Influenza virus characterization

Summary Europe, November 2022

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Summary

The October 2022 characterisation report[1], was the first report for the 2022-2023 influenza season. As of week 48/2022, 24 737 detections had been reported. Of these detections, 93% were type A viruses, with A(H3N2) dominating (64%) over A(H1N1)pdm09 (36%), and 13% type B of which 321 were ascribed to a lineage, with all being B/Victoria. This represents a 6-fold increase in detections compared to the 2021-2022 season, despite only a modest increase (2%) in the number of samples tested. The epidemic threshold of 10% positivity within sentinel specimens was crossed in week 45/2022 and increased to 20% in week 48/2022.

Seven 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 October 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 November 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 nine 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 36% 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 all recently detected viruses, as assessed from sequence deposition in GISAID's EpiFlu™ database, falling in the 'Bangladesh-like' (3C.2a1b.2a.2) subgroup. 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 23 3C.2a1b.2a.2 test viruses from Germany analysed here, together with those analysed previously from a range of countries.

In Europe and across the world generally, few B/Victoria-lineage viruses have been detected during weeks 40-48/2022. All 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. There were no sequences deposited in EpiFlu™ for either V1A.3 B/Washington/02/2019-like viruses or the variants within this subclade that had emerged in Guatemala, the Netherlands and Zambia for viruses with collection dates after 31 August 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 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 2022-2023 season (weeks 40-48/2022),

[1]Influenza virus characterization: summary report, Europe, October 2022. World Health Organization Regional Office for Europe and European Centre for Disease Prevention and Control; Copenhagen and Stockholm;
2022 (https://apps.who.int/iris/handle/10665/364537, accessed 19 December 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, 1.8%), notably from sentinel sources, but a significant rise in the number of influenza detections (20 373, ~6-fold). In the same period of 2020, during the earlier stages of the COVID-19 pandemic, just 100 843 specimens were tested (~5-fold less) and only 288 influenza detections were reported (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) 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 was similar for the two seasons (2021-2022 and 2022-2023), but with a reduction in dominance of A(H3N2) over A(H1N1)pdm09 viruses in 2022-2023. While the number of influenza B virus detections has increased from 259 to 1 723 (7-fold), the number of viruses ascribed to a lineage has increased from 3.4% to 18.6% 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 nine 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 continues to hover around 7%, just below the epidemic threshold for the Region (Figure 1).

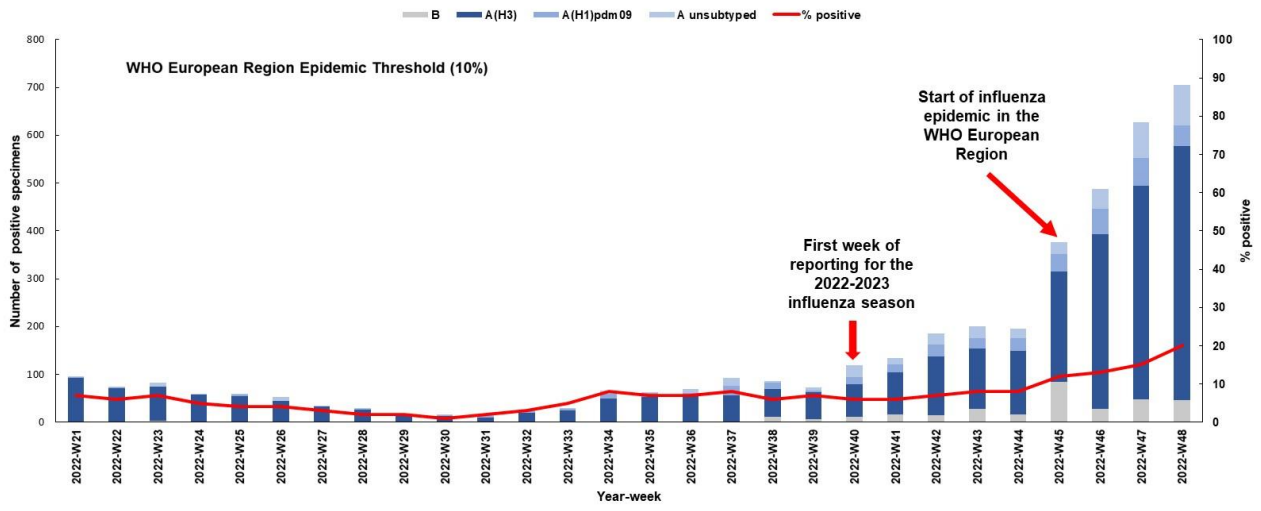
Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2022-2023 season (weeks 40-48/2022)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40-48/2022			Totals*		Cumulative number of detections for weeks 40-48/2021			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**2739**	**20275**	**23014**	**93.0**	**13.4:1**	**210**	**3895**	**4105**	**94.1**	**15.8:1**
A(H1N1)pdm09	299	3223	3522	35.8		3	103	106	4.1	
A(H3N2)	2112	4208	6320	64.2	1.8:1	145	2341	2486	95.9	23.5:1
A not subtyped	328	12844				62	1451	1513		
Influenza B	**291**	**1432**	**1723**	**7.0**		**8**	**251**	**259**	**5.9**	
Victoria lineage	128	193	321	100.0		3	6	9	100.0	
Yamagata lineage	0	0	0			0	0	0		
Lineage not ascribed	163	1239	1402			5	245	250		
Total detections (total tested)	**3 030 (25 886)**	**21 707 (>469 885)**	**24 737 (>495 771)**			**218 (12 121)**	**4 146 (>475 034)**	**4 364 (>487 155)**		

[a] **Numbers taken from Flu News Europe week 48 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 Europe[a]



[a] Figure adapted from FluNewsEurope weeks 36-39/2022 and 48/2022 reports (https://flunewseurope.org/Archives)

Genetic and antigenic characterisation 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 characterisation reports (https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/influenza-virus-characterisation) 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 October 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 30 November 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 November 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 2022-11-30				
	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	2022-11-01	658	595	134
A(H3N2)	2022-09-01	2022-11-01	1299	1156	126
B/Victoria	2022-09-01	2022-11-01	133	119	119
B/Yamagata	2022-01-01	2022-11-01	0	0	0

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

Eleven shipments containing specimens (n = 259: virus isolates and/or clinical specimens) with collection dates after 31 August 2022 were received at the WIC (seven since the October 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 characterisation process at the time of preparing this report.

A total of 31 viruses from the WHO European Region (five A(H1N1)pdm09, 23 A(H3N2 and three B/Victoria-lineage) have been characterised antigenically since the October report (Tables 4, 5 and 6 respectively). Of these, two, 23 and one, 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 / Country/area	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]
2022														
September														
France	7			2	in process	4	in process				1	in process		
Germany	6					5	5				1	1		
Netherlands	5			5	in process									
Norway	14			12	in process	1	in process				1	in process		
Portugal	2					2	in process							
Spain	18			8	in process	10	in process							
October														
France	13			4	in process	6	in process				3	in process		
Germany	18					18	18							
Netherlands	1			1	0									
Norway	35			18	in process	12	in process				5	in process		
Portugal	32			8	in process	24	in process							
Spain	59	39	in process	5	in process	15	in process							
UK (N. Ireland)	14			13		1								
November														
France	5					4	in process				1	in process		
Georgia	10	9	in process	1	in process									
Norway	13			9	in process	4	in process							
Portugal	4			1	in process	3	in process							
Spain	10	10	in process											
TOTAL	259	58	0	85	0	105	23	0	0	0	11	1	0	0
8 Countries/areas		22.4%		32.8%		40.5%			0.0%		4.2%		0.0%	
		95.8%							4.2%					

* 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 2022-12-02

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 recently 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 the October report and earlier ones. 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 October report focused on HA sequences derived from viruses with collection dates after 31 December 2021 for which sequences were submitted to GISAID in the period 29 September to 31 October 2022 (Figure 2a). 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, and virus clusters had emerged defined by amino acid substitutions: (i) **HA1 T216A** often with **D94N** , the cluster showing wide geographic distribution; (ii) **HA1 A48P**, and; (iii) **HA1 K142R**, **D260E** and **HA2 I91V**, **N124H**, frequently with **HA1 P137S**, **T277A** and **HA2 E29D**. Viruses in cluster (iii) with the additional substitutions had recently been detected in countries of the western part of the WHO European Region.

The phylogeny prepared for this 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 2b and Table 2). 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. Viruses within the three clusters (i to iii) identified above have continued to circulate with cluster (iii) viruses dominating and the great majority of these having the additional **HA1 P137S**, **T277A** and **HA2 E29D** amino acid substitutions. Further diversification has 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 have **HA1 T216A** substitutions. Viruses detected recently in the WHO European Region are dispersed throughout the phylogeny.

The panel of post-infection ferret antisera used in HI assays, four raised against subgroup **6B.1A.5a.1** viruses and four against **6B.1A.5a.2** viruses, gives clear discrimination of reference viruses in the two subgroups (Table 4). This discrimination carries through to the five **6B.1A.5a.2** test viruses from the Netherlands analysed since the October report. All fell in virus cluster (iii), and carried the additional **HA1 P137S**, **T277A** and **HA2 E29D** amino acid substitutions, and were recognised well (within fourfold of the homologous titres) by antisera raised against all four **6B.1A.5a.2** reference viruses. Antisera raised against the vaccine viruses, A/Victoria/2570/2029 and A/Sydney/5/2021, recognised the test viruses at titres with twofold of the homologous titres.

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, Oct 2022)

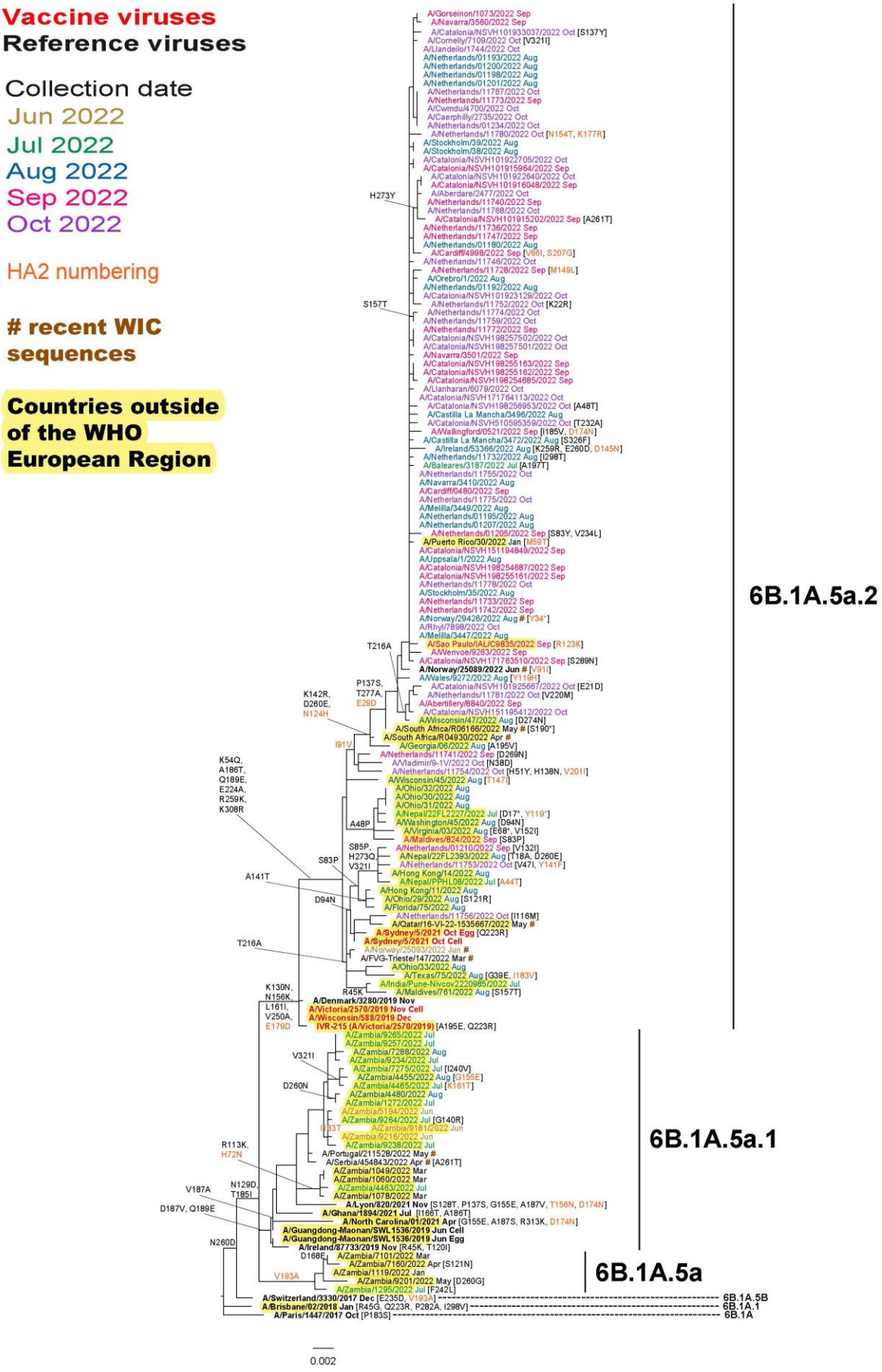


Figure 2b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID/WIC, Nov 2022)

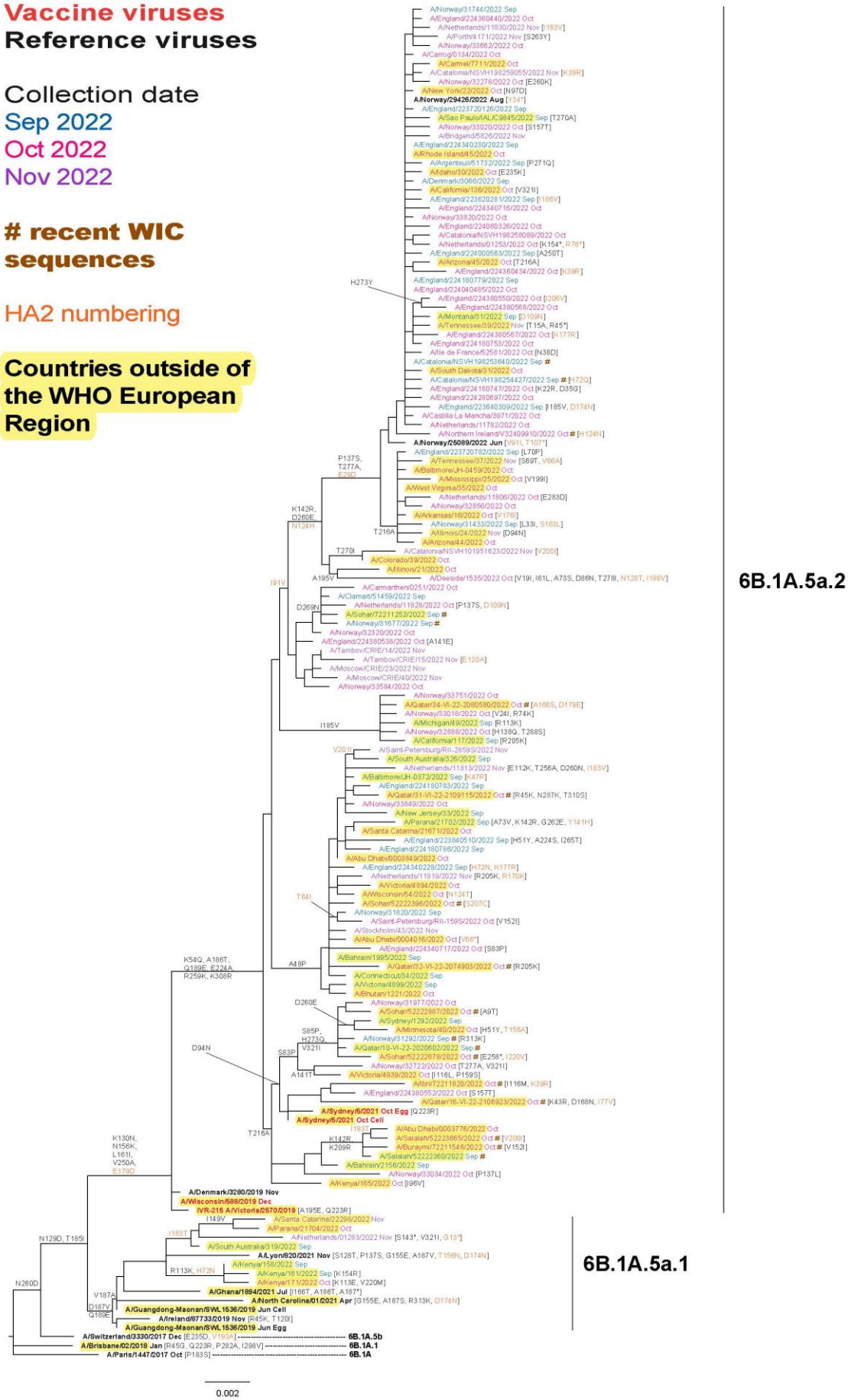


Table 4. Antigenic analysis of influenza A(H1N1)pdm09 viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre								
				Post-infection fereret antisera								
	Passage history			A/G-M SWL1536/19 MDCK	A/G-M SWL1536/19 Egg	A/Ghana 1894/21 Egg	A/Lyon 820/21 Egg	A/Denmark 3280/19 MDCK	A/Nor 25089/22 MDCK	IVR-215 A/Vic/2570/19 Egg	A/Sydney 5/21 Egg
	Ferret number			F09/20	F12/20	F02/22	F06/22	F28/20	F38/22	F37/21	F04/22
	Genetic group			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	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	40	80	40
A/Guangdong-Maonan/SWL1536/2019	6B.1A.5a.1	2019-06-17	E3/E2	1280	1280	640	320	40	<40	80	40
A/Ghana/1894/2021	6B.1A.5a.1	2021-07-21	E2/E1	2560	2560	2560	320	80	<40	160	80
A/Lyon/820/2021	6B.1A.5a.1	2021-11-16	E1/E2	320	320	160	640	40	40	40	40
A/Denmark/3280/2019	6B.1A.5a.2	2019-11-10	MDCK4/MDCK5	80	80	<40	80	2560	1280	2560	1280
A/Norway/25089/2022	6B.1A.5a.2	2022-06-15	MDCK2	<40	40	<40	<40	1280	2560	1280	1280
IVR-215 (A/Victoria/2570/2019)	6B.1A.5a.2	2018-11-22	E4/D7/E2	160	80	40	80	1280	1280	2560	1280
A/Sydney/5/2021	6B.1A.5a.2	2021-10-16	MDCK3/MDCK1	<40	<40	<40	<40	640	320	640	640
A/Sydney/5/2021	6B.1A.5a.2	2022-10-31	E3/E2	80	40	<40	40	640	640	1280	1280
TEST VIRUSES											
A/Netherlands/11714/2022	P137S, K142R	2022-08-03	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	640
A/Netherlands/11699/2022	P137S, K142R	2022-08-11	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	1280
A/Netherlands/11711/2022	P137S, K142R	2022-08-17	MDCK-MIX1/MDCK1	<40	40	<40	<40	1280	1280	1280	1280
A/Netherlands/11733/2022	P137S, K142R	2022-09-06	MDCK-MIX1/MDCK1	<40	40	<40	<40	1280	1280	1280	1280
A/Netherlands/11742/2022	P137S, K142R	2022-09-20	MDCK-MIX1/MDCK1	<40	<40	<40	<40	640	1280	1280	640
					Vaccine NH 2020-21					Vaccine SH 2021 NH 2021-22	Vaccine SH 2023
										SH 2022 NH 2022-23	

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

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 was based on HA sequences derived from viruses with collection dates after 31 August 2022 made available in GISAID and generated at the WIC from 29 September to 31 October 2022 (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; (iii) **D53G** commonly with **D104G** and **K276R**, and further subdivision into clusters defined by either **T135A** (resulting in loss of a glycosylation site) and **T167S** or **E50K** and **R299K**; (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 (largely dominated by viruses detected in Catalonia and wider regions of Spain but with significant contributions from the Netherlands and Wales, together with small numbers from other countries, e.g., Ireland, the Russian Federation and Sweden) 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. Relatively few sequences from viruses detected in countries outside of the WHO European Region had been submitted to GISAID, but these also fell in subgroups (i), (ii) and (iii).

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 November 2022 and is dominated by sequences derived from viruses detected in countries outside of the WHO European Region (Table 2 and Figure 3b). The two phylogenies show similar profiles with the four subgroups identified above (i to iv) represented but with different relative proportions. Subgroups (i) and (ii) are 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) are from viruses detected in the WHO European Region. There is further diversification occurring in all four subgroups and a new subgroup has emerged in South Africa defined by HA1 amino acid substitutions **E83K**, **K121E**, **S205F**, **A212T** and **R261Q**.

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

[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

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 with single A(H3N2) viruses from Croatia and the Netherlands failing to yield a sufficient HA titre with guinea pig RBCs to allow HI analysis.

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 23 A(H3N2) 'Bangladesh-like' (**3C.2a1b.2a.2)** test viruses from Germany with collection dates after 31 August 2022, fully characterised antigenically since the October report, are shown in Table 5. The test viruses fell within the four genetic subgroups identified above (i, n=12; ii, n = 3; iii, n = 6; iv, n = 2) and were recognised with twofold of the homologous titre by the antiserum raised against cell culture-propagated A/Thuringen/10/2022 (subgroup (i)). The remaining reference **3C.2a1b.2a.2** viruses against which antisera were raised all contained **HA1 H156S** amino acid substitutions. The antisera raised against current vaccine-related viruses, cell culture-propagated A/Stockholm/5/2021 and egg-propagated A/Darwin/9/2021, recognised all but one of the test viruses at titres within fourfold of the homologous titres, most within twofold. Of the antisera raised against the subgroup (ii) reference viruses: that against A/Ghana/1724/2022 had a low homologous titre (80) such that fold-changes with test viruses could not be ascertained; that against cell culture-propagated A/Norway/24873/2021 performed as well as that raised against A/Darwin/9/2021, while; that raised against egg-propagated A/Norway/24873/2021 had a high homologous titre (1280) and 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 = 3) and subgroup (iv) (n = 1) viruses recognised those test viruses with **HA1 H156S** substitutions well (within fourfold of homologous titres) but that raised against cell culture-propagated A/Norway/28542/2022 (with **HA1 E50K** and **R299K** substitutions compared to the other two subgroup (iii) viruses) performed slightly less well. The antiserum raised against cell culture-propagated A/Poland/97/2022 performed similarly to that raised against cell culture-propagated A/Norway/28542/2022.

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.

[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, Oct 2022)

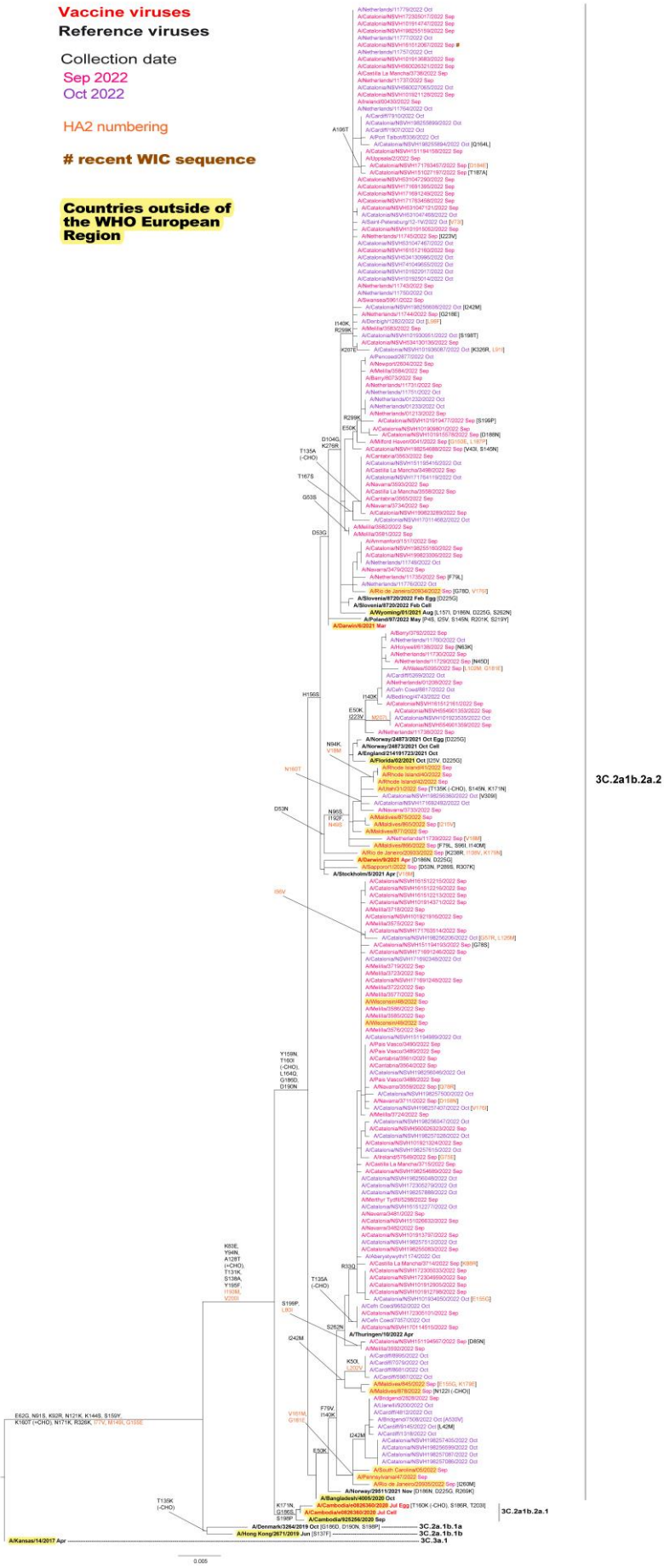


Figure 3b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID/WIC, Nov 2022)

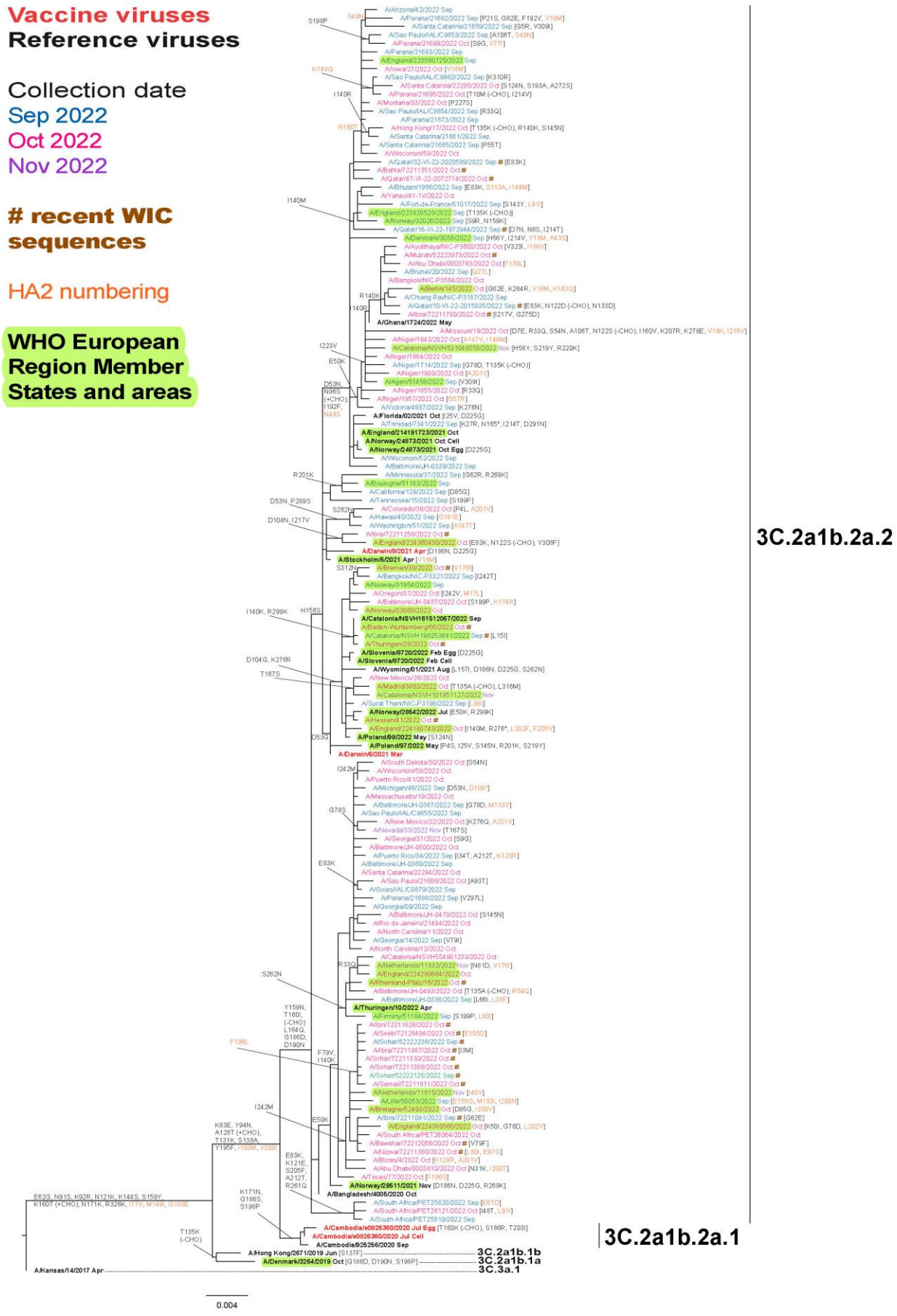


Table 5. Antigenic analysis of influenza A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre														
				Post-infection ferret antisera														
				A/Cambodia/925256/2020	A/Cambodia/e0826360/2020	A/Thuringen/10/22	A/Stockholm/5/21	A/Darwin/9/21	A/Norway/24873/21	A/Norway/24873/21	A/Ghana/1734/22	A/Poland/97/22	A/Sthr/8720/2022	A/Poland/99/22	A/Nor/2854/22	A/Catalonia/NSVH161512067/22
	HA1 substitutions compared to A/Bangladesh/4005/2020*			SIAT5	E5/E2	P1/SIAT2	SIAT0/SIAT3	E3/E4	SIAT2	E3 (An/2Al)	SIAT2	SIAT2	SIAT1/MDCK1/SIAT2	SIAT2	SIAT1/SIAT2	SIAT2	SIAT1/SIAT2	SIAT2
	Passage history			SIAT	Egg	SIAT	SIAT	Egg	SIAT	Egg	SIAT	SIAT	SIAT	SIAT	SIAT	SIAT	SIAT	SIAT
	Ferret number			F03/21	F10/21	F36/22	F35/21	F39/21	F10/22	F11/22	F63/22	F39/22	F2/4/22	F44/22	F4/2/22	F41/22	F41/22	F41/22
	Genetic group			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.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2
REFERENCE VIRUSES																		
A/Cambodia/925256/2020		2020-09-25	SIAT5	640	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A/Cambodia/e0826360/2020		2020-07-16	E5/E2	320	2560	160	320	640	80	160	<40	160	160	320	80	40	40	40
A/Thuringen/10/2022	E5K, F79V, I140K, S262N subgroup (i)	2022-04-01	P1/SIAT2	40	160	160	320	160	80	80	<40	80	160	80	80	80	80	80
A/Stockholm/5/2021	H156S	2021-04-16	SIAT0/SIAT3	160	160	160	1280	640	160	320	<40	640	320	1280	320	160	160	160
A/Darwin/9/2021	H156S (D198N, D225G)	2021-04-17	E3/E4	160	640	320	1280	640	320	320	80	640	640	1280	640	160	320	320
A/Norway/24873/2021	D53N, N65S(+cho), H156S, I192F subgroup (ii)	2021-10-24	SIAT2	80	80	160	640	320	320	640	80	320	320	640	160	160	160	160
A/Norway/24873/2021	D53N, N65S(+cho), H156S, I192F (D225G) subgroup (ii)	2021-10-24	E3 (An/2Al)	160	320	320	1280	1280	640	1280	80	640	1280	1280	640	320	320	320
A/Ghana/1724/2022	E5K, D53N, N65S(+cho), I140K, H156S, I192F, I223V subgroup (i)	2022-05-18	SIAT2	80	80	160	640	640	320	320	80	320	320	1280	320	320	320	320
A/Poland/97/2022	P3S, I23V, D53G, S146N, H156S, R201K, S211Y	2022-05-09	SIAT2	160	160	160	1280	640	320	640	80	1280	640	2560	1280	640	640	640
A/Slovenia/872/2022	D53G, D104G, H156S, K276R subgroup (iii)	2022-02-10	SIAT1/MDCK1/SIAT2	80	80	160	640	640	320	160	<40	640	640	640	320	640	640	640
A/Poland/99/2022	D53G, D104G, S124N, H156S, K276R subgroup (iii)	2022-05-10	SIAT2	80	40	160	640	640	160	160	<40	640	640	1280	320	640	640	640
A/Norway/2854/2022	E5K, D53G, D104G, H156S, K276R, R299K subgroup (iii)	2022-07-12	SIAT2	40	80	160	1280	640	320	320	80	1280	640	2560	2560	640	640	640
A/Catalonia/NSVH161512067/2022	D53G, D104G, I140K, H156S, K276R, R299K subgroup (iv)	2022-09-14	SIAT1/SIAT2	40	80	160	640	320	160	160	<40	640	640	1280	320	640	640	640
TEST VIRUSES																		
A/Rheinland-Pfalz/16/2022	Subgroup (i) E5K, F79V, I140K, S262N	2022-10-04	P1/SIAT1	160	160	640	640	320	320	160	40	160	160	320	160	80	80	80
A/Niedersachsen/49/2022	E5K, F79V, I140K, D142M	2022-09-26	P1/SIAT1	160	160	320	640	320	160	320	40	160	160	160	160	80	80	80
A/Hessen/46/2022	R33Q, E5K, F79V, I140K, S262N	2022-10-17	P1/SIAT1	80	160	640	320	320	80	160	<40	80	80	160	40	40	40	40
A/Baden-Wurttemberg/67/2022	R33Q, E5K, F79V, I140K, S262N	2022-10-17	P1/SIAT1	80	160	320	320	320	80	160	<40	80	80	80	80	80	80	80
A/Hessen/40/2022	R33Q, E5K, F79V, D101E, I140K, S262N	2022-09-29	P1/SIAT1	80	160	320	320	320	80	160	40	80	80	320	80	80	80	80
A/Rheinland-Pfalz/17/2022	R33Q, E5K, F79V, D101E, I140K, S262N	2022-10-04	P1/SIAT1	80	160	160	160	160	80	160	<40	80	160	80	80	80	80	80
A/Hessen/42/2022	R33Q, E5K, F79V, D101E, I140K, S262N	2022-10-05	P1/SIAT1	40	320	320	320	160	80	80	<40	80	160	160	80	80	80	80
A/Rheinland-Pfalz/18/2022	R33Q, E5K, F79V, D101E, I140K, S262N	2022-10-11	P1/SIAT1	80	160	160	320	160	80	160	<40	80	160	80	80	80	80	80
A/Berlin/150/2022	E5K, F79V, T135A(-cho), I140K, S262N	2022-10-07	P1/SIAT1	160	160	640	640	640	320	160	<40	160	160	160	160	80	80	80
A/Hessen/45/2022	E5K, F79V, T135A(-cho), I140K, S262N	2022-10-13	P1/SIAT1	320	320	640	640	320	320	320	40	160	160	160	160	80	80	80
A/Thuringen/30/2022	E5K, F79V, T135A(-cho), I140K, S262N	2022-10-17	P1/SIAT1	80	160	320	320	320	80	80	<40	80	80	80	80	80	80	40
A/Nordrhein-Westfalen/40/2022	E5K, D77X, F79V, T135A(-cho), I140K, S262N	2022-10-13	P1/SIAT1	80	160	320	320	320	160	80	<40	80	80	80	80	80	80	40
A/Berlin/145/2022	Subgroup (ii) E5K, D53N, G62E, N96S(+cho), I140K, H156S, I192F, I223V, K264R	2022-09-15	P1/SIAT1	40	80	160	320	320	640	80	160	160	320	320	160	160	160	160
A/Bremen/29/2022	E5K, D53N, S92N, N96S(+cho), N122Q(-cho), I140K, H156S, I192F, I223V	2022-10-04	P1/SIAT1	80	320	320	640	640	320	1280	160	320	320	640	320	160	160	160
A/Bremen/28/2022	S47L(-cho), E5K, D53N, S92N, N96S(+cho), N122Q(-cho), I140K, H156S, I192F, I223V	2022-10-04	P1/SIAT1	160	320	640	640	1280	640	1280	160	320	320	1280	320	320	320	320
A/Hessen/41/2022	Subgroup (iii) D53G, D104G, H156S, K276R	2022-10-06	P1/SIAT1	40	40	80	640	640	80	160	<40	320	640	1280	320	640	640	640
A/Nordrhein-Westfalen/36/2022	E5K, D53G, D104G, H156S, K276R	2022-09-26	P2/SIAT1	40	80	80	640	640	160	320	40	320	640	2560	1280	640	640	640
A/Berlin/146/2022	E5K, D53G, D104G, H156S, K276R, R299K	2022-10-12	P1/SIAT1	80	40	160	640	640	160	320	80	640	640	2560	2560	640	640	640
A/Bremen/27/2022	D53G, D104G, H156S, K276R, S312N	2022-10-04	P1/SIAT1	160	80	320	1280	1280	160	640	40	640	640	2560	640	1280	1280	1280
A/Bremen/30/2022	D53G, D104G, H156S, K276R, S312N	2022-10-12	P1/SIAT1	80	80	160	640	640	320	320	40	640	1280	2560	640	1280	1280	1280
A/Nordrhein-Westfalen/37/2022	E5K, D53G, D104G, H156S, S262N, K276R, R299K	2022-09-29	P1/SIAT1	40	40	160	640	640	160	320	40	640	640	2560	1280	640	640	640
A/Baden-Wurttemberg/66/2022	Subgroup (iv) D53G, D104G, I140K, H156S, K276R, R299K	2022-10-10	P1/SIAT1	80	80	160	640	640	320	320	160	640	640	2560	640	1280	1280	1280
A/Thuringen/28/2022	D53G, D104G, I140K, H156S, K276R, R299K	2022-10-17	P1/SIAT1	80	80	160	640	320	160	160	<40	320	640	1280	320	640	640	640
					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)

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 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**. 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 October report, was based on sequences from viruses with collection dates after 31 December 2021 that were submitted to GISAID in the period 29 September to 31 October 2022 (Figure 4a). All viruses were **V1A.3** subclade represented by **B/Washington/02/2019**. Overall, the great majority of viruses fell 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 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). **V1A.3a.2** virus clusters defined by specific **HA1** amino acid substitutions had emerged some of which were country specific, e.g., viruses with **A202V** substitution often with **A154T** in Lao People's Democratic Republic. Sequences submitted by the Netherlands split between the **V1A.3a.2** subgroup and subclade **V1A.3** with the latter viruses having **HA1 K75E**, **E128K**, **T155A** and **G230N** substitutions, first detected in viruses in Kenya, but with an additional **HA1 G184R** substitution (Figure 4a). These **V1A.3** viruses have spread throughout the Netherlands and **B/Catalonia/NSVH534128932/2022** represents the second reported detection of such viruses outside of the Netherlands. Further diversity within the **B/Washington/02/2019 V1A.3** subclade was observed with viruses carrying **HA1** substitutions of (i) **N223K** (resulting in loss of a glycosylation site) and either **K52N** (detected in Zambia) or **T73I** (detected in Guatemala) or (ii) **K75E**, **E128K**, **T155A** and **A202V** (detected in Zambia). Sequences derived from **V1A.3a.2** viruses were still in the majority, notably so for those with collection dates in September and October reported by the Netherlands, Spain and Wales.

The phylogeny generated for this November report contains HA sequences from viruses with collection dates after 31 August 2022 that were submitted to GISAID in November 2022 (Figure 4b). The phylogeny has the same structure as that generated for the October report but all the recently detected viruses have HAs that fall in the **V1A.3a.2** subgroup. While sequences from only 119 individual viruses had become available (Table 2), viruses in this subgroup have continued to evolve and virus clusters have emerged 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 WHO Collaborating Centres for Influenza Research and Response have shown the **V.1A.3a** 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 characterisation, 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

[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

B/Austria/1359417/2021, the recommended vaccine virus for southern hemisphere 2022 and 2023, and northern hemisphere 2022-2023 influenza seasons [2, 4, 3].

Three B/Victoria-lineage test viruses have been characterised antigenically at the WIC since the October report (Table 6). 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, had low homologous titres of 160 and 80 respectively. The antiserum raised against the egg-propagated virus, which had lost a glycosylation sequon at **HA1** position **197** (**NET** → **NEA**) due to an egg-adaptation **T199A** substitution recognised neither of the **V1A.3** test viruses well. The antiserum raised against the cell culture-propagated virus did not recognise B/Netherlands/11591/2022 well which was probably related to the test virus having an additional **HA1 D129N** substitution. B/Berlin/6/2022 was recognised well by all the antisera raised against **V1A.3a.2** reference viruses.

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 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.

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 not LAIV-related.

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

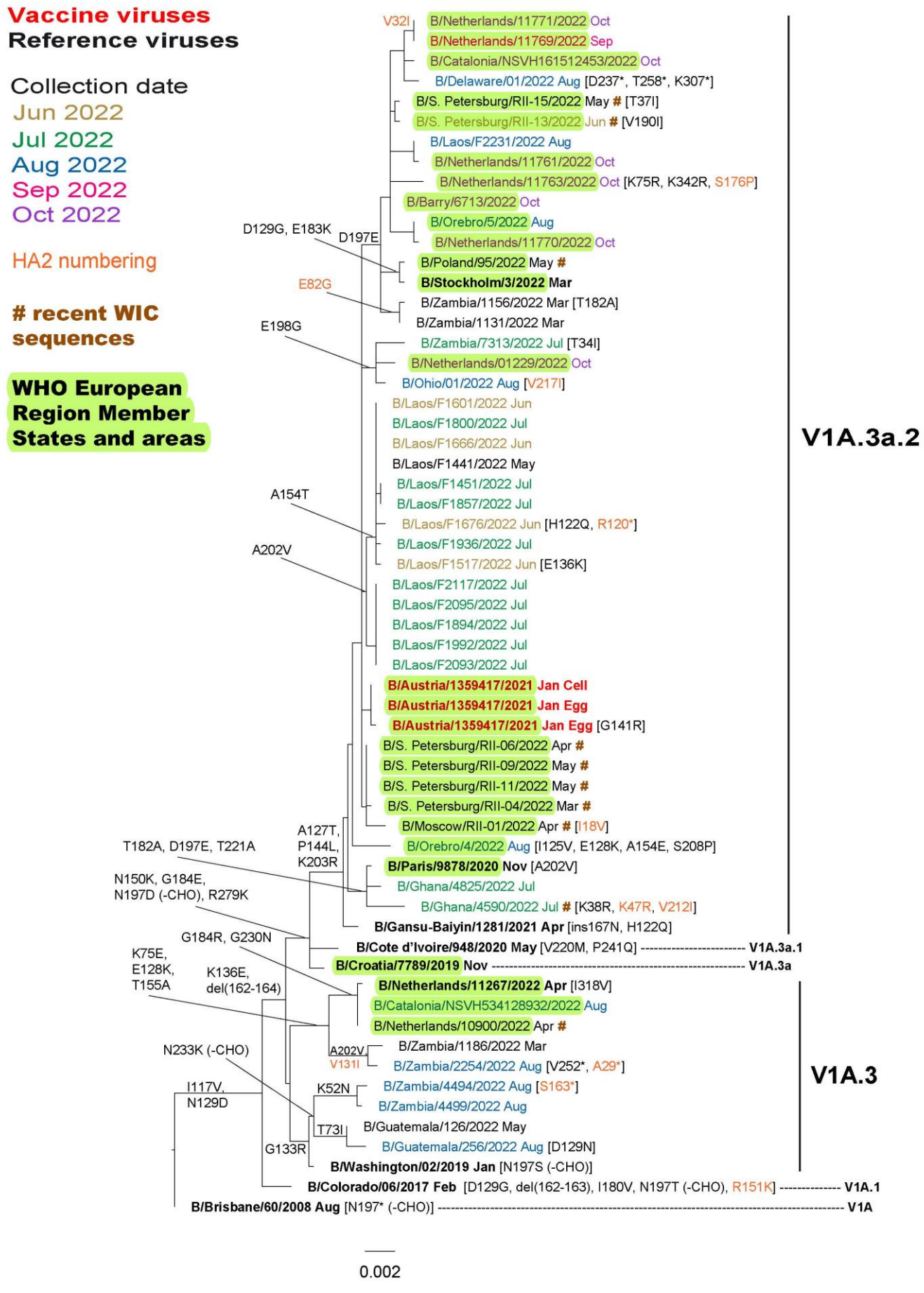


Figure 4b. Phylogenetic comparison of B/Victoria-lineage HA genes (GISAID/WIC, Nov 2022)

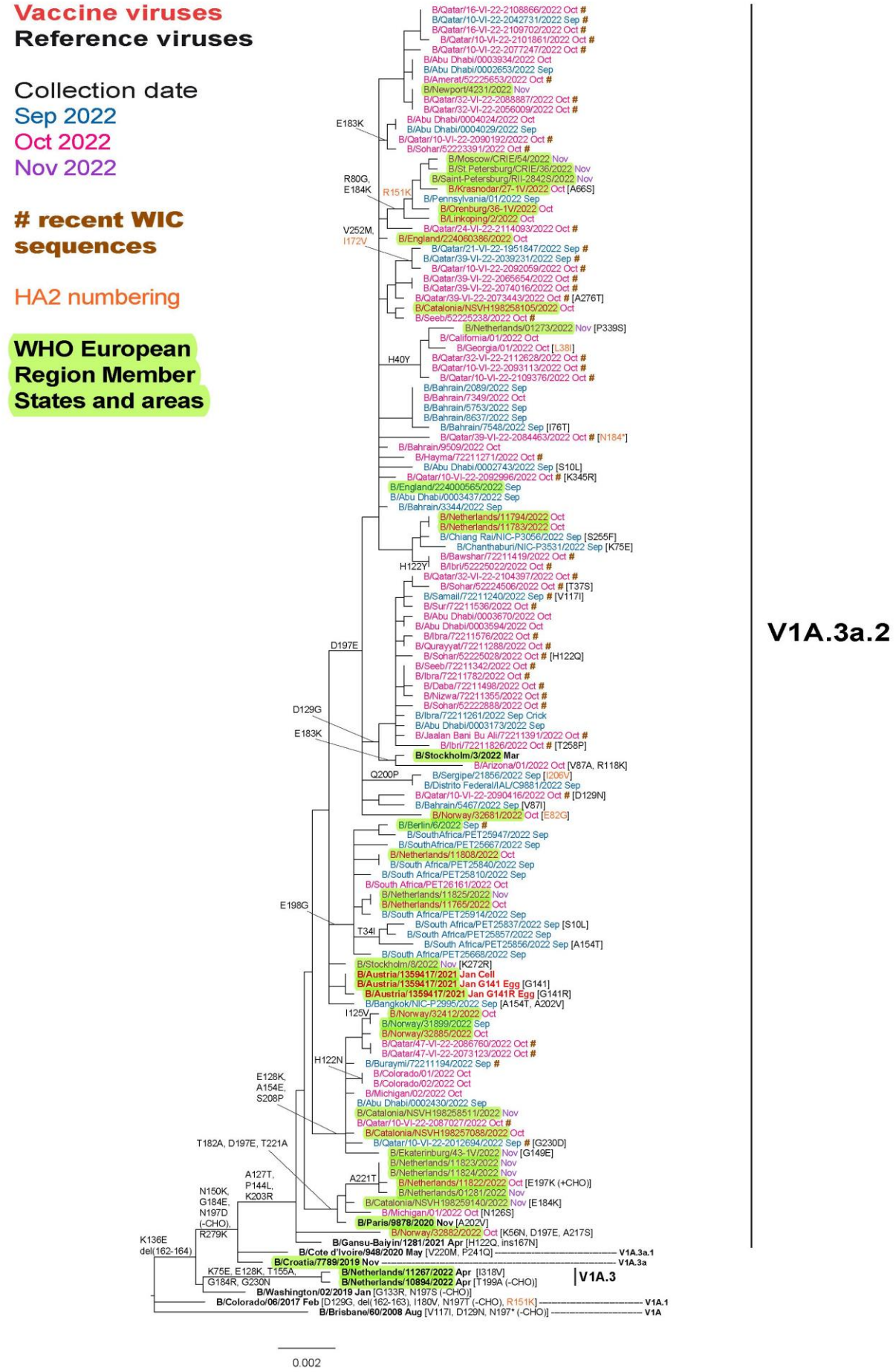


Table 6. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre									
				Post-infection ferret antiserum									
				B/Bris	B/Colorado	B/Wash'ton	B/Neth	B/Neth	B/Stock	B/Austria	B/Austria	B/Austria
				60/08	06/17	02/19	11267/22	10894/22	3/22	1359417/21	1359417/21	1359417/21
			Passage history	Egg	Egg	Egg	MDCK	Egg	MDCK	MDCK	Egg G141	Egg G141R
				Sh 539, 540, 543, 544, 570, 571, 574[1]								
			Ferret number		F44/18	F20/20	F29/22	F37/22	F28/22	NIB F01/21	F15/21	F44/21
			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	1280	320	40	<40	160	<40	<40	<40
B/Colorado/06/2017		V1A.1	2017-02-05	E5/E2	640	320	40	<40	80	<40	<40	<40
B/Washington/02/2019		V1A.3	2019-01-19	E3/E3	640	<40	40	<40	160	<40	<40	<40
B/Netherlands/11267/2022	G184R	V1A.3	2022-04-14	MDCK-MIX/MDCK2	<40	<40	<10	160	<80	<40	<40	<40
B/Netherlands/10894/2022	G184R, V351A (T199A [-cho])	V1A.3	2022-04-02	E4	160	<40	<10	40	80	<40	<40	<40
B/Stockholm/3/2022	D197E, D129G, E183K	V1A.3a.2	2022-03-22	SIAT1/MDCK3	320	<40	<10	<40	<80	1280	640	320
B/Austria/1359417/2021	A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	SIAT1/MDCK4	160	<40	<10	<40	<80	640	640	320
B/Austria/1359417/2021 Isolate 2	A127T, P144L, K302R (G141)	V1A.3a.2	2021-01-09	E3/E5	640	<40	<10	80	<80	1280	1280	640
B/Austria/1359417/2021 Isolate 2	A127T, P144L, K302R (G141R)	V1A.3a.2	2021-01-09	E3/E5	160	<40	<10	40	<80	640	640	2560
TEST VIRUSES												
B/Netherlands/11591/2022	G184R, D129N, V351I	V1A.3	2022-05-17	MDCK-MIX1/MDCK1	<40	<40	<10	<40	<80	<40	<40	<40
B/Netherlands/11678/2022	G184R, V351I	V1A.3	2022-06-30	MDCK-MIX1/MDCK2	<40	<40	<10	160	<80	<40	<40	<40
B/Berlin/6/2022		V1A.3a.2	2022-09-15	P1/MDCK1	320	<40	<10	<40	<80	640	640	320

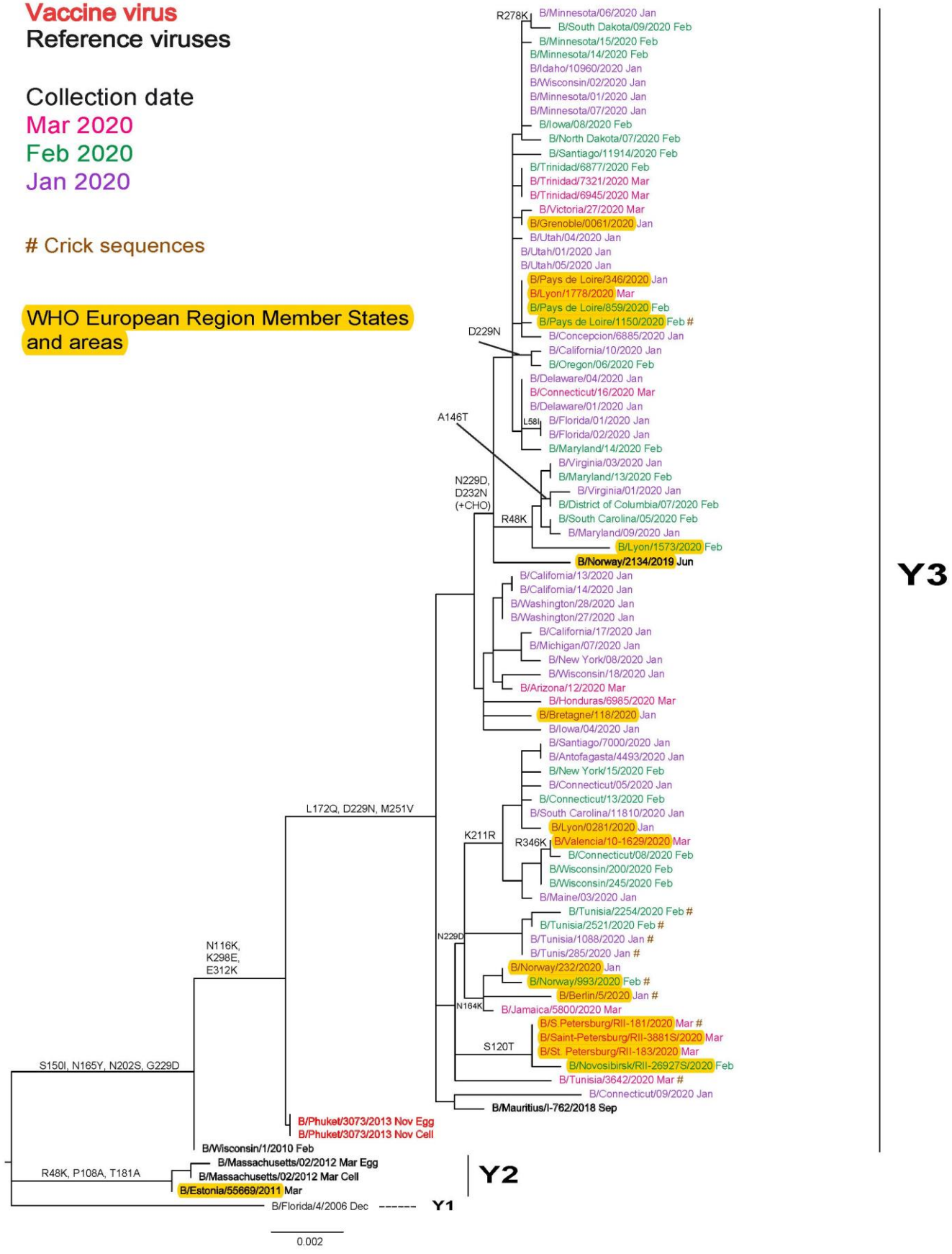
Vaccine
SH 2022
NH 2022-23
SH 2023

Vaccine
NH 2021-22

< 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 characterisation

302 viruses detected over the course of the 2022-2023 season (weeks 40-48/2022) were genetically characterised:

- Of 130 A(H1N1)pdm09 viruses, all but one belonged to clade 6B.1A.5a.2 with 80 represented by A/Norway/25089/2022, 48 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 144 A(H3N2) viruses, 138 belonged to the 'Bangladesh-like' clade (3C.2a1b.2a.2) with 64 represented by A/Slovenia/8720/2022, 63 represented by A/Bangladesh/4005/2020 and 11 represented by A/Darwin/9/2021. Six viruses were not attributed to a clade.
- Of 28 B/Victoria-lineage viruses, 16 were clade V1A.3a.2 represented by B/Austria/1359417/2021. Twelve viruses were not assigned to a clade.

Antiviral susceptibility

Up to week 48/2022, 429 viruses were assessed for susceptibility to neuraminidase (NA) inhibitors (NAIs): 137 A(H3), 129 A(H1)pdm09 and 27 B virus were assessed genotypically, and 125 A(H3), 8 A(H1)pdm09 and 3 B viruses were assessed phenotypically. Susceptibility to the PA inhibitor baloxavir marboxil (BXM) was assessed genotypically for 174 viruses: 95 A(H3), 56 A(H1)pdm09 and 23 B viruses. Phenotypically no viruses exceeded IC_{50}-fold-change thresholds for reduced susceptibility to NAIs and, genotypically, no markers associated with reduced susceptibility to NAIs or BXM were identified.

At the WIC, 12 influenza viruses detected within the WHO European Region during the 2022-2023 season have been assessed phenotypically against oseltamivir and zanamivir: 11 A(H3N2) and one 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 06 December 2022). The assessment published on 11 November 2022 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 28 September 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 11 November 2022. Since the previous risk assessment on 05 October 2022, four detections of A(H5) viruses in humans were reported to WHO [7].

Viet Nam reported a laboratory-confirmed case of A(H5) infection in a four-year-old girl who was severely ill and admitted to hospital on 07 October 2022, information was made available up to 14 October but no

indication of outcome was given. The girl had contact with backyard poultry some of which were sick and had died. Epidemiological investigation was conducted: no further cases were detected in family members, there were no reports of illness and death in other poultry flocks, and environmental samples tested negative for A(H5) viruses.

China reported a laboratory-confirmed case of A(H5N1) infection in a 38-year-old female who was hospitalized on 25 September with severe pneumonia resulting in death on 18 October. This was the first A(H5N1) zoonotic case to have been reported by China since 2015 and while the woman had been exposed to backyard poultry there were no reports of cases among family members.

Spain notified WHO of two detections of avian A(H5N1) viruses in males from Guadalajara. The first was from a 19-year-old male in a sample collected on 23 September and the second from a 27-year-old male in a sample collected on 13 October. Both cases worked at the same poultry farm where avian A(H5N1) had been confirmed in sick poultry. Both patients were asymptomatic and subsequent samples tested for influenza were negative. There was no indication of transmission to close contacts of the individuals. It is probable that the detections in these two cases were related to upper respiratory tract exposure due to working in an A(H5N1) contaminated environment, rather than true infection. The first case of human infection with an A(H5N1) virus in the western area of the WHO European Region was detected in England in January 2020 and a report into the investigation of this case has been published [10].

The latest collaborative report from ECDC and the European Food Safety Authority (EFSA), reported 788 highly pathogenic avian influenza (HPAI) A(H5) detections between 11 June and 09 September 2022, 56 in poultry, 710 in wild birds and 22 in captive birds [9]. Detections occurred in 16 European countries and high mortality was observed in colony-breeding seabird species along the northwest coast of Europe involving HPAI A(H5N1). Overall, the HPAI epidemic season in 2021-2022 is the largest so far observed in Europe with 2 467 outbreaks in poultry and 47.7 million birds culled, 187 outbreaks in captive birds, and 3 573 detections in wild birds. Genetic analyses indicated that the circulating viruses belonged to clade 2.3.4.4b. Such viruses have been circulating in Europe since October 2020 and now exist as seven genotypes, three of which were identified over the summer period. 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 23 November 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 26 October 2022 a total of 1 184 HPAI outbreaks (166 H5Nx, 1 014 H5N1, three H5N2 and one HPAI not confirmed as H5) and a single low pathogenic avian influenza (LPAI) outbreak had been reported [11].

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 05 October 2022, a single zoonotic case of A(H9N2) infection in China had been reported to WHO [7]. The case involved a three-year-old boy who developed mild illness on 20 September 2022 who recovered without need for hospitalization. Exposure history was unknown at the time of reporting, but no cases were reported among family members and environmental samples tested negative for influenza. 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 [12].

Other influenza zoonotic events

Since the previous WHO update on 05 October 2022 four cases of human infection with swine viruses were reported [7].

Brazil reported a case of A(H1N1)v infection in a 60-year-old female living in a rural area where pigs were raised who developed fever, cough and other symptoms on 05 September, requiring hospitalization on 11 September. The patient recovered and no human-to-human transmission was identified.

The Netherlands reported a case of A(H1N2)v infection in a young adult woman who worked as an administrative assistant on a pig farm and had contact with piglets from 27-29 September. She developed fever and chills on 01 October and sought healthcare but has since recovered. None of her contacts developed symptoms in a 14-day follow-up period. Samples taken from pigs that had mild respiratory symptoms were found to have A(H1N2) viruses with the same sequence as that found in the zoonotic case and there were no markers associated with increased virulence in humans detected.

The United States, Centers for Disease Control and Prevention (CDC) reported two human cases of A(H3N2)v infection, both of which were in children less than 18 years of age. The case detected in Michigan developed symptoms in week 36/2022 and that in New Mexico in week 41/2022 and both individuals had exposure to swine before illness onset. Neither person was hospitalized and the Michigan-case individual has recovered while the New Mexico-case individual was reported as recovering. No human-to-human transmission of A(H3N2)v associated with these events had been 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 08 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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