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# Report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza during 2022

Tanya R Diefenbach-Elstob, Presa Chanthavanh, Monica E Bobbitt, Sook Kwan Brown, Cleve Rynehart, Natalie Spirason, Heidi Peck, Yi-Mo Deng, Clyde Dapat, Kanta Subbarao, Ian G Barr

## Abstract

As part of its role in the World Health Organization's (WHO) Global Influenza Surveillance and Response System (GISRS), the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne received a record total of 12,073 human influenza positive samples during 2022. Viruses were analysed for their antigenic, genetic and antiviral susceptibility properties. Selected viruses were propagated in qualified cells or embryonated hen's eggs for potential use in seasonal influenza virus vaccines.

In 2022, influenza A(H3N2) viruses predominated over influenza A(H1N1)pdm09 and B viruses, accounting for 77% of all viruses analysed. The majority of A(H1N1)pdm09, A(H3N2) and influenza B viruses analysed at the Centre were found to be antigenically and genetically similar to the respective WHO recommended vaccine strains for the southern hemisphere in 2022. Of 3,372 samples tested for susceptibility to the neuraminidase inhibitors oseltamivir and zanamivir, two A(H1N1)pdm09 viruses showed highly reduced inhibition against oseltamivir.

Keywords: influenza; vaccine; GISRS; surveillance; laboratory; annual report; WHO

## Introduction

The WHO Collaborating Centre for Reference and Research on Influenza in Melbourne (the Centre) is part of the World Health Organization (WHO) Global Influenza Surveillance and Response System (GISRS). GISRS is a global network of laboratories that was established in 1952 to monitor changes in influenza viruses circulating in the human population, with the aim of reducing influenza's impact through the use of vaccines and antiviral drugs.<sup>1,2</sup> The Centre in Melbourne, designated by WHO in 1992, is one of five Collaborating Centres (with the others located in Atlanta, Beijing, London, and Tokyo). These Centres monitor antigenic and genetic changes in circulating human influenza

viruses, and participate in making the WHO bi-annual recommendations on which influenza strains should be included in the vaccine for the upcoming influenza season in the northern or southern hemisphere. This report summarises the results of virological surveillance activities undertaken at the Centre in Melbourne during 2022. Influenza activity during 2020–2021 was sharply reduced by government restrictions and non-pharmaceutical interventions that were introduced in response to the coronavirus disease 2019 (COVID-19) pandemic, with only 21,350 laboratory-confirmed influenza notifications in 2020 and 749 notifications in 2021, compared to 313,658 notifications in 2019.<sup>3,4</sup>

However, there was substantially increased influenza activity during 2022, with 233,370 notifications.<sup>4</sup>

Two types of influenza viruses (A and B) cause significant disease in humans. Influenza A viruses are further classified into subtypes, based on their haemagglutinin (HA) and neuraminidase (NA) surface proteins. Globally, there are currently two influenza A subtypes circulating in the human population: A(H1N1)pdm09 and A(H3N2). Influenza B viruses are not classified into subtypes, although in past years there have been two distinct co-circulating lineages: B/Victoria/2/1987 (B/Victoria lineage) and B/Yamagata/16/1988 (B/Yamagata lineage). However, no B/Yamagata viruses have been isolated since March 2020, and it has been postulated that during the COVID-19 pandemic this lineage may have become extinct.<sup>5</sup> Influenza C viruses are also detected each year from humans, but these viruses do not cause severe disease and are not a major focus of influenza surveillance.

## Methods

The Centre receives influenza-positive samples for surveillance purposes from submitting laboratories predominantly in Australia and other countries in the Asia-Pacific region. This report includes all surveillance samples received by the Centre in 2022; thus a small number of viruses collected in earlier years are included.

## Virus isolation

All A(H1N1)pdm09 and influenza B original clinical specimens and viral isolates received at the Centre were passaged in cell culture using Madin-Darby Canine Kidney (MDCK) cells, while all A(H3N2) original clinical specimens and viral isolates were passaged in MDCK-SIAT-1 cells.<sup>6</sup> Untyped influenza A viruses were passaged in MDCK-SIAT-1 cells. A subset of influenza positive original clinical samples were directly inoculated into eggs and a qualified cell line to generate potential candidate influenza vaccine viruses.

## Antigenic analysis

The antigenic properties of influenza viral isolates were analysed using the haemagglutination inhibition (HI) assay as previously described,<sup>7</sup> and a subset of A(H3N2) viruses were additionally analysed by the focus reduction assay (FRA). HI assays were performed manually, or using the TECAN Freedom EVO200 robot platform which incorporates a camera (SciRobotics, Kfar Saba, Israel) and imaging software (FluHema™) for analysis. In HI assays, viruses were tested for their ability to agglutinate red blood cells (RBC) in the presence of receptor-destroying enzyme (RDE)-treated post-infection ferret antisera raised against several reference viruses. Turkey RBCs were used for A(H1N1)pdm09 and B viruses, and guinea pig RBCs were used for A(H3N2) viruses. Isolates were identified as antigenically similar to the reference strain if the test samples had a titre that was no more than fourfold higher than the titre of the homologous reference strain. In 2022, results were reported with reference to the viruses that were recommended for inclusion in the 2022 southern hemisphere influenza vaccine (Table 1).

In recent years (including 2022), HI assays involving A(H3N2) viruses have been performed in the presence of 20 nM oseltamivir carboxylate (OC) to reduce non-specific binding of the NA protein.<sup>8</sup> The addition of OC can reduce the number of influenza virus isolates that could be tested by HI, as viruses may lose the ability to bind RBC. This was not the case in 2022, as none of the H3N2 viruses lost their ability to bind to RBC, and all isolates were able to be tested using the HI assay. The Centre still tested a subset of H3N2 viruses using the FRA, a microneutralisation assay which is more sensitive than the HI assay and does not require binding to RBCs.<sup>6</sup> The FRA utilised the same ferret antisera as the HI assay and was performed as previously described,<sup>7</sup> but with 1.2% Avicell RC591 (IMCD Mulgrave, Australia) replacing carboxymethyl cellulose.

**Table 1: Southern hemisphere influenza vaccine reference strains used for reporting purposes by the Centre, 2022**

Subtype/lineage	2022 cell	2022 egg
A(H1N1)pdm09	A/Victoria/2570/2019 (H1N1pdm09)-like	A/Victoria/2570/2019 (H1N1pdm09)-like
A(H3N2)	A/Darwin/6/2021 (H3N2)-like	A/Darwin/9/2021 (H3N2)-like
B/Victoria lineage	B/Austria/1359417/2019 (B/Victoria lineage)-like	B/Austria/1359417/2019 (B/Victoria lineage)-like
B/Yamagata lineage	B/Phuket/3073/2013 (B/Yamagata lineage)-like	B/Phuket/3073/2013 (B/Yamagata lineage)-like

## Genetic analysis

For influenza-positive samples that failed to grow in MDCK cells, real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to determine the influenza type/subtype/lineage using the CDC Influenza Virus Real-Time RT-PCR PCR kit.<sup>i,9,10</sup> A subset of influenza viruses underwent genetic analysis by sequencing of viral RNA genes, usually of HA and NA genes as well as, for influenza A viruses, the matrix gene. Whole genome sequencing (WGS) of a smaller subset of viruses was performed by next generation sequencing (NGS).

For sequencing, RNA was extracted from isolates or original clinical specimens using either a manual QIAGEN QIAamp Viral RNA kit or the automated QIAGEN QIAxtractor platform. For Sanger sequencing, RT-PCR with the BIOLINE MyTaq one step reverse transcription PCR kit and gene-specific primers (primer sequences available on request) were used to amplify the HA, NA, and matrix protein (MP) genes, and then sequencing was carried out on purified PCR products using an Applied Biosystems 3500XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as previously

described.<sup>11</sup> Sequence assembly was performed using Geneious Prime software version 9.0.4 (Biomatters Ltd, Auckland, New Zealand).

NGS was used for either WGS or sequencing of the HA, NA, and MP genes for influenza A, or the HA and NA genes for influenza B, as described previously.<sup>12,13</sup> NGS was conducted using an Illumina iSeq 100 according to the manufacturer's recommendations. Sequence data were analysed using an adapted pipeline IRMA.<sup>13,14</sup> Phylogenetic analysis was performed using the Augur pipeline.<sup>15</sup> In this pipeline, trees were constructed using the IQ-TREE 2,<sup>16</sup> with 1,000 bootstrap replicates and generalised-time reversible (GTR) model; trees were visualised using ggtree.<sup>17</sup> Some viruses were sequenced externally with results provided by the submitting laboratory.

HA clade nomenclature was updated for A(H1N1)pdm09 and A(H3N2) viruses during 2022. Consequently, all clades were verified against data uploaded to Nextclade, and/or against internal or external sequencing results (for viruses not uploaded to Nextclade).

## Antiviral drug susceptibility testing

Circulating viruses were tested for their susceptibility to the currently used neuraminidase inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza). Virus isolates were tested for antiviral susceptibility using a fluorescence-based neuraminidase inhibition assay (NAI) with the substrate MUNANA<sup>ii</sup>.

i The CDC Influenza Virus Real-Time RT-PCR Influenza A/B Typing Panel (RUO) (Catalog No. FluRUO-01), FR-198, was obtained through the International Reagent Resource, Influenza Division, WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention, Atlanta, GA, USA. Website: <https://www.internationalreagentresource.org/>.

ii 20-(4-methylumbelliferyl)-a-D-N-acetylneuraminic acid.

The susceptibility of test viruses to the antiviral drugs was measured in terms of the drug concentration needed to reduce the influenza neuraminidase enzymatic activity by 50% ( $IC_{50}$ ) and was compared to values obtained with reference viruses of the same subtype or lineage. NAI assays were performed as previously described,<sup>18</sup> with the incorporation of a robotic platform by TECAN EVO200 and Infinite 200 Pro for liquid handling and fluorescence measurements respectively (Tecan Australia). For reporting purposes, highly reduced susceptibility of influenza A viruses has been defined by WHO as a  $\geq 100$ -fold increase in  $IC_{50}$  compared to normally susceptible influenza A viruses in an NAI assay.<sup>19</sup> For influenza B viruses, this figure was a  $\geq 50$ -fold-increase compared to normal influenza B viruses.<sup>19</sup> However, it should be noted that the relationship between the  $IC_{50}$  value and the clinical effectiveness of a neuraminidase inhibitor is not yet well understood and a small or moderate reduction in inhibition may not be clinically significant.

Viruses found to have highly reduced susceptibility to either oseltamivir or zanamivir underwent genetic analysis using pyrosequencing, Sanger sequencing or NGS to determine the presence of amino acid substitutions in the NA protein that are associated with reduced inhibition by neuraminidase inhibitors. For example, a change from histidine to tyrosine at position 275 (H275Y) of the NA protein of A(H1N1)pdm09 viruses is known to significantly reduce inhibition by oseltamivir, as does the H273Y NA mutation in B viruses.<sup>18</sup> Pyrosequencing was also performed on original clinical specimens of selected A(H1N1)pdm09 viruses to detect the presence of the H275Y mutation if no isolate was available for phenotypic testing. Pyrosequencing was performed as previously described,<sup>20</sup> using the MyTaq One-Step RT-PCR Kit (QIAGEN, Hilden, Germany) for virus amplification, with pyrosequencing reactions performed using the PyroMark instrument (QIAGEN, Hilden, Germany).

Genetic evidence of reduced susceptibility to baloxavir was identified using pyrosequencing

(for I38 mutations of the PA gene only),<sup>21</sup> or Sanger sequencing or NGS of the PA gene, as well as from mutations identified in external sequencing results provided by submitting laboratories.

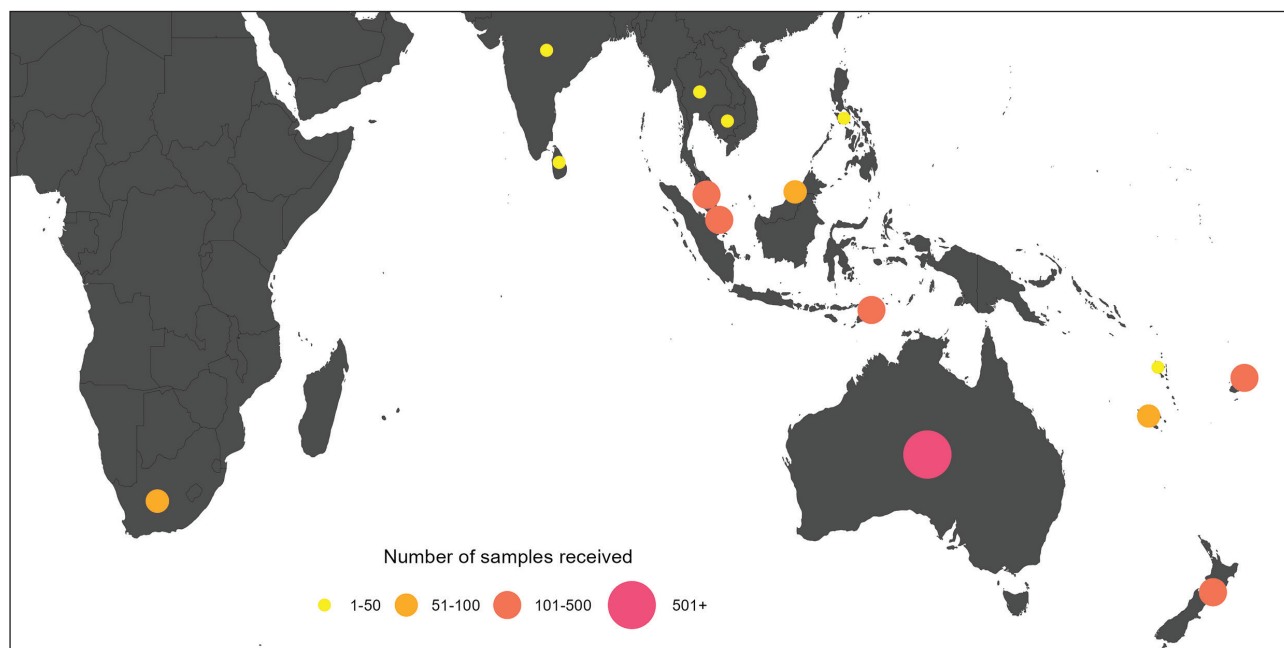
## Candidate vaccine strains

The viruses used to produce human influenza vaccines are required by regulatory authorities to be isolated and passaged in embryonated hen's eggs or qualified cell lines<sup>22–24</sup> directly from human clinical respiratory samples. The Centre has undertaken primary isolation of selected viruses from clinical samples directly into eggs, using previously-described methods.<sup>25</sup> Briefly, the viruses were inoculated into the amniotic cavity of embryonated eggs and once virus growth was established, isolates were passaged in the allantoic cavity until a sufficient titre was obtained. Egg incubation conditions differed slightly, with A(H1N1)pdm09 and A(H3N2) viruses incubated at 35 °C for three days, and influenza B viruses incubated at 33 °C for three days. In addition, selected clinical samples were inoculated into the qualified cell line MDCK 33016PF (Seqirus Limited, Holly Springs, NC, USA)<sup>26</sup> and incubated at 35 °C for three days, with viral growth monitored by haemagglutination of turkey or guinea pig RBC. These isolates were then analysed by HI assay, real time RT-PCR and genetic sequencing using the methods described above.

## Results

During 2022, the Centre received 12,073 samples (including 11,152 clinical specimens, 538 virus isolates, 379 specimen and isolate pairs, and four RNA/DNA samples) from 43 laboratories in 15 countries (Figure 1). Australian laboratories sent the highest number of samples to the Centre ( $n = 10,691$ ; 88.6%), followed by laboratories in Timor-Leste ( $n = 330$ ; 2.7%), Malaysia ( $n = 209$ ; 1.7%), and Fiji ( $n = 203$ ; 1.7%). Of the 12,073 samples received, 11,828 (98%) were collected in 2022. The majority of samples collected in 2022 were received by the Centre in May and June ( $n = 6,725$ , 57%),

Figure 1: Geographic distribution of influenza laboratories sending viruses to the Centre in 2022



with 80% ( $n = 9,409$ ) received during the April to July period, somewhat earlier than the typical influenza season for temperate regions in Australia. Figure 2 depicts the weekly temporal distribution of samples sent to the Centre in 2022 by type and subtype/lineage. During 2022, A(H3N2) was the predominant circulating strain, followed by A(H1N1)pdm09 and B/Victoria. No lineage-confirmed influenza B samples were identified as B/Yamagata viruses.

Overall, isolation and re-passaging was attempted for 8,146 (67%) of the samples received, yielding 4,499 isolates (overall isolation rate of 55%). Isolation rates were lower for clinical specimens (51%) than for virus re-isolations (94%). Of the viruses for which type and subtype could be confirmed, isolation rates by cell propagation were 71% (3,513/4,948) for influenza A(H3N2), 70% (817/1,166) for A(H1N1)pdm09 and 53% (144/273) for B/Victoria. However, 0.8% (27/3,513) of A(H3N2) and 1.8% (15/817) of A(H1N1)pdm09 virus isolates did not reach sufficient titres for antigenic analysis.

A total of 4,328 viral isolates were successfully characterised by HI assay, and compared to the 2022 reference viruses (Table 2). In addition, real-time RT-PCR was attempted on 848

samples to determine their type/subtype or lineage. Sanger sequencing and/or NGS techniques were used to attempt sequencing of the HA genes of 2,371 viruses. The full genomes of 738 viruses were obtained using NGS. Of the samples for which antigenic or genetic analysis was undertaken ( $n = 5,390$ ), influenza A(H3N2) viruses predominated, comprising 77% ( $n = 4,137$ ) of viruses received and analysed.

### A(H1N1)pdm09

Of the 774 A(H1N1)pdm09 isolates analysed by HI assay using ferret antisera in 2022, the majority (87%) were antigenically similar to the cell-propagated vaccine reference strain A/Victoria/2570/2019 (Table 2). There were 15 A(H1N1)pdm09 viruses isolated by cell culture that did not reach sufficient titres for antigenic analysis, and a further two viruses were successfully isolated but did not reach sufficient titres when tested by HI assay.

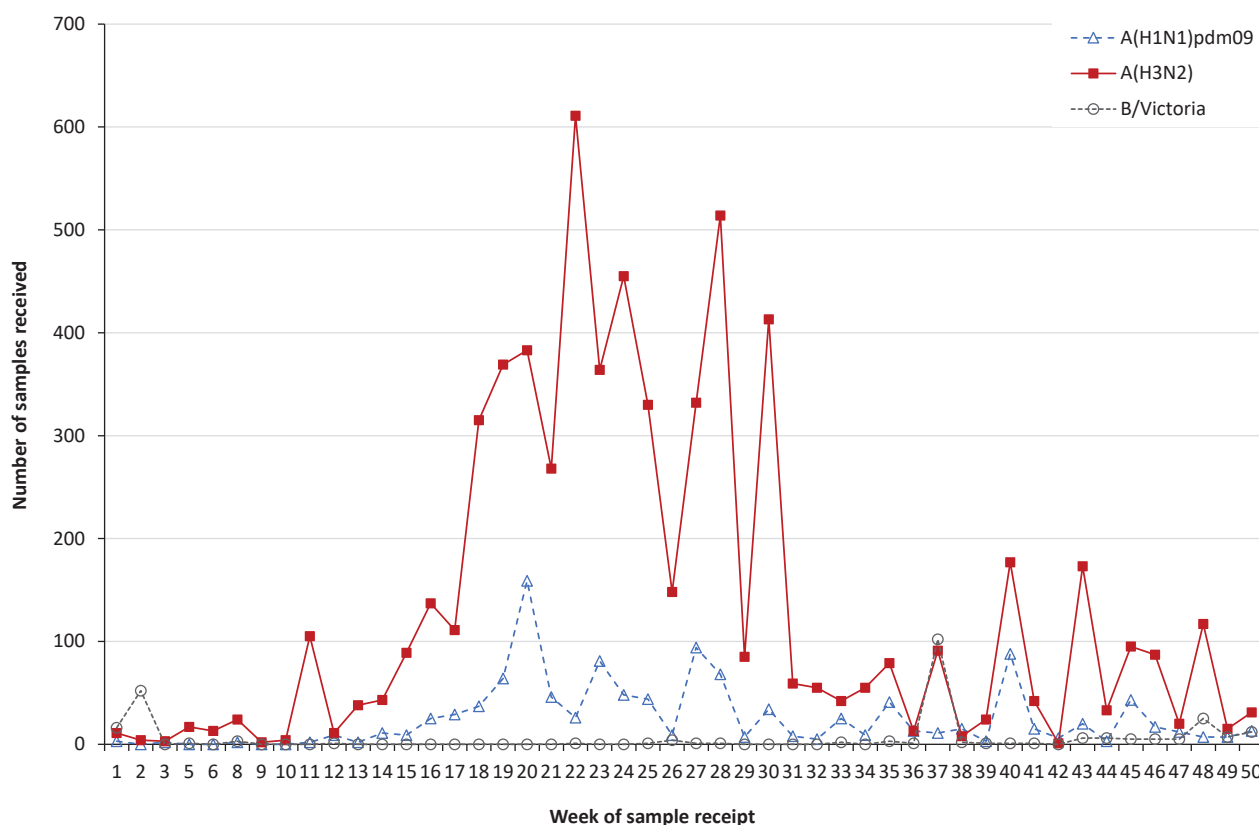
Table 2: Antigenic analysis of viruses received by the Centre in 2022, by geographic region of origin

Region	A(H1N1)pdm09 <sup>a</sup> reference strain:		A(H3N2) <sup>a</sup> reference strain:		B/Victoria reference strain:		B/Yamagata reference strain:	
	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)
Africa	23	13 (36)	7	13 (65)	22	0 (0)	—	—
Australasia	628	91 (13)	2,929	15 (1)	33	0 (0)	—	—
South Asia	17	0 (0)	14	0 (0)	7	4 (36)	—	—
South East Asia	2	0 (0)	356	29 (8)	64	0 (0)	—	—
South Pacific	—	—	61	0 (0)	—	—	—	—
<b>Total</b>	<b>670</b>	<b>104 (13)</b>	<b>3,367</b>	<b>57 (2)</b>	<b>126</b>	<b>4 (3)</b>	<b>—</b>	<b>—</b>

<sup>a</sup> Note that a small number of A(H1N1)pdm09 and A(H3N2) virus isolates that were obtained could not be analysed by HI assay due to low haemagglutination assay (HA) titre in turkey red blood cells (for A(H1N1)pdm09) or guinea pig red blood cells in the presence of oseltamivir (for A(H3N2)).



Figure 2: Number of samples received at the Centre by week of sample receipt



Sequencing was attempted on 655 viruses, with results obtained for 609 viruses. Sequencing and phylogenetic analysis of HA genes from 610 viruses (including one virus sequenced externally) showed that the A(H1N1)pdm09 viruses received during 2022 and able to be sequenced fell into the 6B.1A.5 clade, and into subclades 6B.1A.5a.1 (n = 82; 13%), 6B.1A.5a.2a (n = 498; 82%), and 6B.1A.5a.2a.1 (n=30; 5%) (Figure 3), while the 2022 southern hemisphere vaccine reference strain, A/Victoria/2570/2019, was in subclade 6B.1A.5a.2.

Twenty A(H1N1)pdm09 viruses were inoculated into eggs for isolation of candidate vaccine viruses, with 17 (85%) successfully isolated. Successful isolations included seven viruses from genetic subclade 6B.1A.5a.1, three from 6B.1A.5a.2a, and seven from 6B.1A.5a.2a.1. Twenty-two viruses were inoculated into the qualified cell line MDCK 33016PF, of which 18 (82%) grew successfully, consisting of seven viruses from genetic subclade 6B.1A.5a.1, seven from 6B.1a.5a.2a, and four from 6B.1A.5a.2a.1.

Of the 797 A(H1N1)pdm09 viruses tested, two viruses (both from Australia) exhibited highly reduced inhibition by oseltamivir and had the NA H275Y mutation (known to cause high-level inhibition). No viruses exhibited reduced susceptibility to zanamivir. Furthermore, no viruses were identified with genetic evidence of baloxavir resistance (based on sequencing of 383 viruses for I38 mutations and of 268 viruses for E23 mutations).

Figure 3: Phylogenetic tree of haemagglutinin (HA) genes of A(H1N1)pdm09 viruses received by the Centre during 2022

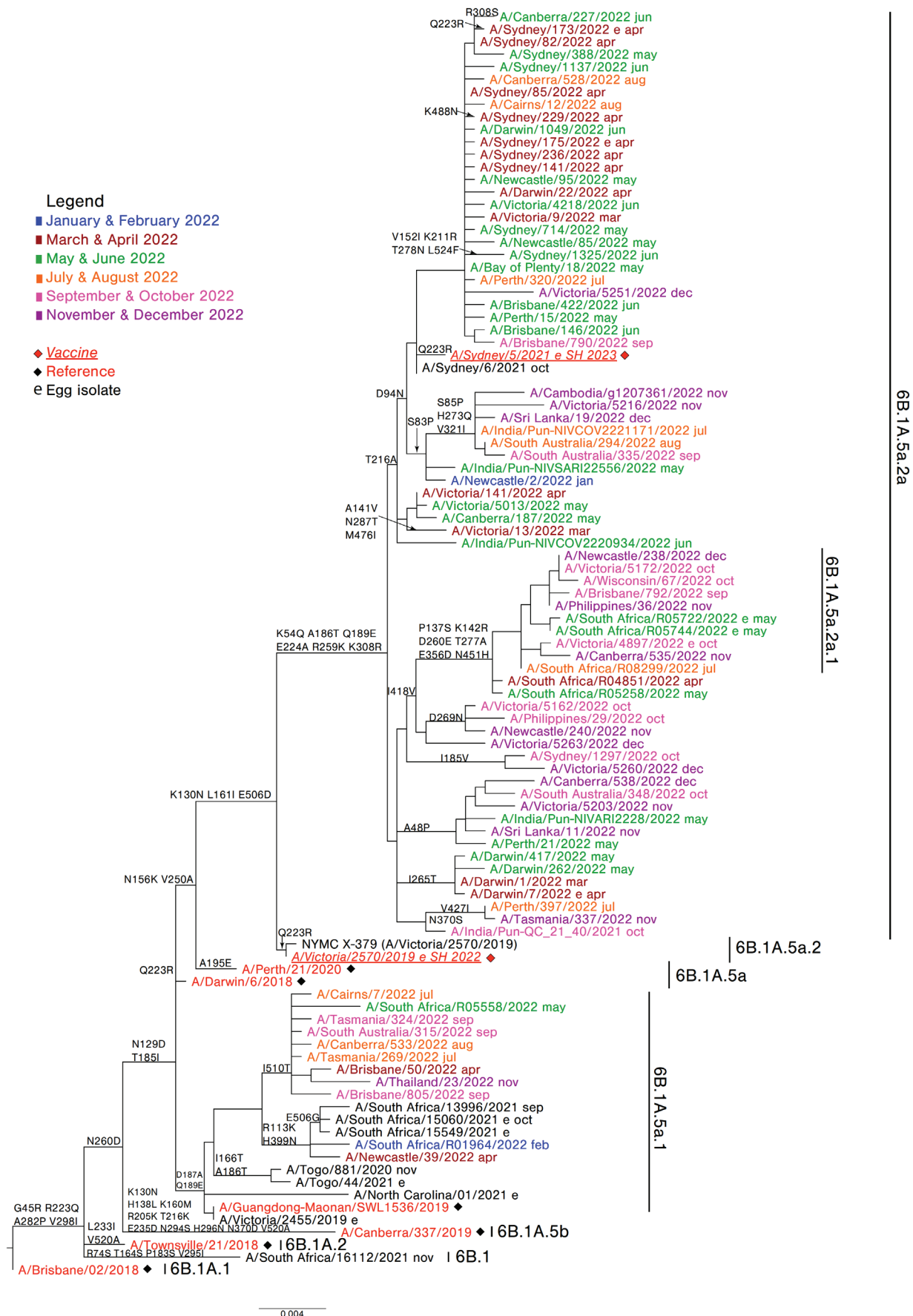


Figure 4: Phylogenetic tree of haemagglutinin (HA) genes of A(H3N2) viruses received by the Centre during 2022

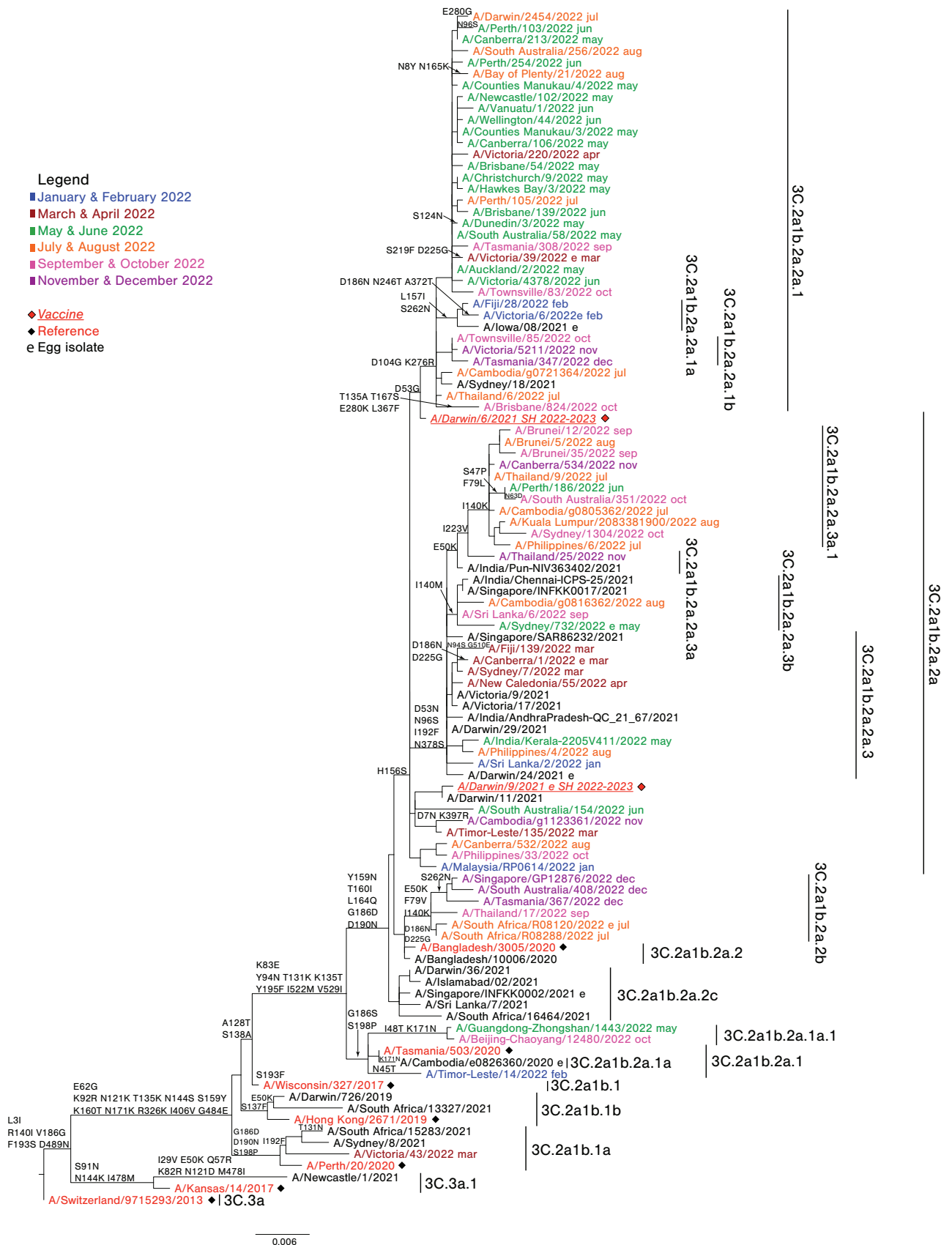
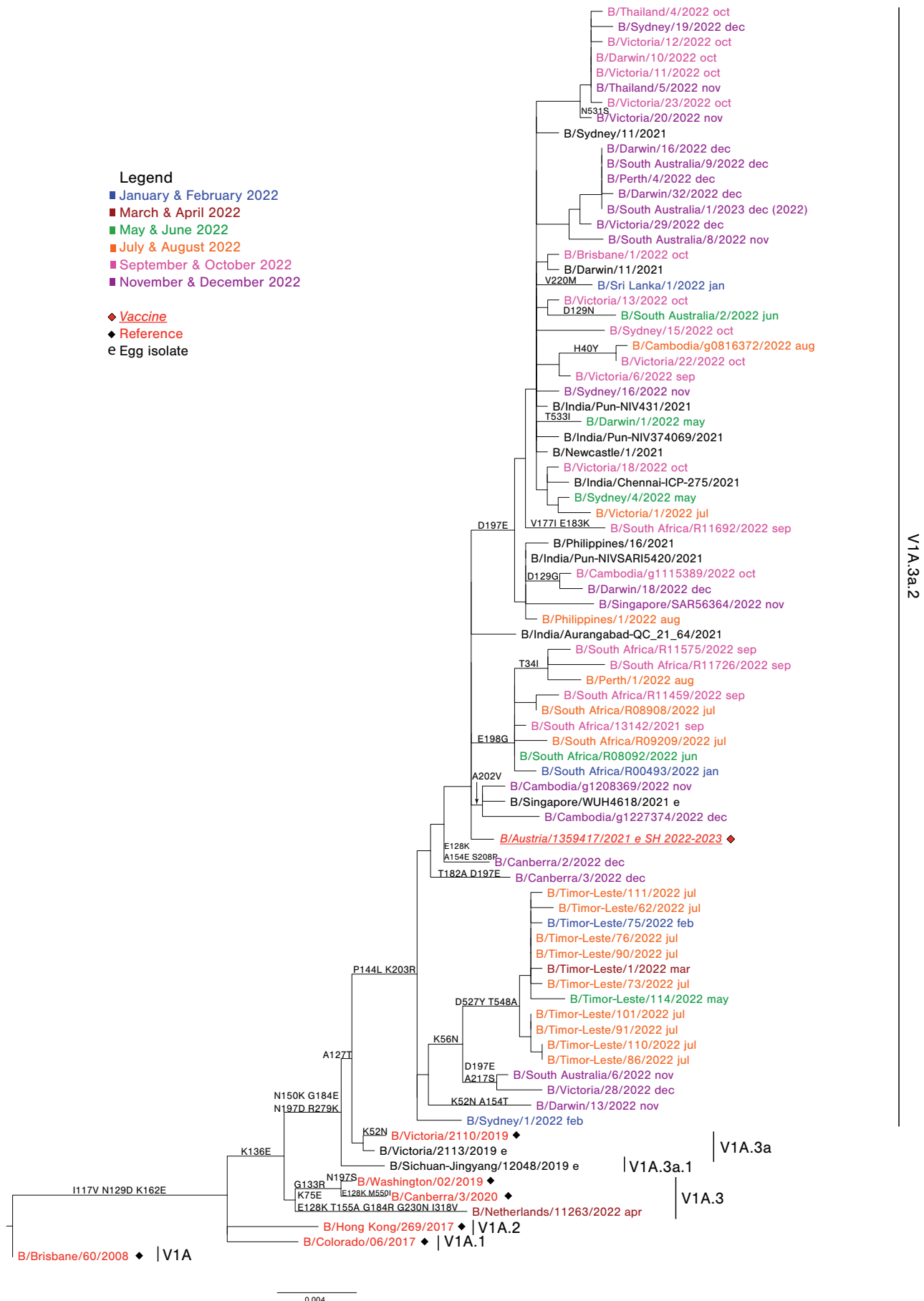


Figure 5: Phylogenetic tree of haemagglutinin (HA) genes of B/Victoria-lineage viruses received by the Centre during 2022



## A(H3N2)

Antigenic analysis of 3,424 A(H3N2) subtype isolates using the HI assay showed that 98% were antigenically similar to the cell-propagated reference strain A/Darwin/6/2021 (Table 2). There were 27 A(H3N2) viruses isolated by cell culture that did not reach sufficient titres for antigenic analysis, and a further six viruses were successfully isolated but did not reach sufficient titres when tested by HI assay in the presence of OC.

A total of 108 A(H3N2) viruses that could not be characterised by HI assay were analysed using FRA. The FRA indicated that five of these viruses (5%) showed greater than fourfold difference in titre compared to the cell-propagated reference strain A/Darwin/6/2021, while 19 (18%) of these viruses had a greater than fourfold difference in titre compared to the egg-propagated reference strain A/Darwin/9/2021.

Sequencing was attempted on the HA genes of 1,592 A(H3N2) viruses, with 1,366 yielding results (Figure 4). Phylogenetic analysis of the HA gene of 1,411 viruses sent to the Centre (including viruses sequenced externally) indicated that the majority of circulating viruses fell into subclade 3C.2a1b.2a.2a.1 (n = 1,022; 72%), while the 2022 southern hemisphere vaccine reference strain A/Darwin/6/2021 was in subclade 3C.2a1b.2a.2a. The remaining viruses fell into subclades 3C.2a1b.1a (n = 1; 0.1%), 3C.2a1b.2a.1 (n = 16; 1%), 3C.2a1b.2a.2a (n=81, 6%), 3C.2a1b.2a.2a.1a (n = 32; 2%), 3C.2a1b.2a.2a.1b (n = 14; 1%), 3C.2a1b.2a.2a.2 (n = 3; 0.2%), 3C.2a1b.2a.2a.3 (n = 96; 7%), 3C.2a1b.2a.2a.3a (n = 41; 3%), 3C.2a1b.2a.2a.3a.1 (n = 66; 5%), 3C.2a1b.2a.2a.3b (n = 10; 0.7%), 3C.2a1b.2a.2b (n = 18; 1%), and 3C.2a1b.2a.2c (n = 11; 0.8%).

Eighteen viruses were inoculated into eggs, of which 11 (61%) grew successfully, consisting of two viruses from genetic subclade 3C.2a1b.2a.2a.1, two from 3C.2a1b.2a.2a.1a, three from 3C.2a1b.2a.2a.3, one from 3C.2a1b.2a.2a.3a.1, one from 3C.2a1b.2a.2a.3b, one from 3C.2a1b.2a.2b, and one from 3C.2a1b.2a.2c. Thirty-one viruses were

inoculated into the qualified cell line MDCK 33016PF, of which 24 (77%) grew successfully, with one virus from genetic subclade 3C.2a1b.1b, one from 3C.2a1b.2a.2a, five from 3C.2a1b.2a.2a.1, two from 3C.2a1b.2a.2a.1a, seven from 3C.2a1b.2a.2a.3, three from 3C.2a1b.2a.2a.3a.1, three from 3C.2a1b.2a.2a.3b, one from 3C.2a1b.2a.2b, and one from 3C.2a1b.2a.2c.

None of the 2,432 A(H3N2) viruses tested with the NAI assay showed highly reduced susceptibility to oseltamivir or zanamivir. Likewise, no viruses were identified with genetic evidence of resistance to baloxavir (based on sequencing of 649 viruses for I38 mutations and of 453 viruses for E23 mutations).

## Influenza B

A total of 130 B/Victoria viruses were characterised by HI assay (Table 2), with 97% (n = 126) found to be antigenically similar to the cell-propagated B/Austria/1359417/2021 vaccine virus.

Sequencing was attempted on 119 B/Victoria viruses, with 96 yielding results. Phylogenetic analysis on the HA gene of 149 viruses (including viruses sequenced externally) indicated that all circulating B/Victoria viruses received by the Centre fell into clade V1A.3a.2, which included the 2022 vaccine strain B/Austria/1359417/2021.

Egg and cell inoculation were not attempted for any B/Victoria-lineage viruses.

Of the 143 B/Victoria viruses tested with the NAI assay, none showed highly reduced susceptibility to oseltamivir or zanamivir, and no viruses were identified with genetic evidence of resistance to baloxavir (based on testing of 51 viruses for I38 mutations and of 44 viruses for E23 mutations).

## Discussion

During 2022, the Centre received the highest number of samples since annual reporting commenced in 1997.<sup>27–48</sup> Substantially more samples were received than during 2020 and 2021, which were heavily impacted by the global reduction in influenza activity that occurred following the emergence of SARS-CoV-2 and the COVID-19 pandemic and the introduction of non-pharmaceutical interventions by many countries.<sup>3,47,49</sup> The high number of samples received in 2022 correlated with the large number of laboratory-confirmed influenza notifications in Australia ( $n = 233,370$ ) during the 2022 Australian influenza season.<sup>4</sup> The 2022 Australian influenza season was characterised by early activity, with a sharp increase in notifications in May, peaking in June, and subsequent low levels of activity for the remainder of the year.<sup>50</sup> Consequently, the Centre saw a dramatic increase in samples received during May and June, with 57% of all samples received during those two months alone.

Geographically, the largest number of notifications of laboratory-confirmed influenza was recorded in New South Wales, which saw 107,521 notifications during the May–July period.<sup>4</sup> Lower numbers of notifications were made in Queensland ( $n = 39,882$ ) and Victoria ( $n = 32,796$ ) for the same period.<sup>4</sup> However, notifications increased earlier in Victoria, which saw 1,729 notifications in April compared to 1,288 in July. The timing of the influenza season was similar in South Australia, where there were 10,171 notifications during the May–July period.<sup>4</sup> There were a large number of notifications in the Northern Territory during May and June ( $n = 4,447$ ), while influenza occurred later in Western Australia, with 3,798 notifications in June and 6,336 in July.<sup>4</sup> In the Australian Capital Territory, most notifications were made in May and June ( $n = 1,543$ ), while in Tasmania most influenza occurred during June and July ( $n = 2,352$ ).<sup>4</sup>

The predominant circulating virus reported to the Australian National Notifiable Diseases

Surveillance System (NNDSS) was influenza A (82.7%), with A(H3N2) predominant in samples that could be subtyped.<sup>50</sup> This is in agreement with samples from Australia received by the Centre, with A(H3N2) viruses accounting for the majority of viruses analysed. Globally, influenza A(H1N1)pdm09, A(H3N2), and influenza B viruses co-circulated in varying proportions, although influenza A was predominant.<sup>51,52</sup> During the February to August period, influenza A(H3N2) was predominant in the temperate zones of the southern hemisphere.<sup>51</sup>

In general, older populations tend to be more affected by influenza in years when A(H3N2) viruses predominate, with younger populations usually more affected by A(H1N1)pdm09.<sup>53</sup> However, in 2022, higher notification rates were observed among children aged 5–9 years (2,154 notifications per 100,000 population) and < 5 years (1,859 per 100,000 population), while the lowest notification rates were among adults aged 70–74 years (320 per 100,000 population) and those aged 65–69 years (335 per 100,000 population).<sup>50</sup> Possible reasons for this pattern include lower levels of influenza among older populations due to lockdowns, restricted visiting, and non-pharmaceutical interventions in aged care facilities associated with the COVID-19 pandemic, as well as effectiveness of the influenza vaccine in a population with high vaccine uptake. There were 308 influenza-associated deaths notified to the NNDSS, with a median age at death of 82 years (range 1–106 years).<sup>50</sup>

Antigenic analysis of influenza A(H1N1)pdm09 viruses indicated that the majority of viruses (87%) displayed similar antigenic characteristics to the cell-propagated vaccine strain, A/Victoria/2570/2019. There were two distinct genetic subclades among circulating A(H1N1)pdm09 viruses, 6B.1A.5a.1 and 6B.1A.5a.2, with ongoing diversification of viruses in the 6B.1A.5a.2 subclade. As a result, the 2023 southern hemisphere A(H1N1)pdm09 vaccine component was updated to an A/Sydney/5/2021-like virus.<sup>51</sup>

Most influenza A(H3N2) viruses received by the Centre were antigenically similar to the cell-propagated vaccine reference strain, A/Darwin/6/2021, and were part of the same genetic group (3C.2a1b.2a.2), although most viruses fell into the minor subclade 3C.2a1b.2a.2a.1. In spite of the overall genetic diversity seen amongst A(H3N2) viruses received, there was no change to the recommended vaccine strain for 2023, as the existing A/Darwin/6/2021 strain gave the best overall coverage of the different clades.<sup>51</sup>

All influenza B isolates that were analysed at the Centre belonged to the B/Victoria lineage. Most of these viruses were antigenically similar to the B/Austria/1359417/2021 vaccine strain, and all belonged to the same genetic subgroup (V1A.3a.2). There was no change to the recommended vaccine strain for B/Victoria for 2023.<sup>51</sup> Globally, no B/Yamagata viruses have been detected since March 2020, and it is hypothesised that this strain is now extinct.<sup>5,52,54,55</sup> However, B/Phuket/3073/2013 (B/Yamagata-lineage)-like virus continued to be recommended for quadrivalent vaccines in case there are remaining isolated pockets with ongoing circulation of B/Yamagata-lineage viruses.<sup>51</sup>

With the ongoing evolution of seasonal influenza viruses and the absence of an effective universal vaccine, it is essential that influenza surveillance and regular updating of seasonal influenza vaccines continues. The work performed at the Centre in Melbourne contributes to the ongoing efforts of the WHO GISRS to better control the disease burden of influenza, and to provide effective countermeasures, such as vaccines, to reduce the impact of influenza on the human population.

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