2022	D53G, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-09-02	SIAT1	<40	40	80	160	320	40	80	320	320	320
A/Catalonia/NSVH172304934/2022	D53G, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-09-13	SIAT1	40	80	80	320	320	40	160	320	320	320
A/Catalonia/NSVH531046263/2022	D53G, G78D, D104G, I140K, H156S, K276R, R299K	3C.2a1b.2a.2	2022-09-08	SIAT1	<40	40	80	160	160	40	80	160	320	160
						Vaccine NH 2021-22			Vaccine SH 2022 NH 2022-23 SH 2023					

< relates to the lowest dilution of antiserum used
ND = Not Done

* For 3C.2a1b.2a.2 viruses HA1 substitutions compared to A/Bangladesh/4005/2020 are shown as related to HA phylogenies (Figures 3a and 3b)

Table 5-3. Antigenic analysis of influenza A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre										
				Post-infection ferret antisera										
	HA1 substitutions compared to A/Bangladesh/4005/2020	Passage history Ferret number Genetic group		A/Camb 9252256/20 SIAT F03/21 3C.2a1b.2a.1	A/Camb e0826360/20 Egg F10/21 3C.2a1b.2a.1	A/Thuringen 10/22 SIAT F36/22 3C.2a1b.2a.2	A/Stock 5/21 SIAT F35/21 3C.2a1b.2a.2	A/Darwin 9/21 Egg F39/21 3C.2a1b.2a.2	A/Norway 24873/21 SIAT F10/22 3C.2a1b.2a.2	A/Norway 24873/21 Egg F11/22 3C.2a1b.2a.2	A/Poland 97/22 SIAT F39/22 3C.2a1b.2a.2	A/Slov 8720/2022 SIAT F24/22 3C.2a1b.2a.2	A/Catal NSVH161512067/22 SIAT F41/22 3C.2a1b.2a.2
REFERENCE VIRUSES														
A/Cambodia/9252256/2020		3C.2a1b.2a.1	2020-09-25	SIAT5	320	160	<40	160	160	<40	<40	80	<40	<40
A/Cambodia/e0826360/2020		3C.2a1b.2a.1	2020-07-16	E5/E2	160	1280	80	160	320	40	80	80	80	<40
A/Thuringen/10/2022	E50K, F79V, I140K, S282N subgroup (i)	3C.2a1b.2a.2	2022-04-01	P1/SIAT2	40	160	320	320	320	80	160	80	160	80
A/Stockholm/5/2021	H156S	3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	80	160	80	640	640	80	160	320	320	160
A/Darwin/9/2021	H156S (D186N, D225G)	3C.2a1b.2a.2	2021-04-17	E3/E4	160	640	320	1280	1280	320	320	640	640	320
A/Norway/24873/2021	D53N, N96S(+cho), H156S, I192F subgroup (ii)	3C.2a1b.2a.2	2021-10-24	SIAT3	80	80	160	640	320	160	320	320	160	160
A/Norway/24873/2021	D53N, N96S(+cho), H156S, I192F (D225G) subgroup (ii)	3C.2a1b.2a.2	2021-10-24	E3/E1	80	320	320	1280	640	320	640	640	640	160
A/Poland/97/2022	P3S, I25V, D53G, S145N, H156S, R201K, S219Y	3C.2a1b.2a.2	2022-05-09	SIAT2	80	160	80	1280	640	160	320	640	320	640
A/Slovenia/8720/2022	D53G, D104G, H156S, K276R subgroup (iii)	3C.2a1b.2a.2	2022-02-10	SIAT1/MDCK1/SIAT3	40	80	80	640	320	160	160	320	640	640
A/Catalonia/NSVH161512067/2022	D53G, D104G, I140K, H156S, K276R, R299K subgroup (iv)	3C.2a1b.2a.2	2022-09-14	SIAT1/SIAT2	40	80	80	320	320	160	160	640	640	320
TEST VIRUSES														
	Subgroup (i)													
A/Burgos/271/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-14	SIAT1	160	160	320	160	160	160	160	80	80	40
A/Burgos/272/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-15	SIAT1	80	80	160	160	160	80	160	40	80	40
A/Burgos/245/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-18	SIAT1	160	80	320	320	320	160	160	80	80	80
A/Burgos/244/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-18	SIAT1	160	80	320	320	320	160	160	80	80	80
A/Burgos/243/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-18	SIAT1	320	80	320	320	320	160	160	80	160	160
A/Burgos/242/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-18	SIAT1	80	40	320	320	320	160	80	40	80	40
A/Castilla La Mancha/4129/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-19	SIAT1	40	40	320	160	80	40	80	40	40	40
A/Pais Vasco/4012/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-19	SIAT1	80	40	320	320	160	80	160	40	80	80
A/Pais Vasco/4010/2022	R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-19	SIAT1	320	80	640	640	320	160	320	160	80	80
A/Burgos/249/2022	P21L, R33Q, E50K, F79V, I140K, S262N	3C.2a1b.2a.2	2022-10-16	SIAT1	40	80	160	160	160	80	80	40	40	40
A/Burgos/260/2022	R33Q, E50K, F79V, Y105H, I140K, S262N	3C.2a1b.2a.2	2022-10-14	SIAT1	40	80	160	160	80	80	80	40	40	40
A/Burgos/246/2022	R33Q, E50K, F79V, I140K, I242M, S262N	3C.2a1b.2a.2	2022-10-17	SIAT1	160	80	320	320	160	160	160	80	80	80
A/Burgos/262/2022	R33Q, E50K, F79V, I140K, S262N, I300T(+cho)	3C.2a1b.2a.2	2022-10-14	SIAT1	80	320	320	320	320	160	320	80	80	40
	Subgroup (ii)													
A/Aragon/4055/2022	E50K, D53N, N96S(+cho), I140K, H156S, I192F, I223V	3C.2a1b.2a.2	2022-10-18	SIAT1	80	40	160	320	320	320	320	160	160	160
						Vaccine NH 2021-22			Vaccine SH 2022 NH 2022-23 SH 2023					

< relates to the lowest dilution of antiserum used

* For 3C.2a1b.2a.2 viruses HA1 substitutions compared to A/Bangladesh/4005/2020 are shown as related to HA phylogenies (Figures 3a and 3b)

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 B/Brisbane/60/2008-like antigenically. However, three genetic groups (described below with amino acid substitutions/deletions relative to B/Brisbane/60/2008 indicated) containing deletions of HA gene codons emerged and displaced viruses with full-length HAs. Viruses in these groups were/are antigenically distinct from B/Brisbane/60/2008 and each other (as noted in the September 2018 characterization 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**. No detections of viruses in this group have been reported recently.
- 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, represented by **B/Hong Kong/269/2017**. No detections of viruses in this group have been reported recently.
- 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 first recommended for use in the 2020 southern hemisphere season and thereafter up to the 2021-2022 northern hemisphere season.

The phylogeny generated for the November report, was based on sequences from viruses with collection dates after 31 August 2022 that were submitted to GISAID in November 2022 (Figure 4a). All viruses were **V1A.3** subclade represented by **B/Washington/02/2019**, falling 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, with such viruses being detected in China in the early months of 2022) 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). All recently detected viruses (n = 119) had HAs that fell in the **V1A.3a.2** subgroup and viruses in this subgroup had continued to evolve leading to emergence of virus clusters defined by specific **HA1** amino acid substitutions, for example: **T182A**, **D197E** and **A221T**; **E128K**, **A154E** and **S208P**; **E198G**; **D129G** and **D197E**; **H40Y**; **R80G** and **E184K**; and **E183K**.

The phylogeny generated for this December report contains HA sequences from viruses with collection dates after 31 August 2022 that were submitted to GISAID in December 2022 (Figure 4b). The phylogeny has the same structure as that generated for the November report with all the recently detected viruses having HAs that fall in the **V1A.3a.2** subgroup. Sequences derived from recently detected viruses in WHO European Region countries fall in virus clusters defined by: **T182A**, **D197E** and **A221T** with some viruses showing reversion at position **221**; **E128K**, **A154E** and **S208P**, some with additional substitutions; **E198G**; **R80G** and **E184K**; and **E183K**. Viruses detected in China in September 2022 carried **HA1 H112Q** amino acid substitution.

The WHO Collaborating Centres for Influenza Research and Response have shown the **V.1A.3a.1** group viruses with additional HA1 substitutions to be antigenically distinct from one another. While relatively few B/Victoria-lineage viruses have been available for detailed antigenic characterization, those characterised 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 2021-2022 northern hemisphere vaccine virus [1]. However, the **V1A.3a.2** viruses were recognised well (with HI titres of at least 160 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 2023, and northern hemisphere 2022-2023 influenza seasons [2, 4, 3].

[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

Six B/Victoria-lineage test viruses were characterised antigenically at the WIC since the November report (Tables 6-1 and 6-2). All test viruses were recognised poorly by antisera raised against former vaccine viruses, B/Colorado/06/2017 and B/Washington/02/2019. The antisera raised against the **V1A.3** reference viruses, cell culture-propagated B/Netherlands/11267/2022 and egg-propagated B/Netherlands/10894/2022, only recognised the older **V1A.3** test virus (B/Netherlands/11473/2022) well. The five **V1A.3a.2** test viruses were recognised well, within twofold of homologous titres, by three of four antisera raised against **V1A.3a.2** reference viruses, while that raised against B/Austria/1359417/2021 with **HA1 G141R** amino acid substitution recognised the test viruses at titres of at least 160.

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 in GISAID as of 30 November 2022. Figure 5 is repeated from the September 2021 report. All sequences fell in genetic clade **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 was recommended for inclusion in quadrivalent vaccines for the 2021-2022 and 2022-2023 northern and, 2022 and 2023 southern hemisphere seasons [1, 3, 2, 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**), had occurred. As noted in previous characterization 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.

A concerted effort by all NICs of GISRS is required to identify B/Yamagata-lineage viruses for detailed characterization to determine if there are any in circulation that are not LAIV-relatedẓ

Figure 4a. Phylogenetic comparison of B/Victoria-lineage HA genes (GISAID/WIC, Nov 2022)

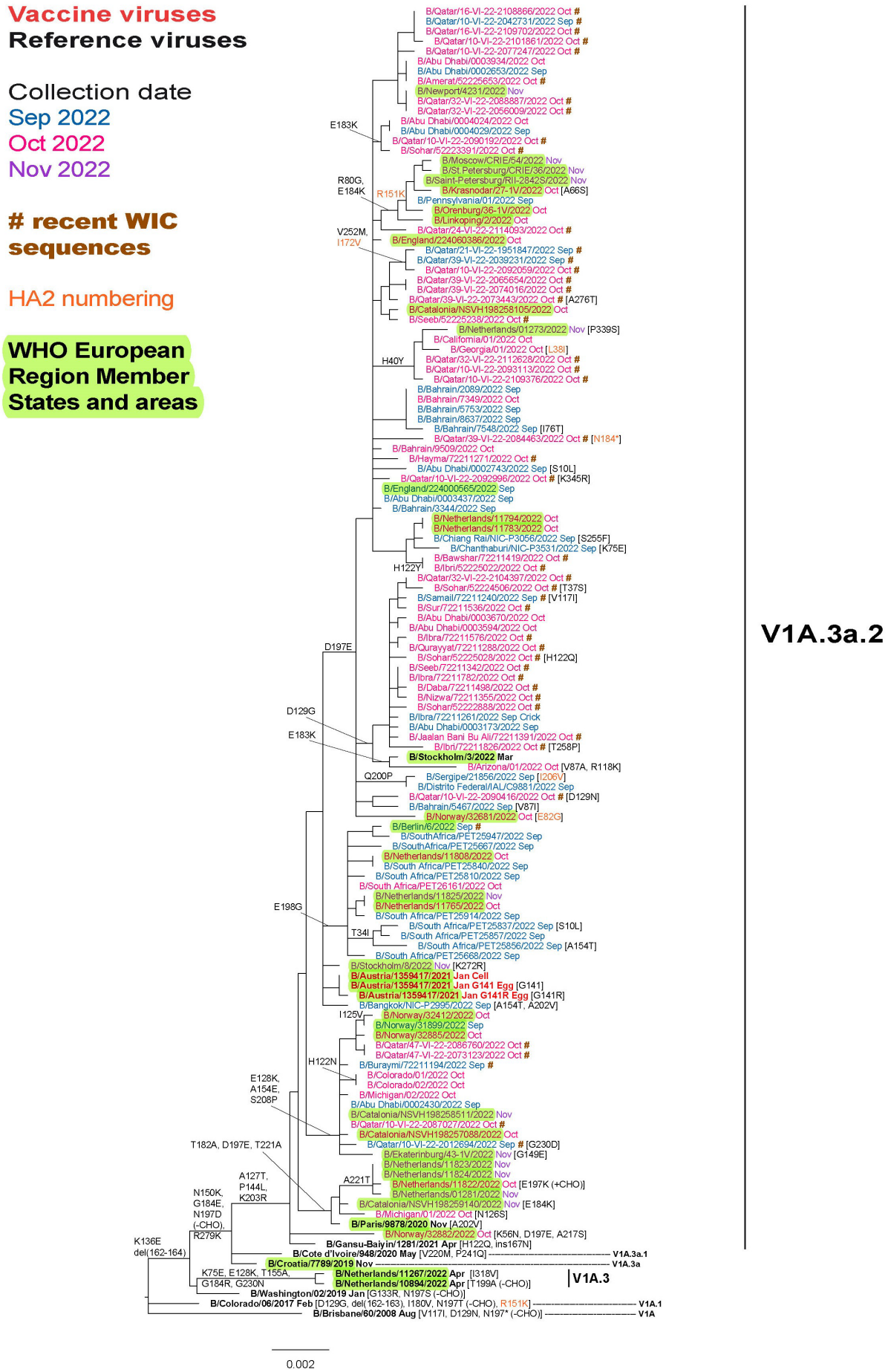


Figure 4b. Phylogenetic comparison of B/Victoria-lineage HA genes (GISAID/WIC, Dec 2022)

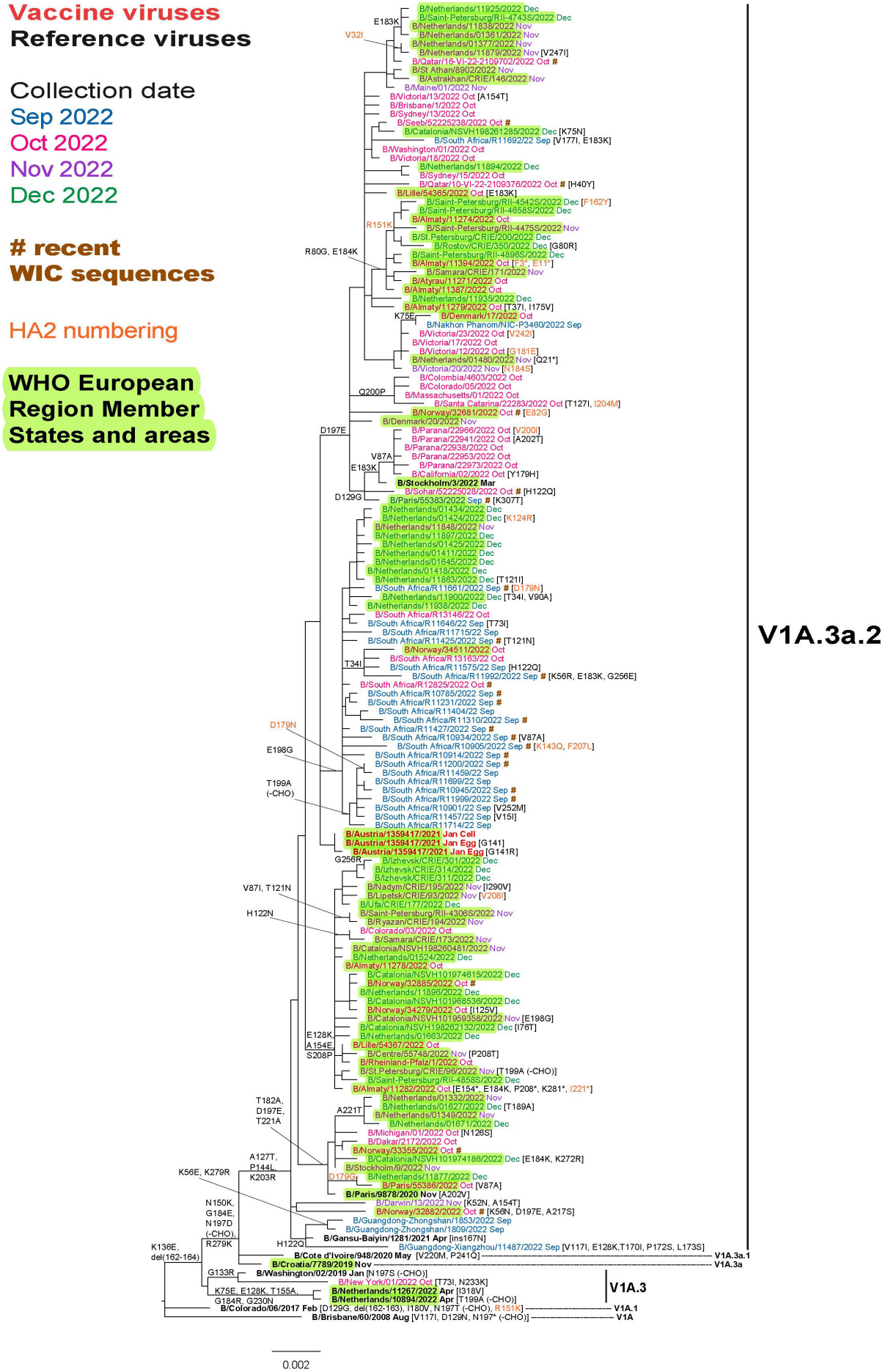


Table 6-1. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

Viruses	Other information	Passage history	Collection date	Passage history	Haemagglutination inhibition titre								
					Post-infection ferret antiserum								
					B/Bris 60/08 Egg Sh 539, 540, 543, 544, 570, 571, 574[1]	B/Colorado 06/17 Egg	B/Washton 02/19 Egg	B/Neth 11267/22 MDCK	B/Neth 10894/22 Egg	B/Austria 1359417/21 MDCK	B/Austria 1359417/21 Egg G141	B/Austria 1359417/21 Egg G141R	B/Stock 3/22 MDCK
		Ferret number			F44/18	F20/20	F29/22	F37/22	NIB F01/21	F15/21	F44/21	F28/22	
		Genetic group			V1A	V1A.1	V1A.3	V1A.3	V1A.3	V1A.3a.2	V1A.3a.2	V1A.3a.2	V1A.3a.2
REFERENCE VIRUSES													
B/Brisbane/60/2008		V1A	2008-08-04	E4/E4	2560	320	40	<40	160	<40	<40	<40	<40
B/Colorado/06/2017		V1A.1	2017-02-05	E5/E2	1280	640	80	<40	160	<40	<40	<40	<40
B/Washington/02/2019		V1A.3	2019-01-19	E3/E3	640	<10	80	40	160	<40	<40	<40	<40
B/Netherlands/11267/2022	G184R	V1A.3	2022-04-14	MDCK-MIX/MDCK2	<40	<10	<10	160	<40	<40	<40	<40	<40
B/Netherlands/10894/2022	G184R, T199A (-cho), V318I	V1A.3	2022-04-02	E4	320	<10	<10	40	80	<40	<40	<40	<40
B/Austria/1359417/2021	A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	SIAT1/MDCK4	320	<10	<10	40	<40	1280	1280	640	640
B/Austria/1359417/2021 Isolate 2	A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	E3/E5	640	<10	<10	80	<40	2560	1280	640	1280
B/Austria/1359417/2021 Isolate 2	A127T, P144L, K302R (G141R)	V1A.3a.2	2021-01-09	E3/E5	320	<10	<10	80	<40	2560	1280	5120	640
B/Stockholm/3/2022	D197E, D129G, E183K	V1A.3a.2	2022-03-22	SIAT1/MDCK3	640	<10	<10	40	<40	1280	640	320	1280
TEST VIRUSES													
B/Norway/30217/2022		V1A.3a.2	2022-08-18	MDCK1	640	<10	<10	80	<40	2560	1280	320	640
B/Norway/30216/2022		V1A.3a.2	2022-08-20	MDCK1	640	<10	<10	40	<40	2560	1280	640	640
							Vaccine NH 2021-22					Vaccine SH 2022 NH 2022-23 SH 2023	

< relates to the lowest dilution of antiserum used
[1] hyperimmune sheep serum; ND = Not Done

Table 6-2. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

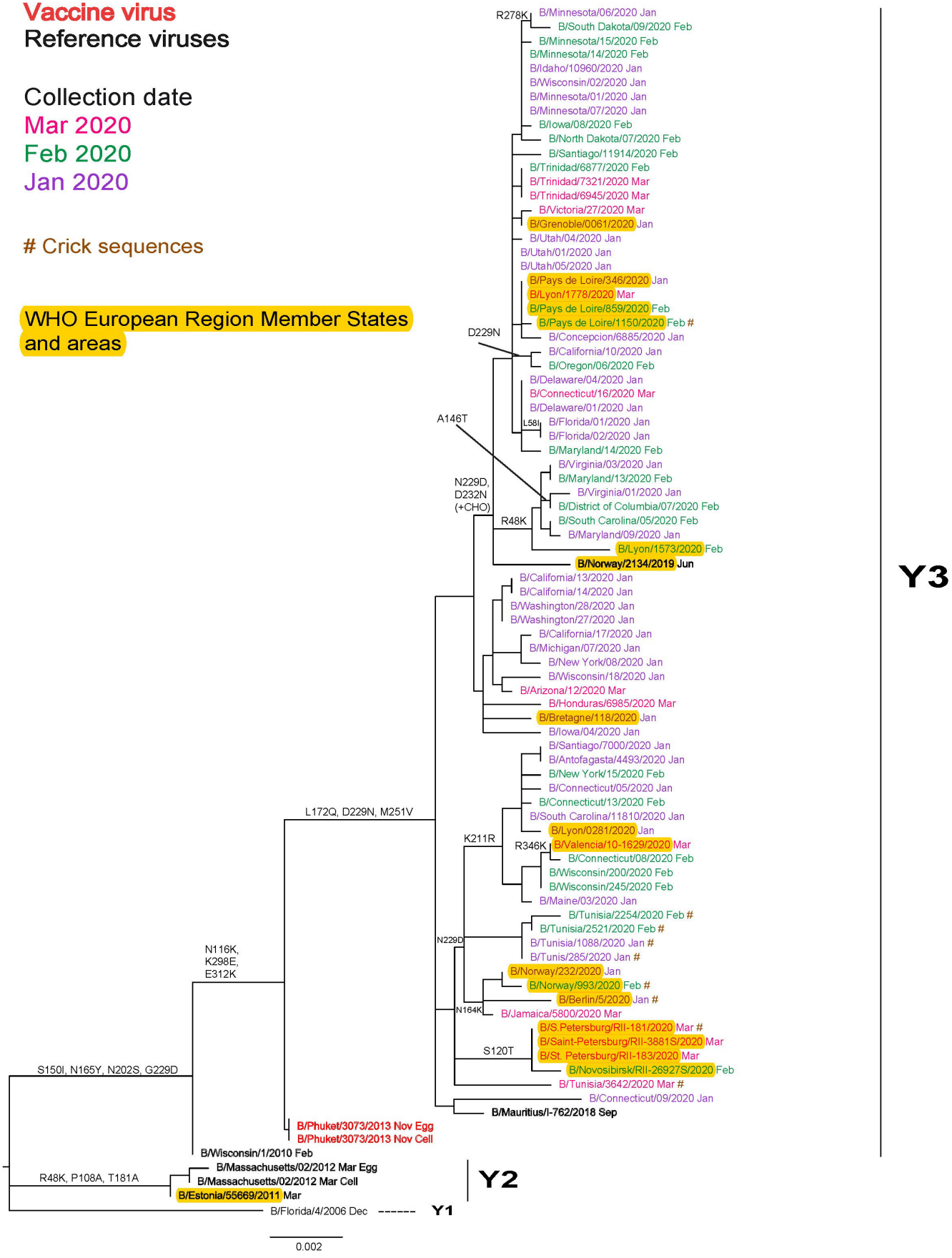
Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre									
				Post-infection ferret antiserum									
				B/Bris 60/08 Egg Sh	B/Colorado 06/17 Egg 539, 540, 543, 544, 570, 571, 574[1]	B/Wash'ton 02/19 Egg F20/20	B/Neth 11267/22 MDCK F29/22	B/Neth 10894/22 Egg F37/22	B/Austria 1359417/21 MDCK NIB F01/21	B/Austria 1359417/21 Egg G141 F15/21	B/Austria 1359417/21 Egg G141R F44/21	B/Stock 3/22 MDCK F28/22
	Passage history			V1A	V1A.1	V1A.3	V1A.3	V1A.3	V1A.3a.2	V1A.3a.2	V1A.3a.2	V1A.3a.2
	Ferret number											
	Genetic group											
REFERENCE VIRUSES												
B/Brisbane/60/2008	V1A	2008-08-04	E4/E4	1280	320	20	<40	80	<40	<40	<40	<40
B/Colorado/06/2017	V1A.1	2017-02-05	E5/E2	1280	640	40	<40	160	<40	<40	<40	<40
B/Washington/02/2019	V1A.3	2019-01-19	E3/E3	640	320	80	<40	160	<40	<40	<40	<40
B/Netherlands/11267/2022 G184R	V1A.3	2022-04-14	MDCK-MIX/MDCK2	<40	20	<10	160	40	<40	<40	<40	<40
B/Netherlands/10894/2022 G184R, T199A (-cho), V318I	V1A.3	2022-04-02	E4(Am1AL3)	320	160	<10	80	160	<40	<40	<40	<40
B/Austria/1359417/2021 A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	SIAT1/MDCK4	320	80	<10	<40	<40	1280	640	320	640
B/Austria/1359417/2021 Isolate 2 A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	E3/E5	640	80	<10	40	<40	2560	1280	640	640
B/Austria/1359417/2021 Isolate 2 A127T, P144L, K302R (G141R)	V1A.3a.2	2021-01-09	E3/E5	160	40	<10	40	<40	1280	640	2560	640
B/Stockholm/3/2022 D197E, D129G, E183K	V1A.3a.2	2022-03-22	SIAT1/MDCK3	320	40	<10	<40	<40	640	640	320	640
TEST VIRUSES												
B/Netherlands/11473/2022 G184R, V318I	V1A.3	2022-04-25	MDCK3	640	320	<10	80	320	<40	<40	<40	<40
B/Paris/55383/2022	V1A.3a.2	2022-09-21	MDCK1/MDCK1	320	80	<10	<40	<40	1280	640	320	1280
B/Paris/55386/2022	V1A.3a.2	2022-10-14	MDCK1/MDCK1	640	40	<10	<40	<40	640	640	160	320
B/Provence_Alpes_Cote_Azur/54364/2022	V1A.3a.2	2022-10-25	MDCK1/MDCK1	640	80	<10	<40	<40	1280	1280	640	640

Vaccine NH 2021-22

Vaccine SH 2022 NH 2022-23 SH 2023

< relates to the lowest dilution of antiserum used
[1] hyperimmune sheep serum; ND = Not Done

Figure 5. Phylogenetic comparison of B/Yamagata-lineage HA genes (GISAID, September 2021)



Summaries of data submitted to TESSy

Genetic characterization

856 viruses detected over the course of the 2022-2023 season (weeks 40-52/2022) were genetically characterised:

- Of 248 A(H1N1)pdm09 viruses, all but one belonged to clade 6B.1A.5a.2 with 143 represented by A/Norway/25089/2022, 103 by A/Sydney/5/2021 and 1 by A/Victoria/2570/2019. One was a clade 6B.1A.5a.1 virus represented by A/Guangdong-Maonan/SWL1536/2019.
- Of 551 A(H3N2) viruses, 540 belonged to the 'Bangladesh-like' clade (3C.2a1b.2a.2) with 188 represented by A/Slovenia/8720/2022, 332 represented by A/Bangladesh/4005/2020 and 20 represented by A/Darwin/9/2021. Eleven viruses were not attributed to a clade.
- Of 57 B/Victoria-lineage viruses, 38 were clade V1A.3a.2 represented by B/Austria/1359417/2021. Nineteen viruses were not assigned to a clade.

Antiviral susceptibility

Up to week 52/2022, 906 viruses were assessed for susceptibility to neuraminidase (NA) inhibitors (NAIs): 420 A(H3), 234 A(H1)pdm09 and 53 B virus were assessed genotypically, and 161 A(H3), 32 A(H1)pdm09 and 6 B viruses were assessed phenotypically. Susceptibility to the PA inhibitor baloxavir marboxil (BXM) was assessed genotypically for 516 viruses: 345 A(H3), 124 A(H1)pdm09 and 47 B viruses. Phenotypically one A(H1)pdm09 virus exceeded the IC_{50}-fold-change threshold associated with reduced susceptibility to NAIs and, genotypically, no markers associated with reduced susceptibility were identified.

At the WIC, 93 influenza viruses detected within the WHO European Region during the 2022-2023 season have been assessed phenotypically against oseltamivir and zanamivir: 15 A(H1N1)pdm09, 73 A(H3N2) and five B/Victoria-lineage. All showed Normal Inhibition (NI) by both NAIs and no NA amino acid markers associated with reduced inhibition were observed following gene sequencing. Similarly, no markers associated with reduced inhibition by BXM were identified following PA gene sequencing.

Animal influenza and zoonotic events

Influenza A(H7N9) virus

On 1 April 2013, the WHO Global Alert and Response System [5] 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 caused some zoonoses, although few human cases were reported during the 2017-18 season [6]. Current risk assessments for influenza at the human-animal interface can be found on WHO's website https://www.who.int/teams/global-influenza-programme/avian-influenza/monthly-risk-assessment-summary (accessed 13 January 2023). The assessment published on 05 January 2023 contains a link to WHO information on A(H7N9) viruses after there being no publicly available reports from animal health authorities in China or other countries on influenza A(H7N9) virus detections in animals for an extending period of time [7]. On 01 June 2022 the Food and Agricultural Organization of the United Nations announced that it was discontinuing monthly A(H7N9) updates as there had been no notifications of avian infections since October 2020. The most recent human case was detected in mid-March 2019 [8]. The latest overview of avian influenza by ECDC in collaboration with the European Food Safety Authority and the EU Reference Laboratory for Avian Influenza was approved on 15 December 2022 and can be found on ECDC's website [9].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 05 January 2023. Since the previous risk assessment on 11 November 2022, China reported a single case of human infection with an influenza A(H5N6) virus to WHO [7]. The case involved a 54-year-old male from Hunan province who was hospitalized on 05 November in a critical condition with pneumonia. He had been working in a restaurant preparing food but the source of exposure to the virus had not been identified and no further cases were reported among family members.

The latest collaborative report from ECDC and the European Food Safety Authority (EFSA), reported 1 162 highly pathogenic avian influenza (HPAI) A(H5) detections between 10 September and 02 December 2022, 398 in poultry, 613 in wild birds and 151 in captive birds [9]. Detections occurred in 27 European countries but with a decrease in colony-breeding seabird species and an increase in the number of detections in waterfowl compared to earlier periods. It is suspected that waterfowl might be more involved than seabirds in the incursion of HPAI virus into poultry establishments. The viruses detected since the September 2022 report (clade 2.3.4.4b) have fallen into 11 genotypes, three of which circulated in summer months while eight represented emergent genotypes. Overall, the HPAI epidemic season in 2021-2022 is the largest so far observed in Europe with 2 520 outbreaks in poultry and 50 million birds

dead/culled, 227 outbreaks in captive birds, and 3 867 detections in wild birds. The risk of human infection was assessed as low for the general population in EU/EEA countries, and low to medium for occupationally exposed persons.

According to reports compiled by the Food and Agricultural Organization of the United Nations (FAO) as of 22 December 2022, various highly pathogenic avian influenza (HPAI) subtypes continued to be detected in wild and/or domestic birds in Africa, Americas, Asia and Europe, and since 23 November 2022 a total of 1 118 HPAI outbreaks (45 H5Nx, 1 069 H5N1, three H5N2 and one HPAI not confirmed as H5) and three low pathogenic avian influenza (LPAI) outbreak had been reported [10]. In addition, nine un-subtyped and un-pathotyped avian influenza-positive events were reported, eight in Japan and one in Viet Nam.

HPAI A(H5) viruses have also been detected in wild mammal species in Europe and North America, with some viruses showing genetic markers of adaptation to replication in mammals.

Influenza A(H9N2) virus

Since the previous WHO risk assessment on 11 November 2022, three zoonotic cases of A(H9N2) infection in China had been reported to WHO [7]. The cases involved a 58-year-old male who developed severe symptoms and was hospitalised on 19 October 2022, a 5-year-old female who developed mild symptoms on 23 October 2022 and a 3-year-old male who developed mild symptoms on 13 November 2022. The three cases were detected through influenza-like illness (ILI) surveillance and were not linked. While both males were exposed at live poultry markets, the exposure history of the female was unknown. No cases were reported among family members. Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa.

Public Health England published an updated risk assessment for avian influenza A(H9N2) in August 2021 [11].

Other influenza zoonotic events

Since the previous WHO update on 11 November 2022 one case of human infection with a swine virus was reported [7]. On 24 September 2022 a 7-year-old female (a member of a family owning a pig barn in Changhua County, Taiwan) developed ILI and tested positive for influenza A. The patient did not require hospitalization and after treatment with oseltamivir her symptoms subsided on 28 September. The virus responsible was subsequently (11 October 2022) identified as Eurasian avian-like influenza A(H1N2)v belonging to clade 1C. The patient had no direct exposure to pigs, and samples other family members and pigs at the pig barn all tested negative for influenza by PCR. Serum samples from all family members, except the case, tested negative by hemagglutination assay for antibodies to the A(H1N2)v virus identified.

WHO Collaborating Centre reports

A description of results generated by the London WHO Collaborating Centre at the WIC and used at the September 2022 WHO VCM (19-22 September 2022 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 30 December 2022).

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. Sequences for many viruses from non-WHO Europe 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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[5] All references accessed on 16 January 2023.