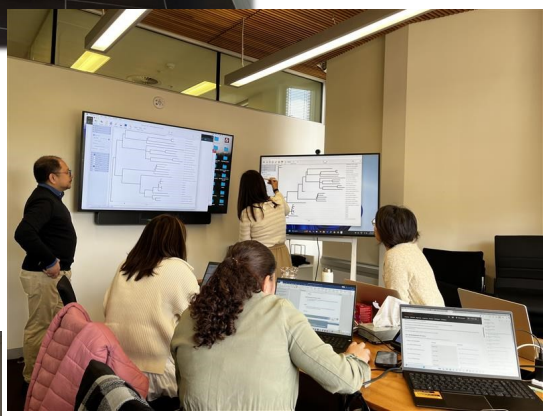


# Annual Report 2024

WHO Collaborating Centre for Reference and Research on Influenza
VIDRL

Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

# Contents

# About the Centre

The WHO Collaborating Centre for Reference and Research on Influenza at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne is part of the World Health Organisation Global Influenza Surveillance and Response System (WHO GISRS). The network was established in 1952 to monitor the frequent changes in influenza viruses with the aim of reducing the impact of influenza through the use of vaccines containing currently circulating strains. Together with WHO Collaborating Centres in Atlanta, Beijing, London and Tokyo, the Centre is responsible for analysing influenza viruses currently circulating in the human population in different countries around the world. The Centre in Melbourne was first designated as a Collaborating Centre in 1992, the third such Centre in the world.

## *Terms of Reference*

Under its designation as a WHO Collaborating Centre for Reference and Research on Influenza, the Centre's Terms of Reference (for 2024-2028) are:

1. To obtain, isolate and preserve representative viruses from outbreaks and sporadic cases of influenza, and characterise their antigenic, genetic and drug sensitivity properties as requested by the WHO.
2. To collect epidemiological information on the prevalence of influenza, especially in countries and areas in the Region, under WHO's leadership.
3. To exchange information and materials (including viruses and antisera) with other WHO Collaborating Centres for Influenza, with Essential Regulatory Laboratories and with Veterinary Laboratories to assist WHO in developing recommendations on viruses to be included in seasonal and potential pandemic influenza vaccines (according to the Pandemic Influenza Preparedness Framework requirements).
4. Under WHO's coordination, to provide training and laboratory support to WHO National Influenza Centres and other laboratories, especially those in the developing world, in specialised techniques for diagnosis, isolation and characterisation of influenza viruses, according to their needs.
5. Under WHO's leadership to undertake research to improve the detection, prevention and treatment of influenza as prioritized by the WHO Research Agenda for influenza.

## *Governance*

The Centre is supported by the Australian Government Department of Health and Aged Care through a funding agreement between the Commonwealth and Melbourne Health, and reports directly to the Department as well as to WHO.

## *Contact information*

WHO Collaborating Centre for Reference and Research on Influenza (VIDRL)
Peter Doherty Institute for Infection and Immunity
792 Elizabeth Street, Melbourne, VIC 3000, Australia
Phone: +613 9342 9300 Fax: +613 9342 9329
Email: Enquiries@influenzacentre.org
Website: http://www.influenzacentre.org

# Highlights of 2024

## Surveillance

The Centre received and processed a total of **12,390 samples** in 2024, of which **87.2% were tested**. Of viruses tested, approximately **44.1%** were **A(H3N2) viruses.**

## First human case of A(H5N1) in Australia

The Centre confirmed the first human case of **A(H5N1)** in Australia, a clade **2.3.3.1a** virus associated with a traveller returning to Australia from India.

## Hosting the WHO Consultation on the Composition of Influenza Virus Vaccines for the Southern Hemisphere 2025

The Centre hosted the **WHO Consultation on the Composition on Influenza Virus Vaccines for the Southern Hemisphere 2025** at the Peter Doherty Institute.

## Research, publications and grants

The Centre further developed its research program during 2024, with Centre staff involved as authors on **56 papers** in peer-reviewed journals. Centre staff were awarded several research grants, including **$3,034,704** from the Novo Nordisk Foundation and **$587,911** from the Cumming Global Centre for Pandemic Therapeutics.

# Director’s report

I present the 2024 Annual Report of the WHO Collaborating Centre for Reference and Research on Influenza. The Centre has continued to fulfil its commitments to the WHO, National Influenza Centres in the region, and to the Commonwealth Government, as well as participating in training and research activities.

Australia experienced its highest recorded influenza season to date, with over 365,000 laboratory-confirmed cases nationwide (source: NINDSS). The Centre also received and processed over 12,390 influenza samples from laboratories in Australia and in 23 other countries. The majority of viruses analysed at the Centre in 2024 were influenza A/H1N1pdm09 and A/H3N2 viruses, with relatively low numbers of influenza B viruses received. The Centre continued to conduct antigenic and genetic characterisation of viruses and noted an increase in genetic diversification of the A/H1, A/H3, and B/Victoria lineage HA genes in viruses received. Notably, B/Yamagata lineage viruses have not been detected globally since 2020. The Centre also continued routine testing of influenza viruses to determine their sensitivity to neuraminidase inhibitors and to the polymerase inhibitor baloxavir marboxil.

During 2024 the Centre continued to work on isolation of cell-based and egg-based viruses for vaccine production. For quadrivalent egg-based vaccines, three of the four prototype strains recommended for the 2024-2025 Northern Hemisphere and two of the four prototype strains recommended for the 2025 Southern Hemisphere influenza vaccines were derived at the Centre.

Highly pathogenic avian influenza A(H5Nx) viruses continued to circulate in 2024, with A(H5N1) clade 2.3.4.4b viruses detected in wild birds, poultry, and mammals in all continents except Australia. The first confirmed human case of A(H5N1) in Australia, a clade 2.3.3.1a virus associated with a traveller returning to Australia from India, was also detected and characterised at the Centre. Overall, the Centre continues to monitor influenza viruses with pandemic potential, seeks to obtain new viruses as they are detected, and confirms that methodologies are established to allow for their detection and characterisation at the Centre.

Centre staff participated in in-person training in several countries, including in India and the Philippines. In addition, we hosted visitors from China, Indonesia, Pakistan, Philippines, and Vietnam for training in serologic and molecular techniques. Centre staff also contributed to a total of 56 original research papers, reviews, and reports. In 2024, research at the Centre was supported by grants from a variety of sources, including the NHMRC (MRFF), the Cumming Global Centre for Pandemic Therapeutics, Novo Nordisk Foundation and NIH (USA) for work on influenza and SARS-CoV-2.

We are very grateful to Dr Chuan Lim, the Acting Director of VIDRL, and to many other members of VIDRL staff, especially Matthew Killen, Dallas Wilson, Ann Cornish, and Claudine Rensburg for their support of the Centre’s work at every level during 2024. The continuing support and counsel of the Office of Health Protection in the Australian Government Department of Health and Aged Care are deeply appreciated.

I am particularly grateful to Prof Kanta Subbarao for her leadership as Centre Director for the past seven years and for her dedication, expertise, and unwavering commitment, particularly during the COVID-19 pandemic. Finally, I would like to thank all the staff and students of the Centre for their excellent work through 2024. It is a privilege to work with the Centre staff and I look forward to working with our partners in 2025 and onwards.

*Prof Patrick Reading*
*Centre Director*

# Surveillance

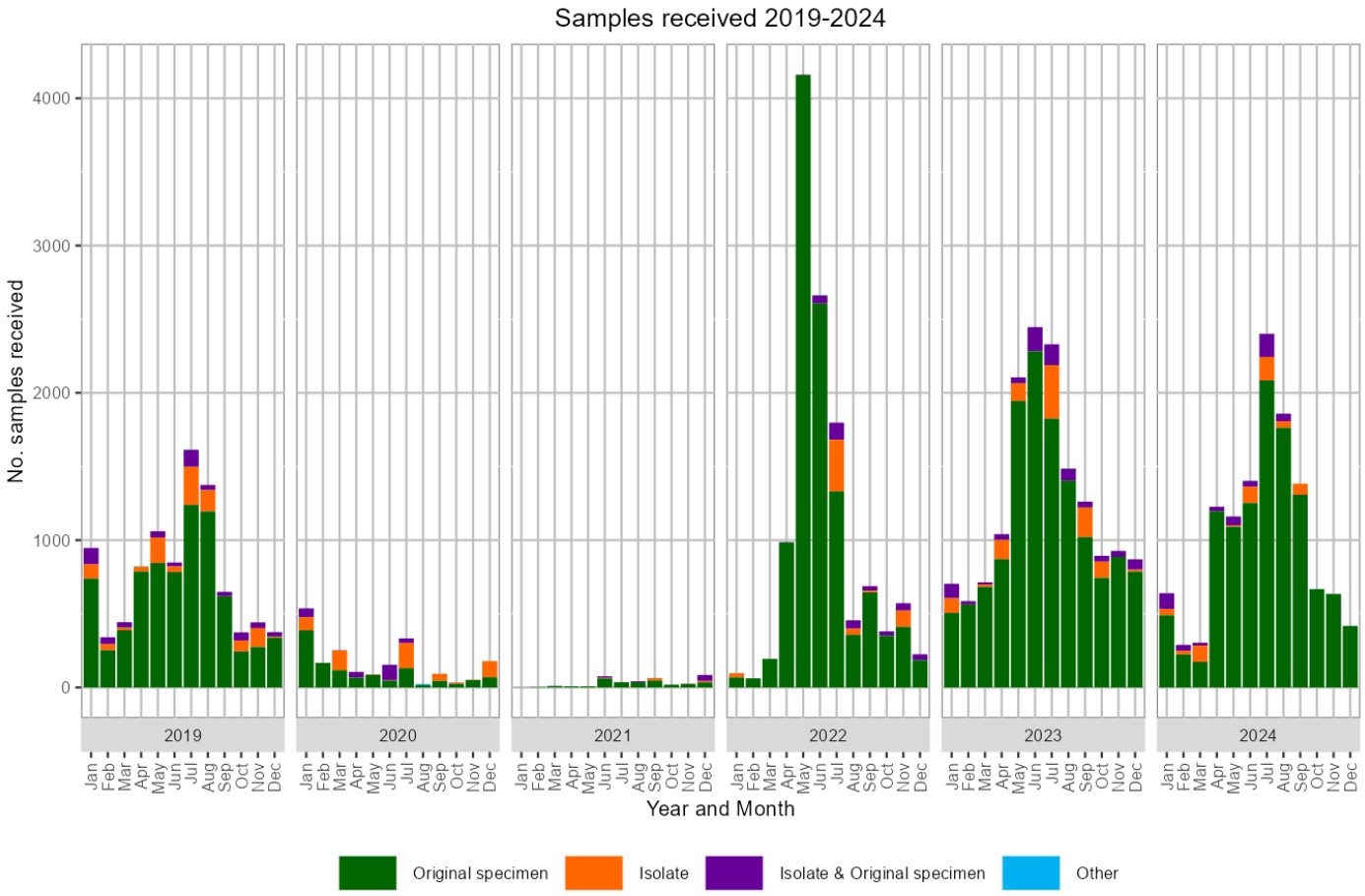
## Introduction

The WHO Collaborating Centre for Reference and Research on Influenza at the Doherty Institute in Melbourne conducts human influenza surveillance for the WHO by analysing influenza samples submitted by WHO National Influenza Centres and other laboratories. There are four other such Collaborating Centres around the world, the others being in Atlanta, Beijing, London and Tokyo. Most of the samples received at the Centre in Melbourne are provided by laboratories in the Asia-Pacific region.

Twice a year (once each for the Northern and Southern Hemispheres), based on data and advice from the five Collaborating Centres and other experts, the WHO makes recommendations on suitable influenza strains to be included in the next seasonal vaccine.

There are two types of influenza virus, type A and type B, which cause significant disease in humans. Two glycoproteins, the haemagglutinin (HA) and the neuraminidase (NA), protrude from the surface of the virus and are used to classify type A viruses into subtypes. There are 18 distinct HA subtypes and 11 NA subtypes of influenza A viruses that exist in nature, usually in wild birds. Influenza B viruses are not classified into subtypes, but rather into lineages based on the genetic and antigenic characteristics of their HA glycoproteins. The B/Victoria lineage currently circulates in humans whereas the B/Yamagata lineage has not been detected since March 2020. Currently, the main influenza viruses circulating in the human population are influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B viruses.

*Figure 1. Samples received by the Centre, 2019-2024*



## Receipt of Influenza Viruses

During 2024, the Centre received 12,390 clinical specimens and/or virus isolates from 67 laboratories in 23 countries (Figures 1 and 2, Table 1). This is similar to the number of samples received by the Centre during 2023 and is consistent with the high number of influenza infections during the 2024 influenza season in Australia. Of samples received by the Centre for which the age of the patient was known, the largest number were from subjects aged between 0-4 years (Figure 3). 3834 samples came from Australian general practitioner-based surveillance systems (Table 2).

## Isolation and analysis of viruses

Original clinical specimens received by the Centre can be genetically analysed through sequencing or real-time reverse-transcription (RT)-PCR. Additionally, they are required for virus isolation in eggs or qualified cell lines, which may serve as potential vaccine strains. For more extensive analyses, the viruses present in the clinical specimens are cultured and isolated in Madin-Darby canine kidney (MDCK) cells.

Of the 12,390 samples received, 10,807 (87.2%) were tested in cell culture and/or analysed by RT-PCR or sequencing. Of the samples tested, 33.3% were identified as A(H1N1)pdm09, 44.1% were A(H3N2) viruses, 16.5% were A unsubtyped, and 5.3% were B/Victoria (Table 2 ). Samples for which a positive cell culture result was obtained with sufficient titre were further analysed by haemagglutination inhibition (HI) assay. For reporting purposes, subtypes and lineages are based on antigenic analysis of the HA and, in some cases, are confirmed by genetic analysis of the HA gene (Table 3).

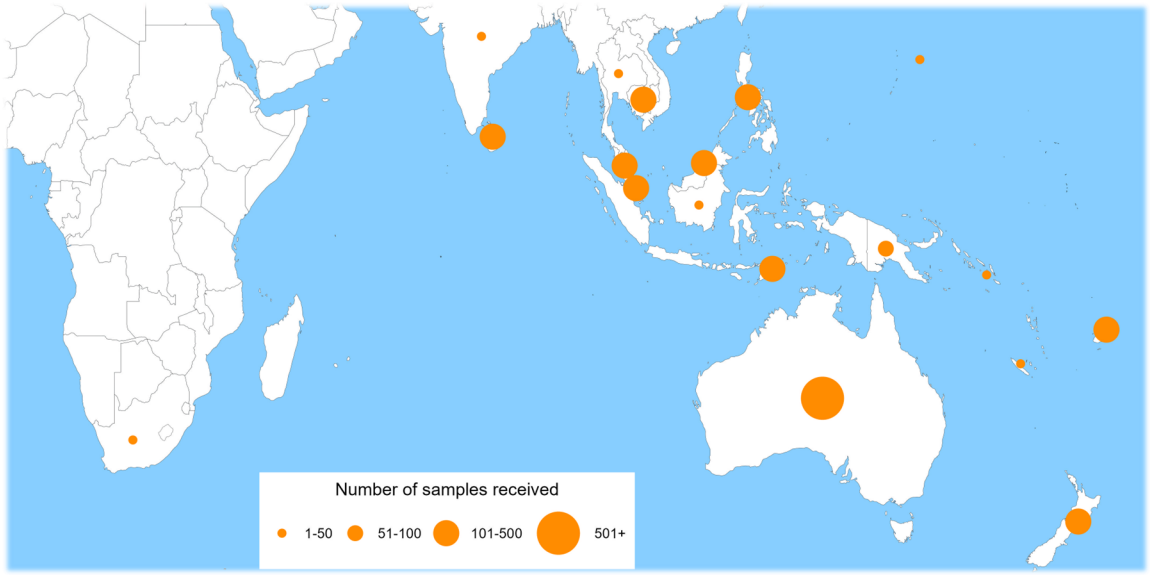



*Figure 2. Geographic spread of laboratories submitting viruses to the Centre in 2024.*

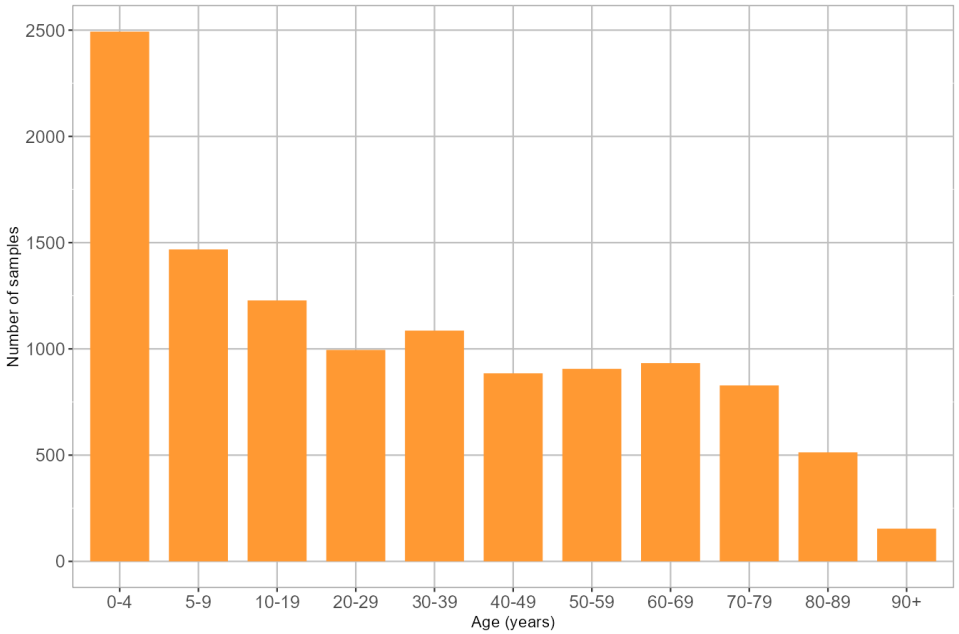



*Figure 3. Age distribution of patients from whom samples were received at the Centre in 2024 and the age is known.*

*Table 1. Samples received by the Centre in 2024, by country.*

| Country | Samples received | | | | % Samples tested |
|---|---|---|---|---|---|
| | Specimens | Isolates | Specimen + Isolate | Other (eg. RNA/DNA/ tissue) | |
| **AUSTRALASIA** | | | | | |
| Australia | 9769 | 0 | 291 | 4 | 99.80 |
| New Zealand | 0 | 202 | 0 | 0 | 100 |
| **SOUTH EAST ASIA** | | | | | |
| Brunei | 331 | 0 | 0 | 0 | 100 |
| Cambodia | 24 | 86 | 0 | 0 | 98.20 |
| Indonesia | 0 | 10 | 0 | 0 | 100 |
| Malaysia | 11 | 220 | 0 | 0 | 100 |
| Philippines | 95 | 0 | 10 | 0 | 100 |
| Singapore | 37 | 0 | 168 | 0 | 100 |
| Thailand | 10 | 10 | 0 | 0 | 100 |
| Timor-Leste | 301 | 0 | 0 | 0 | 100 |
| **SOUTH ASIA** | | | | | |
| India | 0 | 46 | 0 | 0 | 100 |
| Nepal | 15 | 0 | 0 | 0 | 100 |
| Sri Lanka | 125 | 0 | 0 | 0 | 100 |
| **SOUTH PACIFIC** | | | | | |
| Cook Islands | 14 | 0 | 0 | 0 | 100 |
| Fiji | 330 | 0 | 0 | 0 | 100 |
| Kiribati | 11 | 0 | 0 | 0 | 100 |
| New Caledonia | 33 | 0 | 0 | 0 | 100 |
| Papua New Guinea | 75 | 0 | 0 | 0 | 98.70 |
| Samoa | 35 | 0 | 0 | 0 | 100 |
| Solomon Islands | 38 | 0 | 0 | 0 | 100 |
| Tahiti | 32 | 0 | 0 | 0 | 100 |
| Tonga | 18 | 0 | 0 | 0 | 100 |
| **AFRICA** | | | | | |
| South Africa | 0 | 0 | 39 | 0 | 100 |
| **TOTAL** | **11304** | **574** | **508** | **4** | **99.80** |

*Table 2. Samples received from general practitioner-based surveillance systems, namely the Australian Sentinel Practices Research Network (ASPREN) and the hospital-based Influenza Complications Alert Network (FluCAN) in 2024*

| | No. samples received | No. isolates recovered* | Viruses analysed by HI assay* |
|---|---|---|---|
| Australian Sentinel Practices Research (ASPREN) Network | 397 | 248 | 160 |
| Influenza Complications Alert Network (FluCAN) | 3437 | 1857 | 1333 |
| **TOTAL** | **3834** | **2105** | **1493** |

** These numbers do not include samples from which isolates were recovered but did not have sufficient haemagglutination titres to be tested by HI assay.*

*Table 3. Samples successfully isolated and analysed by cell culture and/or RT-PCR assay at the Centre in 2024, by country.*

| Country | Samples tested by cell culture and/or RT-PCR assay | | | | | |
|---|---|---|---|---|---|---|
| | A (H1N1) pdm09 | A (H3N2) | A (H5N1) | A unsubtyped | B/Victoria | B lineage undetermined |
| **AUSTRALASIA** | | | | | | |
| Australia | 2996 | 3952 | 2 | 1785 | 373 | 50 |
| New Zealand | 103 | 90 | 0 | 0 | 9 | 0 |
| **SOUTH EAST ASIA** | | | | | | |
| Brunei | 52 | 83 | 0 | 0 | 25 | 5 |
| Cambodia | 35 | 32 | 0 | 0 | 32 | 0 |
| Indonesia | 3 | 4 | 0 | 0 | 2 | 0 |
| Malaysia | 89 | 128 | 0 | 0 | 7 | 2 |
| Philippines | 7 | 42 | 0 | 0 | 26 | 0 |
| Singapore | 80 | 66 | 0 | 0 | 43 | 0 |
| Thailand | 6 | 7 | 0 | 0 | 6 | 0 |
| Timor-Leste | 19 | 90 | 0 | 0 | 0 | 0 |
| **SOUTH ASIA** | | | | | | |
| India | 28 | 12 | 0 | 0 | 6 | 0 |
| Nepal | 6 | 2 | 0 | 0 | 1 | 0 |
| Sri Lanka | 34 | 53 | 0 | 1 | 29 | 1 |
| **SOUTH PACIFIC** | | | | | | |
| Cook Islands | 6 | 2 | 0 | 1 | 0 | 0 |
| Fiji | 24 | 143 | 0 | 0 | 1 | 1 |
| Kiribati | 0 | 4 | 0 | 0 | 0 | 0 |
| New Caledonia | 4 | 25 | 0 | 0 | 0 | 0 |
| Papua New Guinea | 7 | 16 | 0 | 0 | 7 | 0 |
| Samoa | 11 | 3 | 0 | 5 | 0 | 0 |
| Solomon Islands | 30 | 7 | 0 | 0 | 1 | 0 |
| Tahiti | 23 | 7 | 0 | 0 | 1 | 1 |
| Tonga | 13 | 0 | 0 | 2 | 0 | 0 |
| **AFRICA** | | | | | | |
| South Africa | 26 | 2 | 0 | 0 | 10 | 2 |
| **TOTAL** | 3602 | 4770 | 2 | 1794 | 579 | 60 |

# Antigenic Analysis of Influenza Isolates

## *Background*

The antigenic properties of influenza virus isolates are analysed using the HI assay, in which viruses are tested for their ability to agglutinate red blood cells in the presence of ferret antisera previously raised against reference viruses. A number of A (H3N2) viruses are also analysed antigenically using a microneutralisation assay known as the Focus Reduction Assay (FRA-MN). Subtypes are based on analysis of the HA and, in some cases, are confirmed by genetic analysis of the NA gene.

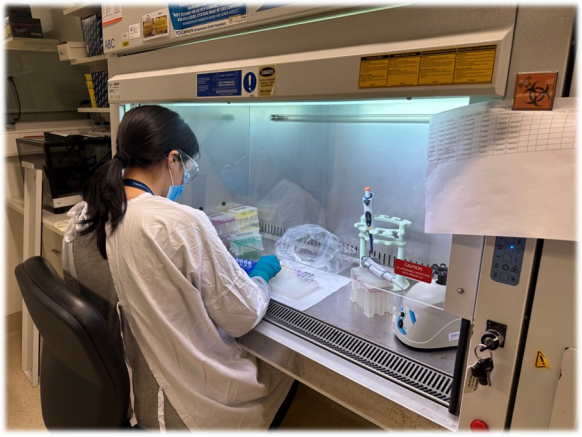


*Figure 4. Influenza subtypes and lineages of samples received in 2024 and characterised by antigenic analysis.*

## *Antigenic analyses in 2024*

A total of 6775 of the samples received at the Centre in 2024 were cultured and isolated in MDCK cells. The largest proportion of viruses were A (H3N2) viruses (50.8%), followed by A(H1N1) pdm09 viruses (42.5%), and B/Victoria viruses (6.5%) (Figure 4). The predominance of A(H3N2) was observed in South East Asia, in Australasia and in the South Pacific region. In Africa, A(H1N1) pdm09 viruses were the most prominent, followed by B/Victoria and then A(H3N2). In South Asia, A (H1N1)pdm09 viruses were the most prominent, followed by A (H3N2) and then B/Victoria.

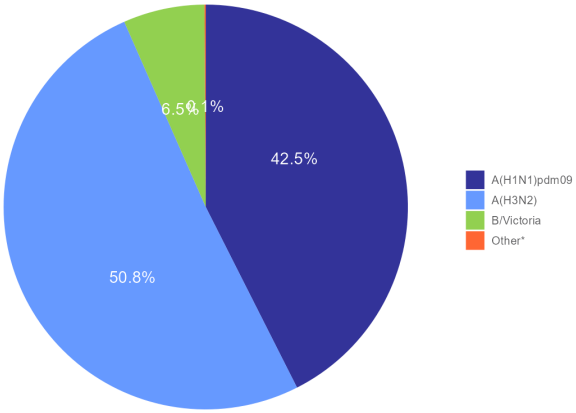
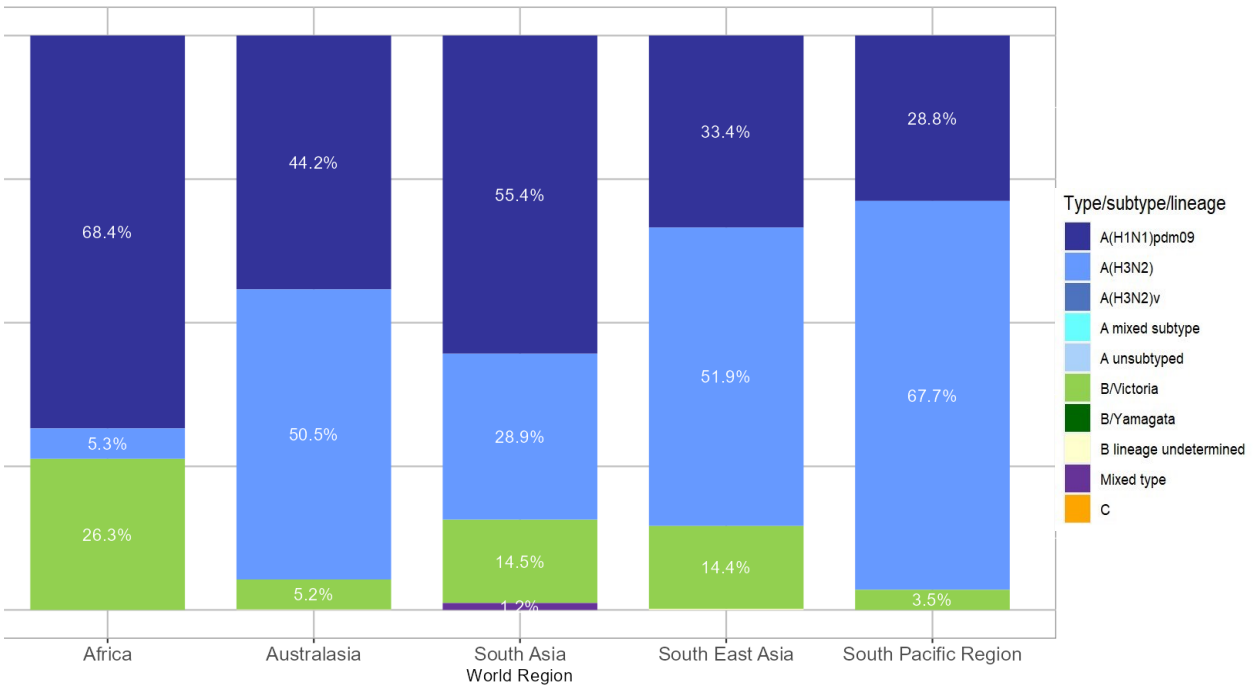



*Figure 5. Influenza subtypes and lineages of isolates received from different world regions during 2024 as determined by antigenic analysis.*



# Genetic Analysis of Influenza Viruses

## *Background*

A subset of all influenza viruses analysed at the Centre undergo genetic analysis by sequencing of viral genes. Determining the amino acid sequence of antigenic regions of the HA and NA glycoproteins provides a sensitive method to examine how these glycoproteins are evolving in circulating influenza viruses. Routine genetic sequencing of the matrix protein (MP) and non-structural protein (NS) genes is also performed. The Centre also routinely sequences the full genomes of a smaller subset of viruses.

Viruses selected to undergo sequencing include those that exhibit evidence of antigenic drift by HI assay, as well as viruses that are generally representative of samples received by the Centre based on geography and date of collection. Sequence data are used to compare viruses from different parts of the world and help to inform the selection of vaccine strains.

In addition to Sanger sequencing, next generation sequencing (NGS) techniques are now routinely employed at the Centre for efficient and cost-effective whole genome sequencing of viruses, and/or selected influenza virus genes. NGS is performed at the Centre using either Illumina or Oxford Nanopore Technologies (ONT) platforms.

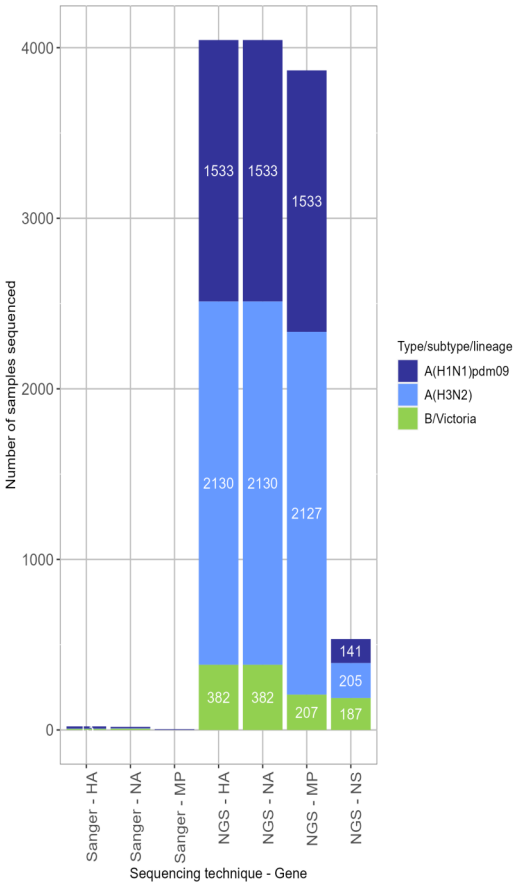



*Figure 6. Sequencing of viruses received at the Centre in 2024. Note that some viruses were analysed by both Sanger sequencing and NGS (Illumina or ONT) and are therefore represented multiple times in this figure.*

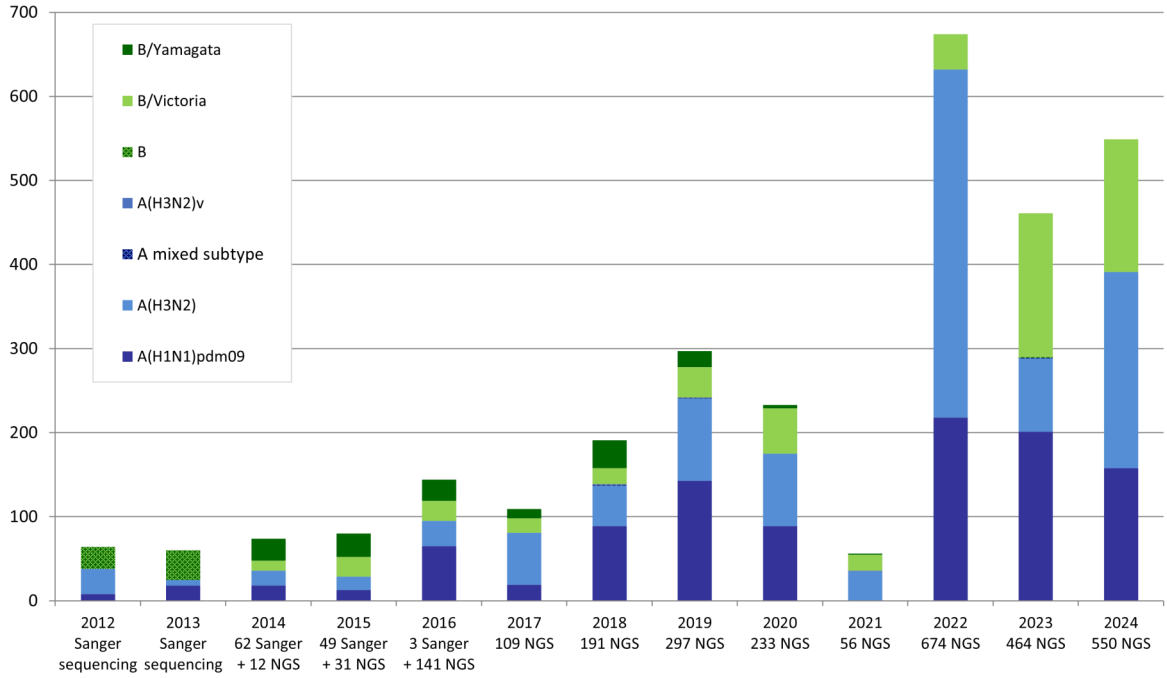
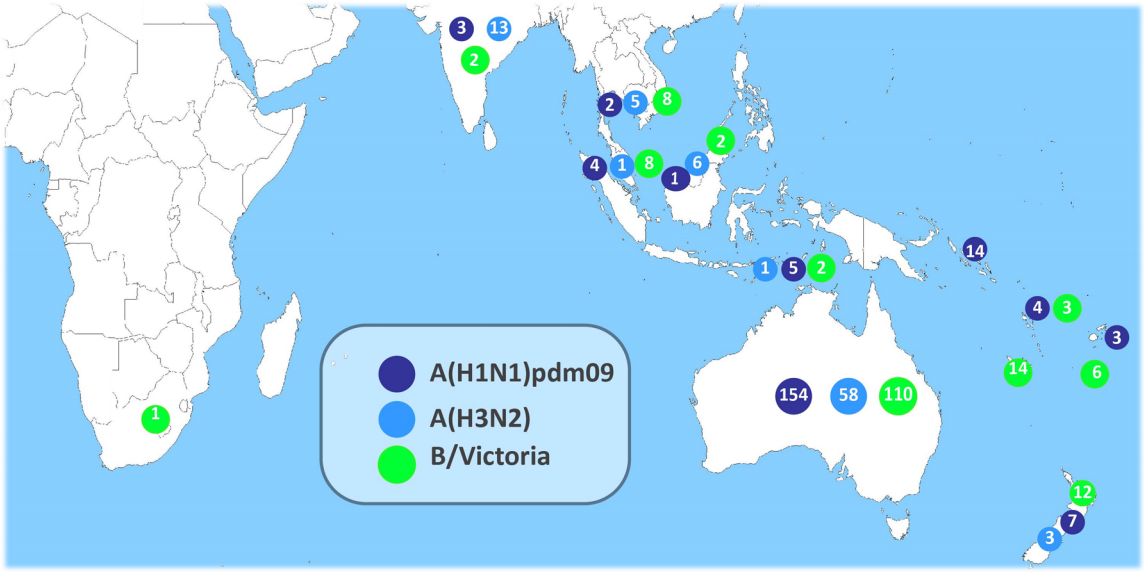



*Figure 7. Number of viruses analysed by full genome sequencing in 2024 using Sanger sequencing and/or NGS (Illumina or ONT).*

## Sequencing in 2024

In 2024, 4088 HA, 4085 NA, 3892 MP and 536 NS genes from 4088 human viruses received at the Centre were analysed by Sanger sequencing or NGS (Figure 6). Of these viruses, full genome sequencing was performed on 550 viruses using NGS techniques (Figures 7 and 8). Viruses were selected for these analyses because they were representative of the viruses received and/or because they displayed unusual properties during antigenic analysis.

*Figure 8. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing using NGS techniques at the Centre in 2024.*




## Submission of Influenza Sequences to GISAID

### Background

Virus sequences generated at the Centre are shared with the global influenza community through the EpiFlu™ database, a publicly accessible international repository of influenza virus sequences developed by the Global Initiative on Sharing All Influenza Data (GISAID) (http://www.gisaid.org).

### Sequences submitted in 2024

A total of 14,356 gene sequences from 3474 human influenza viruses were deposited with GISAID in 2024 (Table 4). The largest number of these sequences were of HA and NA genes, followed by MP and NS genes. Full genomes of 306 influenza viruses (105 A(H1N1)pdm09 viruses, 161 A(H3N2) viruses and 39 B/Victoria viruses) were also represented in the Centre's submissions (data not shown).

*Table 4. Genetic sequences submitted to GISAID by the Centre in 2024*.*

| Type/ Subtype/ Lineage \ Gene | HA | NA | MP | NS | PB1 | PB2 | PA | NP | Total |
|---|---|---|---|---|---|---|---|---|---|
| A(H1N1)pdm09 | 1349 | 1349 | 1315 | 107 | 106 | 107 | 959 | 107 | 5399 |
| A(H3N2) | 1839 | 1817 | 1793 | 171 | 173 | 175 | 1526 | 167 | 7661 |
| B/Victoria | 285 | 283 | 143 | 147 | 84 | 98 | 197 | 51 | 1288 |
| A(H5N1) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 8 |
| **Total** | **3474** | **3450** | **3252** | **426** | **364** | **381** | **2683** | **326** | **14356** |

**Counts include all sequences submitted to GISAID during 2024, which includes viruses received in previous years and viruses sequenced for reference and research purposes.*

## Surveillance Results by Influenza Subtype or Lineage

Viruses were analysed by comparison with reference viruses recommended by WHO for the 2024 Southern Hemisphere vaccines. Using the HI assay, viruses were identified as low-reactors if their titre with the reference antiserum was at least 8-fold lower than the titre of the reference virus. Results of sequencing analysis of the HA gene are also described in the following sections.

### *Influenza A(H1N1)pdm09*

#### *Antigenic analysis*

A total of 2055 A(H1N1)pdm09 isolates were analysed by HI assay in 2024. A small percentage of viruses received from Australasia (1.3%) and South East Asia (3.3%)region were antigenically dissimilar to the cell-grown vaccine A/Victoria/4897/2022 (Figure 9,10, Table 5). All viruses from Africa, East Asia, South Asia and South Pacific region displayed similar antigenic properties to the reference strain.

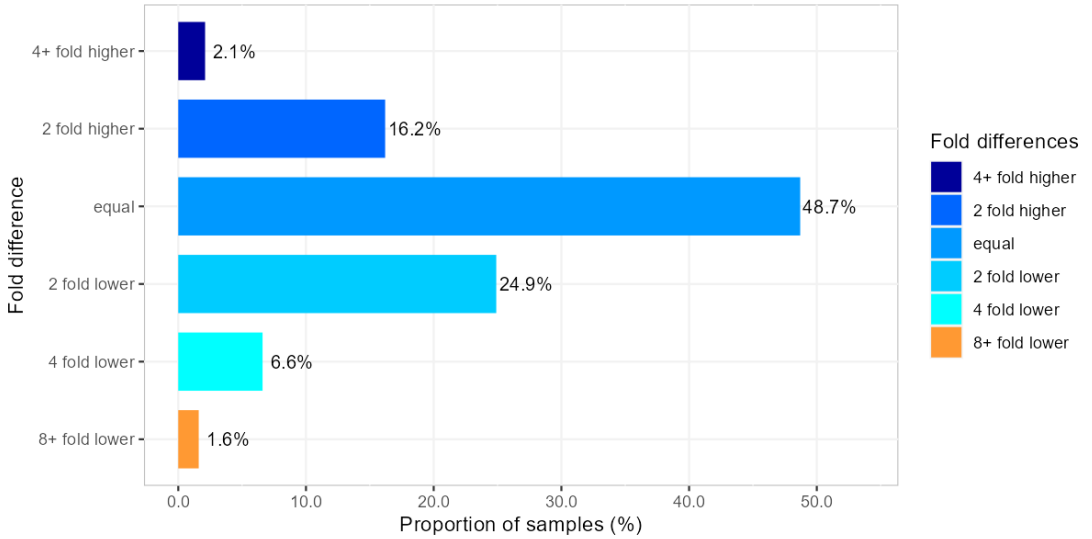
#### *Haemagglutinin gene sequencing*

Sequencing was performed on a total of 1349 HA genes. Phylogenetic analysis showed that the majority of circulating A(H1N1)pdm09 viruses sent to the Centre during 2024 were in subclade 5a2a, which contains the 2024-2025 vaccine strain A/Victoria/4897/2022 (Figure 10).

*Table 5. Antigenic characterisation of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/Victoria/4897/2022 reference virus.*

| | **A(H1N1)pdm09 reference strain:** A/VICTORIA/4897/2022-LIKE | |
|---|---|---|
| **Region** | **Like** | **Low reactor (%)** |
| Africa | 26 | 0 |
| Australasia (AUS + NZ) | 1790 | 24 (1.3%) |
| East Asia | 0 | 0 |
| South Asia | 35 | 0 |
| South East Asia | 121 | 4 (3.3 %) |
| South Pacific Region | 75 | 0 |
| **TOTAL** | 2047 | 28 (1.37 %) |

*Figure 9. Summary of fold differences in HI titres of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/ Victoria/4897/2022 reference virus.*



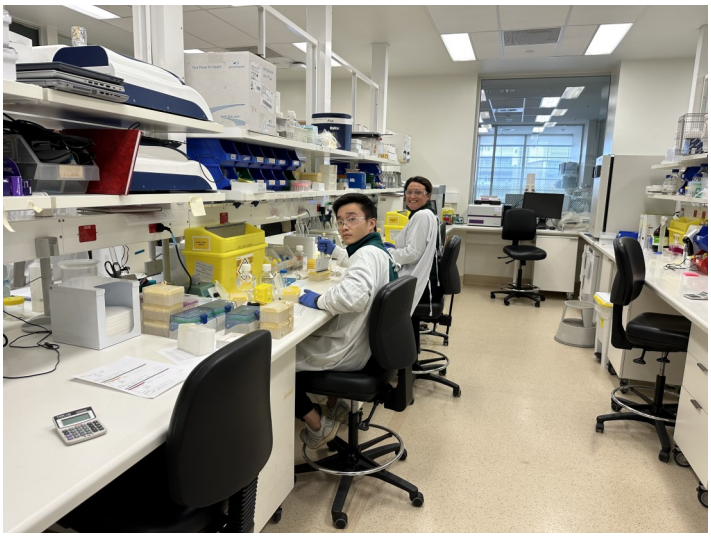
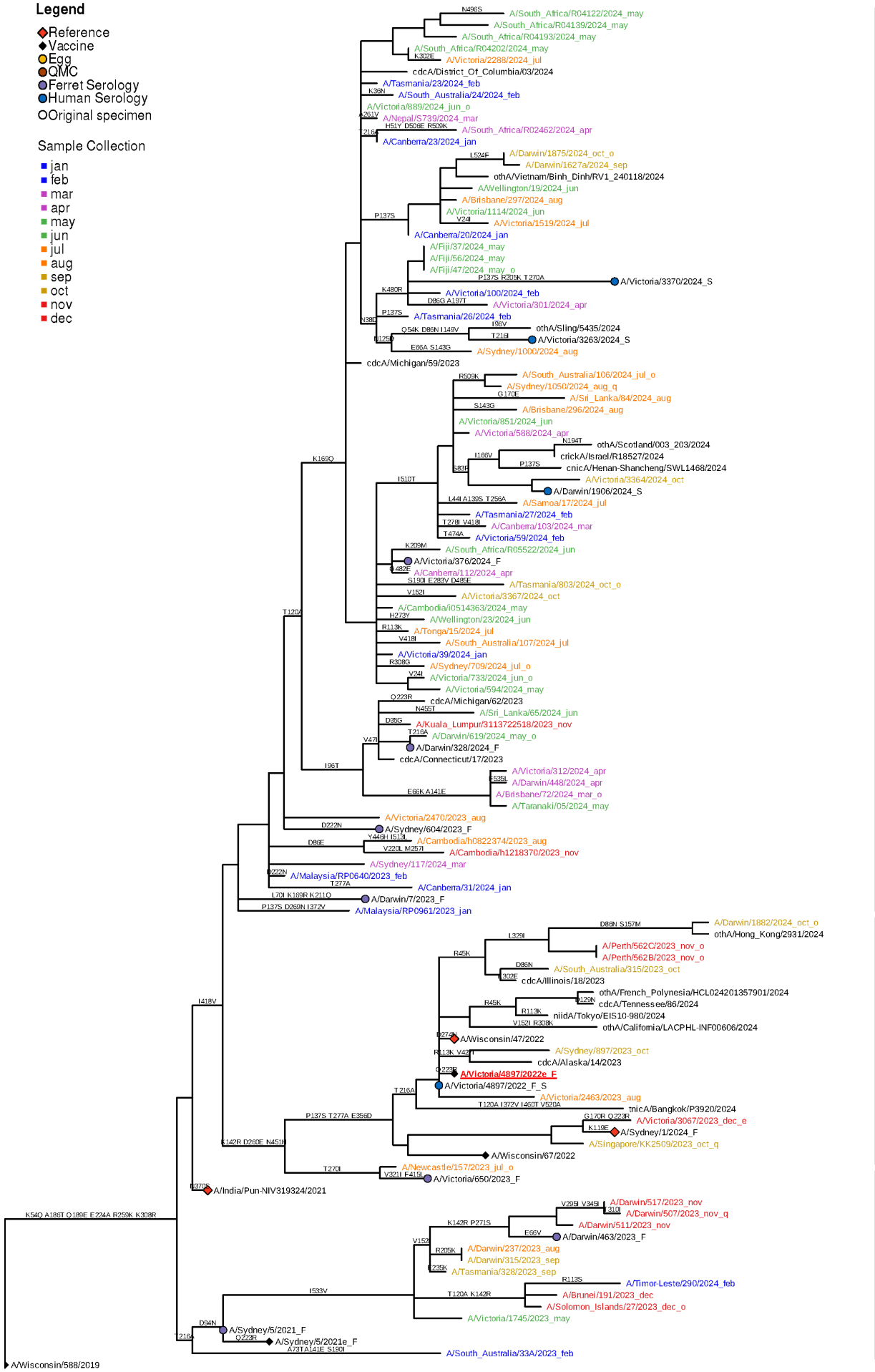



*Figure 11. Phylogenetic tree of representative HA genes of A(H1N1)pdm09 viruses received by the Centre during 2024.*



## *Influenza A(H3N2)*

### *Antigenic analysis*

In the past, evolutionary changes made A(H3N2) viruses more difficult to analyse using the conventional HI assay. To avoid binding of the viral NA protein to red blood cells, it was necessary to add oseltamivir carboxylate. This resulted in some A(H3N2) samples having insufficient haemagglutination titres to conduct the HI assay, leading to the use of additional methods (such as focus reduction microneutralisation assays (FRA-MNs)) to test the antigenic characteristics of these viruses. However, no FRA-MNs were performed for A (H3N2) viruses that were unable to be analysed by HI assay in 2024.

A total of 2673 A(H3N2) isolates were analysed by HI assay in 2024. Of these, 365 viruses were tested against the cell-grown recommended vaccine strain for the 2022 Southern Hemisphere (A/Darwin/6/2021). In these assays, 16.8%, 42.9% and 2.7% of viruses received from Australasia, South Asia and South East Asia, respectively, were antigenically dissimilar to the cell-grown vaccine. In addition, 2308 isolates were tested against the egg-grown recommended vaccine strain for the 2024 Southern Hemisphere (A/ Thailand/8/2022). In these assays, 17%, 7.7%, 3.9% and 1.8% of viruses received from Australasia, South Asia, South East Asia and the South Pacific Region, respectively, were antigenically dissimilar to the egg-grown vaccine.

### *Haemagglutinin gene sequencing*

A total of 1839 HA genes from A(H3N2) viruses were sequenced. Phylogenetic analyses indicate that most circulating viruses fell into clade 2a.3a.1(Figure 13). With this in mind, the A(H3N2) strain recommended for the 2025 Southern Hemisphere vaccine was updated to A/District of Columbia/27/2023.

*Table 6. Antigenic characterisation of A(H3N2) viruses analysed at the Centre compared to cell-grown A/ Darwin/6/2021 and egg-grown A/Thailand/8/2022 reference viruses.*

| | A(H3N2) reference strain: A/Darwin/6/2021 | | A(H3N2) reference strain: A/Thailand/8/2022 | |
|---|---|---|---|---|
| **Region** | **Like** | **Low reactor (%)** | **Like** | **Low reactor (%)** |
| Africa | 0 | 0 | 2 | 0 |
| Australasia | 137 | 23(16.8%) | 1608 | 273(17%) |
| East Asia | 0 | 0 | 0 | 0 |
| South Asia | 7 | 3(42.9%) | 13 | 1(7.7%) |
| South East Asia | 188 | 5(2.7%) | 228 | 9(3.9%) |
| South Pacific Region | 2 | 0 | 171 | 3(1.8%) |
| **TOTAL** | **334** | **31(9.3%)** | **2022** | **286(14.1%)** |

*Figure 12.A. Summary of fold differences in titres of A(H3N2) viruses analysed at the Centre by HI assay compared to the A/Darwin/6/2021.*

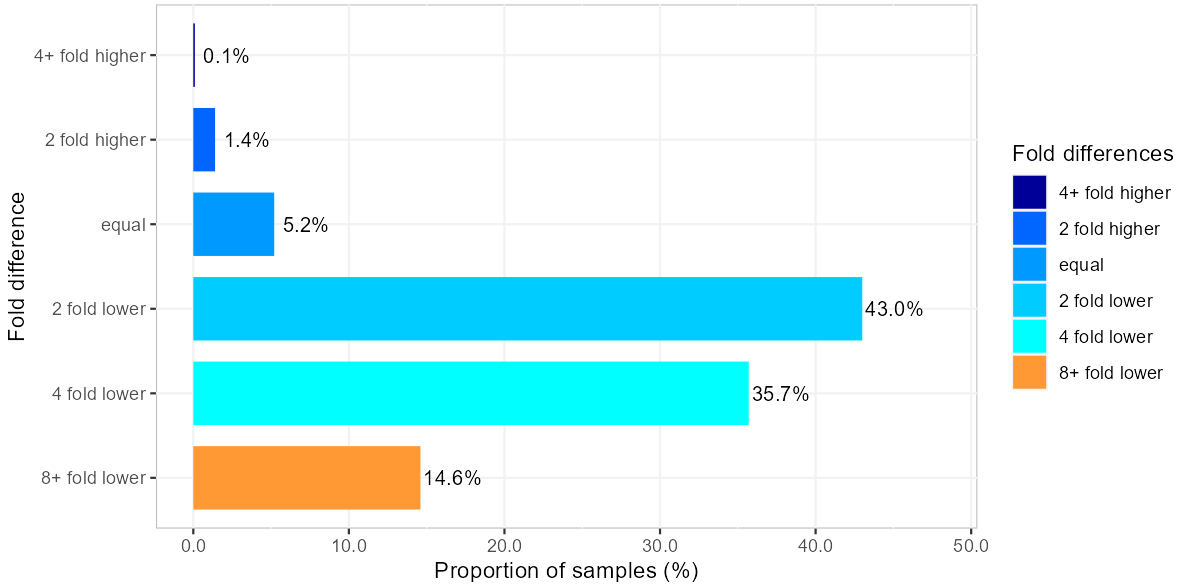



*Figure 12.B. Summary of fold differences in titres of A(H3N2) viruses analysed at the Centre by HI assay compared to the A/Thailand/8/2022 reference virus.*

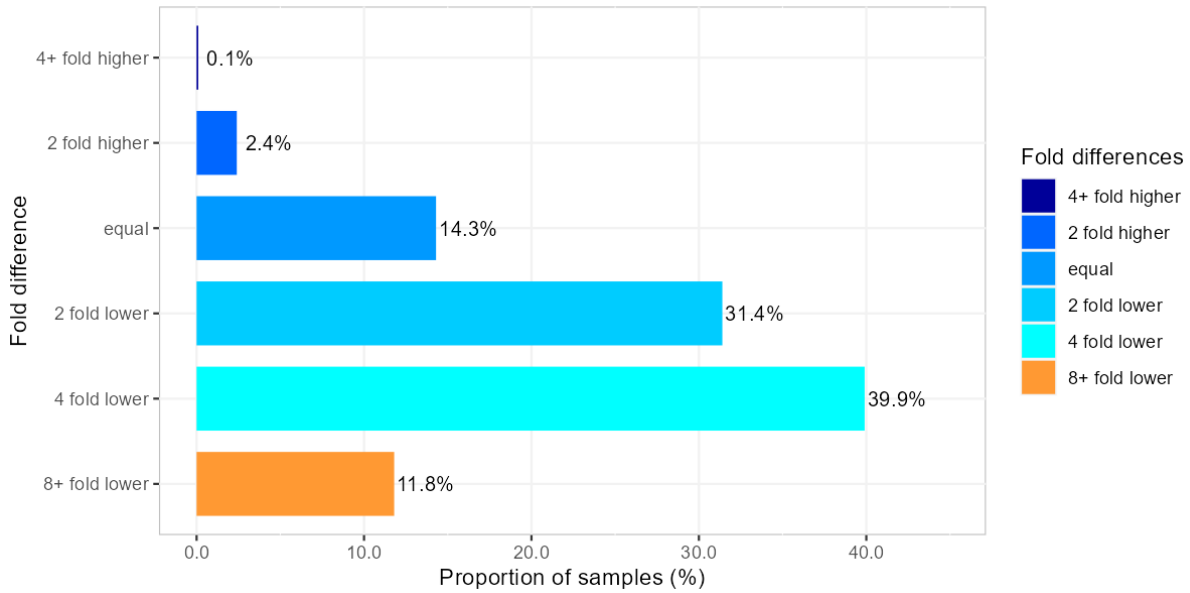
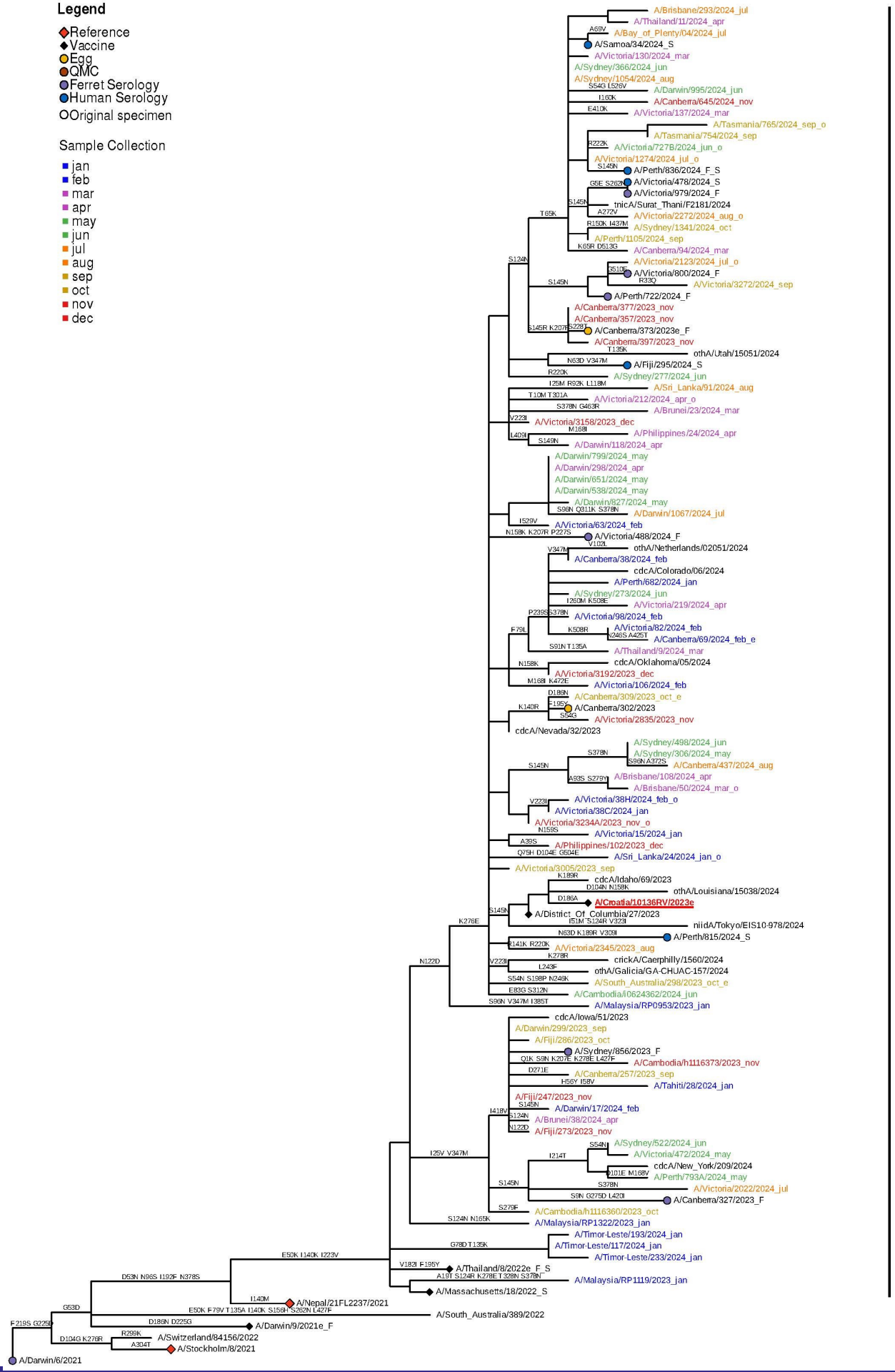


*Figure 13. Phylogenetic tree of representative HA genes of A(H3N2) viruses received by the Centre during 2024.*



## *Influenza B/Victoria*

### *Introduction*

In recent years, there were two antigenically and genetically distinct lineages of influenza B virus in circulation — the B/Victoria/2/87 lineage (represented by the 2022 vaccine strain, B/Austria/1359417/2021), and the B/Yamagata/16/88 lineage (represented by the 2022 vaccine strain B/Phuket/3073/2013). Until 2001, B/Victoria lineage viruses had been restricted to Asia where they tended to alternate in predominance with the B/Yamagata lineage. In 2002, the B/Victoria lineage became the predominant influenza B lineage in most parts of the world. This trend was reversed in 2003 and 2004 when the B/Yamagata lineage predominated. Since then both lineages have co-circulated, with alternating cycles of predominance every few years. However, no B/Yamagata lineage viruses with collection dates after March 2020 have been detected globally, and the Centre did not receive any samples containing B/Yamagata viruses in 2024.

### *Antigenic Analysis*

A total of 444 B/Victoria lineage isolates were analysed by HI assay in 2024. When these viruses were tested against the cell-grown recommended vaccine strain for the 2024 Southern Hemisphere (B/Austria/1359417/2021), no low reactors were detected (Figure 14, Table 7).

### *Haemagglutinin gene sequencing*

A total of 285 HA genes from B/Victoria-lineage viruses were sequenced. Phylogenetic analyses indicate that the majority of circulating viruses fell into clade V1A.3a.2, which contains the recommended vaccine strain for the 2024 Southern Hemisphere (B/Austria/1359417/2021) (Figure 15).

*Table 7. Antigenic characterisation of B/Victoria-lineage viruses received at the Centre during 2024 compared to the B/Austria/1359417/2021 reference virus.*

| | **B/Victoria lineage reference strain: B/Austria/1359417/2021** | |
|---|---|---|
| **Region** | **Like** | **Low reactor (%)** |
| Africa | 10 | 0 |
| Australasia | 291 | 0 |
| East Asia | 0 | 0 |
| SOUTH Asia | 12 | 0 |
| South East Asia | 122 | 0 |
| South Pacific Region | 9 | 0 |
| **TOTAL** | 444 | **0** |

*Figure 14. Summary of fold differences in HI titres of B/Victoria-lineage viruses analysed at the Centre compared to B/Austria/1359417/2021 reference virus.*

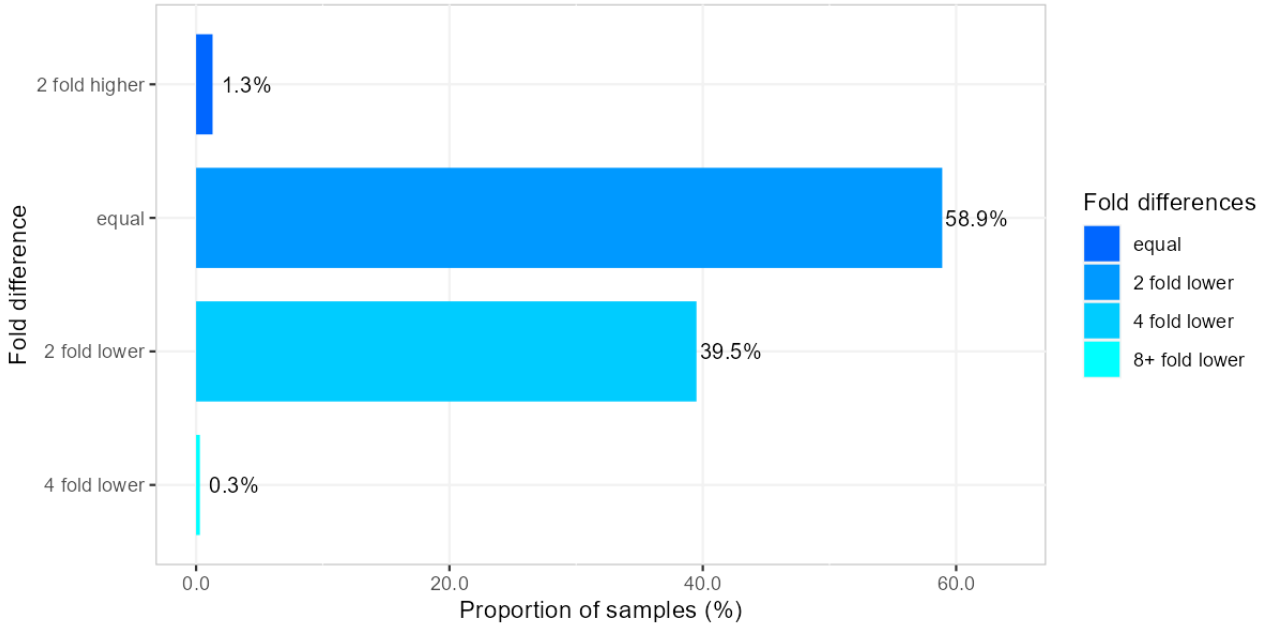
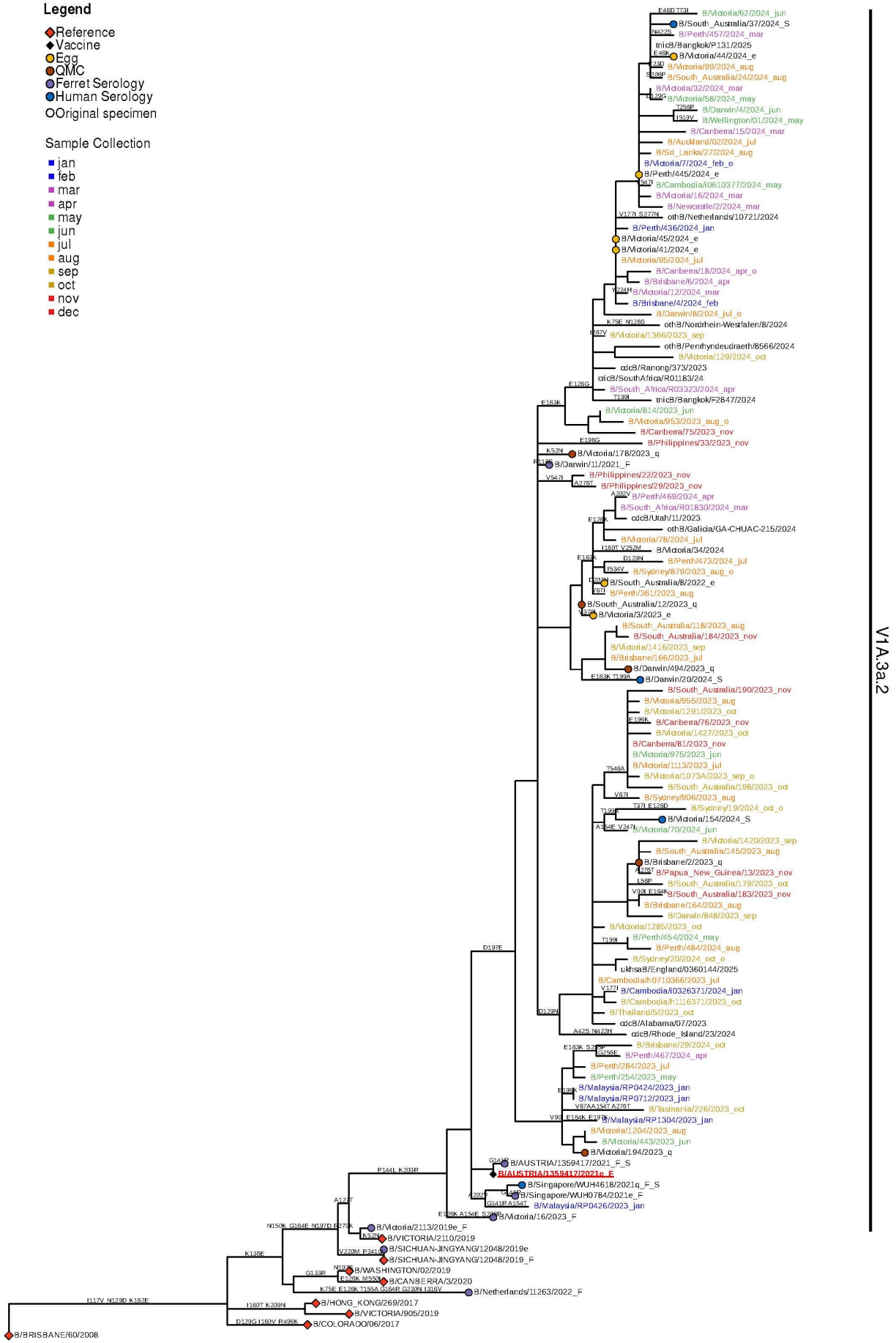



*Figure 15. Phylogenetic tree of representative HA genes of B/Victoria viruses received by the Centre during 2024.*



## Antiviral Drug Resistance Testing

### *Sensitivity to Neuraminidase Inhibitors (NAIs)*

#### *Background*

As influenza viruses continually undergo genetic change, their potential to develop resistance to antiviral drugs is an ongoing concern. To detect the emergence of drug-resistant influenza strains that could present future treatment challenges, viruses are tested for their sensitivity to the currently used neuraminidase inhibitors oseltamivir (Tamiflu), zanamivir (Relenza), laninamivir and peramivir. Laninamivir is not currently approved in Australia but is used in Japan. The Centre has routinely tested and reported the sensitivity of viruses to all four NAIs using the neuraminidase inhibition assay (NAI assay) since 2012. Viruses are routinely screened by an automated NAI assay using a Tecan EVO 200 liquid-handling robot.

The sensitivity of viruses to NAIs is measured according to the concentration of drug required to inhibit 50% of NA activity (IC50). The relationship between the IC50 value and the clinical effectiveness of a NAI against a given virus is not well understood. Further studies would be required to determine whether a virus with an elevated IC50 is clinically resistant.

*Table 8. Viruses received by the Centre in 2024 and tested by NAI assay, by country.*

| | Type/subtype/lineage | | | |
|---|---|---|---|---|
| **Country** | **A(H1N1)pdm09** | **A(H3N2)** | **B/Victoria** | **TOTAL** |
| **Africa** | | | | |
| South Africa | 26 | 2 | 10 | 38 |
| **Australasia** | | | | |
| Australia | 1133 | 1283 | 134 | 2550 |
| New Zealand | 103 | 90 | 9 | 202 |
| **South East Asia** | | | | |
| Brunei | 40 | 75 | 8 | 123 |
| Cambodia | 34 | 32 | 29 | 95 |
| Indonesia | 3 | 4 | 2 | 9 |
| Malaysia | 55 | 72 | 2 | 129 |
| Philippines | 6 | 42 | 21 | 69 |
| Singapore | 80 | 66 | 42 | 188 |
| Thailand | 6 | 7 | 6 | 19 |
| Timor-Leste | 19 | 77 | 0 | 96 |
| **South Asia** | | | | |
| India | 27 | 12 | 4 | 43 |
| Nepal | 6 | 2 | 0 | 8 |
| Sri Lanka | 12 | 10 | 5 | 27 |

*Table 8. Continued below**

| Country | A(H1N1)pdm09 | A(H3N2) | B/Victoria | TOTAL |
|---|---|---|---|---|
| **South Pacific** | | | | |
| Cook Islands | 6 | 2 | 0 | 8 |
| Fiji | 22 | 116 | 1 | 139 |
| Kiribati | 0 | 3 | 0 | 3 |
| New Caledonia | 3 | 25 | 0 | 28 |
| Papua New Guinea | 6 | 15 | 7 | 28 |
| Samoa | 11 | 1 | 0 | 12 |
| Tahiti | 14 | 5 | 1 | 20 |
| Tonga | 11 | 0 | 0 | 11 |
| **TOTAL** | **1623** | **1941** | **281** | **3845** |

*Antiviral resistance analyses in 2024*

NAI assays were used to analyse 3845 viruses for reduced inhibition by the NAIs (Tables 8 and 9). Viruses showing highly reduced inhibition to one or more NAIs underwent further analysis to determine the presence of amino acid substitutions in the NA protein associated with the reduction of inhibition by NAIs.

A total of 22 viruses (16 A(H1N1)pdm09, 5 A(H3N2)and 1 B/Victoria) had highly reduced inhibition by one or more of the NAIs. These viruses underwent further analysis to determine the presence of amino acid substitutions in the NA protein that were associated with the reduction of inhibition by NAIs (Table 10), for example histidine to tyrosine at position 275 (H275Y) of the NA protein of A(H1N1)pdm09 viruses, which reduces inhibition by oseltamivir, or the equivalent H273Y mutation in B viruses.

*Table 9. Neuraminidase inhibitor sensitivity of viruses received by the Centre in 2024.*

| Type/Subtype/ Lineage | No. tested | Oseltamivir | | Peramivir | | Laninamivir | | Zanamivir | |
|---|---|---|---|---|---|---|---|---|---|
| | | RI* | HRI* | RI* | HRI* | RI* | HRI* | RI* | HRI* |
| **A(H1N1)pdm09** | 1623 | 1(0.06%) | 16(0.99%) | 2(0.12%) | 15(0.92%) | 2(0.12%) | 0 | 3(0.18%) | 0 |
| **A(H3N2)** | 1941 | 0 | 5(0.26%) | 0 | 0 | 0 | 0 | 5(0.26%) | 0 |
| **B/Victoria** | 281 | 1(0.36%) | 0 | 1(0.36%) | 1(0.36%) | 1(0.36%) | 0 | 0 | 1(0.36%) |
| **TOTAL** | **3845** | **2(0.05%** | **21(0.55%)** | **3(0.08%)** | **16(0.42%)** | **3(0.08%)** | **0** | **8(0.21%)** | **1(0.03%)** |

** Based on IC50, the NAI sensitivity of each strain is classified as the following: Normal inhibition = IC50 values are within or close to the median IC50 of type/subtype-matched viruses tested at the Centre during the past year. Reduced inhibition (RI) = IC50 values are 10- to 100-fold above the median value of viruses with normal inhibition (5- to 50-fold for influenza B viruses). Highly reduced inhibition (HRI) = IC50 values are greater than 100-fold above the median value of viruses with normal inhibition (above 50-fold for influenza B viruses).*

*Table 10. Characteristics of viruses received by the Centre during 2024 with highly reduced inhibition by NAIs.*

| Type/Subtype/ Lineage | Country of submitting laboratory | NAI(s) with highly reduced inhibition (marked with *) | | | | |
|---|---|---|---|---|---|---|
| | | Oseltamivir | Peramivir | Laninamivir | Zanamivir | Mutations detected |
| A(H1N1)pdm09 | Singapore | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Australia | * | * | | | H275Y |
| A(H1N1)pdm09 | Thailand | * | * | | | H275Y |
| A(H1N1)pdm09 | South Africa | * | * | | | H275Y |
| A(H3N2) | Australia | * | | | | Del 245-248 |
| A(H3N2) | Australia | * | | | | Del 245-248 |
| A(H3N2) | Australia | * | | | | Del 245-248 |
| A(H3N2) | Australia | * | | | | Del 245-248 |
| A(H3N2) | Australia | * | | | | Del 245-248 |
| B/Victoria | Brunei | | * | | * | G243D |

## *Resistance to Baloxavir Marboxil*

### *Background*

Baloxavir marboxil (XofluzaTM) is an antiviral drug that has had regulatory approval for use in the treatment of influenza in Japan and the USA since 2018, and in Australia since 2020. Baloxavir acts by inhibiting the PA endonuclease of influenza A and B viruses, thereby preventing viral replication in host cells. As part of its antiviral drug resistance surveillance program, the Centre has developed a biological assay to detect and monitor circulating influenza viruses with reduced baloxavir sensitivity.

A subset of viruses received at the Centre are selected as temporally and geographically representative viruses and analysed using a phenotypic focus reduction assay (FRA-BX) to detect reduced sensitivity to baloxavir. Viral isolates showing a significant change in antiviral drug susceptibility in the FRA-BX assay are further analysed by sequencing or pyrosequencing of the PA endonuclease gene for known or novel mutations associated with reduced sensitivity to baloxavir, for example for a change in amino acid position 38 of the PA endonuclease from isoleucine to other residues such as threonine (I38T) or methionine (I38M) which is known to confer resistance to baloxavir. Selected viruses are also screened for mutations in the I38 position of the PA endonuclease, either by whole genome sequencing conducted as part of the Centre's routine genetic analysis, or by pyrosequencing. In 2024, 461 viruses which had been characterized by whole genome sequencing were screened for known antiviral substitutions in the PA gene associated with reduced susceptibility to Baloxavir Marboxil. However, no antiviral resistance substitutions for Baloxavir were detected.

### *Screening for baloxavir resistance in 2024*

Until June 2020, a subset of viruses received at the Centre that had been selected as representative viruses from different time periods and geographic locations were analysed using a focus reduction assay (FRA) to detect a reduction in sensitivity to baloxavir. However, due to a solubility issue associated with the active form (baloxavir acid) in the FRA assay, genotypic assays by sequencing and pyrosequencing of the PA endonuclease gene were primarily used during subsequent years to detect any known or novel mutations associated with reduced sensitivity to baloxavir. Analysis of 3055 viruses by pyrosequencing or sequencing did not identify any viruses with mutations in the I38 position of the PA endonuclease (Table 11).

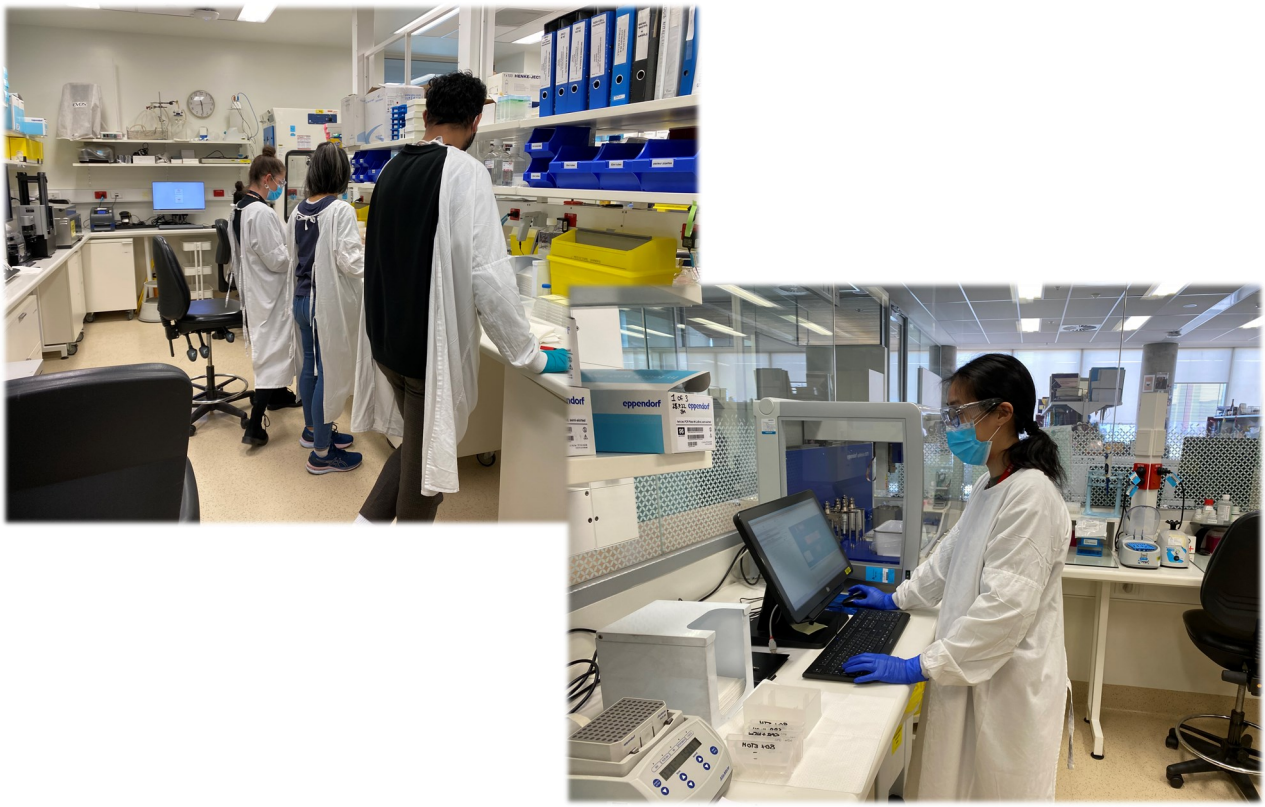


*Table 11. Viruses screened for mutations associated with reduced susceptibility to baloxavir by pyrosequencing/ sequencing during 2024.*

| | Pyrosequencing/ sequencing | | |
|---|---|---|---|
| Country / Type/subtype/ lineage | A(H1N1)pdm09 | A(H3N2) | B/Victoria |
| **Australasia** | | | |
| Australia | 930 | 1536 | 173 |
| New Zealand | 43 | 32 | 9 |
| **South East Asia** | | | |
| Brunei | 15 | 27 | 11 |
| Cambodia | 4 | 15 | 15 |
| Indonesia | 2 | 3 | 2 |
| Malaysia | 5 | 4 | 2 |
| Philippines | 1 | 10 | 0 |
| Singapore | 1 | 1 | 0 |
| Thailand | 6 | 7 | 2 |
| Timor-Leste | 5 | 22 | 0 |
| **South Asia** | | | |
| India | 1 | 2 | 2 |
| Nepal | 6 | 2 | 0 |
| Sri Lanka | 22 | 16 | 7 |
| **South Pacific** | | | |
| Fiji | 15 | 10 | 0 |
| Kiribati | 0 | 4 | 0 |
| New Caledonia | 3 | 2 | 0 |
| Papua New Guinea | 0 | 0 | 3 |
| Samoa | 9 | 3 | 0 |
| Solomon Islands | 1 | 1 | 0 |
| Tahiti | 12 | 4 | 1 |
| Tonga | 13 | 0 | 0 |
| **Africa** | | | |
| South Africa | 26 | 2 | 6 |
| **TOTAL** | **1120** | **1702** | **233** |

## *Resistance to Adamantanes*

### *Background*

The adamantane class of antiviral drugs (amantadine and rimantadine) were previously used to treat cases of influenza A, but are no longer recommended due to the almost universal adamantane resistance amongst circulating influenza A strains in recent years. All five WHO Collaborating Centres continue to screen submitted viruses for the most common resistance-conferring mutation, serine to alanine at position 31 (S31N), in the influenza A M2 protein

### *Screening for adamantane resistance in 2024*

Sequencing was used to analyse 3269 influenza A viruses, which were representative of those submitted to the Centre during 2024 (Figure 12). All of the sequenced influenza A viruses, with the exception of one isolate, carried the MP-S31N mutation which confers amantadine resistance.

*Figure 12. Viruses received by the Centre during 2024 and screened for adamantane resistance.*

| | Type/subtype/lineage | | | |
|---|---|---|---|---|
| **Country** | **A(H1N1)pdm09 Tested** | **A(H1N1)pdm09 Resistant** | **A(H3N2) Tested** | **A(H3N2) Resistant** |
| **Australasia** | | | | |
| Australia | 1139 | 1139 | 1623 | 1623 |
| New Zealand | 43 | 43 | 32 | 32 |
| **South East Asia** | | | | |
| Brunei | 18 | 17* | 36 | 36 |
| Cambodia | 39 | 39 | 28 | 28 |
| Indonesia | 2 | 2 | 3 | 3 |
| Malaysia | 15 | 15 | 11 | 11 |
| Philippines | 5 | 5 | 29 | 29 |
| Singapore | 3 | 3 | 1 | 1 |
| Thailand | 6 | 6 | 5 | 5 |
| Timor-Leste | 13 | 13 | 35 | 35 |
| **South Asia** | | | | |
| India | 1 | 1 | 2 | 2 |
| Nepal | 6 | 6 | 2 | 2 |
| Sri Lanka | 24 | 24 | 30 | 30 |
| **South Pacific** | | | | |
| Fiji | 15 | 15 | 32 | 32 |
| Kiribati | 0 | 0 | 5 | 5 |
| New Caledonia | 3 | 3 | 0 | 0 |
| Samoa | 9 | 9 | 2 | 2 |
| Solomon Islands | 1 | 1 | 0 | 0 |
| Tahiti | 12 | 12 | 0 | 0 |
| **Africa** | | | | |
| South Africa | 26 | 26 | 0 | 0 |
| **TOTAL** | **1393** | **1392** | **1876** | **1876** |

## Candidate Vaccine Strains

*Background*

The Centre collaborates closely with the other WHO Collaborating Centres and vaccine manufacturers to ensure the suitability of candidate strains for inclusion in seasonal vaccines. Selected original clinical specimens containing potential vaccine strains are used to isolate viruses in eggs and in Qualified MDCK cells. (QMCs) in laboratories designed for this purpose under conditions consistent with current internationally accepted regulatory requirements for influenza vaccine viruses. These isolates are then analysed by HI assay and genetic sequencing.

*Isolation of viruses in eggs in 2024*

In 2024, a total of 38 viruses were successfully isolated in eggs at the Centre, representing an overall isolation rate of 62.0% (Tables 12 and 14).

*Table 12. Virus isolation in eggs at the Centre in 2024.*

| Type/subtype | Isolates attempted | Isolates obtained | Success rate (%) |
|---|---|---|---|
| A(H1N1)pdm09 | 30 | 16 | 53% |
| A(H3N2) | 28 | 18 | 64% |
| B/Victoria | 4 | 4 | 100% |
| **Total** | **62** | **38** | **61%** |

*Isolation of viruses in cells in 2024*

In 2024, a total of 54 viruses were successfully isolated in QMCs at the Centre, representing an overall isolation rate of 43% (Tables 13 and 15).

*Table 13. Virus isolation in cells at the Centre in 2024.*

| Type/subtype | Isolates attempted | Isolates obtained | Success rate (%) |
|---|---|---|---|
| A(H1N1)pdm09 | 73 | 25 | 34% |
| A(H3N2) | 53 | 29 | 55% |
| B/Victoria | 0 | 0 | 0% |
| **Total** | **126** | **54** | **43%** |

*Table 14. Potential candidate vaccine strains isolated in eggs at the Centre in 2024.*

| A(H1N1)pdm09 | A(H3N2) |
|---|---|
| A/Darwin/422/2023 | A/Canberra/302/2023 |
| A/Darwin/512/2023 | A/Canberra/309/2023 |
| A/Sydney/1/2024 | A/Victoria/2776/2023 |
| A/Victoria/3067/2023 | A/South Australia/298/2023 |
| A/Canberra/4/2024 | A/Canberra/373/2023 |
| A/Victoria/3102/2023 | A/Canberra/331/2023 |
| A/South Australia/27/2024 | A/Victoria/2997/2023 |
| A/Victoria/102/2024 | A/Victoria/131/2024 |
| A/Darwin/370/2024 | A/Canberra/69/2024 |
| A/Darwin/553/2024 | A/Victoria/488/2024 |
| A/Darwin/480/2024 | A/Perth/722/2024 |
| A/Singapore/ IMH0025/2024 | A/Singapore/MOH0334/2024 |
| A/Singapore/ SAR3644/2024 | A/Victoria/756/2024 |
| A/Darwin/1191/2024 | A/Sydney/982/2024 |
| A/Darwin/1187/2024 | A/Victoria/2159/2024 |
| A/Sydney/1327/2024 | A/Sydney/997/2024 |
| | A/Tasmania/780/2024 |
| | A/Canberra/614/2024 |

| B/Victoria |
|---|
| B/Victoria/44/2024 |
| B/Victoria/41/2024 |
| B/Victoria/45/2024 |
| B/Perth/445/2024 |

*Table 15. Potential candidate vaccine strains isolated in cells at the Centre in 2024.*

| A(H1N1)pdm09 | A(H3N2) |
|---|---|
| A/Victoria/32/2024 | A/Sydney/877/2023 |
| A/Tasmania/27/2024 | A/Sydney/879/2023 |
| A/South Australia/27/2024 | A/Sydney/860/2023 |
| A/Canberra/103/2024 | A/Tasmania/356/2023 |
| A/Darwin/370/2024 | A/South Australia/320/2023 |
| A/Darwin/307/2024 | A/South Australia/337/2023 |
| A/Darwin/448/2024 | A/Victoria/2730/2023 |
| A/Victoria/347/2024 | A/Canberra/359/2023 |
| A/Brisbane/296/2024 | A/Victoria/3052/2023 |
| A/Sydney/1050/2024 | A/Canberra/397/2023 |
| A/Tasmania/665/2024 | A/Victoria/3058/2023 |
| A/Tasmania/667/2024 | A/Perth/722/2024 |
| A/Victoria/3215/2024 | A/Perth/741/2024 |
| A/Sydney/1327/2024 | A/Victoria/384/2024 |
| A/Darwin/1774/2024 | A/Victoria/756/2024 |
| A/Sydney/1356/2024 | A/Sydney/161/2024 |
| A/Victoria/32/2024 | A/Victoria/800/2024 |
| A/Tasmania/27/2024 | A/Victoria/840/2024 |
| A/South Australia/27/2024 | A/Victoria/962/2024 |
| A/Canberra/103/2024 | A/Victoria/1362/2024 |
| A/Darwin/370/2024 | A/Perth/836/2024 |
| A/Darwin/307/2024 | A/Perth/842/2024 |
| A/Darwin/448/2024 | A/Sydney/695/2024 |
| A/Victoria/347/2024 | A/Singapore/MOH0326/2024 |
| | A/Singapore/MOH0333/2024 |
| | A/Singapore/MOH0334/2024 |
| | A/Victoria/2335/2024 |
| | A/Victoria/3087/2024 |
| | A/Victoria/3118/2024 |

## Preparation and Analysis of Vaccine Seed Viruses

The Centre exchanges candidate vaccine viruses that have been isolated in eggs, as well as post-infection ferret antisera raised against these and other reference viruses, with the other WHO Collaborating Centres to enable direct comparison of strains isolated in the five Centres. During 2024, 7 candidate vaccine viruses that had been received from other WHO Collaborating Centres and laboratories were passaged in eggs at the Centre (Table 16).

Selected candidate vaccine strains isolated in eggs are made available to the three laboratories that undertake virus reassortment for WHO — Seqirus, the National Institute for Biological Standards and Control (NIBSC, UK) and New York Medical College (NYMC, USA) — where they are reassorted with established egg-adapted strains to produce potential vaccine seed strains. The reassortant vaccine seed viruses are returned to the Centre, where they are analysed by HI assay and genetic sequencing to ensure that key antigenic and genetic properties of the vaccine virus have been retained. The vaccine seed viruses are distributed to other WHO Collaborating Centres and vaccine manufacturers worldwide through Essential Regulatory Laboratories at the Therapeutic Goods Administration (Australia), NIBSC and the Centre for Biologics Evaluation and Research, Food and Drug Administration (USA).

*Table 16. Potential candidate vaccine viruses from other WHO Collaborating Centres isolated at the Centre during 2024.*

| A(H3N2) |
|---|
| A/Sichuan-Fucheng/11625/2022 |
| A/Zhejiang-Gongshu/1920/2022 |
| A/Switzerland/8649/2023 |
| A/District of Columbia/27/2023 |
| A/Croatia/10136RV/2023 |
| NYMCX-425A (A/Croatia/10136RV/2023) |
| **H5N1** |
| (NIBRG-301) A/duck/Vietnam/NCVD-1584/2012 |

## Serological Analyses

*Background*

Antigenic changes in circulating influenza viruses are also monitored by the extent to which they are inhibited by antibodies produced by subjects who have been immunised with current inactivated seasonal influenza vaccines. Twice a year the WHO Collaborating Centres and Essential Regulatory Laboratories in the WHO surveillance network exchange panels of sera collected from subjects pre- and post-influenza vaccination. These panels are analysed using the HI assay against the current vaccine and representative influenza strains in preparation for the biannual WHO Consultations on the Composition of Influenza Vaccines (Table 17).

*Serum panel analyses in February 2024*

For the February vaccine composition meeting, the Centre analysed serum panels from the following age groups: paediatric (0-36 months), paediatric (3-8 years), paediatric (9-17 years), adults (18-64 years), older adults (51-64 years), and elderly adults (>65 years) who had received the 2022-2023 Northern Hemisphere seasonal quadrivalent inactivated egg-based, cell-based, or recombinant influenza vaccines, in the USA. The Centre also analysed serum panels for paediatric (1-10 years), adults (18-64 years) and elderly adults (>65 years) that had received the 2022-2023 Northern Hemisphere seasonal quadrivalent inactivated egg vaccine in China, and one panel of adults (18-64 years) that had the Northern Hemisphere seasonal quadrivalent inactivated egg vaccine in the UK.

A(H1N1)pdm09: When compared to post-vaccination geometric mean HI titres (GMT) against either egg-propagated A/Victoria/2570/2019 or cell-propagated A/Wisconsin/588/2019-like vaccine antigens, there were significant reductions in most serum panels against most recent A(H1N1)pdm09 viruses of subclades 5a.2a and 5a.2a.1, as well as some viruses of 5a.1.

## Serological Analyses (continued)

A(H3N2): Using HI and virus neutralisation (VN) assays, when compared to GMTs against cell culture-propagated A/Darwin/6/2021-like viruses, post-vaccination GMTs against recent A(H3N2) viruses belonging to clades 2a (2a.1b, 2a.3a.1), 2b and 1a.1 were not significantly reduced in most panels. Reductions of GMTs were observed when compared to egg-propagated A/Darwin/9/2021-like reference viruses.

B/Victoria: Post-vaccination HI GMTs against recent B/Victoria lineage viruses in the 3a.2 clade were not significantly reduced when compared to either cell- or egg-propagated B/Austria/1359417/2021-like viruses. Significant reductions in GMTs were observed in some sera panels for B/Victoria lineage viruses in the 1A.3 clade.

B/Yamagata: No serology studies were performed for B/Yamagata lineage viruses.

*Serum panel analyses in September 2024*

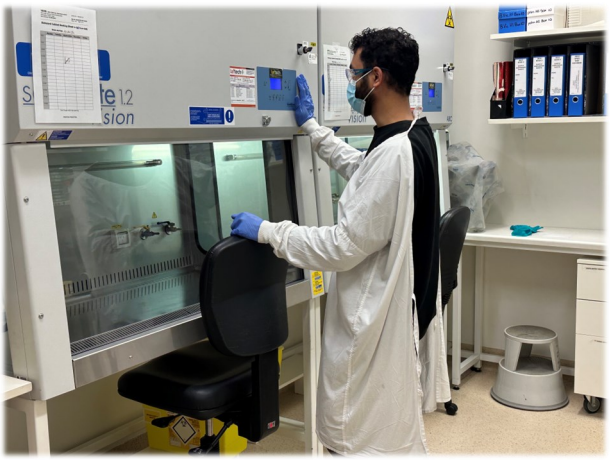
For the September vaccine composition meeting, the Centre analysed serum panels from a paediatric cohort (1-10 years), adults (18-64 years) and elderly (>65 years) who had received either the 2023 Southern Hemisphere seasonal quadrivalent inactivated egg- or cell-based vaccine in Australia.

A(H1N1)pdm09: Human serology studies using these serum panels showed no significant reductions in post-vaccination HI GMTs for the majority of recently circulating, representative A (H1N1)pdm09 5a.2a viruses when compared to cell- or egg -propagated A/Sydney/5/2021-like viruses. There were significant reductions in post vaccination GMTs in some sera panels against recent A (H1N1)pdm viruses belonging to subclade 5a.2a.1.

A(H3N2): When compared to GMTs against cell culture-propagated A/Darwin/6/201-like vaccine viruses, there were significant reductions in some sera panels against A(H3N2) viruses in the more recent 2a.1b, 2a.3a.1, and 2b subclades. These reductions were more pronounced when compared to egg-propagated A/Darwin/9/2021 vaccine virus.

B/Victoria: There were no significant reductions in post-vaccination HI GMTs against the majority of recent B/Victoria lineage viruses from the 3a.2 subgroup when compared to the egg- or cell culture-propagated B/Austria/1359417/2021 vaccine viruses. Significant reductions were detected with most serum panels for viruses from clade 1A.3.

B/Yamagata: No serology studies were performed for B/Yamagata lineage viruses.



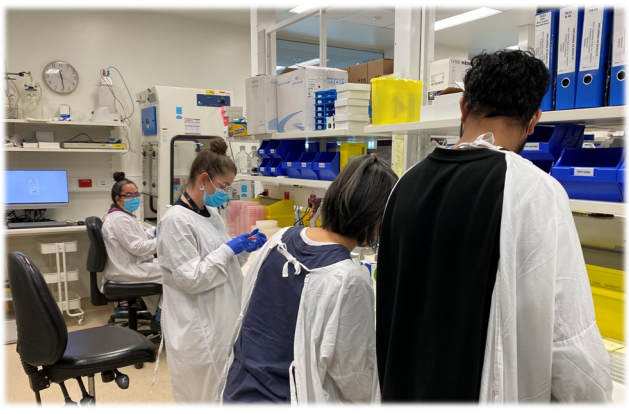


*Table 17. Representative and vaccine candidate strains used for serological analyses during 2024.*

| FEBRUARY |
|---|
| **A(H1N1)pdm09** |
| A/Victoria/4897/2022 |
| A/Victoria/4897/2022 |
| A/Darwin/441/2023 |
| A/Darwin/463/2023 |
| A/Canberra/366/2023 |
| A/Wisconsin/47/2022 |
| A/Wisconsin/47/2022 |
| **A(H3N2)** |
| A/Darwin/9/2021 |
| A/Darwin/11/2021 |
| A/Thailand/8/2022 |
| A/Sydney/856/2023 |
| A/Darwin/344/2023 |
| A/Sydney/878/2023 |
| A/Canberra/302/2023 |
| A/Victoria/2906/2023 |
| A/Victoria/3099/2023 |
| A/Canberra/382/2023 |
| A/Brunei/111/2023 |
| **B/Victoria** |
| B/Singapore/WUH4618/2021 |
| B/Austria/1359417/2021 |
| B/Victoria/1459/2023 |
| B/Canberra/77/2023 |
| B/Victoria/1259/2023 |
| B/South Australia/190/2023 |

| SEPTEMBER |
|---|
| **A(H1N1)pdm09** |
| A/Georgia/12/2022 |
| A/Victoria/4897/2022 |
| A/Victoria/1429/2024 |
| A/Victoria/1959/2024 |
| A/Taranaki/13/2024 |
| A/Victoria/102/2024 |
| A/Singapore/KK1423/2024 |
| A/Victoria/317/2024 |
| A/Auckland/11/2024 |
| A/Darwin/370/2024 |
| A/Darwin/375/2023 |
| **A(H3N2)** |
| A/Sydney/1304/2022 |
| A/Thailand/8/2022 |
| A/Singapore/MOH0334/2024 |
| A/Sydney/711/2024 |
| A/Victoria/131/2024 |
| A/Darwin/1067/2024 |
| A/Victoria/2997/2023 |
| A/Victoria/800/2024 |
| A/Sydney/761/2024 |
| A/Victoria/958/2024 |
| A/Victoria/478/2024 |
| **B/Victoria** |
| B/Singapore/WUH4618/2021 |
| B/Austria/1359417/2021 |
| B/Victoria/70/2024 |
| B/Darwin/6/2024 |
| B/Victoria/34/2024 |

## Recommendations on Influenza Vaccines

### *WHO Consultations on the Composition of Seasonal Influenza Vaccines*

The antigenic, genetic, antiviral resistance and serological data generated from the Centre's surveillance activities are incorporated into detailed dossiers for use at the WHO Consultations on the Composition of Influenza Vaccines in February (for the Northern Hemisphere) and September (for the Southern Hemisphere).

The Centre Director and Deputy Director participate in preparatory teleconferences and then meet at the face-to-face Consultation with WHO, representatives from the other WHO Collaborating Centres and the four Essential Regulatory Laboratories (Center for Biologics Evaluation and Research, US Food and Drug Administration, USA; National Institute for Biological Standards and Control, UK; National Institute of Infectious Diseases, Japan; Therapeutic Goods Administration, Australia). Vaccine effectiveness estimates were also presented by the Centre's senior epidemiologist in person at the Consultation in September. Consultations are also attended by observers from the World Organisation for Animal Health (WOAH), the University of Cambridge, several WHO National Influenza Centres and other relevant organisations. In 2024 WHO made the recommendations reported below.

**WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2024-2025, Geneva, Switzerland, 23 February 2024**

The WHO recommends that trivalent vaccines for use in the 2024-2025 northern hemisphere influenza season contain the following:

**Egg-based vaccines**

- an A/Victoria/4897/2022 (H1N1)pdm09-like virus;
- an A/Thailand/8/2022 (H3N2)-like virus; and
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus

**Cell culture- or recombinant-based vaccines**

- an A/Wisconsin/67/2022 (H1N1)pdm09-like virus;
- an A/Massachusetts/18/2022 (H3N2)-like virus;
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus;

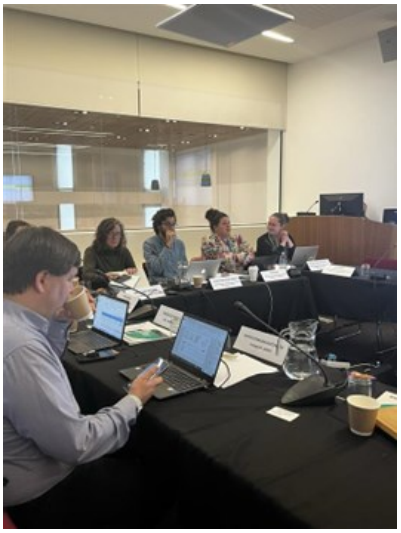
For quadrivalent egg- or cell culture-based or recombinant vaccines for use in the 2024-2025 northern hemisphere influenza season, the WHO recommends inclusion of the following as the B/Yamagata lineage component:

- a B/Phuket/3073/2013 (B/Yamagata lineage)-like virus.

## The WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2025, hosted at the Centre (September 23-26, 2024

In September 2024, the Centre hosted the WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2025 at The Peter Doherty Institute. Professor Ian Barr (Centre Deputy Director) acted as host and temporary advisor and Heidi Peck (Serology Head) presented a serology report from the Centre. Professor Patrick Reading, Dr Jessica Miller, Dr Tanya Diefenbach-Elstob, Dr Yi-Mo Deng, Ms Katie Milne and Ms Symone Mercuri were official attendees at the consultation. Several other Centre staff attended the consultation as observers.

The Centre welcomed 45 guests from around the world, including from other WHO Collaborating Centres, Essential Regulatory Laboratories, WHO NICs and Regional Offices, and other relevant organisations, to attend the meeting. Visitors also toured the facilities at Centre during the consultation.



**WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2025 Melbourne, Australia 27 September 2024**

The WHO recommends that **trivalent** vaccines for use in the 2025 southern hemisphere influenza season contain the following:

**Egg-based vaccines**

- an A/Victoria/4897/2022 (H1N1)pdm09-like virus;
- an A/Croatia/10136RV/2023 (H3N2)-like virus; and
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus.

**Cell– or recombinant-based vaccines**

- an A/Wisconsin/67/2022 (H1N1)pdm09-like virus;
- an A/District of Columbia/27/2023 (H3N2)-like virus; and
- a B/Austria/1359417/2021 (B/Victoria lineage)-like virus.

For quadrivalent egg- or cell culture-based or recombinant vaccines for use in 2025 southern hemisphere influenza season, the WHO recommends inclusion of the following B/Yamagata lineage component:

- a B/Phuket/3073/2013 (B/Yamagata lineage)-like virus.

In addition to the overall recommendations as described above, WHO lists candidate vaccine viruses (CVVs) that may be suitable for inclusion in vaccines. These CVVs, which are listed on the WHO website, are antigenically similar to the recommended vaccine strains. In 2024, the following candidate vaccine viruses, which were originally isolated at the Centre in either eggs or cells, were listed by WHO as being suitable for vaccine use following the indicated meeting.

| Type/Subtype/ Lineage | Egg-derived CVVs | Cell-derived CVVs |
|---|---|---|
| **A(H1N1)pdm09** | A/Victoria/4897/2022 (Feb, Sep) | |
| **A(H3N2)** | A/Brisbane/837/2022 (Feb)<br>A/Croatia/10136RV/2024 (Sep)<br>A/Perth/722/2024 (Sep) | A/Victoria/800/2024 (Sep)<br>A/Darwin/11/2021 |
| **B/Victoria** | | B/Singapore/WUH4618/2021(Feb. Sep)) |
| **B/Yamagata** | B/Phuket/3073/2013 (Feb, Sep) | B/Singapore/INFTT-16-0610/2016 (Feb, Sep)<br>B/Singapore/INFKK-16-0569/2016 (Feb/Sep)<br>B/Brisbane/9/2014 (Feb/Sep) |

## *Australian Seasonal Influenza Vaccine Recommendation*

Whereas the WHO makes recommendations on suitable viruses for inclusion in seasonal influenza vaccines, in individual countries the decision on the composition of vaccines is made by national or regional authorities. In Australia, the Therapeutic Goods Administration makes the decision on the advice of the Australian Influenza Vaccine Committee (AIVC). The Centre Director and Deputy Director both serve on the AIVC.

The AIVC met on 16 October 2024 and recommended that the following viruses be used for influenza vaccines in the 2025 Southern Hemisphere influenza season:

**Egg-based trivalent vaccines:**

• an A/Victoria/4897/2022 (H1N1)pdm09-like virus;

• an A/Croatia/10136RV/2023 (H3N2)-like virus; and

• a B/Austria/1359417/2021 (B/Victoria lineage)-like virus.

**Cell- or recombinant-based trivalent influenza vaccines:**

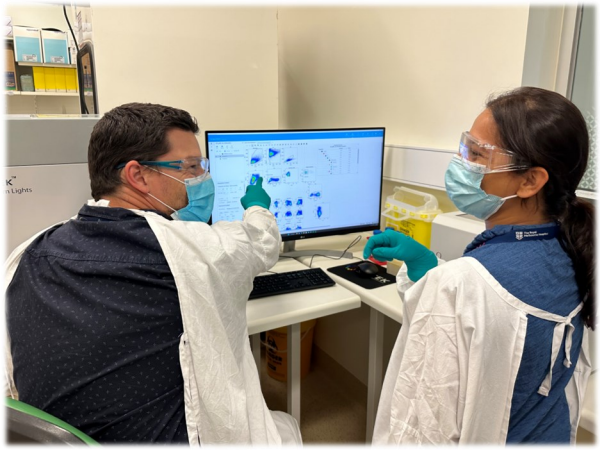
• an A/Wisconsin/67/2022 (H1N1)pdm09-like virus;

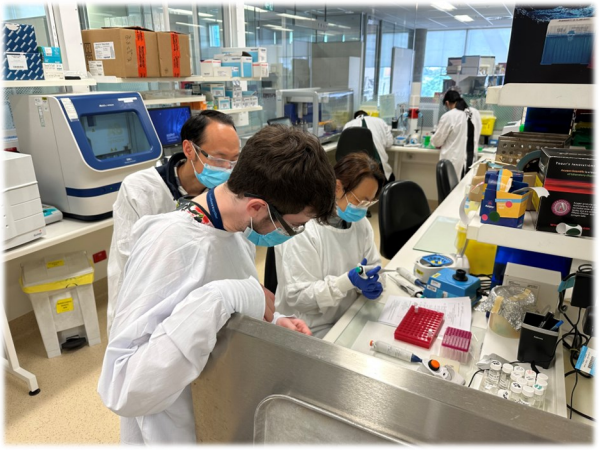
• an A/District of Columbia/27/2023 (H3N2)-like virus; and

• a B/Austria/1359417/2021 (B/Victoria lineage)-like virus;

The recommendation for the B/Yamagata lineage component of quadrivalent influenza vaccines remains unchanged from previous recommendations:

. a B/Phuket/3073/2013 (B/Yamagata lineage)-like virus.





# Training

## Training and Support of National Influenza Centres

The Centre provides support in the form of training and advice to WHO National Influenza Centres (NICs) and other diagnostic laboratories, especially in the Asia-Pacific region. Strengthening technical capabilities and infrastructure for surveillance work in regional laboratories increases their capacity to detect and characterise circulating influenza viruses and to identify viruses with pandemic potential, thus further supporting the GISRS surveillance network. Centre staff are involved in the training of visiting scientists at the Centre, participating in regional workshops and visiting laboratories to provide direct assistance in strengthening surveillance capabilities.

### *Training Programs and Visits to Regional Laboratories*

**Clyde Dapat,** together with Ammar Aziz from the Translational Diagnostics Laboratory at VIDRL and Wytam-ma Wirth from the Microbiological Diagnostic Unit (MDU) Public Health Laboratory, were engaged by the WHO Country Office in the Philippines to deliver a training course in bioinformatic analysis of SARS-CoV-2 and influenza in Manila between 14-19th April 2024. There were 18 participants from national and regional public health laboratories across the country. Participants received lectures and performed practical exercises on quality assessment of NGS data, genome assembly, annotation, phylogenetics, and submission of sequence data to genetic databases.

**Patrick Reading,** together with Jean Moselen from the Translational Diagnostics Laboratory at VIDRL, conducted a one week training at the Molecular Laboratory, Tungaru Central Hospital, Tarawa in Kiribati between 20-24th April 2024. Training was provided in multiplex PCR techniques. This training aimed to assist the team to set up the molecular laboratories for future testing needs.

**Clyde Dapat and Yi-Mo Deng** delivered the International Influenza and RSV Genetic Sequencing Workshop in Pune, India, between 3-7th June 2024. The workshop, hosted at the National Institute of Virology in Pune, was attended by 13 participants from WHO SEARO countries including Bangladesh, Bhutan, India, Indonesia, Nepal, and Sri Lanka. Participants received training in PCR, library preparation, next-generation sequencing using ONT technologies, and bioinformatics analysis on whole genome sequence data.

## *Centre-based Training*

**Clyde Dapat, Xiaomin Dong, and Steven Edwards** delivered bioinformatics training to scientists from the National Institute of Heath, Pakistan, between from 24-28th June 2024. The training covered topics such as quality assessment of sequence data, genome assembly of influenza and RSV, genome annotation, and phylogenetic analysis.

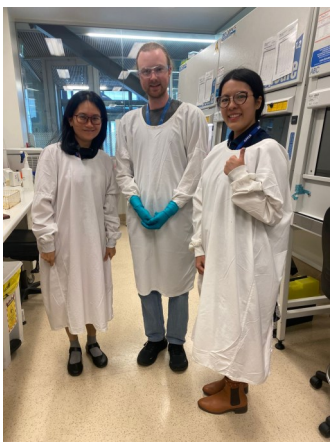
**Clyde Dapat, Yi-Mo Deng, Xiaomin Dong, Steven Edwards and Rachel Wordsworth** were involved in training scientists from The Research Institute for Tropical Medicine (RITM), Philippines from 4-11th October 2024. The workshop focussed on the use of ONT technologies for whole genome sequence of RSV in clinical samples. The training also covered topics such as quality assessment of sequence data, genome assembly of RSV, genome annotation, and phylogenetic analysis.

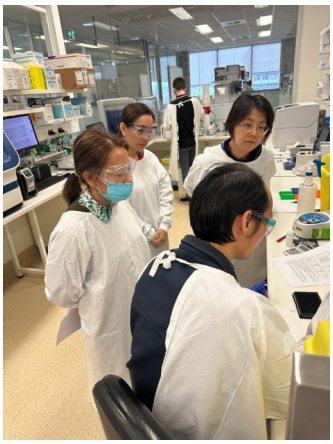
**Heidi Peck, Malet Aban and Leah Gillespie** were involved in training scientists from the NIC at the Pasteur Institute, Ho Chi Minh City, Vietnam from 4-17th October 2024. The workshop covered topics such as mammalian cell culture, influenza virus isolation and influenza virus serology.

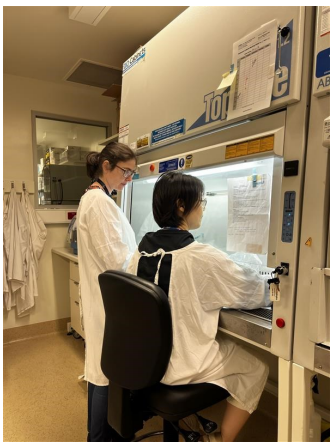
**Saira Hussain** hosted and trained a scientist from the National Institute for Communicable Diseases (NICD), South Africa on 4th October 2024. The training focussed on the use of neuraminidase inhibition (NAI) assays for influYi-Mo Deng coordinated the training a scientist from Beijing CDC from 20th October-19th November 2024. A number of Centre staff were involved in this training, which focused on different aspects of RSV genomic surveillance.

**Yi-Mo Deng** coordinated the training a scientist from Beijing CDC from 20th October-19th November 2024. A number of Centre staff were involved in this training, which focused on different aspects of RSV genomic surveillance.

**Yi-Mo Deng, Michelle Wille Clyde Dapat, Xiaomin Dong, and Steven Edwards** were involved in training a scientist from Nanjing CDC from 7th October–20th December 2024. The training focused on NGS, phylogenetic analyses, bioinformatics, serological analysis of influenza viruses and detection of avian influenza viruses.







# Research

The Centre continues to develop and expand its research interests across a range of projects, both within the Centre and with external collaborators.

## Antivirals and Viral Fitness

### *Centre staff and students*

Saira Hussain (Senior Scientist), Harry Stannard (PhD candidate), Ashwin Muraleetharan (Research assistant), Melkamu Tessema (Post doc), Nikita Deshpande (Routine NAI surveillance testing)

Centre collaborators: Kanta Subbarao, Ian Barr, Patrick Reading

### *Research overview*

Our research mainly focuses on the evaluation of the effectiveness of approved and investigational influenza antivirals as well as the risk of the emergence of drug resistant viruses which may spread widely amongst the community. We also study the viral fitness of different drug resistant variants. This information provides insights into the likelihood that such viruses could spread amongst the community. We are developing new tools/models for antiviral efficacy screening which are biologically relevant, low cost and reduce the number of animals in research, detailed below.

### *Collaborators*

Lorena Brown, Jenny Mckimm-Breschkin, Jessica Neil, Brad Gilbertson, Matthew Gartner, Charlie-McKenzie Kludas, Rubaiyea Farrukee and Sarah Londrigan (University of Melbourne); Regis Grailhe (Translation Research Division, Institute Pasteur Korea); Jessica Belser (CDC); Sebastian Maurier Stroh (National University of Singapore); Larisa Gubareva (CDC); Emi Takashita (NIID, Tokyo), Andrew Mehle (University of Wisconsin); Seema Lakdawala (Emory University School of Medicine), Paul Digard (The Roslin Institute, University of Edinburgh), Elena Govorkova (St Jude's Childrens Research Hospital, US), Aus Bio Ltd. (Australia), F. Hoffmann-La Roche Ltd, SK Bioscience (Republic of Korea).

### *Highlights and developments 2024*

Development of investigational antivirals (PAD grant)

The WHOFLU antivirals group, along with collaborators from Aus Bio Ltd. and The University of Melbourne, were successful in 2023 in obtaining funding through the Pandemic Antiviral Discovery initiative (PAD) from the Novo Nordisk foundation (NNF) to complete the preclinical development of the lead candidate as a long-acting pan-influenza antiviral drug for both prevention and treatment of influenza, with a focus on countering influenza strains with pandemic potential. The project is led by Kanta Subbarao and co-investigators from the Centre are Ian Barr and Saira Hussain. The WHOFLU antiviral group will be working to determine antiviral efficacy in vitro and in vivo (ferrets) of the Aus Bio compounds compared with licensed influenza antivirals as well as performing studies to determine generation of drug resistant mutants. The group on-boarded new staff in 2024 to work on this project, Ashwin Muraleetharan (March 2024, continued into 2025) and Melkamu Tessema (March 2024-Dec 2024). Melkamu worked with Saira to determine in vitro toxicity in relevant adherent mammalian cell lines of the Aus Bio candidate prodrugs (3x) and their active compound, alongside currently licensed antiviral drugs. The drugs performed well for toxicity barring one of the prodrugs, which was quickly discounted due to toxicity, poor solubility and limited antiviral efficacy in mice. For in vitro and in vivo antiviral efficacy testing, Harry, Ashwin and Melkamu have plaque purified, sequence confirmed and determined infectious titres for a number of contemporary seasonal influenza A and B viruses. Melkamu worked with Saira to compare different assays for measuring antiviral efficacy in vitro of the active compound MD378, alongside licensed antivirals, against seasonal influenza A and B viruses. Three different assays were compared, namely a multi-cycle replication virus yield assay, a plaque reduction assay and a focus reduction assay (FRA).

## Antivirals and Viral Fitness (continued)

After number of preliminary experiments, the high throughput FRA assay was chosen for subsequent assays, given it was associated with reduced drug and consumables costs, as well as experimental time. Ashwin and Saira worked together to demonstrate reproducibility in the FRA assay for in vitro testing of Aus Bio drugs and optimise focus forming unit (FFU) sizes for the different seasonal influenza types/ subtypes using different overlay conditions to allow for accurate IC50 determination within the range of drug concentrations used in the assay. They tested a number of different seasonal influenza A(H1N1) pdm09, A(H3N2) and B/Victoria-lineage wild-types and oseltamivir, zanamivir and baloxavir-resistant mutants to show that the active AusBio compound MD378, showed superior efficacy against licensed neuraminidase inhibitors (NAIs) and in several cases, the licensed polymerase inhibitor (PAI), baloxavir. Animal viruses (potential pandemic) will be tested in 2025 such as swine, LPAI and HPAI viruses.

In 2025, the group will determine in ferrets the prophylactic and therapeutic activity of the lead prodrug (determined by mouse studies) against seasonal influenza wild type and drug resistant isolates and animal viruses, compared to licensed NAIs and baloxavir.

Assays/Tools development for future antiviral and virus fitness studies:

Ferret Precision-Cut Lung Slices: Development of a high-throughput ex-vivo model to study influenza virus infection

Saira Hussain was successful as co-investigator on a grant obtained from the Cumming Global Centre Foundation Grants in 2023 for the development of a comprehensive suite of human and animal in vitro respiratory tract models for evaluating viruses with pandemic potential. The project is in collaboration with Jessica Neil, Kanta Subbarao and other investigators at the University of Melbourne, Monash University and the University of Adelaide. Specifically, Saira Hussain and the antivirals group have been working in 2024 on developing ferret precision cut lung slices (PCLS) as a surrogate model for influenza infection and antiviral efficacy studies. Saira, Harry and Ashwin adapted a protocol for preparation of mouse PCLS for their ferrets, resulting in thousands of slices (8 mm diameter, 250 µm depth) that can be cultured in 48 well dishes for up to 3 weeks. They demonstrated successful infection of PCLS harvested from different ferret lobes with a contemporary seasonal influenza A(H1N1)pdm09 strain and several strains of H5N1 (HPAI) (the latter studies performed by Brad Gilbertson, the University of Melbourne). For H5N1, the virus replication kinetics were similar to that observed using human air liquid interface (ALI) cultures. The group also demonstrated antiviral efficacy of NAIs and baloxavir against seasonal influenza in ferret PCLS, and plan to develop a high throughput luminescent reporter virus-based assay for antiviral screening in ferret PCLS. The group are also investigating replication of a range of H3 and B influenza virus isolates in ferret PCLS ex vivo (including serially passaged viruses with adaptative mutations), as contemporary strains of these do not replicate in the ferret lung in vivo. Further microscopy studies will determine if cell types maintained in ferret PCLS ex vivo cultures are similar in architecture to the ferret lung and investigate localisation of influenza infection in the ferret PCLS versus the ferret lungs in vivo. Cell viability assays measuring metabolic activity (such as Cell-Titer Glo and Alamar Blue) of ferret PCLS cultures over time gave variable results, possibly due to variability between PCLS in cell numbers, cell types and sectioning. In 2025, the group will investigate the use of microscopy assays to quantify cell death by different pathways primary necrosis, necroptosis, apoptosis – loss of membrane integrity by staining with propidium iodide or red-fluorescent ethidium homodimer-1 (DNA binding), loss of metabolic activity by staining with green-fluorescent calcein-AM to indicate intracellular esterase activity. The group will also try different methods for cryopreservation of PCLS.

Harry Stannard PhD: Further development of the ferret model of influenza A virus infection and its utility to explore novel therapeutic treatments

Harry Stannard began his PhD in July 2023, with his prospective thesis titled, ‘Further development of the ferret model of influenza A virus infection and its utility to explore novel therapeutic treatments.’ Under the supervision of Patrick Reading, Saira Hussain and Ian Barr from the Centre, Harry will explore multiple avenues for the improvement of the ferret model for influenza antiviral efficacy studies, with a particular focus on exploring lower respiratory tract viral load and disease kinetics, in A(H1N1)pdm09 as well as for the first time A(H3N2) and B/Victoria lineage viruses. He has worked to identify more contemporary influenza virus strains that demonstrate robust influenza disease, transmission, and lower respiratory tract (LRT) involvement in the ferret and can be used for future antiviral studies.

## Antivirals and Viral Fitness (continued)

In 2023, Harry identified contemporary H1pdm09 isolates that demonstrate improved replication in ferret lungs compared with the 2009 viruses. In 2024, Harry went on to serially passage contemporary seasonal influenza H3 and B viruses in the ferret lungs (and newly established ferret PCLS model) to adapt them to grow in ferret lungs ex vivo. He observed changes in the glycosylation of HA of H3 and B viruses being serially passaged in ferret lungs/PCLS, similar to what has been observed for mouse lung adapted viruses. However, although these serially passaged A(H3N2) and B isolates, as well as reverse genetics generated viruses mimicking these HA glycosylation changes, showed improved replication in ferret PCLS compared to their precursor wild type isolates, they have not yet demonstrated robust replication in ferret lungs in vivo, for use in an antiviral efficacy model.

In 2025, Harry will work on a project (in collaboration with US CDC) to assess if respiratory inhalation of aerosolized virus to inoculate ferrets represents a more advantageous challenge route than standard intranasal inoculation when evaluating antiviral efficacy in ferrets (a study initiated by Saira Husain and Jessica Belser, CDC). He will further investigate the use of aerosol delivery to improve A(H3N2) and B virus replication in ferret lungs (compared with his current intranasal route) for his LRT antiviral model. Hopefully, together with the expertise of international collaborators, can set up an aerosol delivery ferret model at the Doherty institute.

In 2025, Harry will work on setting up live imaging of influenza infection in ferrets. The project is a collaboration between the WHOFLU Centre and Regis Grailhe (Institute Pasteur Korea), who aims to develop a world-leading live-imaging device for ferrets, enabling quantification and location of fluorescent or bioluminescent tagged influenza viruses during the infection, and is funded by SK Bioscience. The aim of this project is to provide improved empirical readouts of influenza viral kinetics in the animal, while reducing the number of animals required in each study. Collaborations with Andrew Mehle (University of Wisconsin) and Seema Lakdawala (Emory University School of Medicine) have enabled the sharing of labelled influenza viruses for use in our live-imaging system, specifically NanoLuciferase (bioluminescence) and mRuby (fluorescent) tagged influenza viruses, respectively. In preparation for the live imaging, Harry has already demonstrated successful in vitro and in vivo (ferrets) replication of these reporter viruses in our lab.

In 2025, Harry will also be testing efficacy of novel host directed antivirals (targeting the interferon-stimulated genes, ISG pathway) in vitro and in vivo (mice and ferrets) against influenza and RSV. He will also be investigating delivery of these compounds in vivo using liquid nanoparticle (LNP) formulations.

Antiviral resistance studies

Multiple Neuraminidase mutations in serial samples from an immunocompromised patient infected with A (H3N2) influenza virus

In 2024, the group identified an interesting patient case from Alfred hospital where a highly immunocompromised patient acquired multiple NA oseltamivir resistant mutations during the course of treatment. A 30-year-old man with cystic fibrosis was infected with influenza A(H3N2) 14 days after living related kidney transplantation. He received several courses of oseltamivir over the next 10 weeks but had persistent symptoms and was unable to clear the virus. Several nose/throat swabs collected over 14 weeks were subject to whole genome sequencing and NAI susceptibility phenotypic testing using the MUNANA assay. WGS of the clinical samples revealed that the patient had acquired a range of changes in the NA gene in several samples over the course of the infection that conferred highly reduced inhibition (HRI) to oseltamivir. These included NA-R292K and NA-E119V substitutions, and a deletion at 245-248 residues. These results were confirmed by the phenotypic assay (IC50 values were 103- to 166-fold higher than the median value). Following these results, treatment with zanamivir was initiated and the influenza infection was cleared. The study highlights that early detection of drug resistance and alternative treatments such as zanamivir, peramivir and baloxavir (a polymerase inhibitor) are most likely to improve clinical outcomes.

## Antivirals and Viral Fitness (continued)

*Cluster of PA-A37T viruses circulating in Tasmania during June-Sept 2024 showed reduced susceptibility to baloxavir*

The group identified a series of A(H3N2) isolates received from Royal Hobart Hospital containing PA-A37T, which has been reported to have emerged during clinical trials of Baloxavir in Japan (2016-2018) but has been extremely rare in seasonal isolates since baloxavir was licensed and used for treatment.

It is unclear whether this mutation has been selected for during baloxavir treatment, as patient travel histories were not available. During her visit to CDC in March 2024, Saira learnt their assay for baloxavir phenotypic testing (Influenza Replication Inhibition Neuraminidase-based Assay- IRINA). Ashwin has set up this assay in our lab. He demonstrated using IRINA that the Tasmania PA-A37T show >3-20-fold elevated IC50s to baloxavir (reduced susceptibility) compared to wild-type matched PA viruses and CDC reference control wild-type virus A/Louisiana/50/2017, similar to results obtained by Emi Takashita (NIID, Japan) using our isolates.

### *Publications and presentations*

In 2024, studies from the Antivirals Group contributed to two peer-reviewed publications in Communicable Diseases Intelligence and PloS Biology, with other manuscripts in preparation or under review. Studies were also presented at a number of conferences, including Options XII for the Control of Influenza, Brisbane, October 2024 and the Australasian Virology Society, Creswick, December 2024.

# Avian influenza

## *Centre staff*

Michelle Wille

## *Research overview*

Avian influenza viruses can pose a threat to humans via direct infection from an avian source. If the virus has the ability to replicate well in humans and transmit, there is potential that such viruses may cause an influenza pandemic. We routinely sample migratory shorebirds and resident ducks in Australia to determine what types of avian influenza viruses are circulating amongst avian populations. The Centre is involved with the characterisation of viruses sampled from birds in Australia, including culture, sequencing and phylogenetic analysis. Furthermore, to understand overall exposure of Australian wild birds to influenza A virus, we are also screening blood samples for antibodies against influenza A viruses. In the case of shorebirds, this will allow us to assess not only the burden of influenza locally, but also provide insight into influenza exposure of these birds while at their northern breeding grounds, and during their annual migration.

## *Collaborators*

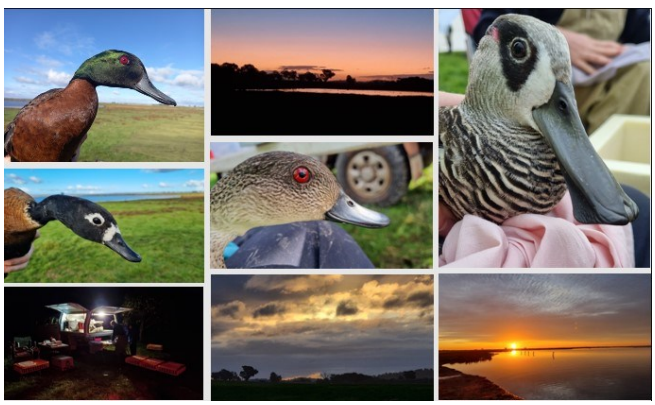
Marcel Klaassen (Deakin University, Victoria); Frank Wong (Australian Centre for Disease Preparedness [ACDP], Geelong VIC); Andrew Breed (Australian Government Department of Agriculture), Paul Eden (National Avian Influenza Wild Bird Program, Wildlife Health Australia).

## *Highlights and developments 2024*

In 2024, we collected and screened 1696  paired swab and serum samples from wild Anseriformes (ducks) and Charadriiformes (shorebirds and terns) in Victoria, New South Wales, Tasmania, South Australia and Western Australia, with 52 influenza A virus detections. Through sequencing, the viruses were shown to comprise H1N7, H1N2, H3N7, H4N7, H7N6 and H12N5 viruses.

In addition to routine sample collection, we provided support to Agriculture Victoria through sampling of wild ducks following the outbreaks of HPAI H7N9 and H7N3 in poultry. While we did not detect any HPAI H7 in wild ducks, we did recover a LPAI H7N6 genome and isolate. Detailed virology, serology and statistical analysis of the national dataset were undertaken to support the outbreak response.

We also performed heightened surveillance from September – December, coinciding with the return of migration birds to rule out HPAI incursion. All swab samples were negative for HPAI and none of the serum samples which were positive by ELISA were positive by HI when using a 2.3.4.4b antigen. Our enhanced surveillance program was funded by Department of Agriculture, Fisheries and Forestry, administered by Wildlife Health Australia.



*Birds tested  play an important role in the maintenance of AIV in Australia. : Photo by Michelle Wille*

# Early Recognition and Response to Influenza Infection

## *Centre staff*

Patrick Reading, James Barnes

## *Research overview*

Our research, which is undertaken at the Centre and at the University of Melbourne, investigates how the body first recognises and responds to infections with influenza and other respiratory viruses. We employ in vitro studies using human proteins and cells, as wells as in vivo studies using mouse and ferret models of infection. We are also interested in assessing novel treatment and vaccine platforms for influenza and other respiratory viruses in vitro and in animal models of infection.

Our current studies are focused on:

I. How different cell types in the respiratory tract sense and respond to influenza virus infection,

II. Identifying specific host proteins that are expressed in virus-infected cells and can interfere with the entry, replication and/or release of influenza and other respiratory viruses (restriction factors),

III. Understanding the similarities and differences between restriction factors expressed in humans compared to those expressed in other species susceptible to influenza virus infections, and

III. Utilizing approaches to induce host innate immunity, including an array of restriction factors, to limit the impact of subsequent infection with influenza or other respiratory viruses.

## *Collaborators*

Keith Chappell, Daniel Watterson, Paul Young (University of Queensland); Nathan Bartlett (University of Newcastle); Daniel Steinfort (Royal Melbourne Hospital); Andrew Brooks, Justine Mintern, Stephen Kent, Linda Wakim, Georgia Deliyannis, Carol Hartley and Joanne Devlin (The University of Melbourne), Gunther Hartmann and Eva Bartok (University Hospital, Bonn, Germany)

## *Highlights and developments 2024*

A major focus of our research is understanding and characterising particular intracellular proteins (termed restriction factors) that are expressed or induced in host cells, which can block the replication of influenza and/or other respiratory viruses. We utilise approaches to overexpress or delete putative restriction factors to determine their role in blocking virus replication and to characterise their mechanism/s of antiviral activity against influenza virus and respiratory syncytial virus (RSV).

Working with collaborators at University Hospital, Bonn in Germany we have also been using synthetic RNA molecules that target specific intracellular pattern recognition receptors to stimulate host innate immunity and to provide protection against subsequent influenza and respiratory syncytial virus (RSV) infections in mouse and ferret models of infection. In 2024, we are now investigating the potential of mRNA-mediated delivery of particular restriction factors as a novel antiviral treatment. In addition to humans, we are also studying restriction factors expressed by mammalian species susceptible to influenza infections (e.g. ferrets, mink, cattle and other species) which may play a role in limiting interspecies transmission and/or driving virus evolution.

In 2024, research contributed to four peer-reviewed publications, in Cell, PLoS Pathogens, Science Reports, Science Advances, and Communication Biology. Prof. Reading is co-lead of a research group at the University of Melbourne consisting of two post-doctoral scientists, four Ph.D. students and one Master of Biomedical Science student. Dr Reading also supervises James Barnes, a research assistant based at the Centre, who has been investigating restriction factors expressed in ferrets and cattle to assess their antiviral activity against influenza and other respiratory viruses.

# Epidemiology

## *Centre staff*

Jessica Miller (started August 2024), Tanya Diefenbach-Elstob, Jessie Goldsmith (PhD student, UoM), Arseniy Khvorov (ended April 2024)

## *Research overview*

Our work primarily focuses on using surveillance data to examine fluctuations in influenza activity and vaccine effectiveness across populations and seasons. We have been working with influenza sentinel surveillance systems operating in Australia to estimate influenza vaccine effectiveness in the community and provide ongoing estimates to government and WHO. We also conduct various simulation studies and meta-analysis/meta-regression to understand the validity of study designs used to estimate vaccine effectiveness.

The team collaborates on studies exploring the validation of various data source used to capture influenza and RSV cases. This includes examining the validity of thresholds used to determine excess influenza-associated mortality (Jessie Goldsmith) and of RSV syndromic surveillance data in Victoria (Tanya Diefenbach-Elstob).

The team continues to work closely with the WHO and other partners on influenza burden of disease studies and provides technical support to member states in the SEAR and WPR.

## *Collaborators*

VE studies: Monique Chilver (University of Adelaide); Allen Cheng (Monash)

VE hospital studies: FluCAN and PAEDS collaborators (Allen Cheng, Philip Britton, Christopher Blyth, Kristine Macartney, Tom Kotsimbos) on behalf of the FluCAN investigators and PAEDS network.

VE paediatric studies: Sheena Sullivan (Monash); Patricia Campbell and Katherine Gibney (UoM); Janet Strachan (Vic DoH); Stephen Lambert (QLD DoH).

RSV validation studies: Rob Moss (UoM); Sheena Sullivan (Monash); Janet Strachan (Vic DoH); Kara Martin (Vic DoH)

The following collaborations are in the early phase and project plans are currently being formulated:

Avian Influenza studies: David Brett-Major and Jana Broadhurst (University of Nebraska Medical Center); Alicia Arnott, Kathryn Edenborough, Adrian Mercato (VIDRL)

Severity studies: David Price (VIDRL); Allen Cheng (Monash)

## Epidemiology (continued)

We continued to work with the Australian Sentinel Practices Research Network (ASPREN) and the Influenza Complications Alert Network (FluCAN) to evaluate the effectiveness of Australian quadrivalent inactivated seasonal influenza vaccines in 2024. HI assay and sequencing data generated by the Centre were used to inform VE estimates, and patient information obtained from the surveillance programmes was used to inform selection of viruses for sequencing (e.g., vaccination status). As always, all ASPREN samples were received at the Centre. In addition, in 2024, 72% of FluCAN 2024 samples were received, reflecting enhanced efforts to ensure these samples are included in the Centre's virological surveillance, and to improve the quality of vaccine effectiveness estimates possible through that network.

The epidemiology group compiled the Global Influenza Vaccine Effectiveness Reports for the 2024 and 2024/2025 influenza seasons. The 2024 report was presented by Sheena Sullivan at the WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2025 (held in September 2024), and by Jessica Miller at the WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2025-2026 (held in February 2025).

The group continued their work on vaccine effectiveness in various populations. Jessie Goldsmith continued to work on vaccine effectiveness in children and progressed a systematic review on vaccine efficacy and effectiveness by dose for children less than 9 years of age in the first year of vaccine, which has been submitted for publication. She continued analyses of influenza vaccine effectiveness in 2022 among children born during the COVID-19 pandemic when influenza was temporarily eliminated in Australia (2020 and 2021). This project uses linked health administrative data from Victoria and Queensland. Jessie Goldsmith continued to explore potential biases in test-negative studies and has been co-leading a systematic review and meta-analysis to understand the underlying sources of heterogeneity in vaccine effectiveness studies for COVID-19 vaccines. Preliminary results were presented at the OPTIONS XII conference and the manuscript is currently being drafted. Tanya Diefenbach-Elstob continued to work with FluCAN and the Paediatric Active Enhanced Disease Surveillance (PAEDS) collaborators to describe vaccine effectiveness in Australian sentinel hospitals.

The group continued to describe the disease burden of influenza and RSV using hospital and unlinked routinely collected health data from Victoria and Queensland.

Tanya Diefenbach-Elstob continued to co-lead work on RSV and the validation of RSV syndromic surveillance data in Victoria, using linked and unlinked health administrative data from the Victorian Department of Health.

We continued working on other serological studies (see Human Immunity to Influenza). In these studies, the epidemiology group worked to develop tools to better analyse antibody titre data (Arseniy Khvorov).

<u>The following abstracts were presented at conferences:</u>

Jessie Goldsmith. Using data from the COVID period to improve our understanding of the burden of influenza mortality. Oral presentation at OPTIONS XII for the Control of Influenza, Brisbane 2024

Christy Vu, Jessie Goldsmith. Influenza vaccine effectiveness by subtype: a systematic review and meta-analysis of test-negative design studies. Poster presentation at OPTIONS XII for the Control of Influenza, Brisbane 2024

Diefenbach-Elstob T, Peck H, Deng Y, Dapat C, Britton PN, Blyth C, Macartney K, Kotsimbos T, Barr I, Cheng AC, on behalf of the FluCAN investigators and PAEDS network. Influenza in Australian FluCAN and PAEDS sentinel hospitals in 2023. Poster presentation at OPTIONS XII for the Control of Influenza, Brisbane 2024

<u>The following manuscripts have been submitted for publication:</u>

Diefenbach-Elstob T, Chilver M, Spirkoska V, Carville K, Dapat C, Turra M, Tran T, Deng Y, Peck H, Barr I, Stocks N, Sullivan SG. Influenza vaccine effectiveness in Australia during 2017-2019.

Jessie Goldsmith. Influenza vaccine effectiveness for children in the first year of vaccination by number of doses received: a systematic review and meta-analysis

# Immunity to Respiratory Viruses

## *Centre staff and student*

Annette Fox, Louise Carolan, Sheena Sullivan, Stephany Sanchez, Yi Liu, Anastasia Jessica Hadiprodjo, Ziheng Zhu, Arada Hirankitti

## *Research overview*

A key goal of our work is to identify strategies to improve the immunogenicity and, in turn, effectiveness of seasonal influenza vaccines. It is challenging to induce long-term immunity against highly mutable viruses such as influenza due to immune escape, and to a propensity for antibody levels to decline with successive exposures to variant influenza virus strains. We have established several human influenza cohorts to investigate how immune responses evolve through successive exposures to different vaccine strains +/- interim infections. We also initiated a clinical trial to understand how different vaccine formulations perform in the context of repeated vaccination. A range of techniques have been developed to understand whether B cell and antibody responses are induced against variant epitopes versus epitopes that are largely conserved with past strains. This includes modelling of antibody titres against a large array of viruses; flow cytometric characterization of B cell reactivity and cross-reactivity to hemagglutinins representing different strains; single cell B cell receptor sequencing and antibody production; and reverse genetics to generate viruses with mutations of selected antigenic sites.

## *Collaborators*

Adam Kucharski (London School of Hygiene and Tropical Medicine),Barnaby Young (National Centre for Infectious Diseases (NCID) and Tan Tock Seng Hospital, Singapore), Andrew Ward (Scripps, San Diego, USA), Rogier van Doorn (Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam); Le Quynh Mai (National Institute of Hygiene and Epidemiology, Hanoi, Vietnam); Scott Boyd (Stanford University, Stanford CA, USA); Mark Thompson (Centre for Disease Control, Atlanta, USA); Derek Smith (Centre for Pathogen Evolution, Infectious Diseases Research Centre, Cambridge University, Cambridge, UK); Alain Townsend (Weatherall Institute of Molecular Medicine, Oxford University, Oxford UK); Maryna Eichelberger (US Food and Drug Administration, Silver Spring MD, USA; Kim Jacobson (Monash University); Katherine Kedzierska (The University of Melbourne); David Price (The University of Melbourne, VIDRL); Adam Wheatley (The University of Melbourne); Ben Cowling (Hong Kong University)

## *Highlights and developments 2024*

### *Influenza vaccine immunogenicity in health care workers*

During 2024 we continued to characterize immune responses to influenza vaccination among hospital workers participating in a longitudinal cohort study established in 2020 by Drs Sheena Sullivan (formerly of the Epidemiology unit) and Annette Fox (Immunology unit) at the Centre, and Adam Kucharski (London School of Hygiene and Tropical Medicine). The primary aims of the study are to determine the effect of repeated influenza vaccination on vaccine immunogenicity and to investigate immunological mechanisms underlying effects. A secondary objective was to investigate effects of repeated vaccination on vaccine effectiveness. Participants were recruited from 6 hospitals across Australia. They were asked to self-report their history of vaccination during five years prior to enrolment and to provide blood before and after vaccination and at the end of the influenza season. A total of 3808 pairs of pre and post vaccination sera were collected over the 4 years of the study (Figure 1A).

## Immunity to Respiratory Viruses (continued)

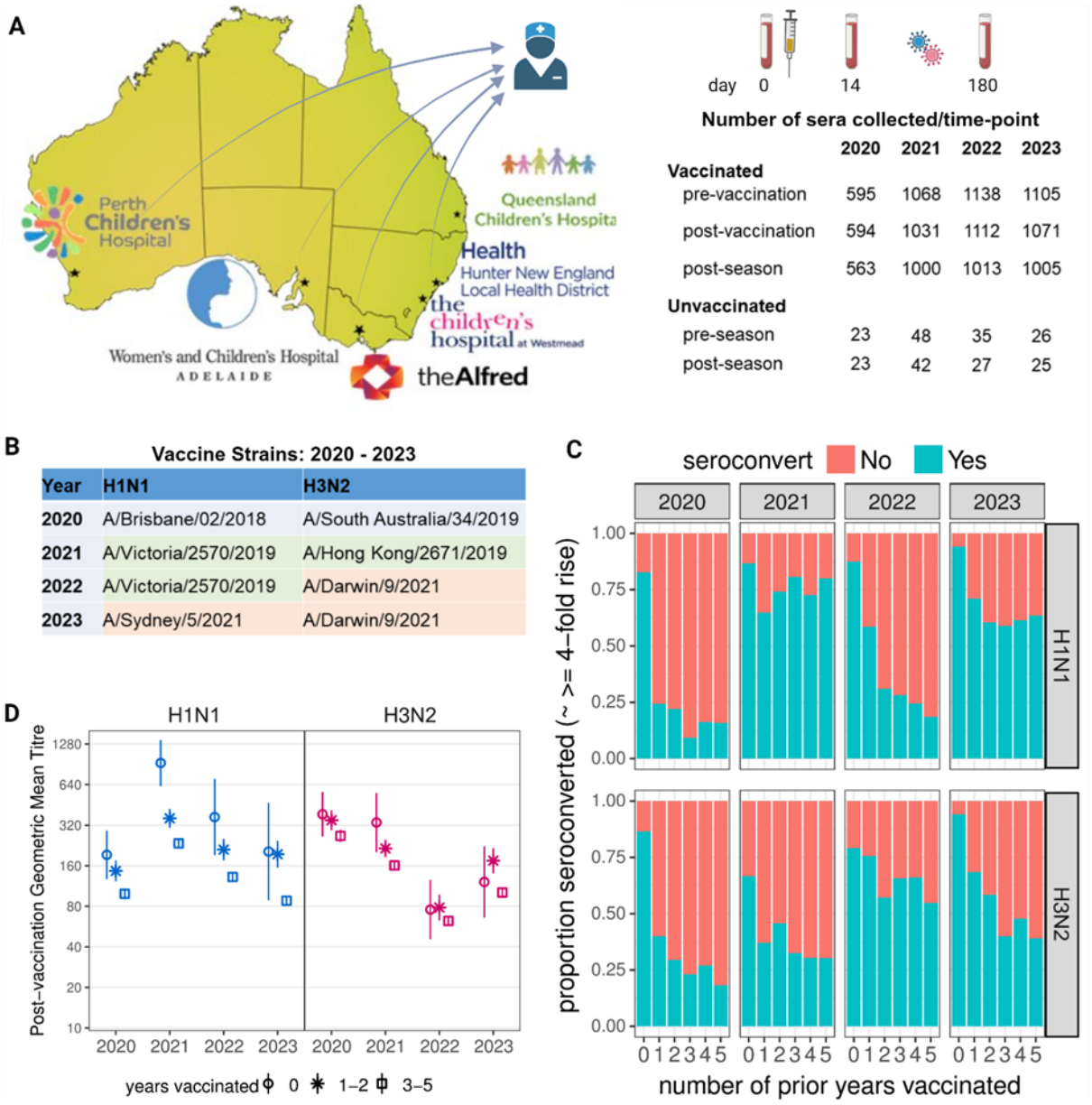



*Figure 1. Health Care Worker Influenza Vaccination Study Overview. (A) Study Design and numbers of participants and sera collected. (B) Influenza A strains included in vaccines each year and used for serology. (C) Proportions seroconverting against vaccine strains by year, subtype and number of prior years vaccinated. Proportions seroconverting decreased with increasing prior vaccination with weaker trends in years when there was substantial antigenic change to vaccine strains. (D) Post-vaccination geometric means titres against influenza A strains in each years vaccine by prior vaccination category. Created with Bioender.*

Sera were titrated by hemagglutination inhibition (HI) assays against viruses representing egg and cell-grown equivalents of the A(H1N1), A(H3N2), and B/Victoria strains in vaccines administered each year (Figure 1B). A (H1N1) vaccine strains changed in 2021 and 2023 and A(H3N2) strains changed in 2021 and 2022. Proportions of participants who seroconverted against vaccine strains decreased with increasing prior vaccination **(Figure 1C).** This trend was stronger in years when there was little or no antigenic change from previous vaccine strains. The impact of prior vaccination on seroconversion reflected lower post-vaccination titres among participants vaccinated 3-5 prior years, particularly compared to participants vaccinated 0 of 5 prior years **(Figure 1D).**

In the last 12 months we have been further exploring the breadth of antibodies induced against A(H3N2) viruses among healthcare workers who participated each of four years since 2020 or three years since 2021. We included all participants who had not been vaccinated for 5 years preceding enrolment (0 prior), and a subset of those vac-

## Immunity to Respiratory Viruses (continued)

cinated 1, 3, or 5 times previously. In total 688 samples from 66 participants were selected for serology against 22 A(H3N2) viruses spanning 2007 to 2022. Examples of antibody titre landscapes before and after vaccination each year from 2020 – 2023 are shown for a typical participant vaccinated 5 years prior to enrolment (Figure 2A-D) and 1 year prior to enrolment (Figure 2E-H). Notably, the participant with fewer prior vaccination at enrolment has higher titres and titre rises to prevailing vaccine strain until their last study year. The participant with 5 prior vaccinations exhibits little antibody rise after vaccination until 2022 when the vaccine strain changes to A/Darwin/9/2021, and their responses is greater again in 2023 when the vaccine strain is A/Darwin/9/2021 again. Analysis of the full dataset is ongoing but preliminary analysis suggest that vaccination is particularly poor at inducing antibodies against prevailing vaccine and circulating strains among repeatedly vaccinated. In addition, results suggest that attenuation associated with repeated vaccination may be alleviated by changes to the vaccine strain, consistent with previously reported observations for A(H1N1) vaccine strain change.

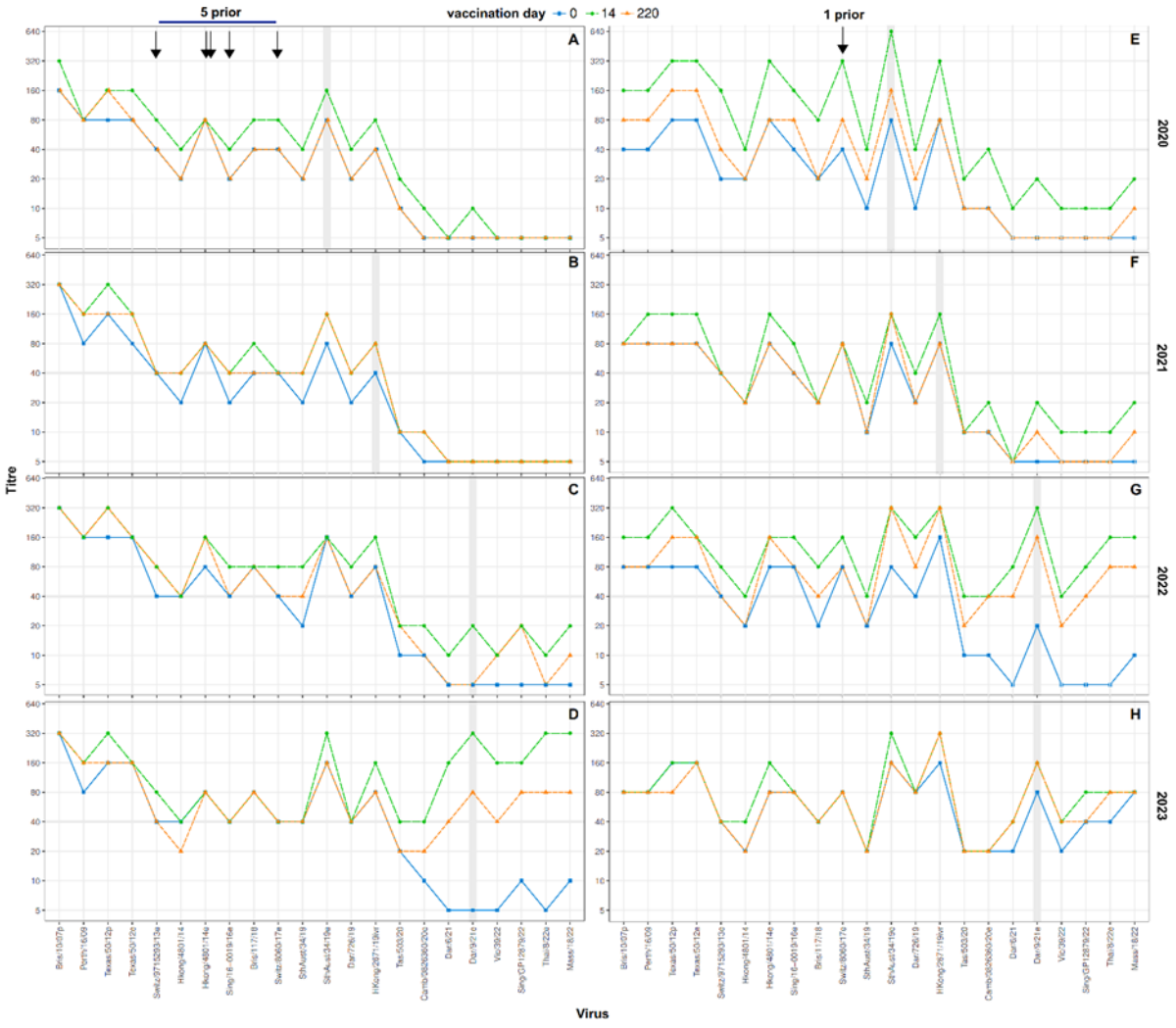



*Figure 2. Evolution of antibody breath against A(H3N2) strains over four years of influenza vaccination (2020-2023). Results are shown for one participant vaccinated 5/5 prior years (A-D) and another vaccinated 1/5 prior years (E-H). Sera collected on days 0, 14, and ~180 of vaccination each year were titrated against 22 viruses spanning 2007-2022 (x-axis). Thick vertical lines indicate the vaccine strain each year. Arrows indicate previously encountered in the previous 5 years.*

## Immunity to Respiratory Viruses (continued)

Our second aim is to investigate mechanisms underlying the effects of repeated vaccination and vaccine strain change on vaccine immunogenicity.

One hypothesis proposes that memory B cells out-compete naïve B cell for resources required to divide and differentiate so that with each new strain encountered antibodies become increasingly focused on epitopes that remain conserved. It is also thought that pre-existing antibodies prevent de novo B cells from participating in germinal centre reactions. To explore these hypothesis we have analysed B cells reactive with one or more HA's representing 2015-2023 vaccine strains in terms of frequency, isotype and phenotype. Samples from 213 participants have been assessed for reactivity against HA's representing five different A(H3N2) viruses. Results show that vaccination induced substantial rises in H3 HA reactive B cell frequencies among vaccine naïve (~ 4-fold rise) but not among repeatedly vaccinated (<2-fold rise) participants (Figure 3). Preliminary analysis also indicates that B cell reactivity is more biased towards prior vaccine strains among repeatedly vaccinated participants compared to those who were previously vaccine naïve.

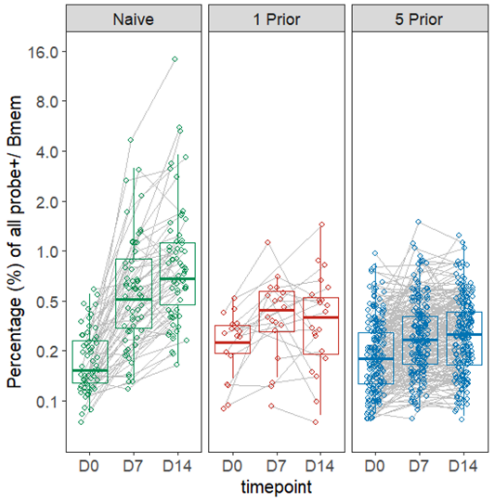



*Figure 3. A(H3N2) hemagglutinin (HA) reactive B cell frequencies induced by vaccination among health care workers who have been vaccinated 0/5 versus 5/5 years previously. PBMCs were stained with HA's representing A/Switzerland/9715293/2013, A/Hong Kong/4801/2014, A/South Australia/34/2019, A/Hong Kong/2671/2019, and A/Darwin/9/2021, each labelled with a unique fluorochrome, then with monoclonal antibodies to detect memory B cells. Plots show the percentages of memory B cells reactive with any of the five HA's. Results are shown for 213 participants including 68 vaccine-naïve, 21 with 1 prior vaccination and 98 vaccinated 5/5 prior years.*

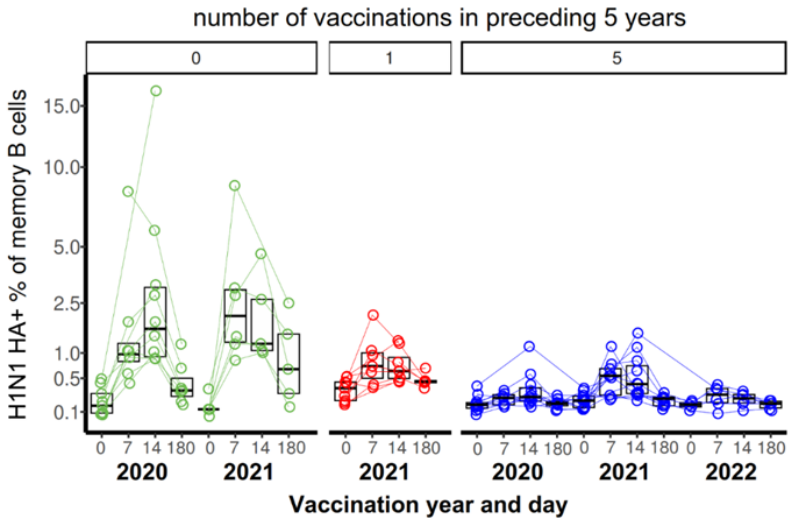



*Figure 4. A(H1N1) hemagglutinin (HA) reactive B cell frequencies induced by vaccination among health care workers who have been vaccinated 0/5, 1/5 or 5/5 years previously. PBMCs were stained with HA's representing A/Brisbane/02/2018, A/Victoria/2570/2019, and A/Sydney/5/2021, as described in Figure 3.*

## Immunity to Respiratory Viruses (continued)

Similar results were obtained from analysis of B cells reactive with A(H1N1) virus HA's representing three different vaccine strains (Figure 4). This analysis also shows that A(H1N1) HA-reactive B cells responses among the repeatedly vaccinated group improved in 2021 when the vaccine strain changed to A/Victoria/2570/2019, consistent with findings for antibody titres (Figure 1).

We have commenced single cell analysis of B cell receptor gene use and gene expression to explore whether repornding cells have B cell receptors that are characteristics of de novo B cells (IgD/M, low somatic hypermutation) or recalled memory B cells (IgG/A, high somatic mutation). We first optimized and validated that B cell reactivity against individual or multiple hemagglutinins (HA) defined by sequencing of oligo-tags replicated reactivity defined by fluorescence signals (Figure 5).

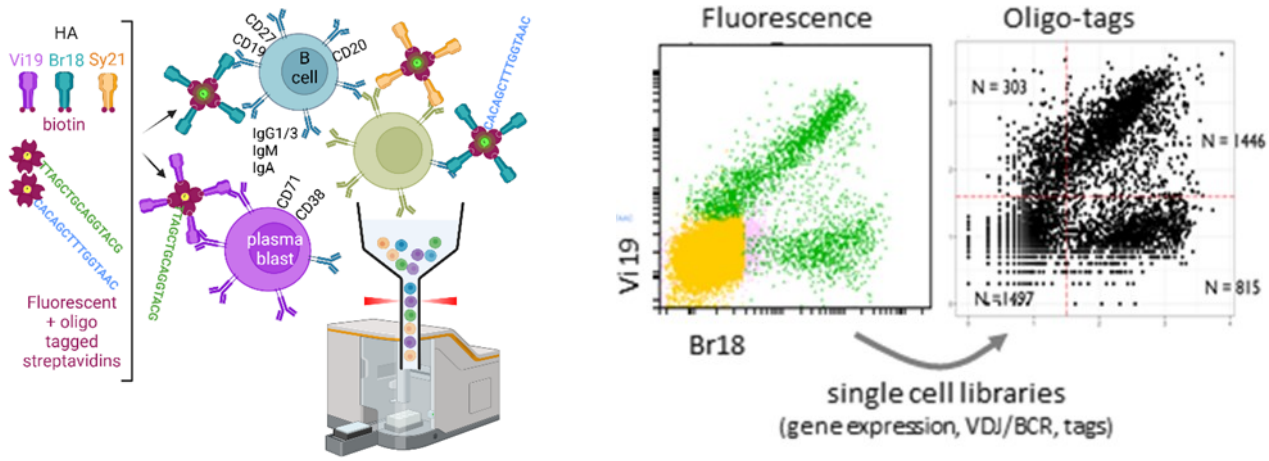



*Figure 5. Characterization of B cell reactivity against two different A(H1N1) virus hemagglutinins (HA) via flow cytometry versus single cell sequencing of oligo-tags. Cells were assessed for binding to fluorescence- and oligo-tagged HA of A/Brisbane/02/2018 (Br18) and A/Victoria/2570/2019 (Vi19) via flow cytometry and single cell sequencing, respectively.*

We have subsequently assessed samples collected across multiple time-points form several participants with different vaccination histories (Figure 6). These participants exhibit different patterns of reactivity with HAs of three different A(H1N1) viruses based on oligo-tag reads per cell. Analysis of B cell receptor gene use by HA-reactivity and prior vaccination status is ongoing.

# Immunity to Respiratory Viruses (continued)

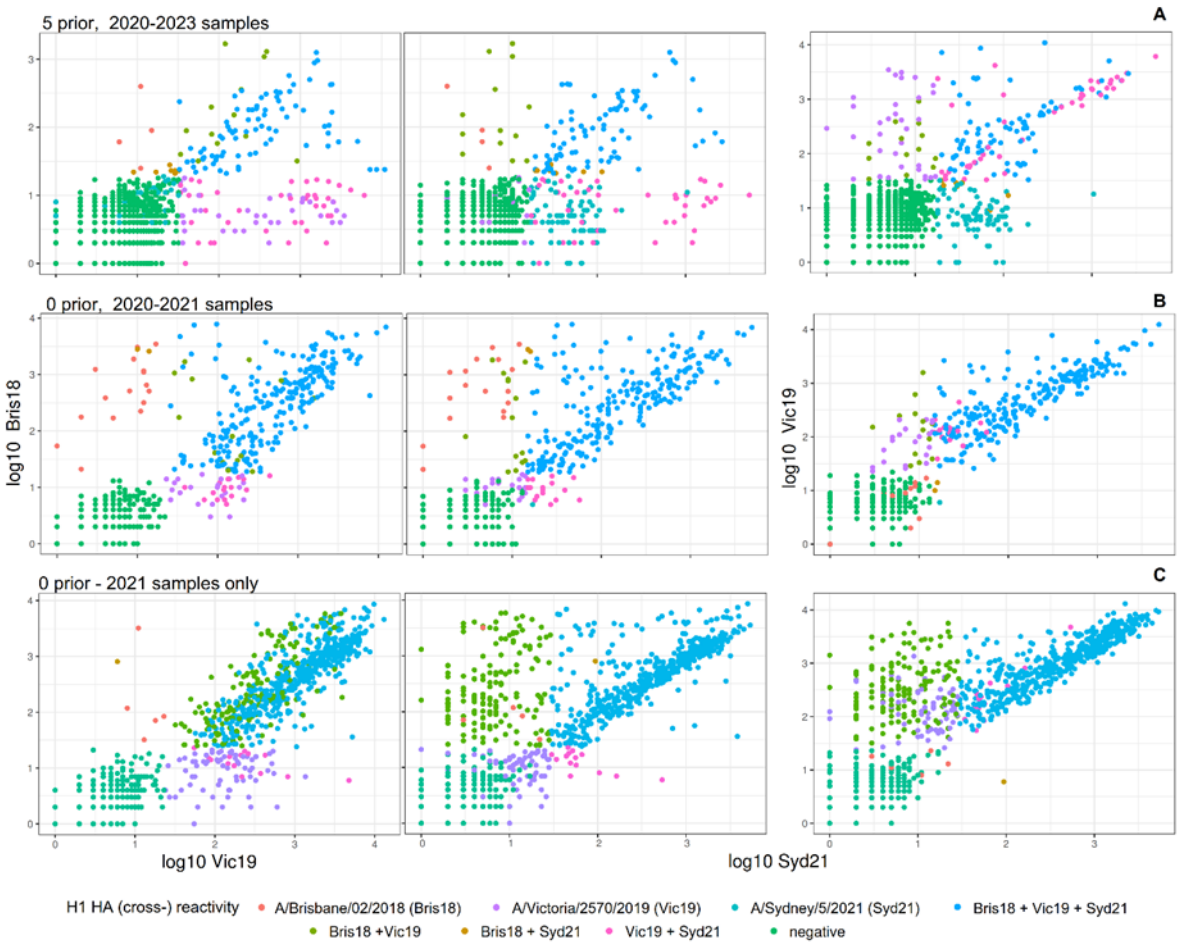



*Figure 6. B cell reactivity against three different A(H1N1) virus hemagglutinins (HA) based on single cell sequencing of oligo-tags that were unique to each HA. Samples collected pre and post vaccination for up to four years from three participants were assessed. Each row shows a different participant with individual plots showing different combinations of HA proteins on the x and y axes. Colours indicate the combination of HA reactivities of each cell (see legend).*

## Study of early life imprinting of influenza immunity

As per previous reports, we conducted a pilot study in infants and young children to examine how first exposure to influenza through vaccination versus infection affects subsequent antibody responses to vaccination. In 2019-202, we enrolled children aged 6-60 months including nine who had prior influenza A infection when A(H3N2) predominated; all had antibodies against A(H3N2) at enrolment. We also enrolled eight children who had been vaccinated in the year preceding enrolment; all had antibodies against A(H3N2), A(H1N1) or both at enrolment. Having established that antibody titres against A(H3N2) virus hemagglutinin and neuraminidase were markedly higher among infection-primed compared to vaccination-primed children, we set out to further define epitopes recognized in 2024. We extended serology to assess antibodies against the stem of A(H3N2) virus hemagglutinin (HA). We developed an ELISA using recombinant HA stem protein provided by Adam Wheatley and verified that this protein is recognized by anti-stem monoclonal antibodies indicating that it is correctly folded. We found that vaccination induced H3 HA stem binding antibodies in most influenza primed children, and that titres correlated strongly with hemagglutination inhibiting (HI) antibody titres. Similarly, analysis of plasma antibody binding to recombinant HA by electron microscopy by collaborators at Scripps, US shows substantial antibody binding to stem. Peripheral blood mononuclear cells (PBMCs) were isolated from children's blood samples. To date, we have performed single cell B cell receptor sequence and gene expression analysis on samples from five children. Fluorescence- and oligo-tagged H1, H3 and N2 proteins were used to sort B cells.

## Immunity to Respiratory Viruses (continued)

Around 0.4% of memory B cells within children's PBMC samples recognized one of the influenza proteins used. Sequences were obtained for 2753 sorted influenza reactive B cells, which clustered discretely according to H1, H3 and N2 oligo-tags. Preliminary analysis indicates that first year responses of infection-primed children were dominated by non-switched, H3-reactive B cells with low somatic hypermutation. H1-reactive and class-switched B cells were more common in previously vaccinated children and exhibited more somatic hypermutation. Only 10% of clones were expanded. The largest clones were from an infection-primed child, bound H3 or N2, and included IgD, M and G isotypes. Expanded H1-reactive clones were detected in vaccination primed children across successive years of vaccination. These preliminary results indicate that antibody responses and the composition of B cells responding to vaccination differ between infection- versus vaccination-primed children.

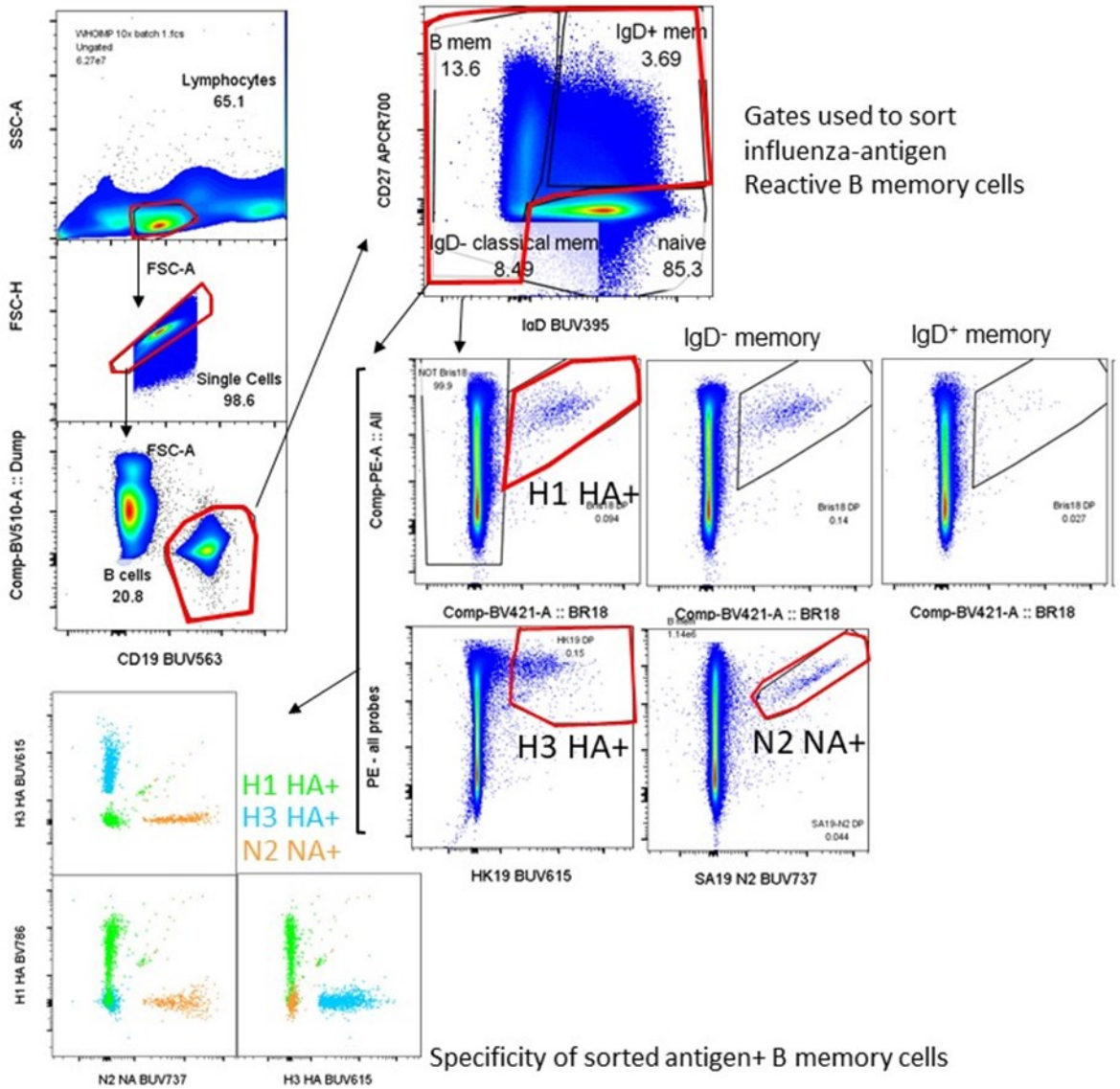



*Figure 7. Gates used to sort B cells reactive with HA's of H1N1 or H3N2 or with neuraminidase (NA) of H3N2 within 24 pooled samples from five children. Each protein was labelled with streptavidin-PE with a unique oligo-tag per protein, and also with an additional unique streptavidin-fluorochrome to detect double positive cells.*

## Immunity to Respiratory Viruses (continued)

### Clinical Trial Comparing the Immunogenicity of Three Influenza Vaccine Formulations in Healthy Adults with Infrequent Versus Frequent Prior Vaccination

In collaboration with Barnaby Young from Singapore we initiated a clinical trial to compare three influenza vaccine formulations with different manufacturing processes: 1) egg-grown; 2) cell-grown; and 3) recombinant protein. Healthy adults were recruited in Singapore and stratified into two groups by their influenza vaccination history: ) frequently vaccinated (3 or more vaccinations) and B) infrequently vaccinated (0 or 1 prior vaccinations). Following stratification participants were randomized 1:1:1 to receive recombinant (QIV-R), cell-grown (QIV-C) or egg-grown (QIV-E). A total of 362 participants were enrolled between September and December 2022, 120+/vaccine of whom 60 each per vaccine were infrequently and frequently vaccinated. Bleeds were performed pre-vaccination and ~ 14, 180 and 365 days after vaccination. Follow-up was completed by December 2023. A total pr 1400 sera were collected and sent to our lab. Sera have been assessed by HI assay against egg- and cell-grown vaccine strains as well as an extended set of A(H3N2) viruses spanning 2007 to 2022. Adjusted GMTs against cell-grown A(H3N2) were nearly 3-fold higher following QIV-R (GMT=100, 95% CI 84 to 120) compared with QIV-C (GMT=35; 95%CI 29 to 43; p<0.001) or QIV-E (GMT=36, 95%CI 29 to 43; p<0.001). Adjusted GMTs were similar for infrequent and frequent vaccinees who received QIV-R (GMT=110, 95%CI: 84 to 150 v GMT=93, 95%CI 70-120), but were higher among infrequent than frequent vaccinees who received QIV-E (GMT=50, 95%CI 38-66 v GMT=25, 95%CI: 19-34) or QIV-C (GMT=53, 95%CI 40-70 v GMT=24, 95%CI 18-32). The breadth of antibody recognition across A(H3N2) strains was also substantially greater among QIV-R recipients (Figure 8). Breakthrough influenza illnesses were detected in 5/120 QIV-E, 4/120 QIV-C and 1/119 QIV-R vaccinees. In summary, QIV-R induced substantially greater A(H3N2)-reactive antibodies, overcame attenuation associated with repeated vaccination and demonstrated lower risk of infection. QIV-R may be preferable for use in younger adult populations that require annual influenza vaccination.

## Immunity to Respiratory Viruses (continued)

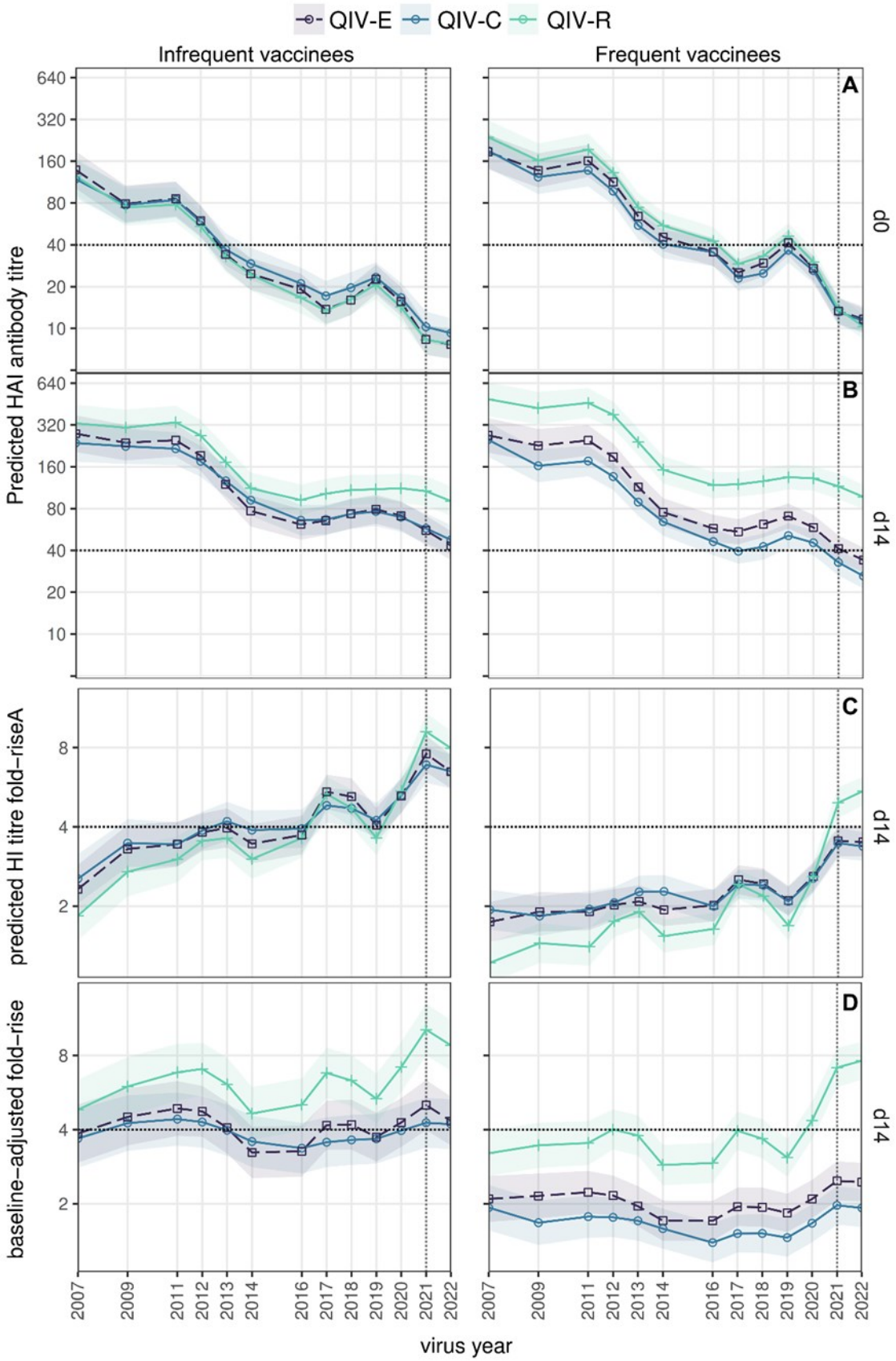



*Figure 8. Fitted antibody titre and titre rise landscapes across (AH3N2) viruses comparing QIV-E, QIV-C, and QIV-R and infrequent versus frequent vaccinees. Generalized additive models were used to fit titres and titre rises against A(H3N2) viruses grouped by year.*

## Collaborative Agreements

The Centre is party to two Collaborative Research and Development Agreements (CRADA's) with industry bodies. As with all potential collaborations with the commercial sector, these agreements have undergone review to ensure that they support the Centre's objective of advancing global public health, have scientific merit and adhere to the principles of neutrality, transparency, independence and accountability.

### CRADA with the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) (2012-2024)

**Centre staff:** Several staff are involved in this CRADA

**Overview:** This project aims to enhance the number and geographic range of influenza vaccine viruses isolated in eggs as candidates for reassorting and commercial influenza vaccine manufacture.

**Highlights and developments 2024:**

An egg isolate of A(H1N1)pdm09 was derived at the Centre (A/Victoria/4897/2022) and was used as the A(H1N1)pdm09 egg-derived vaccine component for the Southern Hemisphere (SH) influenza vaccine in 2024. This strain was also recommended for the Northern Hemisphere (NH) influenza vaccine for 2024-2025.

An egg isolate of A(H3N2) was derived at the Centre (A/Thailand/8/2022(H3N2)). This was used as the A (H3N2) egg-derived vaccine component for the SH influenza vaccine in 2024. This strain was also recommended for the NH influenza vaccine for 2024-2025.

For the full recommendation for the SH 2025 vaccine, click here.

For the full recommendation for the NH 2024-2025 vaccine, click here.

### CRADA with Seqirus (formerly BioCSL) (2021-2025)

**Centre staff:** Several staff are involved in this CRADA

**Overview:** The Centre continues to isolate and evaluate various seasonal influenza virus cell isolates derived from the use a proprietary qualified Seqirus cell line (MDCK 33016PF). Virus cell isolates were evaluated as potential cell culture candidate vaccine viruses (cc-CVV) based on their antigenic properties, genetic sequence and growth properties.

**Highlights and developments 2024:**

A qualified cell isolate of A(H3N2) was derived at the Centre (A/Sydney/1304/2022)) was a WHO approved like-strain for the A(H3N2) cell-derived vaccine component for the SH influenza vaccine in 2024. In addition, B/Singapore/WUH4618/2021 derived at the Centre was also a a WHO approved like-strain for B/Austria/1359417/2021 (B/Victoria lineage)-like virus for the 2024 SH vaccine. Qualified cell isolates of B/ Yamagata lineage were isolated at the Centre (B/Singapore/INFTT-16-0610/2016, B/Singapore/INFKK-16 -0569/2016,and B/Brisbane/9/2014) which were B/Phuket/3073/2013-like viruses, were available for use in the 2024 SH and the 2024-5 NH vaccines.

For the full list of candidate vaccine viruses, click here.

## Research Funding and Awards

Centre staff members are Chief, Co-, or Associate Investigators in grants administered across 2024 (includes those awarded outside of 2024):

**Annette Fox, Sheena Sullivan** and Adam Kucharski (London School of Hygiene and Tropical Medicine) are Principal Investigators and Project Directors on a US National Institute of Allergy and Infectious Diseases (NIAID), **National Institute of Health (NIH) grant project** "Does repeated influenza vaccination constrain influenza immune responses and protection?" The grant, totalling USD $4.2 million is awarded for the period 3 July 2019 – 30 June 2024. The grant will be administered by the University of Melbourne and the work will be undertaken at the Centre, the University of Melbourne, University of Western Australia, Alfred Hospital, University of Queensland, Sydney Children's Hospital Network, University of Adelaide and University of Newcastle. Kanta Subbarao is a Co-Investigator on the project. In addition, a USD $700,000 supplement to this project was given to investigate COVID-19 outcomes.

**Kanta Subbarao** is Chief Investigator for the $1,800,000 **NHMRC Investigator Grant** titled, 'Translating virus biology and host immunity for influenza control' for the period 2020-2025. The grant is administered by the University of Melbourne.

**Annette Fox** is the is the project lead for the US $549,030 **NIH/NIAID via the NIAID Centers of Excellence for Influenza Research and Response (CEIRR)** grant titled 'Dissecting influenza antibody evolution through successive exposures in early life' for the period 2023-2025. The grant is administered by The Royal Melbourne Hospital and Scripps.

**Kanta Subbarao** and **Saira Hussain** are Co-Investigators for the $587,911 **Cumming Global Centre Foundation Grants** project titled "Development of human and animal in vitro respiratory tract models for risk assessment of viruses with pandemic potential " for the period 2024-2027. The grant is administered by the University of Melbourne and the Royal Melbourne hospital.

**Kanta Subbarao** is Co-Investigator for the $2,911,774.24 **NHMRC Medical Research Future Fund (MRFF)** project titled, 'Bringing Optimised COVID-19 vaccine Schedules To ImmunoCompromised populations (BOOST-IC)', for the period 2022-2024. The grant is administered by the University of Melbourne.

**Kanta Subbarao** is Lead Investigator for the $998,339 **NHMRC MRFF** project titled, 'Aerosol Infection Research: Better mOdels to Reduce iNdoor Exposure (AIRBORNE)' within the MRFF 2021 program 'COVID-19 Treatment Access and Public Health Activities', for the period 2022-2025. The grant is administered by the University of Melbourne.

**Kanta Subbarao** is Associate Investigator for the $4,157,377.94 **NHMRC Medical Research Future Fund (MRFF)** project titled, 'The Platform trial In COVID-19 vaccine BOOsting (PICOBOO)', for the period 2022-2026. The grant is administered by the University of Melbourne.

**Kanta Subbarao** is a Lead Investigator **and Ian Barr** and **Saira Hussain** are Co-Investigators for the $3,034,704 **Pandemic Antiviral Discovery (PAD) initiative and the Novo Nordisk Foundation** project titled "Development of a Novel Long Acting Pan-Influenza Antiviral Drug" for the period 2024-2026. The grant is administered by the University of Melbourne and the Royal Melbourne Hospital.

## Research Students

### *PhD Candidates*

**Ms Jessie Goldsmith** continued her PhD candidature from July 2022 at the University of Melbourne. Her project is titled, 'What can we learn about influenza as Australia's COVID-19 suppression strategy ends?', under the supervision of **Sheena Sullivan**, Katherine Gibney, and Trish Campbell.

**Harry Stannard** continued his PhD candidature from July 2023 at the University of Melbourne, under the supervision of **Patrick Reading, Saira Hussain** and **Ian Barr**. His thesis title is 'Further development of the ferret model of influenza virus infection and its utility to explore novel therapeutic treatments.'

**Ziheng (Annie) Zhu** continued her PhD candidature from September 2022 at the University of Melbourne, under the supervision of **Annette Fox, Sheena Sullivan, Stephany Sanchez,** and **Yi Liu**. Her thesis is titled, 'When and why do memory B cells dominate responses to variant influenza virus strains?'.

### *Masters Students*

**Jiaheng Chen** completed a Master of Biomedical Science research project with the University of Melbourne under the supervision of **Patrick Reading**, titled 'Investigating the antiviral activity of ferret interferon-inducible transmembrane proteins against respiratory viruses' (Feb 2023 – Oct 2024).

### *Work experience students*

Fathima Shyma Murshideen from Deakin University was hosted at the Centre from 4-20th March 2024 as part of the undergraduate work experience program at the Deakin School of Biomedical Sciences.

VIDRL work experience program:

Edwin Howell from Cornish College was at the Centre from 13-17th May 2024.

Tyler Miet from University High School and Jade De Run from Methodist Ladies College were at the Centre from 24-28th June 2024.

AJ Armedilla from Baybrook College was at the Centre from 29th July-2th August 2024.

We also conducted laboratory tours for 70 1st year Bachelor of Science students from the University of Melbourne on 9th October 2024.

# Communications and Advisory Activities

The Centre actively contributes to the knowledge and understanding of influenza in scientific and public health domains through many different forums. Centre staff members participate in WHO meetings and workshops to support the ongoing work and growth of WHO GISRS, as well as providing advice on influenza to the Australian Government. Centre staff members publish peer-reviewed journal papers and present numerous talks and posters.

## Publications and Reports

The Centre continued to build its research and surveillance profile with the publication of 56 original research papers, reviews and reports in 2024.

### *Centre Publications 2024*


1. Adair, A., Tan, L. L., Feng, J., Girkin, J., Bryant, N., Wang, M., Mordant, F., Chan, L.-J., Bartlett, N. W., **Subbarao, K.**, Pymm, P., & Tham, W.-H. 2024. Human coronavirus OC43 nanobody neutralizes virus and protects mice from infection. Journal of Virology, Jun 13;98(6):e0053124. doi: 10.1128/jvi.00531-24. Epub 2024 May 6. PMID: 38709106; PMCID: PMC11237593

2. **Barr IG.** Influenza in Australia before, during and after the COVID-19 pandemic. Microbiology Australia. 2024 Oct 45, 188-192. doi: https://doi.org/10.1071/MA24052

3. **Barr IG, Subbarao K.** Implications of the apparent extinction of B/Yamagata-lineage human influenza viruses. NPJ Vaccines. 2024 Nov 16;9(1):219. doi: 10.1038/s41541-024-01010-y. PMID: 39550399; PMCID: PMC11569178.

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10. Edler, P., Schwab, L. S. U**., Aban, M., Wille, M., Spirason, N., Deng, Y. M.,** Carlock, M. A., Ross, T. M., Juno, J. A., Rockman, S., Wheatley, A. K., Kent, S. J**., Barr, I. G.,** Price, D. J., & Koutsakos, M.

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18. Islam A, **Wille M,** Rahman MZ, Porter AF, Hosaain ME, Hassan MM, Shirin T, Epstein JH, Klaassen M. Phylodynamics of high pathogenicity avian influenza virus in Bangladesh identifying domestic ducks as the amplifying host reservoir. Emerg Microbes Infect. 2024 Dec;13(1):2399268. doi: 10.1080/22221751.2024.2399268. Epub 2024 Sep 10. PMID: 39207215; PMCID: PMC11389634.

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*Centre Publications 2024 (continued)*


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25. Lu C, **Barr IG,** Lambert S, Mengersen K, Wang L, Yang W, Li Z, Vardoulakis S, Bambrick H, Hu W. Shifts in seasonal influenza patterns in Australia during and after COVID-19: A comprehensive analysis. J Infect Public Health. 2024 Dec 16;18(1):102620. doi: 10.1016/j.jiph.2024.102620. Epub ahead of print. PMID: 39708760.

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*Centre Publications 2024 (continued)*


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51. **Wille M,** Broz I, Cherrington T, Crawley A, Farrugia B, Ford M, Frost M, Grimsey J, Kirkland PD, Latimore S, Lynch SE, Martin S, Matereke C, Mee PT, Neave MJ, O'Dea M, Read AJ, O'Riley K, Stevens V, Thayaparan S, Zufan S, Ban de Gouvea Pedroso S, Grillo V, Breed AC**, Barr IG**, Holmes EC, Klaassen M, Wong FYK. Contrasting dynamics of two incursions of low-pathogenicity avian influenza virus into Australia. Virus Evol. 2024 Sep 10;10(1):veae076. doi: 10.1093/ve/veae076. PMID: 39416286; PMCID: PMC11482279.

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54. **Wille M,** Dewar ML, Claes F, Thielen P, Karlsson EA. A call to innovate Antarctic avian influenza surveillance. Trends Ecol Evol. 2024 Nov 29:S0169-5347(24)00278-7. doi: 10.1016/j.tree.2024.11.005. Epub ahead of print. PMID: 39616087.

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56. Zhong S, Ng TWY, Skowronski DM, Iuliano AD, Leung NHL, Perera RAPM, Ho F, Fang VJ, Tam YH, Ip DKM, Havers FG, Fry AM, Aziz-Baumgartner E, **Barr IG**, Peiris M, Thompson MG, Cowling BJ. Influenza A(H3N2) Antibody Responses to Standard-Dose Versus Enhanced Influenza Vaccine Immunogenicity in Older Adults and Prior Season's Vaccine Status. J Infect Dis. 2024 May 15;229(5):1451-1459. doi: 10.1093/infdis/jiad497. PMID: 37950884; PMCID: PMC11095559.

## Presentations

Centre staff members presented talks and posters at numerous events during 2024, including national and international conferences, WHO meetings, educational lectures and research seminars.

### *ORAL PRESENTATIONS*

| Event/Institute; Location, date | SPEAKER, Title(s) |
|---|---|
| Obstacles, Opportunities, Pathways & Synergies (OOPS) Seminar, 25 June 2024, Doherty Institute Melbourne, Australia. | SAIRA HUSSAIN: Development of a ferret precision cut-lung slice model to study influenza virus infection. |
| Centre for Infectious Diseases and Microbiology (CIDM) – Public Health, Avian influenza webinar, 7 June 2024, webinar. | MICHELLE WILLE: Panzootic HPAI H5N1 clade 2.3.4.4b – how did we find ourselves here? |
| Australian Veterinary Poultry Association Scientific Meeting 2024, 15 May 2024,Queensland Australia. | MICHELLE WILLE: Risk and consequences of HPAI incursion to Australia |
| ETNTAC Seabird Workshop, June 2024, webinar. | MICHELLE WILLE: Avian influenza threat: resources available for planning and preparedness |
| International Forum on the Ecology and Evolution of Avian Influenza, 25 June 2024, webinar. | MICHELLE WILLE: Evolutionary ecology of avian influenza viruses in Australia, and how this may inform our preparation for HPAI |
| Wildlife Health Forum Victoria, 11 June 2024, Melbourne, Australia. | MICHELLE WILLE |
| New South Wales Department of Health [Hunter Valley] One Health Meetings, 30 May 2024, webinar. | MICHELLE WILLE: A brief overview of the HPAI panzootic and what we have learned about LPAI in Australia |
| Victorian Department of Health Weekly Surveillance Meetings, June 2024, webinar. | MICHELLE WILLE: A brief overview of the HPAI panzootic |
| How to protect wildlife from avian flu in UNESCO World Heritage sites, Biosphere Reserves and Ramsar sites, 13 May 2024, webinar. | MICHELLE WILLE: The plight of wildlife in the current avian influenza panzootic |
| Emory University Infectious Disease Across Scales seminar, April 2024, webinar. | MICHELLE WILLE: Avian influenza virus across scales |
| International Association of Antarctica Tour Operators (IAATO) Town Hall HPAI, 8 April 2024, webinar. | MICHELLE WILLE: Avian influenza in Antarctica |
| UNESCO WesternPort BioSphere, 21 June 2024, Melbourne, Australia. | MICHELLE WILLE: The plight of wildlife in the current avian influenza panzootic |
| The World Influenza Conference, 6-7 July 2024, Hainan China. | YI-MO DENG: Advances in laboratory detection technology for respiratory pathogens |

## *ORAL PRESENTATIONS (continued)*

| Event/Institute; Location, date | SPEAKER, Title(s) |
|---|---|
| AIID Action seminar series, 7 August 2024, Melbourne, Australia. | MICHELLE WILLE: H5N1 Pandemic Preparedness |
| AIID Action seminar series, 7 August 2024, Melbourne, Australia. | IAN BARR: H5N1 and the Melbourne WHO Collaborating Centre for Reference and Research on Influenza activities |
| ASM Vic Branch Public Health Night 2024, 28 August 2024, Melbourne, Australia. | MICHELLE WILLE: Avian Influenza: a One Health problem |
| Biodiversity Council H5N1 HPAI Webinar, 26 August 2024, webinar. | MICHELLE WILLE: The plight of wildlife in the current avian influenza panzootic |
| Queenstown Pathogen Genomics Meeting, 3-6 September 2024, New Zealand. | MICHELLE WILLE: Evolutionary ecology of avian influenza viruses in Australia, and how this may inform our preparation for HPAI |
| The 5th Beijing Forum on Global Health, 14 Sep 2024, Beijing, China. | YI-MO DENG: How NGS reshaped public health responses to respiratory pathogen outbreaks |
| Viral Infectious Diseases theme: Obstacles, Opportunities, Pathways & Synergies (OOPS) Seminars, 23 September 2024, Melbourne Australia. | ANNIE ZHU: Unpacking the attenuating effects of repeated influenza vaccination (A/H3N2) |
| Options XII for the Control of Influenza, 29 September to 2 October 2024, Brisbane, Australia. | IAN BARR: Predicting the unpredictable: advancements in influenza surveillance |
| | MICHELLE WILLE: Emergence and spread of high pathogenicity avian influenza (HPAI) H5 in wildlife of South America and Antarctica |
| | YI MO DENG: Expanded diversity of influenza viruses following the COVID-19 pandemic induced bottleneck in Australia |
| | STEPHANY SANCHEZ OVANDO: Influenza vaccine A (H1N1)pdm09 strain change effects on immunogenicity among repeatedly vaccinated healthcare workers |
| | JESSIE GOLDSMITH: Using data from the COVID period to improve our understanding of the burden of influenza mortality |
| | PATRICK READING: Bovine Myxovirus resistance protein 1 mediates antiviral activity against human and avian influenza A Viruses |

| *ORAL PRESENTATIONS (continued)* | |
|---|---|
| **Event/Institute; Location, date** | **SPEAKER, Title(s)** |
| Pandemic Research Alliance International Symposium 29-30 October 2024, Guangzhou, China. | PATRICK READING: Harnessing Innate Immune Responses to Reduce the Impact of Respiratory Virus Disease |
| Pirbright Institute (UK) seminar series, 7 November 2024, online. | MICHELLE WILLE:HPAI panzootic and impact on wild birds, globally |
| Ecology and Evolution of Influenza Viruses, 18 -19 November 2024, Georgia, USA. | MICHELLE WILLE: Ecology and evolution of avian influenza viruses, from LPAI to HPAI - highlights and lowlights |
| St. Jude Children's Research Hospital seminar series, 21 November 2024, TN, USA. | MICHELLE WILLE: HPAI panzootic and impact on wild birds, globally |
| 12th Australasian Virology Society Meeting 2024, 2-5 December 2024, Creswick, Australia. | ASHWIN MURALEETHARAN: Ferret Precision-Cut Lung Slices: Development of a High-throughput ex-vivo model to Study Influenza Virus Infection |
| The Avian Influenza symposium, 10 December 2024, Cambodia. | IAN BARR: Human Seasonal Influenza Situation and Human Spillovers in Asia Pacific |
| One Health Aotearoa Symposium 03 December 2024, New Zealand. | IAN BARR: Avian Influenza; Internal and external threats to humans and animals |

| *POSTER PRESENTATIONS* | |
|---|---|
| **Event/Institute; Location, date** | **SPEAKER, Title(s)** |
| Asia-Pacific and Vaccine Immunotherapy Congress (APVIC 2024), 14-17 May 2024, Melbourne, Australia. | MALET ABAN: Immunity to zoonotic influenza viruses in individuals vaccinated with seasonal influenza vaccines |
| Asia-Pacific Vaccine and Immunotherapy Congress (APVIC 2024), 14-17 May 2024, Melbourne, Australia. | HARRY STANNARD: Trends in viral replication and lung pathogenesis of influenza A(H1N1)pdm09 viruses from 2009 to 2022 in the ferret model |
| Asia-Pacific Vaccine and Immunotherapy Congress (APVIC 2024), 14-17 May 2024, Melbourne, Australia. | NIKITA DESHPANDE: Characterisation of viral interactions between Influenza, SARS-CoV-2 and other circulating respiratory viruses |
| Options XII for the Control of Influenza, 29 September to 2 October 2024, Brisbane, Australia. | SAIRA HUSSAIN: Multiple neuraminidase mutations in serial samples from an immunocompromised patient infected with A(H3N2)<br><br>ARADA HIRANKITTI: Different B cell receptor sequence and transcriptomic profiles following vaccination with BNT162b2 compared with AZD1222<br><br>MALET ABAN: Immunity to zoonotic influenza viruses in individuals vaccinated with seasonal influenza vaccines<br><br>HARRY STANNARD: Replication of contemporary A (H1N1)pdm09, A(H3N2) and B/Victoria lineage influenza viruses in the upper and lower respiratory tract of ferret<br><br>ANNETTE FOX: Enhanced influenza vaccines extend A (H3N2) antibody reactivity in older adults but attenuating effects of prior vaccination persist<br><br>TASOULA ZAKIS: The circulation of human influenza in Australia post the COVID-19 pandemic (2022-2024)<br><br>MEGAN TRIANTAFILOU: The circulation of human influenza in Australia post the COVID-19 pandemic (2022 -2024)<br><br>JESSIE GOLDSMITH: Variability of influenza vaccine effectiveness (VE) by subtype: a systematic review and meta-analysis of test-negative design studies<br><br>PRESA CHANTHALAVANH: Supporting molecular detection and characterisation of respiratory viruses with epidemic and pandemic potential in the Asia Pacific Region<br><br>HEIDI PECK: Circulation of and seropositivity to influenza B/Yamagata-lineage viruses prior to and after the covid-19 pandemic in Australia<br><br>TANYA DIEFENBACH-ELSTOB: Influenza in Australian FluCAN and PAEDS sentinel hospitals in 2023<br><br>PATRICK READING: Restriction factor SAMHD1 does not inhibit influenza A virus replication in human epithelial- or macrophage-like cells<br><br>MELKAMU TESSEMA: Mouse guanylate-binding proteins of the chromosome 3 cluster do not inhibit influenza A virus in vitro or in vivo |

***POSTER PRESENTATIONS Continued***

| Event/Institute; Location, date | SPEAKER, Title(s) |
|---|---|
| Options XII for the Control of Influenza, 29 September to 2 October 2024, Brisbane, Australia. | JESSICA HADIPRODJO: Influenza vaccine responses among young children first exposed to influenza antigens via infection versus vaccination<br><br>YI LU: Superior immunogenicity of mRNA over adenoviral vectored COVID-19 vaccines translates into stronger B cell responses among vaccinees with break-through infections<br><br>ZIHENG ZHU: Unpacking the attenuating effects of repeated influenza vaccination<br><br>LOUISE CAROLAN: Antibody responses against influenza A decline with successive years of annual influenza vaccination<br><br>IAN BARR: Genetic Surveillance of RSV: Insights from 25 RSV Project Countries, 2019-2023<br><br>XIAOMIN DONG: A rapid and sensitive ONT workflow for RSV whole-genome sequencing of clinical samples<br><br>MICHELLE WILLE: Evolutionary ecology of avian influenza viruses in Australia, and how this may inform our preparation for HPAI<br><br>NIKITA DESPANDE: Comparison of human influenza virus susceptibilities to neuraminidase inhibitors before and after the COVID-19 pandemic in the Australia - Asia Pacific region<br><br>YI-MO DENG/CLYDE DAPAT: Expanded diversity of influenza viruses following the COVID-19 pandemic-induced bottleneck in Australia<br><br>STEPHANY SANCHEZ: Influenza vaccine A(H1N1) pdm09 strain change effect on immunogenicity among repeatedly vaccinate healthcare workers |
| 12th Australasian Virology Society Meeting 2024, 2-5 December 2024, Creswick, Australia. | SAIRA HUSSAIN: Replication of contemporary A(H1N1) pdm09, A(H3N2) and B Victoria lineage influenza viruses in the upper and lower respiratory tract of ferrets.<br><br>SAIRA HUSSAIN: Multiple neuraminidase gene mutations in serial samples from an immunocompromised influenza A(H3N2) infected patient treated with oseltamivir |

## Committees and Advisory Groups

Centre staff members served on the following governing boards, committees and advisory groups during 2024.

**Ian Barr**

Australasian Vaccine & Immunotherapeutics Development Group, *Member*

Australian Influenza Vaccine Committee (Therapeutic Goods Administration), *Member*

Centre of Excellence for Influenza Research and Surveillance) program at St Jude Children's Research Hospital, Scientific Advisory Committee

Doherty Institute PC3 Laboratory Users Group, *Member*

Public Health Laboratory Network (Department of Health and Aged Care), *Committee member*

Influenza and other respiratory viruses, *Editorial Board*

**Yi-Mo Deng**

WHO Working Group for GISRS PCR detection for influenza surveillance, *Member*

**Annette Fox**

International Committee on Advancing Pandemic and Seasonal Influenza Vaccine Preparedness and Response. US National Academy of Medicine. 2020-2021, *Member*

Victorian Infection and Immunity Network, *Committee member*

International Society for Influenza and Other Respiratory Viruses, *Council Member*

**Saira Hussain**

WHO Expert Working Group for GISRS Surveillance of Antiviral Susceptibility, *Member*

**Katie Milne**

Medical Laboratory Quality Network, *Member*

Victorian Infectious Disease Reference Laboratory NATA Action Group*, Member*

**Patrick Reading**

Options for the Control of Influenza XII, Brisbane 2024, *Local organising committee*

Influenza and Other Respiratory Viruses, *Editorial board*

Australian Influenza Vaccine Committee ( Therapeutic Goods Administration), *Member*

Peter Doherty Institute for Infection and Immunity, Global Health Capacity and Capability Building Steering Committee, *Member*

VIDRL Regional and Global Health Steering Committee, *Member*

Pacific Public Health Surveillance Network (PPHSN) Technical Working Group, *Member*

## Committees and Advisory Groups (continued)

**Michelle Wille**

Antarctic Wildlife Health Working Group. Expert Group for Birds and Marine Mammals. Scientific Committee for Antarctic Research, *Member*

Avian Influenza Virus AUSVETPLAN working group, *Member*

Discover Research Steering Committee for Doherty Computational Sciences Initiative, *Member*

Doherty Institute Green DISCO, *Member*

East Asian Australian Flyway Partnership Avian Disease Working Group, *Member*

eMERGE (Early and Mid-career network Committee, Peter Doherty Institute for Infection and Immunity, *Member*

Global Consortium for highly pathogenic avian influenza viruses in seabirds, *Chair*

High Pathogenicity H5N1 Avian Influenza Intersessional Group, Population and Conservation Status Working Group of the Agreement for the Conservation of Albatrosses and Petrels (ACAP), *Member*

MicroSeq Conference Organising Committee, *Member*

National Avian Influenza Wild Bird Surveillance, *Steering Committee*

Victorian Wader Study Group, *Member*

Wildlife Health Australia, *Member*

PLoS Pathogens, *Editor*

Waterbirds, *Editor*

Virology and Molecular Ecology (special issue), *Editor*

**Tanya Diefenbach-Elstob**

National Influenza Surveillance Committee (Department of Health and Aged Care), *Proxy*

The Computational Sciences Initiative Public Health Steering Committee, *Member*

**Jessica Miller**

National Respiratory Infections Surveillance Committee (previously National Influenza Surveillance Committee) (Department of Health and Aged Care), *Member*

Doherty Public Health Leadership Group, *Member*

## Visitors to the Centre

The Centre was pleased to host the following visitors during 2024.

| Date | VISITOR and affiliation |
|---|---|
| 10-11 April 2024 | MELISSA YOW; Visiting Scientist, Tasmania Health, Australia. |
| 6-24 May 2024 | DAVID HODGSON; Visiting Scientist, London School of Hygiene and Trop Medicine, UK. |

## Visitors to the Centre (continued)

| Date | VISITOR and affiliation *Continued* |
|---|---|
| 16 May 2024 | DR SEBASTIAN MAURER STROH; Deputy Director, Bioinformatics Institute A*STAR, Singapore. |
| 22-24 May 2024 | DR ADAM KUCHARSKI; Epidemiologist, London school of Hygiene and Tropical Medicine, UK. |
| 05 June 2024 | DANNY KO; Visiting scientist, NSW Pathology. |
| 24-28 June 2024 | ABDUL AHAD; Visiting scientist, National Institute of Health, Pakistan.<br>NAZISH BADAR; Laboratory Coordinator, National Institute of Health, Pakistan. |
| 04 October 2024 | We welcomed 5 Guests from Hokkaido University, Japan, as part of their visit to the wider Doherty Institute visit. |
| 04-11 October 2024 | JONJEE CALAOR-MORIN. CATHERINE CALZADO-DACASIN, VINA LEA ARGUELLES, Institution Research Institute of Tropical Medicine (RITM DOH), The Philippines. |
| 4-17 October 2024 | THU NGOC NGUYEN and MINH HANG DUONG; Pasteur Institute, Vietnam. |
| 7-11 October 2024 | LORIN ADAMS; The Francis Crick Institute, UK. |
| 4 October 2024 | NICOLE WOLTER and LORENS MAAKE; National Institute for Communicable Diseases of South Africa, South Africa. |
| 07 October – 20 December 2024 | MIN HE; Nanjing CDC, China. |
| 20 October – 19 November 2024 | ZHAO MIN FENG; Beijing CDC, China. |
| 22-27 September 2024 | We welcomed 45 guests from across the world to attend the WHO Consultation on the Composition of Influenza Virus Vaccines for Use in the 2025 Southern Hemisphere Influenza Season which was hosted at Peter Doherty Institute for Infection and Immunity, Melbourne, Australia. |

## Engagement in WHO activities

| Event; Location, Date | Centre staff involved |
|---|---|
| WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2023-2024; Geneva, Switzerland, 19-23 February 2024. | Ian Barr and Kanta Subbarao attended. |
| The Pacific Respiratory Disease Surveillance Meeting; Fiji, 25-28 March 2024. | Attended by Patrick Reading, Michelle Wille and Presa Chanthalavanh.<br><br>Patrick Reading presented a regional update of influenza activity and a tutorial on use of PCR for respiratory virus detection. |
| WHO Information session on HPAI, Webinar, Palau, 4 April 2024. | Michelle Wille presented a talk titled “Bird migration and risk of HPAI introduction to Oceania”. |
| 13th Meeting of the WHO Expert Working Group on Surveillance of Influenza Antiviral Susceptibility (AVWG) for GISRS, Lyon, France, 13-14 June 2024. | Saira Hussain presented the annual update on antiviral susceptibility monitoring and development of Baloxavir phenotypic testing at VIDRL. |
| 14th Meeting of the WHO Working Group for the Molecular Detection and Subtyping of Influenza Viruses and the use of Next Generation Sequencing; Lyon, France, 11-12 June 2024. | Yi Mo Deng provided updates on molecular surveillance work for Influenza and RSV at the WHO CC Melbourne and the New development of NGS and applications for respiratory viruses characterisation. |
| WHO Collaborating Centre for Reference and Research Seminar Series, Melbourne, Australia, 25 July 2024. | Michelle Wille attended. |
| Joint National International ILI/SARI surveillance review and data management training; Indonesia, 29 July - 2 August 2024. | Heidi Peck and Patrick Reading participated in the review.<br><br>Patrick Reading led the laboratory review team. |
| 2024 GISAID Bioinformatics Workshop for respiratory viruses; Brisbane, Australia, 28 Sep 2024. | Yi-Mo Deng gave a talked titled “Quality control is critical for NGS data analysis”. |
| 17th Bi-Regional Meeting of National Influenza Centres and Influenza Surveillance in the WHO’s Western Pacific and South-East Asia Regions; Philippines, 20-22 November 2024. | Ian Barr and Patrick Reading facilitated group work sessions, and Clyde Dapat, Jessica Miller, and Yi-Mo Deng attended.<br><br>Ian Barr presented an update on influenza activity in the Southern Hemisphere. |
| WHO Northern Hemisphere TC1; virtual, 17 December 2024. | Heidi Peck, Ian Barr and Patrick Reading attended. |

## Other Conference Participation and Professional Engagement

Centre staff members also participated in the following events as attendees and/or in other roles during 2024

| Event; Location, date | Centre staff involvement |
|---|---|
| World Health Summit Regional Meeting 2024; Melbourne, 22-24 April 2024. | Vishma Varsani attended. |
| Asia-Pacific Vaccine and Immunotherapy Congress; Melbourne 14-17 May 2024. | Ian Barr was a member of the organising committee and attended. |
| Asia-Pacific Vaccine and Immunotherapy Congress; Melbourne 14-17 May 2024. | Malet Aban, Heidi Peck and Monica Bobbitt attended. |
| Communicable Diseases & Immunisation Conference 2024; Brisbane, 11-13 June 2024. | Tanya Diefenbach-Elstob and Ian Barr attended. |
| Accreditation Matters Conference 2024; Sydney, 25-26 June 2024. | Katie Milne attended. |
| Options XII for the Control of Influenza, 29 September to 2 October 2024, Brisbane, Australia. | Xiaomin Dong, Li Yiu, Patrick Reading, Heidi Peck, Malet Aban, Nikita Deshpande, Tanya Diefenbach-Elstob, Clyde Dapat, Saira Hussain, Harry Stannard, Annette Marsh, Jessica Hadiprodjo, Arada Hirankitti, Stephany Sanchez, Louise Carolan, Ziheng Zhu, Tasoula Zakis, Yi-Mo Deng, Megan Triantafilou, Presa Chanthalavanh, Olivia Lay, Ian Barr, Melkamu Tessema and Jessica Miller attended. |
| 12t$^{h}$ Australasian Virology Society Meeting 2024, 2-5 December 2024, Creswick, Australia. | Patrick Reading, Saira Hussain and Ashwin Muraleetharan attended. |
| The NRISC Annual meeting, 12 November 2024, Canberra, Australia. | Tanya Diefenbach-Elstob and Jessica Miller attended. |
| Pandemic Research Alliance International Symposium 29-30 October 2024, Guangzhou, China. | Patrick Reading attended. |
| AIVC Recommendations for the Composition of Influenza Vaccines for Australia 9 October 2024, Canberra, Australia. | Ian Barr and Patrick Reading attended. |

## Community Engagement

The Director, Deputy Director and other staff members participated in requests from media representatives for interviews and comments throughout 2024.

**Ian Barr**

- Appeared on ABC radio to comment on “Deadly bird flu reaches Antarctica, is Australia next?”, on 27 February 2024.
- Appeared on Channel 9 to comment on “Australia has experience of a surge in summer flu cases” on 7 March 2024.
- Was quoted on Aus Doc “Return to trivalent flu shots ‘likely’ as previously widespread strain disappears” on 7 March 2024. https://www.ausdoc.com.au/news/return-to-trivalent-flu-shots-likely-as-previously-widespread-strain-disappears/?cspt=1710109776%7C99ef4fb75352878048fa2a9fb5bfc377
- Appeared on SBS News to comment on “Why this year's flu vaccine includes a strain that went 'extinct” on 9 March 2024. https://www.sbs.com.au/news/article/why-this-years-flu-vaccine-includes-a-strain-that-went-extinct-in-the-covid-19-pandemic/xihrxws29
- Appeared on The House of Wellness to comment on “The best ways to prepare for this year’s flu season” on 1 April 2024. https://www.houseofwellness.com.au/health/tips/flu-season
- Appeared on Australian Doctor to comment on “GPs warned to brace for another early flu season” on 2 April 2024. https://www.ausdoc.com.au/news/gps-warned-to-brace-for-another-early-flu-season/?cspt=1712531515%7Cd608b0c12ee359458fb3cc67c746ed90
- Appeared on the Allergy and Respiratory Republic to comment on “influenza, COVID-19 and RSV” on 10 April 2024. https://www.puffnstuff.com.au/flu-covid-and-rsv-in-one-place/80990
- Appeared on the Medical Republic to comment on “the flu vaccine uptake” on 24 April 2024. https://www.medicalrepublic.com.au/sluggish-flu-vaccine-uptake-has-experts-worried/106980
- Appeared on The Telegraph to comment on “bird flu vaccine stockpiles” on 14 May 2024. https://www.telegraph.co.uk/global-health/science-and-disease/bird-flu-vaccine-h4n1-influenza/#:~:text=Classical-,There%20might%20be%20stockpiles%2C%20but%20making%20an%20effective%20bird%20flu,t%20be%20quick%20or%20easy&text=Health%20officials%20have%20been%20steadfast,vaccine%20waiting%20in%20the%20wings.
- Wrote an article for Medicine Today regarding “the battle against RSV in Australia begins – new preventives are here” on 25 May 2024. https://medicinetoday.com.au/mt/2024/may/regular-series/battle-against-rsv-australia-begins-new-preventives-are-here
- Appeared on RACGP news to comment on “the concern of avian influenza detected at commercial poultry farm” on The 21 June 2024. https://www1.racgp.org.au/newsgp/clinical/concern-as-avian-influenza-detected-at-commercial#:~:text=Professor%20Ian%20Barr%2C%20Deputy%20Director,is%20endemic%20but%20otherwise%20the
- Was quoted in The Age and Sydney Morning Herald for an article titled, ‘A ferret's sneeze is helping monitor the risk of a bird flu pandemic’. Published 14 August 2024. https://www.smh.com.au/national/a-ferret-s-sneeze-is-helping-monitor-the-risk-of-a-bird-flu-pandemic-20240812-p5k1n4.html
- Was quoted in The Telegraph for an article titled, ‘Bird flu kills 47 tigers, three lions and a panther at Vietnamese zoos’. Published 3 October 2024. https://www.telegraph.co.uk/global-health/science-and-disease/bird-flu-kills-tigers-lions-panther-at-vietnam-zoos/
- Was quoted in Sunday Times for an article titled, ‘CONTAGION UNEASE Health watchdog has warned bird flu could cause pandemic’. Published 1 December 2024. https://readnow.isentia.com/Temp/188956-1137022878/1032556024_20251201.pdf

## Community Engagement (continued)

**Michelle Wille**

- Was quoted on Science News "A bird flu outbreak is sweeping the globe. Its long-term effects are unclear" Published 25 January 2024.
- Appeared on ABC radio to comment on "Deadly bird flu reaches Antarctica, is Australia next?" On 27 February 2024.
- Was quoted on an ABC news page titled "'Unprecedented trail of destruction", On 21 May 2024. https://www.abc.net.au/news/2024-05-21/bird-flu-mutation-reaches-antarctica-australia-outbreak/103844240
- Wrote an article for The Conversation in "Chickens, ducks, seals and cows: a dangerous bird flu strain is knocking on Australia's door" On 22 May 2024. https://theconversation.com/chickens-ducks-seals-and-cows-a-dangerous-bird-flu-strain-is-knocking-on-australias-door-230013
- Appeared on ABC News Tonight and discussed H5N1 poultry outbreaks, On 29 May 2024.
- Was quoted on ABC News in an article titled "Experts warn H5N1 bird flu strain 'likely' to arrive in Australia in spring and could put wild birds such as little penguins at risk". Published 6 July 2024.https://www.abc.net.au/news/2024-07-06/h5n1-birdflu-strain-threat-australian-wildlife-barunguba-penguin/104050166
- Was quoted in The Guardian in an article titled "Egg shortage: why the avian influenza outbreak has left shoppers and farmers shell-shocked". Published 5 July 2024. https://www.theguardian.com/australia-news/article/2024/jul/05/egg-shortage-australia-bird-flu-avian-influenza
- Was quoted in News.com.au in an article titled, "Scientists have confirmed the spread of bird flu in Antarctica which has caused significant mortalities of wild birds and mammals on a global scale". Published 6 July 2024. https://www.news.com.au/technology/science/animals/scientists-have-confirmed-the-spread-of-bird-flu-in-antarctica-which-has-caused-significant-mortalities-of-wild-birds-and-mammals-on-a-global-scale/news-story/613874e2d03fbc4e28c329b738afcf20
- Was quoted in The Balkan Times in an article titled, "Scientists confirm spread of bird flu in Antarctica". Published 7 July 2024. https://www.balkanstimes.eu/scientists-confirm-spread-of-bird-flu-in-antarctica-64114.html
- Was quoted on The Saturday Paper in an article titled, "Authorities race to combat the threat of bird flu". Published 3 August 2024. https://www.thesaturdaypaper.com.au/news/health/2024/08/03/authorities-race-combat-the-threat-bird-flu?cspt=1722813486%7Cb7bdc3addf4712e169d2665f2fc8d10b
- Was quoted on The Sydney Morning Herald in an article titled, "A potential wildlife massacre': Eyes on the sky as virus wings its way towards Australia". Published 28 July 2024. https://www.smh.com.au/politics/federal/a-potential-wildlife-massacre-eyes-on-the-sky-as-virus-wings-its-way-towards-australia-20240725-p5jwgf.html
- Was quoted in The Guardians in an article titled, "When, not if': H5N1 bird flu outbreak could reach Australia this spring, experts warn". Published 12 August 2024. https://www.theguardian.com/australia-news/article/2024/aug/12/bird-flu-outbreak-australia-h5n1-risk
- Was quoted in The Age and Sydney Morning Herald in the article titled "A ferret's sneeze is helping monitor the risk of a bird flu pandemic". Published 14 August 2024.
- Was on ABC Listen in a discussion around "Bird watchers on alert for Bird Flu" Published 12 September 2024. https://www.abc.net.au/listen/programs/pm/bird-watchers-on-alert-for-bird-flu/104345680
- Was quoted in a Sunday Times article tilted "Contagion unease" Published 1 December 2024.

## Website and social media

The Centre website was maintained and updated throughout the year, with information provided on the progress of the influenza season and vaccine recommendations by WHO and the TGA. During 2024, the website was viewed by over 9,000 unique users from 156 different countries. The majority of visits to the website were from Australia, followed by the USA.

The Centre continued to operate its Twitter account in during 2024. The Centre's Twitter profile gained 102 followers during the year, with a total of 1033 followers by 31 December 2024

Scan to access our Centre video

## New staff members

**Dr Jessica Miller**

Head Epidemiologist

**Rachel Wordsworth**

Medical scientist

## Director's departure

The Centre announced the departure of our Director, Professor Kanta Subbarao, who left the Centre on 1/06/2024. She has been a source of inspiration and leadership for all of us. We wish Professor Subbarao all the best as she takes up a role at Laval University in Quebec, Canada.

During her tenure, Professor Subbarao has made remarkable contributions to the field of virology and infectious diseases. Recognized globally for her expertise in pathogenesis and vaccine development for respiratory viruses such as influenza, SARS, MERS, and COVID-19, She was instrumental in advancing research at the Royal Melbourne Hospital and Doherty Institute. Under her leadership, the WHO Collaborating Centre for Reference and Research on Influenza has maintained its excellence in public health and research, playing a crucial role in the Global Influenza Surveillance and Response System (GISRS). Professor Subbarao's pioneering work during the COVID-19 pandemic contributed to the critical research infrastructure at the Doherty Institute, including the development of a widely used neutralizing antibody assay for SARS-CoV-2 and establishment of a Doherty Institute PC3 animal facility for studies in mouse models for SARS-CoV-2.

Her dedication, expertise, and unwavering commitment, particularly during the COVID-19 pandemic, have left an indelible mark on our organization and the global health community. We extend our heartfelt gratitude for her exceptional contributions and wish her continued success in her future endeavours.

Thank you, Professor Subbarao, for your tireless efforts and leadership. You will be deeply missed.

# Management and staff

**Director**
Prof Patrick Reading

**Deputy Director**
Prof Ian Barr

**Administrative officer**
Symone Mercuri

**Quality & Regulatory Officer**
Katie Milne

**Educator/Research scientist**
Prof Patrick Reading

**Laboratory Manager/ Senior Medical Scientist**
Dr Paul Whitney

**Data and Communications officer**
Vishma Varsani

## Surveillance

**ANTIGENIC ANALYSIS**
Head: Heidi Peck
Medical scientists:
Monica Bobbitt
Joelle Dharmakumara
Leah Gillespie
Lani Metuisela
Megan Triantafilou
Tasoula Zakis
Malet Aban
Laboratory Assistant:
Violeta Stojceva

**GENETIC ANALYSIS**
Head: Dr Yi-Mo Deng
Medical scientists:
Dr Xiaomin Dong
Steven Edwards
Hilda Lau
Olivia Lay
Presa Chanthalavanh
Natalie Spirason
Rachel Wordsworth

**ANTIVIRAL DRUG SENSITIVITY**
Head: Dr Saira Hussain
Medical scientist: Nikita Deshpande

**BIOINFORMATICS**
Manager: Dr Clyde Dapat

## Vaccine development

**VACCINE CANDIDATES**
Head: Heidi Peck
Medical scientists:
Hilda Lau
Cleve Rynehart

**CELL-BASED VACCINES**
Head: Heidi Peck
Medical scientists:
Cleve Rynehart

## Research

**IMMUNITY TO RESPIRATORY VIRUSES**
Senior research scientist:
Dr Annette Fox

Post-Doctoral researchers
Dr Yi Liu
Dr Stephany Sanchez

Research assistant
Louise Carolan
Anastasia Jessica Hadiprojo

**ANTIVIRALS AND ANIMAL INFLUENZAS**
Senior research scientist:
Dr Saira Hussain

Research assistants:
Harry Stannard
Melkamu Tessema

**EARLY IMMUNE RESPONSES TO INFLUENZA**
Senior research scientist:
Prof Patrick Reading
Research assistant:
James Barnes

## Epidemiology

Head:
Jessica Miller
Epidemiologists:
Tanya Diefenbach-Elstob
Research assistant:
Arseniy Khvorov
Ph D Candidate:
Jessie Goldsmith