Annual Report 2019

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 2019-2023) 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. 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. To undertake research to improve the detection, prevention and treatment of influenza and to assist WHO and national health authorities in developing and implementing plans for responding to pandemic influenza.
6. To implement activities defined in the Annex 5 of the PIP Framework under the Terms of Reference for WHO Collaborating Centres for Influenza (https://www.who.int/influenza/resources/pip_framework)

Governance

The Centre is supported by the Australian Government Department of Health 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: whoflu@influenzacentre.org
Website: http://www.influenzacentre.org

Highlights of 2019

Surveillance

The Centre received and processed **9283 samples**, of which **98.9% were tested**. This was the highest annual number of viruses received by the Centre since the 2009 pandemic. Of viruses tested, approximately **50%** were **A(H3N2) viruses.**

Research, publications and grants

The Centre further developed its research program during 2019, with Centre staff involved as authors on **40 papers** in peer-reviewed journals. Centre staff were awarded several research grants, including **USD$4.2million** from the **US National Institutes of Health (NIH).**

2009 pandemic special seminar

The Centre held a special seminar to mark the **10 year anniversary** of the **2009 pandemic in Victoria.**

Australian Influenza Symposium

The **13th Australian Influenza Symposium** was held at the **Queensland University of Technology (QUT)** and attended by approximately **200 delegates.**

WHO vaccine strains isolated by the Centre

Two vaccine candidate viruses that were originally isolated in eggs by the Centre were selected for **inclusion in the WHO recommended influenza vaccine strains.**

Routine testing of virus for susceptibility to baloxavir marboxyl

The Centre established **routine testing** of circulating viruses for reduced **susceptibility to baloxavir marboxyl.**

Director's report

It is a pleasure to present the 2019 Annual Report of the WHO Collaborating Centre for Reference and Research on Influenza. Our designation as a WHO Collaborating Centre was renewed in 2019 for five years. The Centre has continued to actively fulfil its commitments to the WHO, National Influenza Centres in the region, and the Commonwealth Government and to participate in training and research activities.

Following a mild influenza season in 2018, the influenza season in 2019 in Australia started earlier and lasted for longer than usual. The 'inter -seasonal' influenza activity that started in December 2018 and extended into early 2019 was followed by a record number of notifications from all jurisdictions. The Centre received and processed more than 9000 influenza samples from 40 laboratories in Australia and 16 other countries during 2019. The largest proportion (>50%) of the samples analysed were influenza A(H3N2) viruses.

As we noted in recent years, there is considerable genetic diversification of the A(H3N2) HA gene. The integration of Next Generation Sequencing (NGS) techniques into routine surveillance activities has resulted in a large annual number of viruses undergoing full genome sequencing. In 2019, the Centre sequenced a total of 297 full genomes and 2204 partial genomes (HA and NA genes plus MP gene for influenza A viruses). The Centre also established routine testing of viruses for reduced susceptibility to baloxavir marboxyl in preparation for potential availability and use of baloxavir in the future.

During 2019 the Centre continued to work on isolation of cell-based and egg-based viruses for vaccine production. Two vaccine candidate viruses that were originally isolated in eggs by the Centre were selected for inclusion in the WHO recommended influenza vaccine strains during 2019. The Centre also continued to monitor potential pandemic influenza viruses and seeks to obtain new viruses as they were detected (such as A(H7N9) viruses), to check reagents and prepare virus and RNA stocks.

Centre staff participated in training international visitors at the Centre as well as at workshops hosted overseas. Centre staff presented at several domestic and international conferences in 2019. The Centre held the 13th Australian Influenza Symposium that was hosted at the Queensland University of Technology (QUT) and attended by approximately 200 delegates. We also held a special seminar at the Doherty Institute to mark the 10 year anniversary of the 2009 pandemic in Victoria that was very well attended.

Centre staff contributed to a total of 40 original research papers, reviews and reports in 2019. Centre staff were successful in obtaining grant funding to support their research from a variety of sources including NHMRC, NIH (USA) and the Coalition for Epidemic Preparedness Innovations (CEPI).

The emergence and spread of the respiratory illness COVID-19 caused by a novel coronavirus is a global public health emergency that will have to be managed alongside seasonal influenza. Some of our staff will be involved in COVID-19 laboratory and research activities while the Centre maintains its focus on influenza.

We are very grateful to Dr Mike Catton, Director of VIDRL, and many other members of VIDRL staff, especially Jane Brewster, Anna Ayres and Dallas Wilson, for their support of the Centre's work at every level during 2019. The continuing support and counsel of the Office of Health Protection in the Australian Government Department of Health are deeply appreciated. Finally, I would like to thank all the staff and students of the Centre for their excellent work in 2019. It is a privilege to work with the Centre staff and I look forward to working with our partners in 2020 and onwards.

Prof Kanta Subbarao
Centre Director

Surveillance

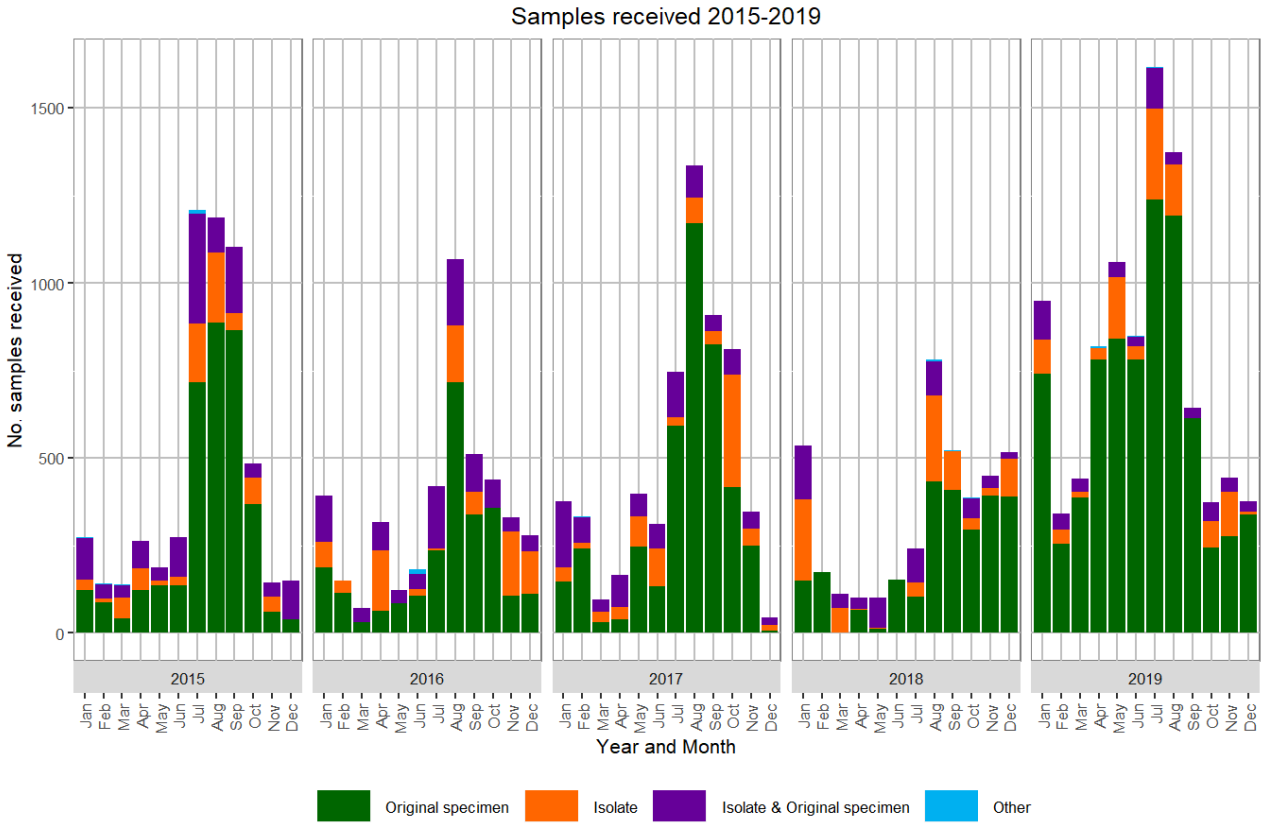
Introduction

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.

Two types of influenza virus, Type A and Type B, cause significant disease in humans. The surface of influenza viruses is coated with two proteins, haemagglutinin (HA) and neuraminidase (NA). There are many subtypes of influenza A viruses, usually of avian origin, with various combinations of 18 antigenically different HA variants and 11 NA variants. Influenza B viruses are not classified into subtypes, however, there are two co-circulating lineages, B/Victoria and B/Yamagata. Currently there are three predominant families of influenza viruses circulating in the human population — influenza A(H1N1)pdm09, influenza A(H3N2) and influenza B.

Figure 1. Samples received by the Centre, 2015-2019



Receipt of Influenza Viruses

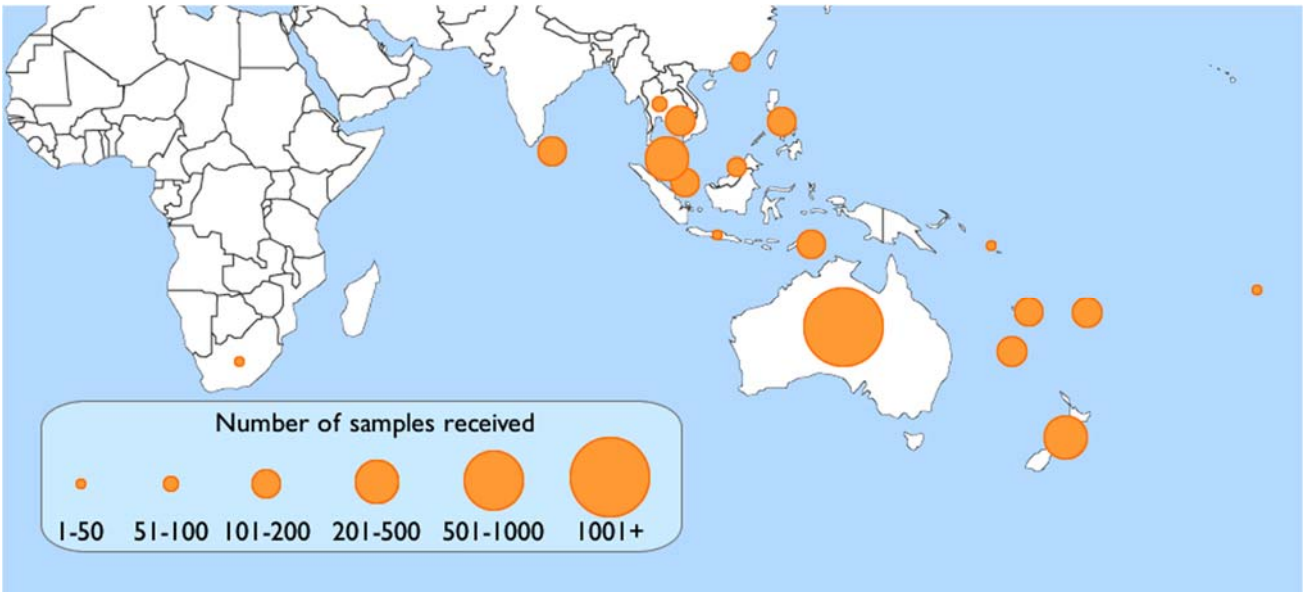
During 2019 the Centre received 9283 clinical specimens and/or virus isolates from 40 laboratories in 17 countries (Figures 1 and 2, Table 1). This is the highest number of samples received by the Centre in an annual period since the pandemic in 2009, and is consistent with the record notifications of laboratory-confirmed influenza during the 2019 Australian influenza season. An unusually large number of samples were received during the first few months of 2019, due to highly elevated levels of influenza in the interseasonal period in Australia (November 2018 to May 2019). Amongst samples received by the Centre for which the age of the patient was known, the largest number were from subjects aged under 5 years (Figure 3). A total of 2340 samples came from Australian general practictioner based surveillance systems (Table 2).

Isolation and analysis of viruses

Original clinical specimens received by the Centre can be genetically analysed by sequencing or real-time RT-PCR and are also required for recovery of egg isolates that may be potential vaccine strains. For more extensive analyses, viruses from original clinical specimens are cultured and isolated in Madin-Darby Canine Kidney (MDCK) cells.

A total of 9177 samples (98.9%) were isolated by culture and/or analysed by real-time reverse-transcription polymerase chain reaction (RT-PCR). 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 NA. Of the samples for which results could be obtained, 50.3% were identified as A(H3N2) viruses, 27.0% were A(H1N1)pdm09 viruses, 17.4% were B/Victoria and 2.1% were B/Yamagata viruses (Table 3).

Figure 2. Geographic spread of influenza laboratories sending viruses to the Centre during 2019.



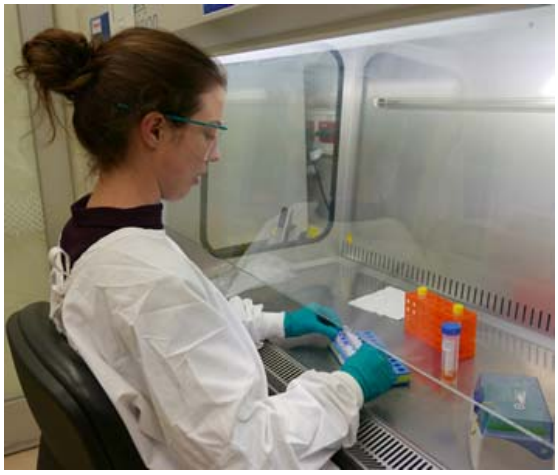


Figure 3. Age distribution of patients from whom samples were received

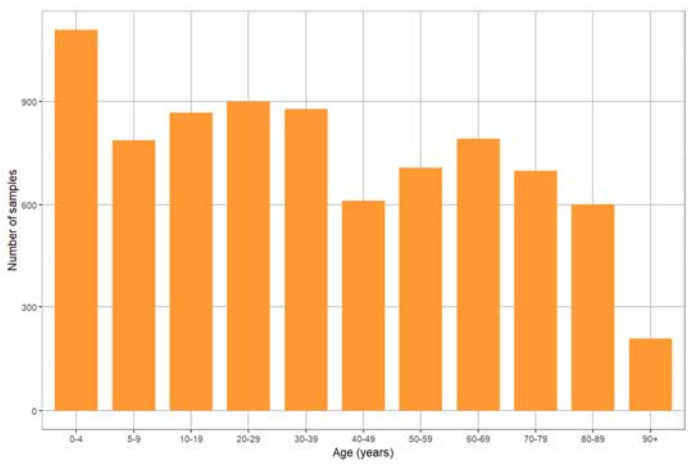


Table 1. Samples received by the Centre in 2019, by country.

Country	Samples received				% Samples tested
	Specimens	Isolates	Specimen + Isolate	Other (eg. RNA/ DNA/tissue)	
AUSTRALASIA					
Australia	6862	187	365	6	97.3%
New Zealand	54	245			100%
SOUTH PACIFIC					
Fiji	169				100%
French Polynesia	19				100%
New Caledonia	145				100%
Solomon Islands	18				100%
SOUTH EAST ASIA					
Brunei	83				100%
Cambodia	61	92			100%
Indonesia	5	18			100%
Malaysia		319			100%
Philippines	71		48		100%
Singapore		8	109		100%
Thailand	11	75			100%
Timor-Leste	119				100%
EAST ASIA					
Macau SAR, China		70			100%
SOUTH ASIA					
Sri Lanka	77		29		100%
AFRICA					
South Africa			18		100%
TOTAL	**7694**	**1014**	**569**	**6**	**98.9%**

Table 2. Samples received from general practitioner based surveillance systems in Australia, 2019

	No. samples received	No. isolates recovered*	Viruses analysed by HI assay
Australian Sentinel Practices Research Network *(ASPREN)*	986	363	199
Victorian Sentinel Practices Influenza Network *(VicSPIN)*	225	181	142
Influenza Complications Alert	1129	692	424
TOTAL	**2340**	**1236**	**765**

** 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 tested by cell culture and/or RT-PCR assay at the Centre in 2019, by country.

Country	Samples tested by cell culture and/or RT-PCR assay							
	A (H1N1)pdm09	A H3N2	A mixed subtype	A unsubtyped	B/Victoria	B /Yamagata	B lineage undetermined	Mixed type (A/B)
AUSTRALASIA								
Australia	1317	3033	25	92	681	51	74	9
New Zealand	43	102			150	1		
SOUTH PACIFIC								
Fiji	34	17	2		96		1	1
French Polynesia	6	2			11			
New Caledonia	71	35	1		27	2		
Solomon Islands					2		8	
SOUTH EAST ASIA								
Brunei	23	9			4	10		
Cambodia	68	13			25	26		
Indonesia	5	5			7	6		
Malaysia	111	99	1		75	11		
Philippines	27	21		2	23	2		
Singapore	35	34			28	20		
Thailand	29	28			21	7		
Timor-Leste	33	16			4			
EAST ASIA								
Macau SAR	34	15			20	1		
SOUTH ASIA								
Sri Lanka	13	24			19	4		
AFRICA								
South Africa	3	15						
TOTAL	**1852**	**3468**	**29**	**94**	**1193**	**141**	**83**	**10**

Antigenic Analysis of Influenza Isolates

Background

The antigenic properties of influenza viral 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.

Antigenic analyses 2019

A total of 9160 isolates that were received at the Centre in 2019 were cultured and isolated in MDCK cells, of which 6103 (66.6%) produced a positive result. The largest proportion of viruses were A(H3N2) (51.5%), followed by A(H1N1) pdm09 viruses (29.1%) (Figure 4). The predominance of A(H3N2) viruses for samples received and successfully isolated by cell culture at the Centre (Figure 5) was observed some world regions (Africa, Australasia, South Asia, while in other regions (East Asia and South East Asia) A(H1N1)pdm09 viruses were predominant. In the South Pacific Region, of viruses which were successfully cultured and isolated in cells, B/Victoria viruses predominated.

Figure 4. Influenza sub/types and lineages of samples received in 2019 and characterised by antigenic analysis.

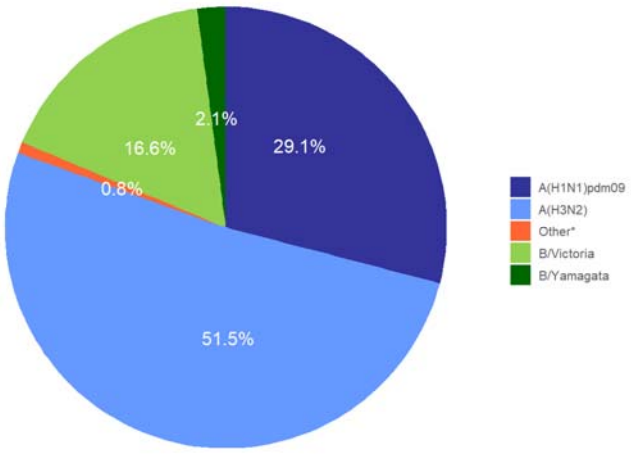
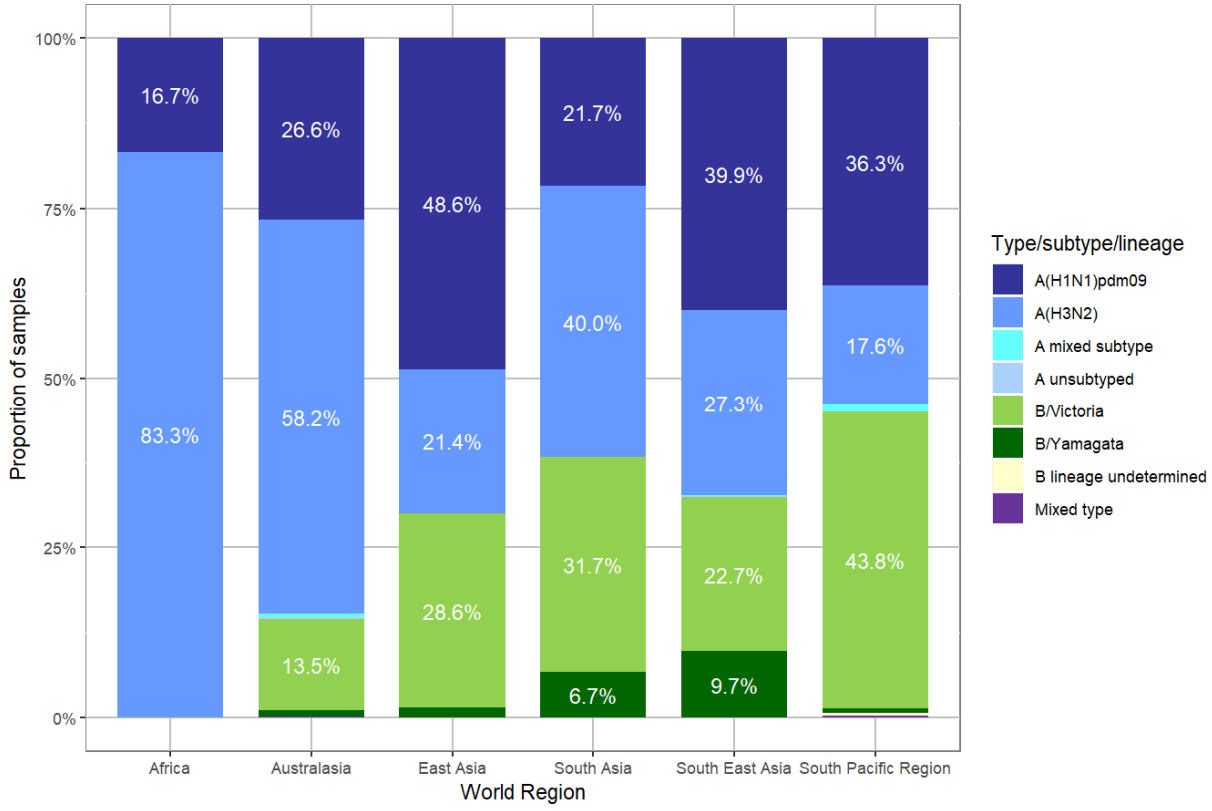


Figure 5. Influenza sub/types and lineages of isolates received from different world regions during 2019 as determined by antigenic analysis.



Genetic Analysis of Influenza Viruses

Background

A subset of all influenza viruses analysed at the Centre undergoes genetic analysis by sequencing of viral genes. Determining the amino acid sequence of antigenic regions of the HA and NA proteins provides a sensitive method to examine the extent and direction of change 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 by 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.

Next generation sequencing (NGS) techniques are now routinely employed at the Centre for efficient and cost-effective sequencing of whole genomes of viruses, and/or selected influenza virus genes.

Figure 6. Sequencing of viruses received at the Centre in 2019. Note that some viruses were analysed by both Sanger sequencing and NGS, and are therefore represented twice in this figure.

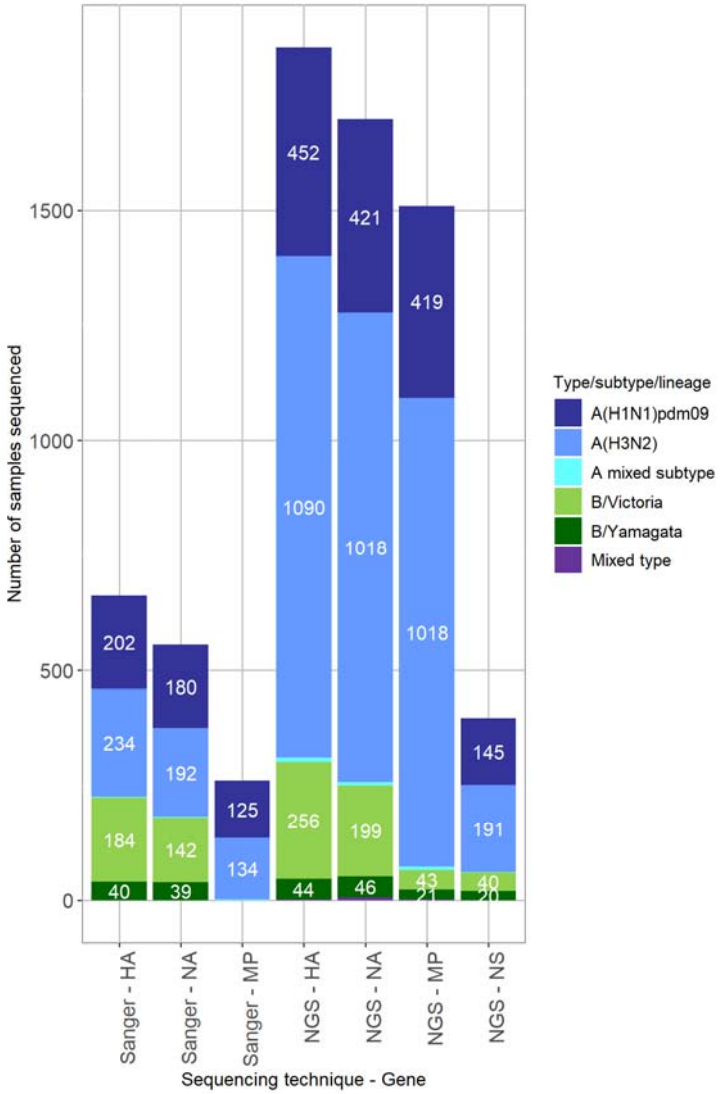
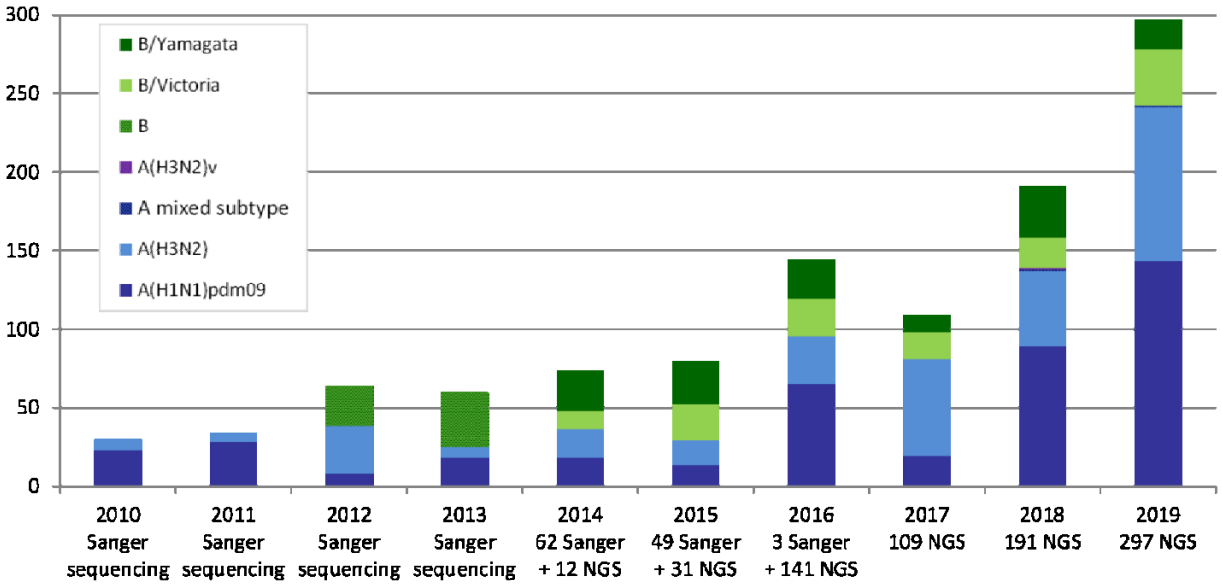


Figure 7. Number of viruses analysed by full genome sequencing 2010-2019 using Sanger sequencing and NGS techniques.



Sequencing 2019

In 2019, 2490 HA, 2226 NA, 1757 MP and 397 NS genes from 2501 human viruses received at the Centre were analysed by Sanger sequencing or NGS (Figure 6). Of these viruses, full genome sequencing was performed on 297 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.

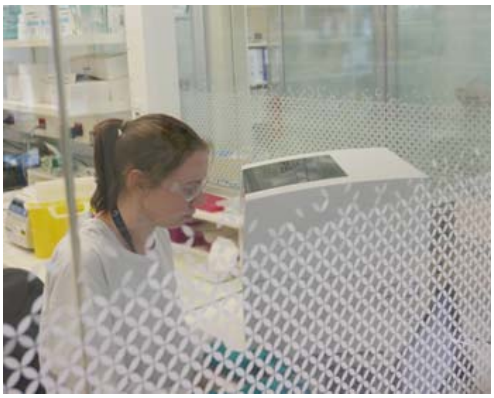
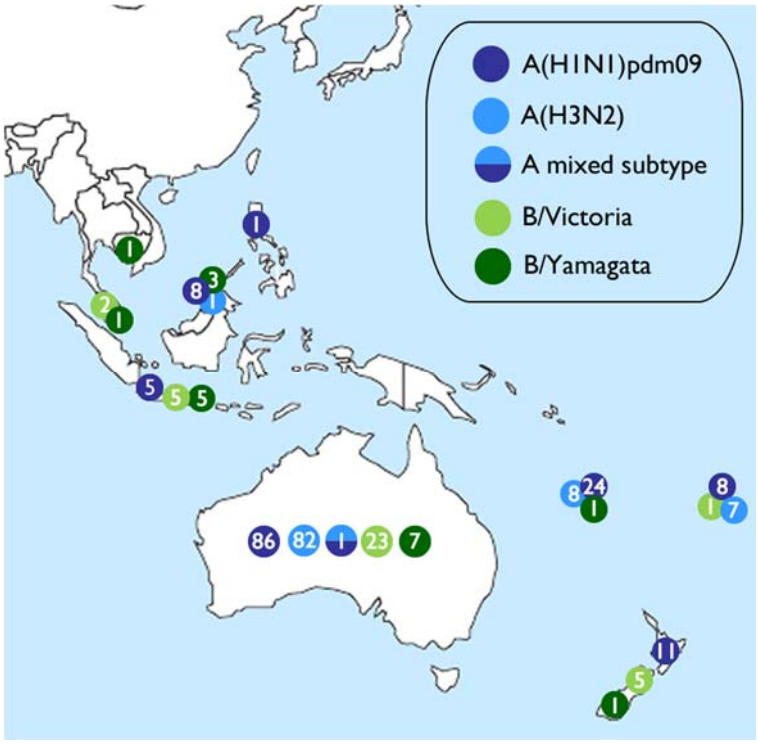


Figure 8. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing using NGS techniques at the Centre in 2019.



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 2019

A total of 7367 gene sequences from 1910 human influenza viruses were deposited with GISAID in 2019 (Table 4). The largest number of these sequences were of HA and NA genes, followed by MP and NS genes. Full genomes of 387 influenza viruses were also represented in the Centre's submissions (data not shown).

Table 4. Genetic sequences submitted to GISAID by the Centre in 2019.*

Gene / Type/ Subtype/ Lineage	HA	NA	MP	NS	PB1	PB2	PA	NP	Total
A(H1N1)pdm09	619	619	566	224	222	223	189	224	**2886**
A(H3N2)	932	932	839	172	167	171	171	173	**3557**
B/Victoria	244	244	11	11	11	11	11	11	**554**
B/Yamagata	115	115	23	26	23	23	23	22	**370**
Total	**1910**	**1910**	**1439**	**433**	**423**	**428**	**394**	**430**	**7367**

*

Counts include all sequences submitted to GISAID during 2019, 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 2019 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 region of the haemagglutinin gene are also described in the following sections.

Influenza A(H1N1)pdm09

Antigenic analysis

A total of 1606 A(H1N1)pdm09 isolates were analysed by HI assay in 2019. Almost all of these viruses (94.5%) displayed similar antigenic properties to the cell-grown vaccine reference strain A/Michigan/45/2015 (Figure 9, Table 5).

Haemagglutinin gene sequencing

Sequencing was performed on a total of 642 HA genes. Phylogenetic analysis showed that the majority of circulating A(H1N1)pdm09 viruses sent to the Centre during 2019 were in subclade 6B.1A which contains the Southern Hemisphere 2020 recommended vaccine strain A/Brisbane/2/2018 (Figure 10).

Table 5. Antigenic characterisation of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/Michigan/45/2015 reference virus.

A(H1N1)pdm09 reference strain: A/Michigan/45/2015		
Region	**Like**	**Low reactor (%)**
Africa	3	0
Australasia	1118	28 (2.4 %)
East Asia	31	3 (8.8 %)
South Asia	13	0
South East Asia	273	26 (8.7 %)
Pacific	80	31 (27.9 %)
TOTAL	**1518**	**88 (5.48 %)**

Figure 9. Summary of fold differences in HI titres of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/Michigan/45/2015 reference virus.

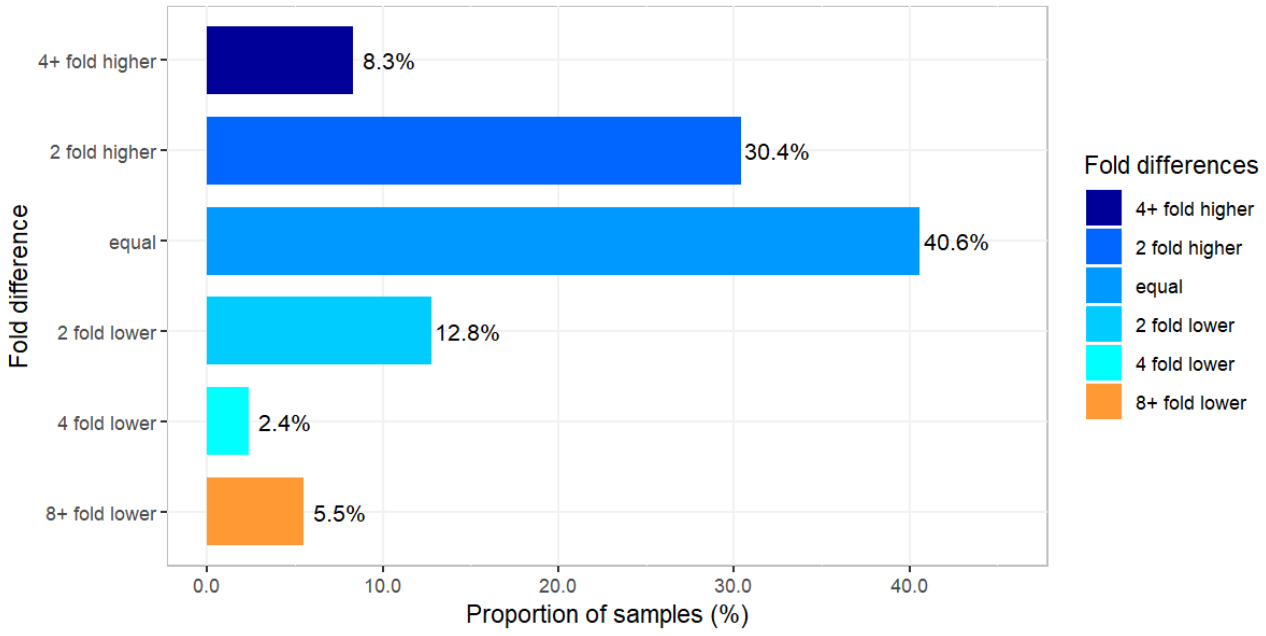
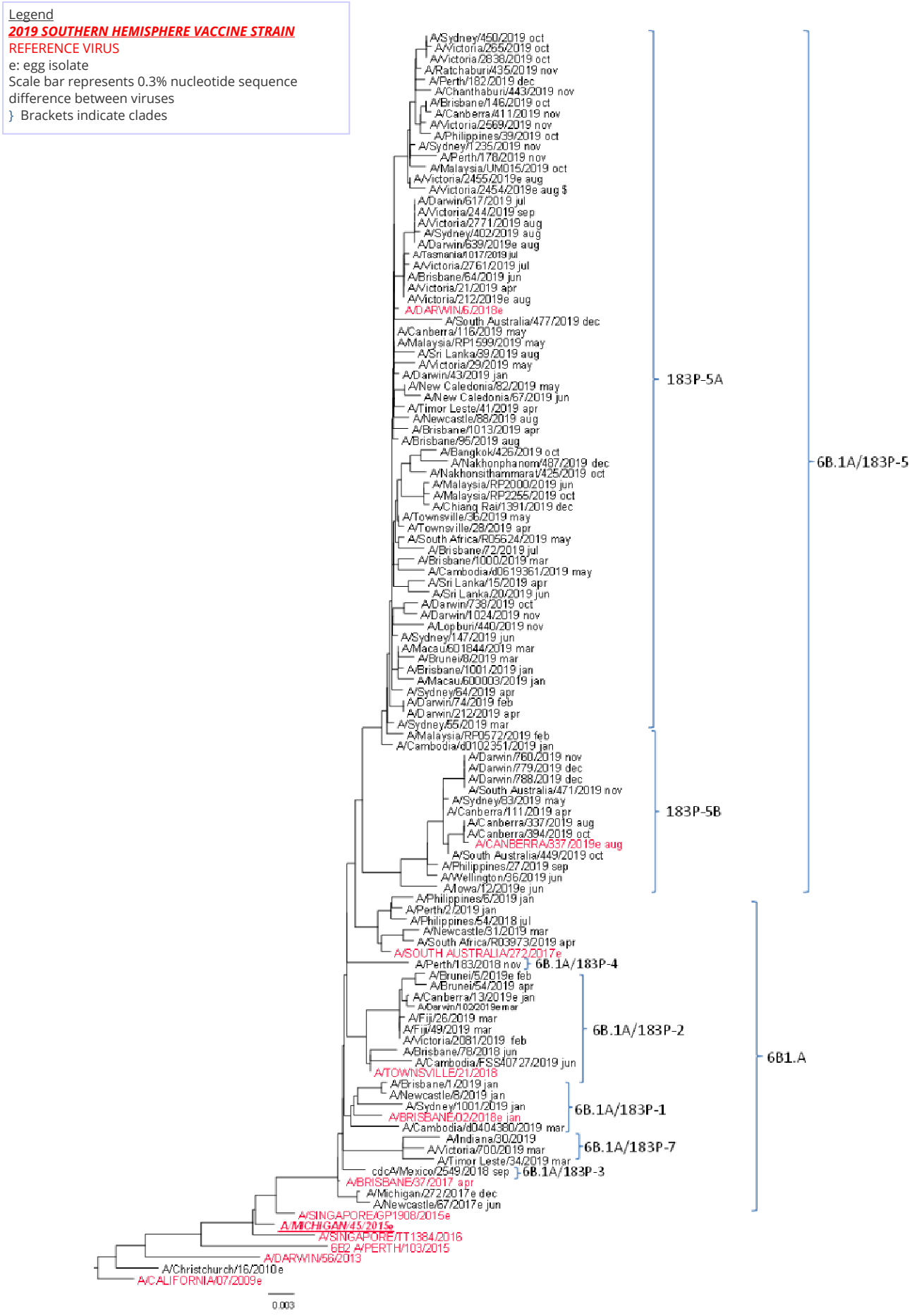


Figure 10. Phylogenetic tree of representative HA genes of A(H1N1)pdm09 viruses received by the Centre during 2019.



Influenza A(H3N2)

Antigenic analysis

In recent years evolutionary changes in A(H3N2) viruses have made it difficult to detect antigenic change using conventional HI assays. To avoid binding of the neuraminidase protein to red blood cells, it has been necessary to add oseltamivir carboxylate to the assay. However, in the presence of oseltamivir, approximately 50% of current A(H3N2) isolates have insufficient haemagglutination titre to conduct the HI assay. Hence only a proportion of A(H3N2) virus isolates are successfully cultured and can be analysed by HI assay. Other assays, such as a focus reduction microneutralisation assay (FRA-MN) are required to test the antigenic characteristics of these viruses. During 2019 FRA-MNs were performed on a regular basis and continue to be integrated into the Centre's routine surveillance activities.

Of 1520 A(H3N2) subtype isolates analysed by HI assay compared to the cell-propagated reference strain A/Switzerland/8060/2017 (Figure 11, Table 6), the majority were antigenically similar to the reference virus when using ferret antisera generated to cell grown A(H3N2) viruses, however a there was a growing proportion of low reactors. A smaller number of A(H3N2) viruses were analysed by HI assay compared to the previous vaccine reference strain A/Singapore/INFIMH-16-0019/2016—none of these viruses were low reactors to the reference strain (Table 6, Figure 12)

A total of 85 A(H3N2) viruses that could not be analysed by HI assay were analysed by FRA-MN. None of these viruses were low reactors to cell-grown A/Switzerland/8060/2017 as analysed by FRA-MN assay (Figure 13).

Haemagglutinin gene sequencing

A total of 1311 HA genes from A(H3N2) viruses were sequenced. Phylogenetic analysis indicate that most circulating viruses fell into clade 3C2.a1b, represented by the new reference strain A/South Australia/34/2019, which was recommended by WHO for inclusion in Southern Hemisphere vaccine in 2020 (Figure 14).

Table 6. Antigenic characterisation of A(H3N2) viruses analysed at the Centre compared to the cell-grown A/Switzerland/8060/2017 reference virus.

	A(H3N2) reference strain: A/Switzerland/8060/2017		A(H3N2) reference strain: A/Singapore/INFIMH-16-0019/2016	
Region	**Like**	**Low reactor (%)**	**Like**	**Low reactor (%)**
Africa	4	11 (73.3 %)	0	0
Australasia	1003	327 (24.6 %)	131	0
East Asia	4	1 (20.0 %)	10	0
South Asia	3	2 (40.0 %)	13	0
South East Asia	65	70 (51.9 %)	31	0
Pacific	25	5 (16.8 %)	0	0
TOTAL	**1104**	**416 (27.4 %)**	**185**	**0**

Figure 11. Summary of fold differences in titres of A(H3N2) viruses analysed at the Centre by HI assay compared to the A/Switzerland/8060/2017 reference virus.

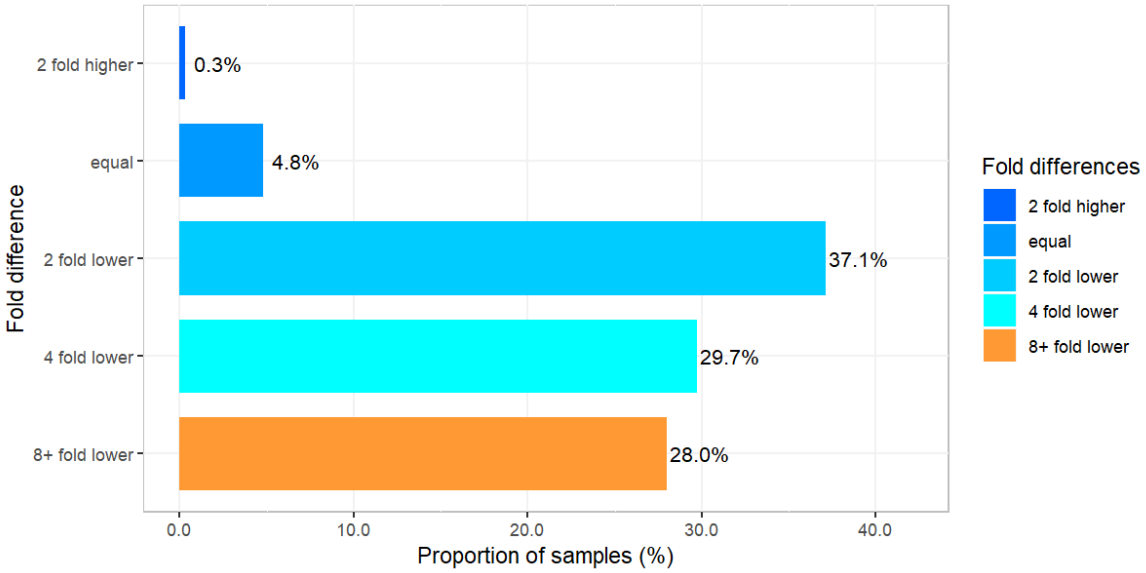


Figure 12. Summary of fold differences in HI titres of A(H3N2) viruses analysed at the Centre compared to the A/Singapore/INFIMH-16-0019/2016 reference virus.

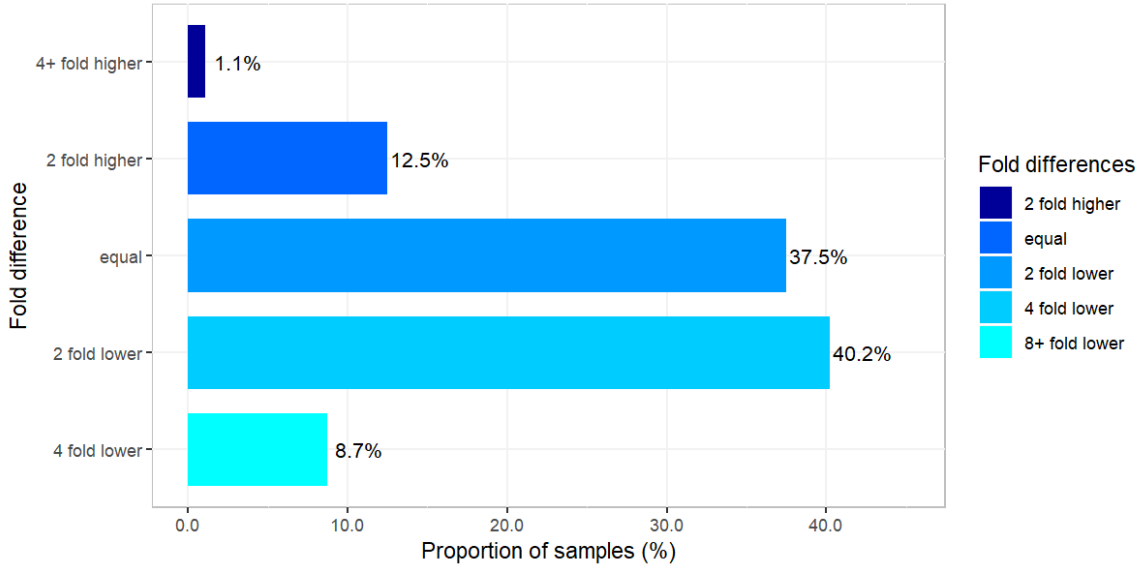


Figure 13. Summary of fold differences in titres of A(H3N2) viruses analysed at the Centre by FRA-MN assay compared to the the A/Switzerland/8060/2017 reference virus.

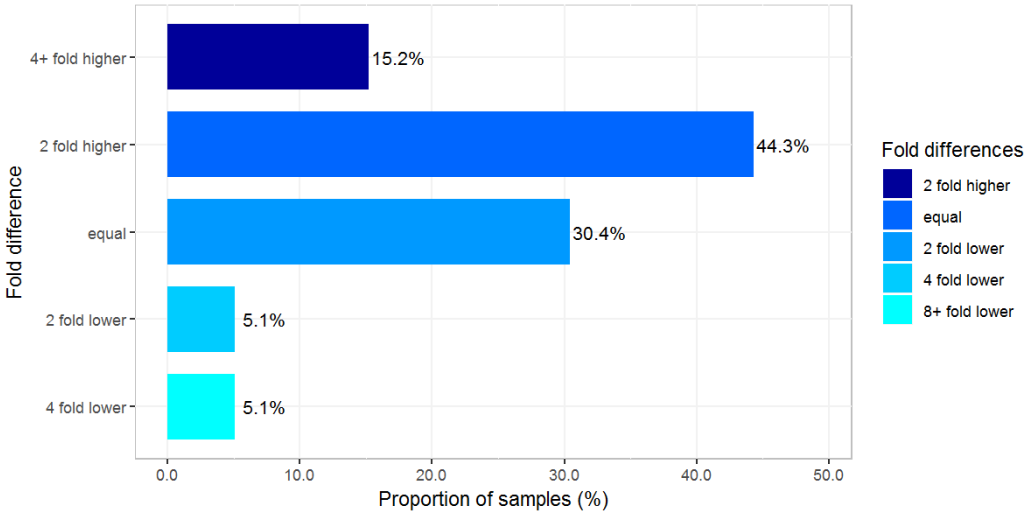
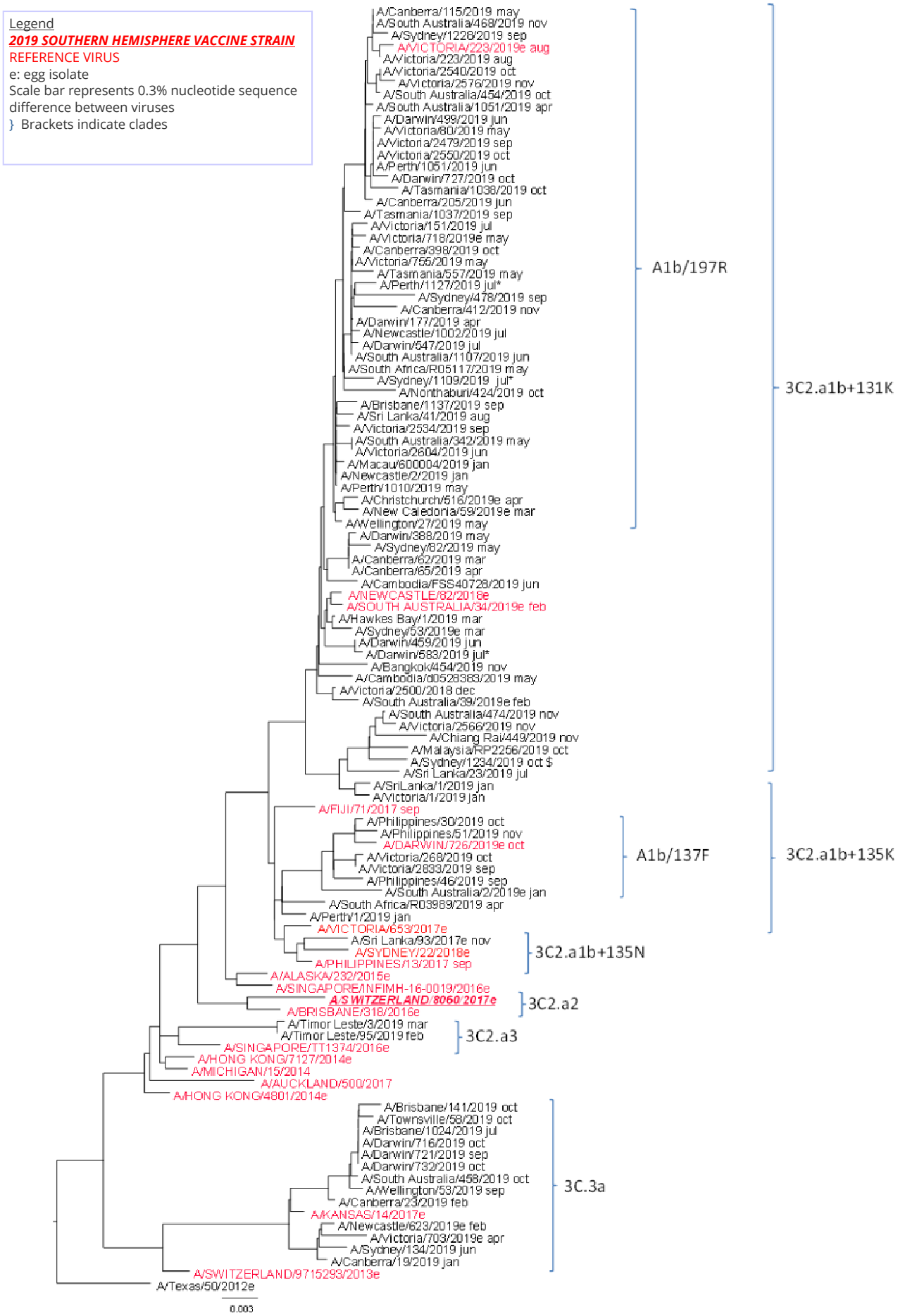


Figure 14. Phylogenetic tree of representative HA genes of A(H3N2) viruses received by the Centre during 2019.



Influenza B/Victoria

Introduction

There are currently two antigenically and genetically distinct lineages of influenza B virus in circulation—the B/Victoria/2/87 lineage (represented by the 2019 vaccine strain B/Colorado/06/2017), and the B/Yamagata/16/88 lineage (represented by the 2015 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. During 2019 the Centre received many more B/Victoria lineage viruses compared to B/Yamagata lineage viruses.

Antigenic Analysis

A total of 849 B/Victoria viruses were analysed by HI assay. While the majority of viruses antigenically similar to the cell-grown reference virus B/Colorado/6/2017, a growing proportion of circulating viruses were low reactors (Table 7, Figure 15). This was consistent with data from other Collaborating Centre, leading to a change to B/Washington/2/2019 in the recommended strain for the B/Victoria component of the southern hemisphere vaccine in 2020.

Haemagglutinin gene sequencing

Phylogenetic analysis of 432 genes from B/Victoria lineage viruses showed the growth of the subclade V1A.3. This subclade contains the new vaccine strain B/Washington/2/2019, which has three amino acid deletions in the HA gene and is genetically distinct from the previous vaccine strain B/Colorado/6/2017 (Figure 16).

Table 7. Antigenic characterisation of B Victoria viruses received at the Centre during 2019 compared to the B/Colorado/6/2017 reference viruses.

B/Victoria lineage reference strain: B/Colorado/6/2017		
Region	**Like**	**Low reactor (%)**
Australasia	478	55 (10.3 %)
East Asia	13	7 (45.0 n%)
South Asia	17	0
South East Asia	112	47 (29.6 %)
Pacific	98	22 (18.3 %)
TOTAL	**718**	**131 (15.4 %)**

Figure 15. Summary of fold differences in HI titres of B/Victoria viruses analysed at the Centre compared to B/Colorado/6/2017 reference virus.

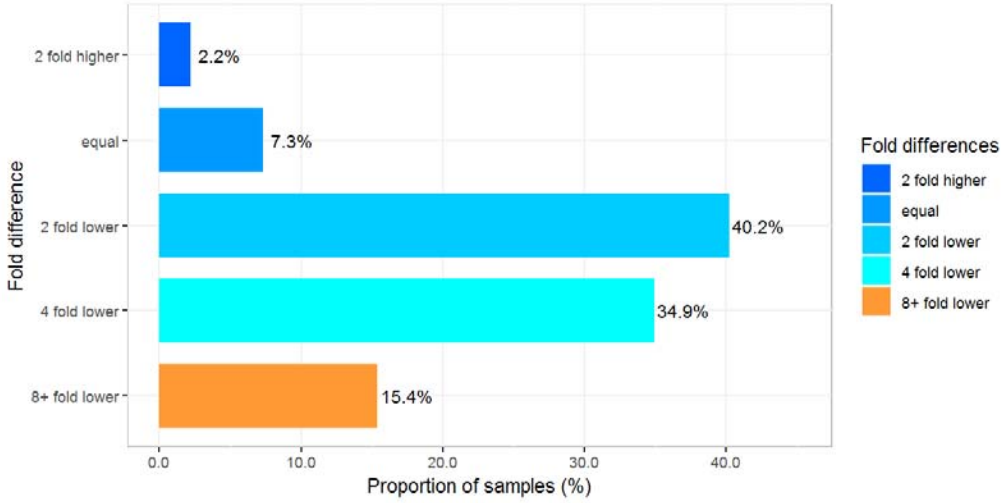
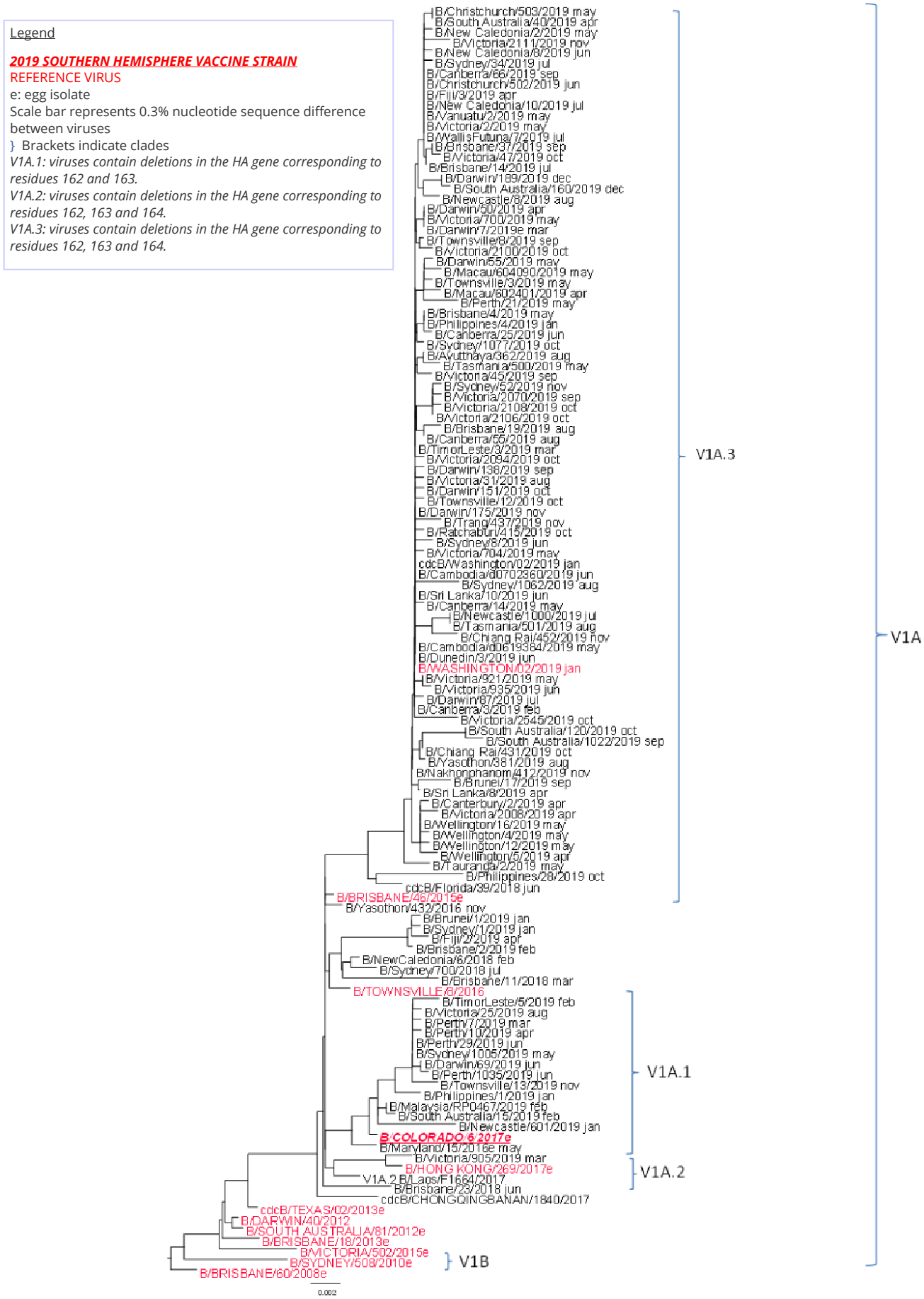


Figure 16. Phylogenetic tree of representative HA genes of B/Victoria viruses received by the Centre during 2019.



Influenza B/Yamagata

Antigenic analysis

A total of 111 B/Yamagata viruses were analysed by HI assay, of which all were antigenically similar B/Phuket/3073/2013 virus grown in cells (Figure 17, Table 8).

Haemagglutinin gene sequencing

Sequencing was performed on 79 B/Yamagata viruses were sequenced. Phylogenetic analysis showed that almost all of these viruses belonged to the clade represented by B/Phuket/3073/2013 (Figure 18).

Table 8. Summary of fold differences in HI titres of B/Yamagata viruses analysed at the Centre compared to the B/Phuket/3073/2013 reference virus.

B/Yamagata lineage reference strain: B/Phuket/3073/2013		
Region	**Like**	**Low reactor (%)**
Australasia	37	0
East Asia	1	0
South Asia	4	0
South East Asia	69	0
Pacific	0	0
TOTAL	**111**	**0**

Figure 17. Summary of fold differences in HI titres of B/Yamagata viruses analysed at the Centre compared to the B/Phuket/3073/2013 reference virus.

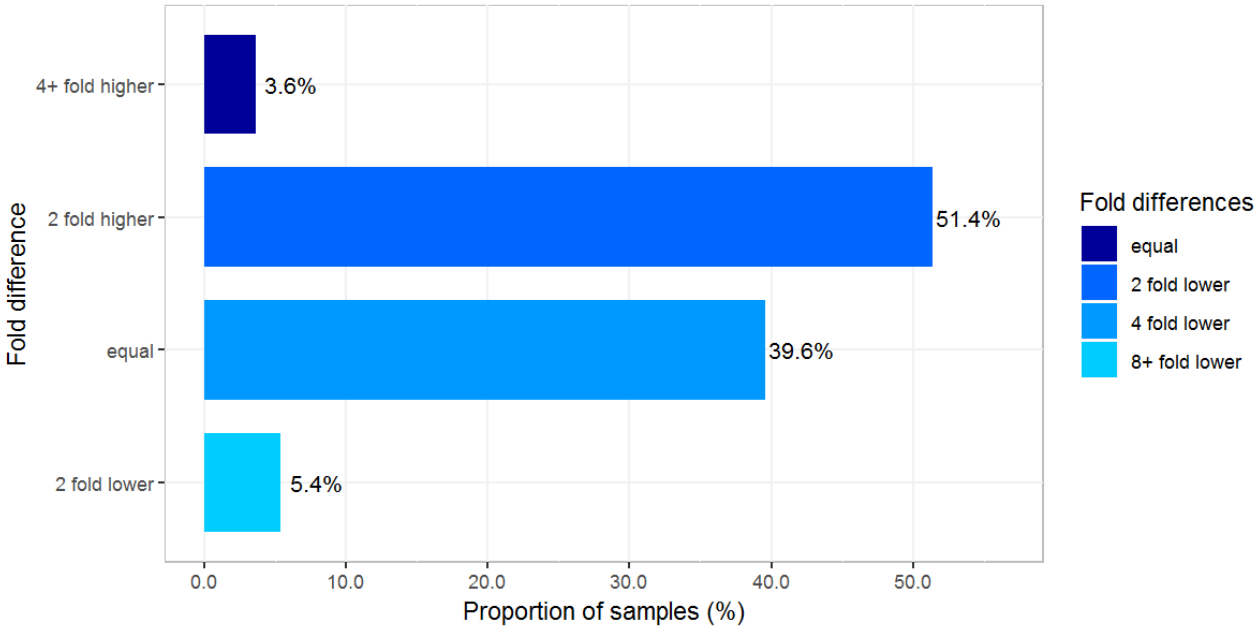


Figure 18. Phylogenetic tree of representative HA genes of B/Yamagata viruses received by the Centre during 2019.

Legend

2019 SOUTHERN HEMISPHERE VACCINE STRAIN
(quadrivalent vaccine only)
REFERENCE VIRUS
e: egg isolate
Scale bar represents 0.3% nucleotide sequence difference between viruses
} Brackets indicate clades

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. The latter two inhibitors are not currently approved in Australia but used in Korea (peramivir), USA (peramivir) and Japan (laninamivir and peramivir) and under clinical trial in many countries around the world. The Centre has routinely tested and reported the sensitivity of viruses to all four NAIs using 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 (IC_{50}). The relationship between the IC_{50} value and the clinical effectiveness of a neuraminidase inhibitor against a given virus is not well understood. Further studies would be required to determine whether a virus with an elevated IC_{50} is clinically resistant.

Table 9. Viruses received by the Centre in 2019 and tested by NAI assay, by country.

Type/ subtype/ lineage / Country	A (H1N1) pdm09	A (H3N2)	A mixed type	B/ Victoria	B/ Yamagata	B lineage undeter-mined	Mixed type (A/B)	TOTAL
Australasia								
Australia	1065	2032	15	496	41		3	**3652**
New Zealand	43	96		150	1			**290**
South Pacific								
Fiji	34	16	2	96		1	1	**150**
French Polynesia	6	2		11				**19**
New Caledonia	71	35	1	25	2			**134**
South East Asia								
Brunei	22	9		4	8			**43**
Cambodia	68	13		25	26			**132**
Indonesia	5	5		6	6			**22**
Malaysia	111	97	1	74	10			**293**
Philippines	27	16		23	2			**68**
Singapore	35	30		28	20			**113**
Thailand	29	28		21	7			**85**
Timor-Leste	9	16		4				**29**
East Asia								
Macau SAR	34	15		20	1			**70**
South Asia								
Sri Lanka	13	24		19	4			**60**
Africa								
South Africa	3	15						**18**
TOTAL	**1575**	**2449**	**19**	**1002**	**128**	**1**	**4**	**5178**

Antiviral resistance analyses 2019

NAI assays were used to analyse 5178 viruses for reduced inhibition by the NAIs (Tables 9 and 10). 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 21 viruses (3 A(H1N1)pdm09 and 18 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 associated with the reduction of inhibition by NAIs (Table 11), for example histidine to tyrosine at position 275 (H275Y) of the neuraminidase protein of A(H1N1)pdm09 viruses, which reduces inhibition by oseltamivir, or the equivalent H273Y mutation in B viruses.

Table 10. Neuraminidase inhibitor sensitivity of viruses received by the Centre in 2019.*

		Oseltamivir		Peramivir		Laninamivir		Zanamivir	
Type/Subtype/ Lineage	No. tested	RI	HRI	RI	HRI	RI	HRI	RI	HRI
A(H1N1)pdm09	1575	1 (0.06%)	2 (0.13%)	2 (0.13%)	2 (0.13%)	1 (0.06%)		1 (0.06%)	1 (0.06%)
A(H3N2)	2449								
A mixed subtype	19								
B/Victoria	1002	14 (1.4%)		14 (1.4%)	18 (1.8%)	6 (0.6%)		10 (1.0%)	3 (0.3%)
B/Yamagata	128			1 (0.78%)					
B lineage undetermined	1								
Mixed type (A/B)	4								
TOTAL	**5178**	**15 (0.29%)**	**2 (0.04%)**	**17 (0.33%)**	**20 (0.39%)**	**7 (0.14%)**	**0**	**11 (0.21%)**	**4 (0.08%)**

Based on IC_{50}, the NAI sensitivity of each strain is classified as the following: **Normal inhibition = IC_{50} values are within or close to the median IC_{50} of type/subtype-matched viruses tested at the Centre during 2018-2019. **Reduced inhibition (RI)** = IC_{50} 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)** = IC_{50} values are greater than 100 fold above the median value of viruses with normal inhibition (above 50 fold for influenza B viruses).*

Table 11. Characteristics of viruses received by the Centre during 2019 with highly reduced inhibition by NAIs.

Type/ Subtype/ Lineage	Country/city of submitting laboratory	NAI(s) with highly reduced inhibition (marked with *)				Mutation(s) detected
		Oseltamivir	Peramivir	Laninamivir	Zanamivir	
A(H1N1)pdm09	Queensland	*	*			H275Y
	Victoria	*	*			H275Y
	Victoria				*	E119G
B Vic	Queensland		*			H134Y, D432G
	Queensland		*			T146P
	Queensland		*			G140R
	Queensland		*			G247D, I361V
	Queensland		*			G140R
	Australian Capital Territory		*		*	E105K
	Brunei		*			E105K
	Malaysia		*			E105K
	Malaysia		*			E105K
	Malaysia		*			T146K
	Malaysia		*		*	E105K
	Malaysia		*			E105K
	Malaysia		*			E105K
	Malaysia		*			H439P
	Malaysia		*		*	T146P,N169S
	Malaysia		*			H101L
	Philippines		*			T146K
	Philippines		*			E105K

Resistance to Baloxavir Marboxil

Background

Baloxavir marboxil (XofluzaTM) is an antiviral drug which has had regulatory approval for use in the treatment of influenza in Japan and the USA since 2018. 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. In 2019, this assay was implemented on a routine basis at the Centre for the first time.

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 or methionine which is known to confer resistance to baloxivir.

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 pyrosequencing.

Screening for baloxavir resistance in 2019

In 2019, a total of 559 viruses were successfully analysed using the FRA-BX assay (Table 12), of which none showed reduced susceptibility to baloxavir. Genetic screening of 345 viruses (Table 12) by pyrosequencing or sequencing identified one A(H3N2) virus from Sydney that contained a valine at the position 38 of the PA endonuclease gene. This mutation is not known to have any impact on the susceptibility of influenza viruses to baloxavir, and analysis using the FRA-BX assay showed that this virus had no reduction in susceptibility to baloxavir.

Table 12. Viruses screened for reduced susceptibility to baloxavir during 2019, by FRA=BX assay and pyrosequencing/sequencing.

	FRA-BX assay						Pyrosequencing/sequencing				
Type/ subtype/ lineage / Country	A(H1N1)pdm09	A(H3N2)	A mixed type	B/Victoria	B/Yamagata	TOTAL	A(H1N1)pdm09	A(H3N2)	B/Victoria	B/Yamagata	TOTAL
Australasia											
Australia	128	169	3	114	45	**459**	7	235	6	1	**249**
New Zealand	4	8		15	1	**28**		4	4		**8**
South Pacific											
Fiji	1			2		**3**	8	4	1		**13**
French Polynesia						**0**		2			**2**
New Caledonia		1				**1**		12		1	**13**
South East Asia											
Brunei	5				2	**7**	3	2			**5**
Cambodia	1					**1**		6		1	**7**
Indonesia				1	5	**6**		4			**4**
Malaysia				4		**4**		9	1	1	**11**
Philippines	5					**5**	1				**1**
Singapore	7	4				**11**		14			**14**
Thailand		4		2	4	**10**		3			**3**
Timor-Leste	2					**2**					**0**
East Asia											
Macau SAR	7	2				**9**					**0**
South Asia											
Sri Lanka	2	4		1	1	**8**					**0**
Africa											
South Africa	1	4				**5**		15			**15**
TOTAL	**163**	**196**	**3**	**139**	**58**	**559**	**19**	**310**	**12**	**4**	**345**

Resistance to Adamantanes

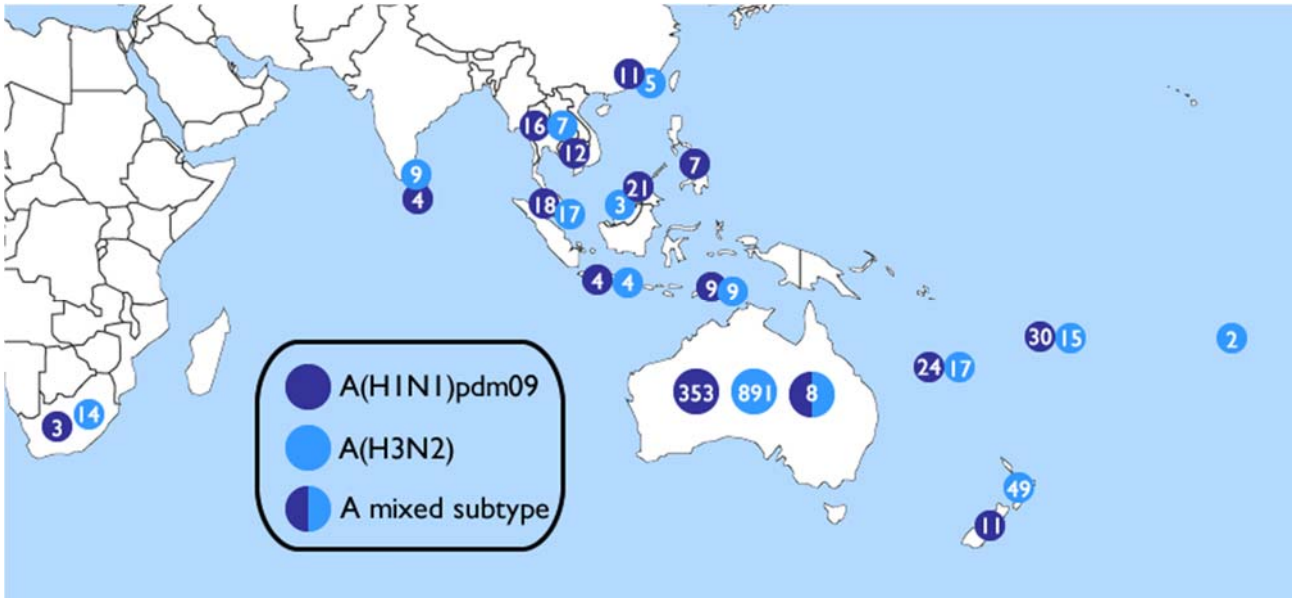
Background

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 2019

Real-time PCR or sequencing was used to analyse 1573 influenza A viruses, which were representative of those submitted to the Centre during 2019 (Figure 19). Almost all of the tested influenza A viruses carried the S31N mutation, indicating that they would be resistant to adamantanes. One A(H1N1)pdm09 virus from the Philippines contained a serine residue at position 31 in the matrix protein, which would render it susceptible to adamantanes.

Figure 19. Geographic spread of viruses received at the Centre during 2019 and screened for adamantane resistance.



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. Regulatory requirements stipulate that viruses used to produce human vaccines are isolated and passaged only in embryonated hens' eggs or primary egg-derived cell cultures. Accordingly, the Centre undertakes primary isolation of selected viruses from clinical samples directly into eggs. These isolates are then analysed by HI assay and genetic sequencing.

Isolation of viruses in eggs in 2019

In 2019, a total of 58 viruses were successfully isolated in eggs at the Centre, representing an overall isolation rate of 66.7% (Tables 13 and 14).

Table 13. Virus isolation in eggs at the Centre in 2019.

Type/subtype	Isolates attempted	Isolates obtained	Success rate (%)
A(H1N1)pdm09	18	16	88.9%
A(H3N2)	46	27	58.7%
B/Victoria	15	11	73.3%
B/Yamagata	8	4	50.0%
Total	**87**	**58**	**66.7%**

Table 14. Potential candidate vaccine strains isolated in eggs at the Centre in 2019 .

A(H1N1)pdm09	A(H3N2)		B/Victoria
A/Darwin/119/2018	A/Newcastle/82/2018	A/Christchurch/515/2019	B/Brisbane/35/2018
A/Darwin/122/2018	A/Newcastle/83/2018	A/Christchurch/516/2019	B/Sri Lanka/38/2018
A/Darwin/123/2018	A/Newcastle/104/2018	A/New Caledonia/59/2019	B/Bangkok/147/2018
A/Darwin/124/2018	A/South Australia/36/2019	A/South Australia/320/2019	B/Victoria/705/2018
A/Darwin/125/2018	A/South Australia/34/2019	A/Canberra/109/2019	B/Brisbane/36/2018
A/Brunei/5/2019	A/South Australia/39/2019	A/Darwin/177/2019	B/Newcastle/62/2018
A/Canberra/13/2019	A/Newcastle/78/2017	A/Victoria/718/2019	B/Darwin/7/2019
A/Newcastle/46/2019	A/South Australia/82/2019	A/Darwin/384/2019	B/Darwin/8/2019
A/Newcastle/48/2019	A/Newcastle/42/2019	A/Darwin/402/2019	B/Darwin/20/2019
A/Darwin/102/2019	A/Sydney/53/2019	A/South Australia/2/2019	B/Christchurch/505/2019
A/Victoria/212/2019	A/Newcastle/623/2019	A/South Australia/4/2019	B/Brisbane/21/2019
A/Victoria/214/2019	A/Victoria/703/2019	A/Victoria/223/2019	**B/Yamagata**
A/Canberra/337/2019	A/South Australia/218/2019	A/Victoria/169/2019	B/Brunei/10/2019
A/Darwin/639/2019	A/Christchurch/514/2019		B/Darwin/58/2019
A/Victoria/2454/2019			B/South Australia/6/2019
A/Victoria/2455/2019			B/Brisbane/1/2019

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 2019, 14 candidate vaccine viruses that had been received from other WHO Collaborating Centres and laboratories were passaged in eggs at the Centre (Table 15).

Selected egg-isolated candidate vaccine strains 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 15. Potential candidate vaccine viruses from other WHO Collaborating Centres isolated at the Centre during 2019.

A(H1N1)pdm09
A/Fujian-Gulou/SWL1884/2018
A/Guangdong-Maonan/SWL1536/2019
A/Iowa/12/2019
A(H3N2)
A/Hong Kong/681/2018
A/Netherlands/10260/2018
A/Abu Dhabi/240/2018
NIB-114 (A/Singapore/GP0454/2018)
NIB-115 (A/Sydney/22/2018)
NYMC X-327(A/Kansas/14/2017)
A/Beijing/2019-15554/2018
B/Victoria
B/Guangdong-Nanshan/38/2018
B/Guangdong-Nanshan/2018-33566/2018
B/Sichuan-Gaoxin/531/2018
B/Washington/02/2019

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 16). Serum panels from children, younger adults (20-64 years old) and older adults (≥ 65 years old) are assessed.

Serum panel analyses in February 2019

In February the Centre analysed serum panels from recipients of seasonal quadrivalent influenza vaccines in China, USA and Europe.

A(H1N1)pdm09: The combined data from all WHO Collaborating Centres and ERLs showed that geometric mean HI titres (GMT) of anti-HA antibodies against recently circulating A(H1N1) pdm09 viruses containing the S183P amino acid substitution in the HA protein were reduced compared to GMTs against the cell-grown vaccine strain A/Michigan/45/2015. Moreover, these reductions were greater when measured against egg-propagated vaccine virus.

A(H3N2): In HI assays, GMTs of antibodies against representative recent A(H3N2) viruses grown in eggs similar to titres against the egg-grown A/Singapore/INFIMH-16-0019/2016 vaccine virus – an exception to this were viruses from clade 3C.3a, which showed significant reductions in titres. In virus neutralisation tests, geometric mean neutralisation titres (GMNT) against recently representative A(H3N2) viruses grown in cells were significantly reduced compared to egg-propagated A/Singapore/INFIMH-16-0019/2016. When compared to cell-grown A/Singapore/INFIMH-16-0019/2016, only viruses from clade 3C.3a showed significantly lower GMNT levels.

B/Victoria: Serum panel analyses showed that GMT of antibodies against recent B/Victoria/2/87 lineage viruses representating the three major genetic groups containing three, two or no amino acid deletions in the HA protein had only small to medium reductions in GMTs compared to vaccine virus B/Colorado/06/2017 grown in both eggs and cell culture

B/Yamagata: GMTs against representative recent B/Yamagata/16/88 lineage viruses were only slightly reduced compared to HI titres to the B/Phuket/3073/2013 vaccine virus grown in cells.

Serum panel analyses in September 2019

In September, the Centre analysed serum panels from recipients of seasonal quadrivalent or trivalent (≥ 65years old in Australia) influenza vaccines in Australia.

A(H1N1)pdm09: The combined data from all WHO Collaborating Centres and ERLs showed that geometric mean HI titres (GMT) of anti-HA antibodies against many recently circulating A(H1N1)pdm09 viruses containing the S183P amino acid substitution in the HA protein were reduced compared to GMTs against the cell-grown vaccine strain A/Michigan/45/2015. Moreover, these reductions were greater when measured against egg-propagated vaccine virus.

A(H3N2): Serology studies using both HI assays and virus neutralization assays showed that GMTs and GMNTs of antibodies against most recent A (H3N2) viruses were reduced compared to titres against egg- and cell-grown vaccine viruses A/ Switzerland/8060/2017 (Southern Hemisphere vaccine virus 2019) and A/ Singapore/INFIMH-16-0019/2016 (Southern Hemisphere vaccine virus 2018).

B/Victoria: Serum panel analyses showed that GMT of antibodies against recent B/ Victoria/2/87 lineage viruses representating the three major genetic groups containing three, two or no amino acid deletions in the HA protein had only small to medium reductions in GMTs compared to the reference virus B/Colorado/06/2017 grown in both eggs and cell culture

B/Yamagata: GMTs against representative recent B/Yamagata/16/88 lineage viruses were only slightly reduced compared to HI titres to the B/Phuket/3073/2013 reference virus grown in cells.

Table 16. Representative and vaccine candidate strains used for serological analyses during 2019. All viruses are egg grown unless indicated otherwise.

FEBRUARY	SEPTEMBER
A(H1N1)pdm09	**A(H1N1)pdm09**
A/Michigan/45/2015*^ (C, E)	A/Michigan/45/2015*^ (C, E)
A/Sydney/181/2018 (C)	A/Darwin/102/2019 (C, E)
A/Tasmania/2/2018 (C)	A/Darwin/122/2018 (C, E)
A/Darwin/6/2018 (C,E)	A/Guangdong-Maonan/SWL1536/2019
A/Perth/148/2018	A/Wisconsin/505/2018 (C)
A(H3N2) (microneutralisation	**A(H3N2) (HI assays)**
A/Singapore/INFIMH-16-0019/2016	A/Switzerland/8060/2017*^ (C,E)
A/Switzerland/8060/2017*^ (C,E)	A/Kansas/14/2017 (C, E)
A/Sydney/22/2018 (C,E)	A/South Australia/2/2019 (C,E)
A/Victoria/28/2018 (C)	A/Sydney/53/2019 (E)
B/Victoria	**B/Victoria**
B/Colorado/6/2017*^ (C,E)	B/Colorado/6/2017*^ (C,E)
B/Sri Lanka/14/2018 (C,E)	B/Victora/705/2018 (C,E)
B/Brisbane/35/2018 (C)	B/Washington/2/2019 (C,E)
B/Yamagata	**B/Yamagata**
B/Phuket/3073/2013* (C,E)	B/Phuket/3073/2013* (C,E)
B/Victoria/704/2018 (C)	B/Darwin/58/2019 (C,E)
B/Christchurch/500/2018 €	
B/Darwin/26/2018	

**Trivalent vaccine strain ^Quadrivalent vaccine strain*

[E]: Egg-grown virus [C]: Cell-grown virus

Note: HI assays for A(H3N2) viruses were performed in the presence of oseltamivir

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 OIE/FAO Network of Expertise on Animal Influenza (OFFLU), the University of Cambridge, several WHO National Influenza Centres and other relevant organisations. In 2019 WHO made the recommendations reported below.

WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2019–2020, Beijing, China, 18 - 20 February 2019

It is recommended that vaccines for use in the 2019–2020 influenza season (northern hemisphere winter) contain the following:

- an A/Brisbane/02/2018^ (H1N1)pdm09-like virus;
- an A/Kansas/14/2017 (H3N2)-like virus;*
- a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and
- a B/Phuket/3073/2013^-like virus (B/Yamagata/16/88 lineage).

It is recommended that the influenza B virus component of trivalent vaccines for use in the 2019-2020 northern hemisphere influenza season be a B/Colorado/06/2017-like virus of the B/Victoria/2/87-lineage.

* The A(H3N2) component was recommended on 21 March 2019.

WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2020, Geneva, Switzerland, 23–26 September 2019

It is recommended that quadrivalent vaccines for use in the 2020 southern hemisphere influenza season contain the following:

- an A/Brisbane/02/2018^(H1N1)pdm09-like virus;
- an A/South Australia/34/2019^ (H3N2)-like virus;
- a B/Washington/02/2019-like (B/Victoria lineage) virus; and
- a B/Phuket/3073/2013^-like (B/Yamagata lineage) virus.

It is recommended that trivalent vaccines for use in the 2020 southern hemisphere influenza season contain the following:

- an A/Brisbane/02/2018 (H1N1)pdm09-like virus;
- an A/South Australia/34/2019 (H3N2)-like virus; and
- a B/Washington/02/2019-like (B/Victoria lineage) virus.

^ *Viruses originally isolated at the WHO Collaborating Centre in Melbourne.*

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 2019 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/Brisbane/02/2018# (Feb, Sept)	
A(H3N2)	A/South Australia/34/2019# (Sept) A/Christchurch/516/2019# (Sept)	A/Newcastle/82/2018 (Sept)
B/Victoria	B/Brisbane/35/2018#(Sept) B/Victoria/705/2018# (Sept)	B/Darwin/7/2019# (Sept)
B/Yamagata	B/Brisbane/9/2014 (Feb, Sept) B/Phuket/3073/2013 (Feb, Sept)	B/Brisbane/9/2014 (Feb, Sept) B/Singapore/INFKK-16-0569/2016 (Feb, Sept) B/Singapore/INFTT-16-0610/2016# (Feb, Sept)

 # Indicates CVVs newly included in the WHO list of viruses suitable for vaccine

Australian Seasonal Influenza Vaccine Recommendation

Whereas 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 AIVC.

The AIVC met on 9 October 2019 and recommended that the following viruses be used for influenza vaccines in the 2020 southern hemisphere influenza season:

Egg based quadrivalent vaccines:

- an A/Michigan/45/2015 (H1N1)pdm09-like virus;
- an A/Switzerland/8060/2017 (H3N2)-like virus;
- a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage); and
- a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage)

Egg based trivalent vaccines:

- an A/Michigan/45/2015 (H1N1)pdm09-like virus;
- an A/Switzerland/8060/2017 (H3N2)-like virus; and
- a B/Phuket/3073/2013-like (B/Yamagata/16/88 lineage)

Preparation and Distribution of Diagnostic Reagents

Reagents for Antigenic Typing of Influenza Viruses

Each year the Centre prepares and distributes kits to regional and reference laboratories to enable influenza preliminary analysis and characterisation of influenza specimens prior to submission of samples to the Centre. The kits contain polyclonal sera and viral antigens for reference influenza strains. During 2019, 20 kits were sent to 9 laboratories in 8 countries. Each kit contained 10 mL each of the reference antigens A/Michigan/45/2015, A/Switzerland/8060/2017, B/Colorado/2/2017 and B/Phuket/3073/2013 and homologous antisera.

Due to the lack of demand for the Centre's kits, this activity will cease after 2019. Leftover 2019 kits that are still within the expiry date will be supplied to laboratories requesting them in 2020 but no new kits will be made.

Recipients of the 2019 Kit

AUSTRALIA: Queensland Health Scientific Services, Brisbane, Queensland; Vaxxas,

CAMBODIA: Institut Pasteur, Phnom Penh

HONG KONG SAR: University of Hong Kong

INDIA: Manipal University, Manipal

MACAU SAR: Public Health Laboratory

PHILIPPINES: Research Institute for Tropical Medicine, Mutinlupa City

SINGAPORE: Singapore General Hospital

THAILAND: Thai National Influenza Center,

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 training visiting scientists at the Centre, participate in regional workshops and visit laboratories to provide direct assistance in strengthening surveillance capabilities.

Training Programs and Visits to Regional Laboratories

Sheena Sullivan and **Olivia Price** visited Male, Maldives on 24–28 February in a collaboration with the Dr Philip Gould (WHO Regional Office for South-East Asia, SEARO) and Dr Faiha Ibrahim (WHO Country office, Maldives) to determine the feasibility of:

1) Describing an influenza outbreak that occurred in 2017.

2) Collecting and analysing influenza denominator data to determine whether there are currently sufficient data to estimate the national influenza burden of disease. (SEARO) and (WHO Country office, Maldives)

Patrick Reading was involved in a mission to Assess the Influenza Laboratory at the Ethiopia Public Health Institute in Addis Ababa, Ethiopia on 2-5 April. He was invited as an expert to conduct an on-site assessment to validate the laboratory for its readiness and capacity to be recognised as a WHO National Influenza Centre.

Patrick Reading was an invited speaker at Pacific Public Health Surveillance Network (PPHSN) Regional Meeting, held in Nadi, Fiji, on June 6.

Patrick Reading visited the University of Malaya, in Kuala Lumpur, Malaysia on 13–24 May. He trained staff in cell culture and virus isolation techniques, as well as assessing molecular techniques used for influenza virus detection and identification. He also presented lectures on influenza surveillance, detection and characterisation, as well as virus sharing.

Photo courtesy of the University of Malaya

Patrick Reading visited the Institute for Medical Research in Kuala Lumpur, Malaysia on 13–24 May. He trained staff in cell culture and virus isolation techniques, as well as assessing molecular techniques used for influenza virus detection and identification. He also presented lectures on influenza surveillance, detection and characterisation, as well as virus sharing.

Photo courtesy of the Institute of Medical Research

Vivian Leung was a facilitator at a Training on Influenza Data Management and Basic Analysis workshop, organised by SEARO and the WHO Indonesia country office, held in Bandung, Indonesia on 17–22 June. She was a facilitator and presented three talks at the workshop, which was attended by approximately 30 staff members from SARI/ILI provincial and national sentinel sites.

Vivian Leung was a facilitator at a Workshop on Pandemic Influenza Severity Assessment (PISA), held in Jakarta, Indonesia on 24–26 June. She assisted with hands-on activities to calculate influenza thresholds. The workshop was attended by 11 participants from central and national sites.

Patrick Reading visited the National Institute of Health Research and Development (NIHRD) in Jakarta, Indonesia on 17–27 June. He trained staff in cell culture and virus isolation techniques, as well as assessing molecular techniques used for influenza virus detection and identification. He also presented lectures on influenza surveillance, detection and characterisation, as well as virus sharing.

Patrick Reading visited Institut Pasteur, Ho Chi Minh City, Vietnam, on 15 – 26 July. He trained staff in cell culture and virus isolation techniques, as well as assessing molecular techniques used for influenza virus detection and identification. He also presented lectures on influenza surveillance, detection and characterisation, and virus sharing.

Yi-Mo Deng and **Naomi Komadina** participated in a WHO GISRS Influenza Bioinformatics workshop with hands-on sessions as instructors in Singapore, on 26–27 August. The workshop was attended by 36 participants from National Influenza Centres, veterinary laboratories in the OFFLU network and the European Centre for Disease Control and Prevention (ECDC). Participants were trained in techniques for analysis and sharing of influenza virus genetic data, including bioinformatics, submission of sequences to public databases and interpretation of influenza virus sequence mutations.

Kanta Subbarao was a presenter at the India Vaccinology Course (INDVAC), in Vellore, India, on 19 - 21 September.

Yi-Mo Deng and **Naomi Komadina** led a training course on bioinformatics and data analysis of influenza viruses in Kajang, Malaysia, on 14–17 October. The workshop, which was attended by 26 participants from the Malaysia Ministry of Health laboratories, was organised by the Malaysian Ministry of Health, the WHO Representative Office for Malaysia, Brunei Darussalam & Singapore and the Centre.

Naomi Komadina participated as a trainer in an NGS Bioinformatics training course, held in Jakarta, Indonesia, on 4–8 November. The course was held by the Bioinformatics Institute (Singapore) and GISAID.

Centre-based Training

Ms Aisha Al Amri and Ms Ahlam Al Amri, from the Central Public Health Laboratory, Muscat, Oman, visited the Centre 8–19 October. They undertook training in virus isolation and serology techniques for analysis of influenza viruses, with a view to sharing their knowledge and skills with other scientists and National Influenza Centres in the Eastern Mediterranean Region of WHO (EMRO) following their time at the Centre.

Dr Fatima El Falaki, from the National Institute of Hygiene, Rabat, Morocco, visited the Centre 4-15 November, and Dr Massab Umair Raja, from the National Institute of Health, Islamabad, Pakistan, visited the Centre 11-22 November. They undertook training in virus isolation and serology techniques for the analysis of influenza viruses, as well as genetic analyses, with a view to sharing their knowledge and skills with other scientists and National Influenza Centres in the Eastern Mediterranean Region of WHO (EMRO).

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

Edin Mifsud, Sook Kwan Leah Brown, Leo Lee, Ankita George, Rubaiyea Farrukee, Paulina Koszalka, Aeron Hurt

Research overview

Our research focuses on improving our understanding of the effectiveness of currently approved influenza antivirals and compounds in late-phase human clinical trials, and the risk that drug resistant viruses may spread widely amongst the community.

In understanding viral fitness, it is important to assess the ability of different drug resistant variants to replicate *in vitro* or *in vivo* and then to assess the ability of the viruses to transmit between ferrets. This information will provide insights into the likelihood that such viruses could spread amongst the community.

A cooperative research and development agreement (CRADA) with Romark Laboratories which commenced in 2016 has continued to investigate *in vitro* and *in vivo* aspects of the repurposed drug nitazoxanide for its effectiveness against human and potentially pandemic avian influenza viruses. In addition a CRADA with Shionogi which commenced in 2018 continued to investigate whether the PA endonuclease inhibitor baloxavir is able to reduce the rate of transmission using the ferret model of infection.

Collaborators

James McCaw and Alex Zarebski (University of Melbourne); Jesse Bloom (Fred Hutchinson Cancer Research Centre, Seattle WA, USA); Jean-Francois Rossignol (Romark Laboratories, Tampa FL, USA); Takao Shishido and Keiko Baba (Shionogi TechnoAdvance Research, Osaka, Japan); Wendy Barclay (Imperial College London, London, United Kingdom).

Highlights and developments 2019

In our baloxavir studies, we found that treating influenza-infected ferrets with baloxavir significantly reduced viral shedding compared to oseltamivir and placebo, and subsequently reduced the risk of virus transmission to untreated ferrets housed in close contact. In collaboration with Shionogi and Imperial College London, our results provide significant supporting evidence for Centerstone, a first-of-its-kind clinical trial on the role of baloxavir to control the spread of influenza from infected patients to household contacts without prophylaxis. A paper describing these results was accepted for publication by PLoS Pathogens. Additionally, we developed a rapid pyrosequencing assay to detect viruses with low baloxavir susceptibility due to PA amino acid substitutions (PA/I38X). We are currently investigating the fitness costs of the PA/I38T substitution emerging in baloxavir-treated patients using competitive fitness experiments in ferrets.

In our studies of the influenza antiviral nitazoxanide, we showed that the combination of nitazoxanide and oseltamivir therapy in ferrets reduced the clinical signs associated with influenza infection and significantly reduced the number of ferrets shedding influenza virus when compared to either nitazoxanide or oseltamivir alone. The results of these studies were accepted for publication in Antiviral Research. Currently, *in vitro* and *in vivo* studies are being carried out to determine if combination therapy can prevent the selection of oseltamivir resistant influenza viruses.

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. As part of ongoing analyses of avian influenza in Antarctica, further samples from penguins in Antarctica were collected by our Chilean collaborators.

Collaborators

Marcel Klaassen (Deakin University, Victoria); Edward Holmes (University of Sydney, Sydney NSW); Daniel González Acuña (University of Concepción, Bío Bío Region, Chile); Frank Wong (Australian Animal Health Labs, Geelong VIC); Andrew Breed (Australian Government Department of Agriculture)

Highlights and developments 2019

In 2019, we collected and screened 824 swab samples from wild Anseriiformes (ducks) and Charadriiformes (shorebirds and terns) in Victoria, Tasmania and Western Australia, with 62 influenza A virus detections (see Table R1). These samples are being characterised and isolated in embryonated hens' eggs and will assist in the understanding of the ecology of avian influenza viruses in Australia. None of the viruses detected contained markers that would indicate they were highly pathogenic. Furthermore, to better understand influenza A infection burden we have collected and screened 687 serum samples for general anti-influenza A antibodies using a commercial NP-ELISA.

Starting at the end of 2019 we have embarked on a large collaborative project with key collaborators at the Australian Animal Health Laboratory (AAHL), Deakin University and University of Sydney and state laboratories across Australia to sequence and analyse original specimens that are positive by rRT-PCR and viral isolates. This project will be critical understanding the ecology and evolution of avian influenza A in Australia. This project is being funded by Wildlife Health Australia through funds provided by the Department of Agriculture.

In addition to classical approaches to screen for and characterise influenza A viruses, we have embarked on a new collaboration to use RNA sequencing (RNA-seq) to assess the total viral burden in Australian wild birds. A critical finding was that the presence of influenza A virus infection is associated with higher overall viral burden and viral diversity in shorebirds and ducks as compared to birds that were not infected with influenza A virus. This research has further allowed us to identify a number of other RNA viruses in wild birds that are important reservoir hosts for influenza A virus.

Table R1. Samples collected from wild birds in 2019

Avian order	**Serum samples**		**Swab samples**	
	Samples collected	*Influenza-positive samples*	*Samples collected*	*Influenza-positive samples*
Anseriformes	115	43	337	15
Charadriiformes	572	127	489	47

Epidemiology

Centre staff

Sheena Sullivan, Vivian Leung, Olivia Price, Arseniy Khvorov (University of Melbourne, UoM), Leslie Dowson (UoM), Angelyna Lee (UoM), Ailin Guan (UoM)

Research overview

We are interested in 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 conduct various simulation studies to understand the validity of vaccine effectiveness estimates for influenza vaccine strain selection.

We are particularly interested in understanding observations that vaccine effectiveness appears to be attenuated among people who are repeatedly vaccinated. To that end we are involved in several sero-epidemiology studies to understand the immunological mechanisms underlying these observations.

Highlights and developments 2019

We continued to work with the Australian Sentinel Practices Research Network (ASPREN), the Victorian General Practice Sentinel Surveillance (VicSPIN) network, and the Influenza Complications Alert Network (FluCAN) to estimate influenza vaccine effectiveness for the WHO Vaccine Consultation Meetings and publish interim estimates for the National Influenza Surveillance Committee. In 2019, we coordinated a report of interim vaccine effectiveness estimates for four southern hemisphere countries: Chile, New Zealand, South Africa and Australia, which was published in *Euro Surveillance*.

The group was successful in securing funding to conduct a large longitudinal cohort study to understand the long-term effects of repeated vaccination in hospital workers, led by Drs Sheena Sullivan and Annette Fox (Immunology unit) at the Centre, and Adam Kucharski (London School of Hygiene and Tropical Medicine). This study will commence in 2020, with recruitment in six Australian cities, and all laboratory analysis to be conducted at the Centre. Within the epidemiology team, two new staff have been recruited for this project: Arseniy Khvorov to lead the statistical analyses and Leslie Dowson to provide project administrative support.

We continued working on other serological studies (see *Human Immunity to Influenza*). In these studies, the epidemiology group is working to develop tools to better analyse antibody titre data, particularly for estimating correlates of protection (Arseniy Khvorov); estimation of average time between influenza infections (Ailin Guan); and antibody landscape analyses of samples from a 2016 study of repeated vaccination among hospital workers.

Continuing work on the burden of disease project undertaken with the Telethon Kids Institute, the University of Western Australia and University of Hong Kong in 2017-2018, we presented the outcomes of this work (Vivian Leung, Options X) and prepared several manuscripts for publication. Further analyses of these data to understand the potential impact of vaccination to mitigate the influenza burden is ongoing. Dr Sullivan was also successful in gaining access to the OptumLabs data (with Annette Regan and Onyebuchi Arah, UCLA) to understand the burden of influenza during pregnancy.

Under Angelyna Lee's lead, the Centre contributed to a bid to CDNA to consider RSV be listed as a notifiable disease, which is under consideration.

Collaborators

VE studies: Monique Chilver (University of Adelaide); Kylie Carville (VIDRL); Benjamin Cowling, Huiying Chua, (University of Hong Kong); Sander Greenland (UCLA); Jill Ferdinands (US CDC)

Burden of disease: Hannah Moore and Tom Snelling (Telethon Kids Institute); George Milne and Joel Kelso (University of Western Australia); Benjamin Cowling and Jessica Wong (University of Hong Kong); Annette Regan, Onyebuchi Arah (UCLA)

Serological studies: Benjamin Teh (Peter Macallum Cancer Centre); Kylie Carville (VIDRL); David Smith (PathWest, Perth); Adam Kucharski (London School of Hygiene and Tropical Medicine); Christopher Blyth (Telethon Kids Institute,); Helen Marshall (Women and Children's Hospital); Allen Cheng (Alfred Hospital); Kristine Macartney (Sydney Children's Hospital Network); Peter Wark (John Hunter Hospital); Julia Clark (Brisbane Children's Hospital); Benjamin Cowling and Wey Wen Lim (University of Hong Kong); Mark Thompson, Min Levine (US CDC); Scott Hensley (University of Pennsylvania)

Human Immunity to Influenza

Centre staff and student

Annette Fox, Louise Carolan, Ryan (Yeu-Yang) Tseng, Sheena Sullivan, Vivian Leung, Maria Auladell Bernat

Research overview

A key goal of our work is to identify strategies to improve the immunogenicity and hence effectiveness of seasonal influenza vaccines. It is challenging to induce long-term immunity against highly mutable viruses such as influenza, not only due to immune escape, but also to a propensity for antibody levels to decline with successive exposures to variant influenza virus strains. This phenomenon was first described in the 1950's and referred to as original antigenic sin. It is thought that immune responses (antibodies or B cells) induced by prior influenza exposures interfere with the development of immunity to new strains. In particular, memory B cells that cross-react with shared epitopes in subsequent strains may out-compete naïve B cells for the resources required for activation, so that responses become focused on epitopes from old strains, and are not updated towards new strains. We have established several human influenza cohorts to document and investigate the effects of prior influenza exposures on influenza vaccine responses, and have developed techniques to explore the specificity of antibody and B cell responses to influenza vaccination.

Collaborators

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)

Human Immunity to Influenza (continued)

Highlights and developments 2019

During 2019 we focused on increasing our capacity to investigate why humans respond variably, and sometimes poorly to influenza vaccination. This has been achieved by (1) securing funding to establish and investigate additional human vaccination cohorts; and (2) developing laboratory analyses to dissect the cellular and molecular processes that shape the immune response to vaccination.

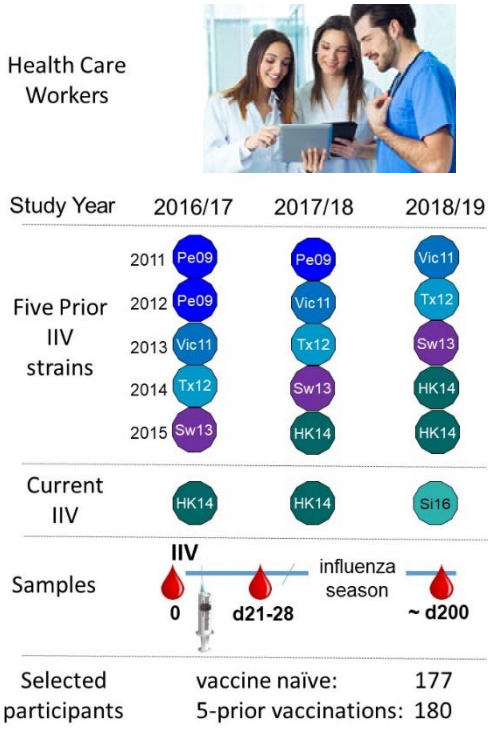


Figure R1. *Studying Effects of Repeated Vaccination in Health Care Workers in Peru and Israel*

A US Centers for Disease Control and Prevention (CDC) funded study is underway to assess sera collected from Health Care Workers (HCW) in Peru and Israel (Figure R1). These vaccine studies, led by Mark Thompson (CDC), were conducted yearly from 2016/17 to 2018/19. To investigate the hypothesis that repeated vaccination promotes focusing of antibody responses on limited epitopes that have been shared between successively encountered vaccine strains we are comparing the breadth of antibodies induced by vaccination among 177 first-time versus 180 highly vaccinated HCW (Figure 1). The HCW selected in each group have similar age and sex distributions, and include three vaccine years, so we can determine how the vaccine strain used may modify repeat vaccination effects. The strain-coverage of antibodies induced by vaccination, and the relative extent of back-boosting versus forward-boosting is being determined by haemagglutination inhibition (HI) assay of 1071 sera (Pre+post-vaccination) against 32-35 well-characterized viruses. A second set of samples from 41 HCW who had little prior vaccination is being assessed to determine if the breadth of antibody response declines during each successive vaccination. A third set of samples from HCW who were infected after vaccination (vaccine failure) is being assessed to determine if interim infection expands the breadth of vaccine responses. We hypothesize that infection alleviates antibody focusing because antigen and inflammation is sufficient in magnitude or duration to induce responses to novel epitopes of new virus strains. We have selected 7 health care workers who had a PCR confirmed A(H3N2) infection in study year 1 and 14 health care workers of similar age, sex, and prior-vaccination history, but with no virologic or serologic evidence of interim A(H3N2) infection as controls. The breath of antibodies induced by vaccination in the year after infection (in cases) will be compared between cases and controls.

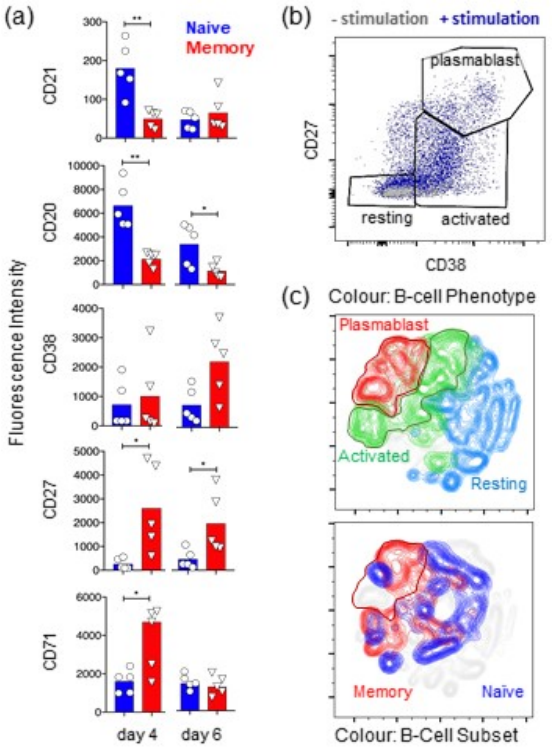


Figure R2. *Naïve and memory B cells become activated but remain phenotypically distinct up to 6 days after robust* in vitro *stimulation*
*(**a**) Fluorescence intensity of indicated surface markers on sorted naïve versus memory human B cells after in vitro stimulation for 4- or 6-days. (**b**) Surface expression of CD27 and CD38 are increased on naïve B cells that have been stimulated compared to unstimulated counterparts. (**c**) Analysis using T-SNE software to cluster cells based on expression of all surface markers used shows that sorted naïve and memory B cells fall into distinct phenotypic clusters up to 6-days after activation, even within regions comprising plasmablasts and activated B cells.*

A five-year study funded by the US National Institute of Health (NIH) is underway to prospectively recruit 1500 health care workers from 6 hospitals across Australia and investigate influenza vaccine immunogenicity and effectiveness. As above, we will use sera to investigate and compare the breadth of antibodies induced by vaccination. To further investigate antibody focusing we have been developing a competition ELISA to detect antibodies in serum that compete with antigenic-site specific mAbs for binding to HA1-subunit proteins of A(H3N2) viruses. mAbs generated by ourselves and others are being produced in house. Sera that predominantly inhibits mAbs against one site will be further tested in HI against wild-type viruses versus viruses that have been engineered to contain substitutions within suspected sites of antibody focusing.

In addition to serological studies we are performing nested studies to investigate the cellular mechanisms that determine the magnitude and breadth of antibodies induced. Specifically, we are collecting peripheral blood mononuclear cells (PBMCs) to determine the ancestry of vaccine-responsive B cells, and investigate the hypothesis that recalled memory B cells dominate responses and monopolize resources needed to generate B cells and antibodies against new strain epitopes. We have identified a panel of lymphocyte cell surface markers that can be used in flow cytometry to distinguish naïve from memory B cells up to 6 days after stimulation (Figure R2, PMC6851823). These markers are being used in conjunction with fluorescent-labelled recombinant-influenza haemagglutinin (HA) probes to detect influenza-reactive B cells and characterize their (cross)-reactivity with prevailing and past strains and their phenotype (Figure R3). Preliminary results for the ex vivo analysis of B cells pre- and post-vaccination support the hypothesis that recalled memory B cells dominate the immune response to H3N2 HA following vaccination (Figure R3).

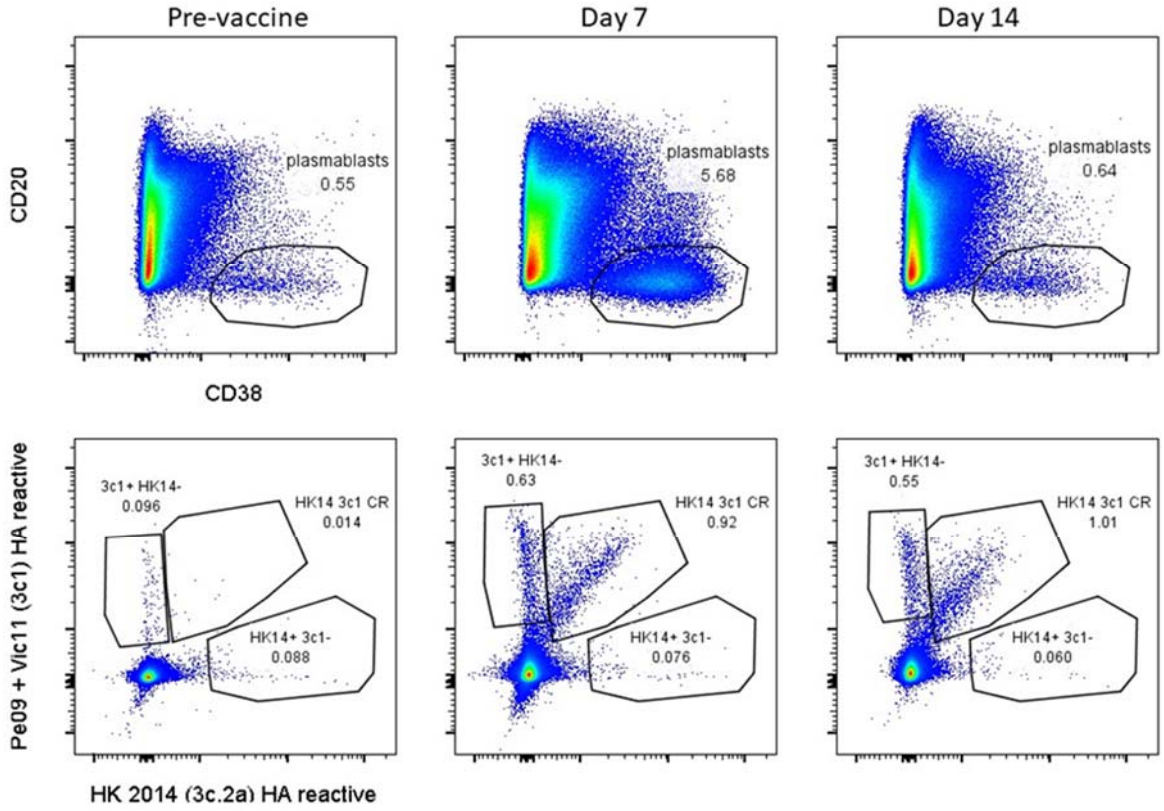


Figure R3. *PBMCs collected pre/post vaccination in 2016 were thawed and directly stained with fluorescent labelled B cell phenotypic markers and influenza-HA probes. Vaccination induced plasmablasts (effector B cells, top panel) and HA-reactive B cells (bottom panel), which predominantly (cross)-reacted (CR) with prior- (Pe-2009+Vic-2011) and vaccine-strain (HK 2014) HA, suggesting that they are recalled memory B cells. Results for one vaccinee are shown. The top panel shows cells in the CD19^{+} B cell gate, the bottom panel shows cells in the IgD-negative B cell gate.*

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.

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 and (iii) utilizing approaches to simulate host innate immunity to limit the impact of subsequent infection with influenza or other respiratory viruses. In addition, we collaborate with researchers at the University of Queensland to develop and assess novel vaccines against influenza and other viruses.

Collaborators

Paul Young (University of Queensland); Nathan Bartlett (University of Newcastle); Kirsten Spann (Queensland University of Technology); Lara Herrero (Griffith University); Daniel Steinfort (Royal Melbourne Hospital); Andrew Brooks, Justine Mintern, Stephen Kent, David Jackson, Lorena Brown, Carol Hartley and Joanne Devlin (The University of Melbourne)

Highlights and developments 2019

During 2019, our research focussed on understanding and characterising particular intracellular proteins (termed restriction factors) that are expressed or induced in host cells and can block the replication of influenza and/or other respiratory viruses. We are now using approaches to ectopically overexpress or delete putative restriction factors to determine their role in blocking virus replication and to characterise their mechanism/s of antiviral activity.

Dr Reading was also Chief Investigator on two new grants which commenced in 2019, (i) NHMRC Development Grant (2019-2021, 'Clamp stabilised vaccines to provide broad spectrum protection against influenza') and (ii) Coalition of Epidemic Preparedness Innovations (2019-2021, 'Rapid response pipeline for stabilised subunit vaccines'). These grants fund research with collaborators at the University of Queensland to investigate novel recombinant vaccines against influenza viruses, as well as other viruses such as respiratory syncytial virus (RSV). James Barnes joined the group as a research assistant in February 2019 and has been developing assays to measure antibody-dependent cell-mediated cytotoxicity to vaccination, as well as assessing novel vaccines in ferret models of infection.

Overall, our research contributed to six peer-reviewed publications during 2019, in journals such as The Journal of Virology, Virology and Frontiers in Immunology. In 2019, Dr Reading leads 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 is co-supervisor of an additional four Ph.D. students enrolled at the University of Melbourne. He is also the Partner Investigator on a successful ARC Discovery Project Grant (2020-2022, 'Harnessing innate immunity to mitigate bovine respiratory disease').

Evolution, Modelling and Serological Responses to Influenza Viruses

Centre staff

Ian Barr, Aeron Hurt, Malet Aban, Yi-Mo Deng, Sheena Sullivan, Annette Fox

Research overview

We are undertaking several collaborative projects both with local and international groups to investigate various aspects of influenza virus evolution and the immune responses to influenza viruses and vaccines.

A project titled "Advanced vaccination and immunity management strategies to protect from influenza virus infection" is funded by the US based CEIRS (Centers of Excellence for Influenza Research and Surveillance) group based at the Mount Sinai Hospital (New York City NY, USA). This project aims to identify future influenza viruses in advance of them becoming widespread. This would enable the generation of vaccine candidate viruses to provide enhanced protection in contrast to the current system whereby vaccine viruses are chosen some 9-10 months in advance of the relevant influenza season. Work has continued using reverse engineered HA mutant influenza viruses with changes introduced by site directed mutagenesis and antibody escape mutants in order to produce viruses that may resemble future circulating influenza viruses. Extensive antigenic testing (using both HI and virus microneutralisation assays) of mutated viruses using a combination of ferret and human antisera has been undertaken.

Another ongoing collaboration is with Vijaykrishna Dhanasekaran and Miguel Lopez (Monash University) and Gene Tan (J. Craig Venter Institute, La Jolla CA, USA). We have continued analyzing sequence data from previous batches of influenza B/Yamagata viruses and are also analysing the full genomes of 804 A(H3N2) viruses collected from Australia in 2017 and 2018. Results from these studies are being compiled for future publications.

In 2018 the Centre, with Dan Layton and Andrew Bean at AAHL, CSIRO, obtained a 3-year post-doctoral position grant from the CSIRO ResearchPlus Program. This enabled Dr Mailing Dai to be employed in mid-2018 and this work has progressed into 2019. The project is investigating the differential responses *in vitro* between avian influenza viruses that cause severe disease and mild disease in humans. A molecular approach has been taken to allow a genome-wide scan of cellular genes to identify those that may account for these differences.

Highlights and developments 2019

Enhanced influenza vaccines may improve protection for older adults, but comparative immunogenicity data are limited. Our objective was to examine immune responses to enhanced influenza vaccines, compared to standard-dose vaccines, in community-dwelling older adults. Mean fold rises (MFR) in haemagglutination inhibition (HI) titres to egg-propagated A(H1N1)pdm09 and A(H3N2) viruses and the MFR in microneutralisation assays to cell-propagated A(H3N2) viruses were statistically significantly higher in the enhanced vaccine groups, compared to the standard-dose vaccine. This is the first years results of a multiyear study which includes several cross-over vaccinations with the different vaccines.

This project resulted in the following publication:

Comparative Immunogenicity of Several Enhanced Influenza Vaccine Options for Older Adults: A Randomized, Controlled Trial. Cowling BJ et al. Clin Infect Dis. 2019 [Epub ahead of print Dec 2019]

Collaborators

Derek Smith and Sam Wilks (Cambridge University, UK); Yoshihiro Kawaoka (The University of Wisconsin, Madison WI, USA and The University of Tokyo, Japan); Ron Fouchier (Erasmus University, Rotterdam, The Netherlands); Edward Bolongia (Marshfield Clinic Research Foundation, Marshfield WI, USA); Vijaykrishna Dhanasekaran and Miguel Lopez (Monash University); Alan Durbin and Gene Tan (J. Craig Venter Institute, Rockville and La Jolla CA, USA); Edward Holmes and Jemma Geohagan (University of Sydney/Macquarie University, NSW); Malik Peiris and Benjamin Cowling (University of Hong Kong, Hong Kong SAR), Dan Layton Andrew Bean and Meiling Dai (AAHL, CSIRO Geelong).

Collaborative Agreements

The Centre is party to four collaborative research and development agreements 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.

Agreement with the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA) (2018-2019)

Centre staff: Hilda Lau, Ian Barr, Heidi Peck, Cleve Rynehart

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

Highlights and developments 2019: A total of 58 egg isolates were obtained from 87 inoculations with original clinical specimens from various geographical locations. Isolation rates varied from 50% to 89% according to virus type/subtype and lineage. Suitable isolates were made available to other laboratories and industry for reassortment and assessment as vaccine candidates.

Cooperative Research and Development Agreement with Seqirus: Development and provision of influenza virus strains isolated on MDCK 33016PF cells for vaccine

Centre staff: Heidi Peck, Cleve Rynehart, Sally Soppe, Ian Barr

Project overview: Using a proprietary Seqirus cell line to isolate influenza viruses, the Centre produces potential candidate vaccine viruses (CVVs) for cell-based influenza vaccine manufacture. A number of original clinical specimens are used to isolate viruses directly into the MDCK33016PF cell line. The resultant isolates undergo analysis of their growth, antigenic characteristics and other properties

Highlights and developments 2019: During 2019, 107 clinical specimens were cultured in MDCK 33016PF cells, of which 68 (64%) produced isolates. The isolates, which comprised A(H1N1) pdm09, A(H3N2), B/Victoria and B/Yamagata viruses, were sent to Seqirus in Holly Springs NC, USA, for further evaluation as potential vaccine candidates produced by cell culture.

All four vaccine components included in the cell-based influenza vaccine are now fully derived from cells. The B/Yamagata component of the Seqirus cell-based vaccine for the Northern Hemisphere 2019-2020 season continues to use B/Singapore/INFTT-16-0610/2016, a cell-derived seed isolated at the Centre.

Agreement with Romark Laboratories: Studies of the influenza antiviral nitazoxanide (2016-2019)

Centre staff: Edin Mifsud, Danielle Tilmanis, Aeron Hurt

Overview: The Centre is evaluating the effectiveness of the influenza antiviral nitazoxanide *in vitro* and *in vivo* (ferret and mouse models) using both seasonal influenza viruses and potentially pandemic viruses influenza vaccines

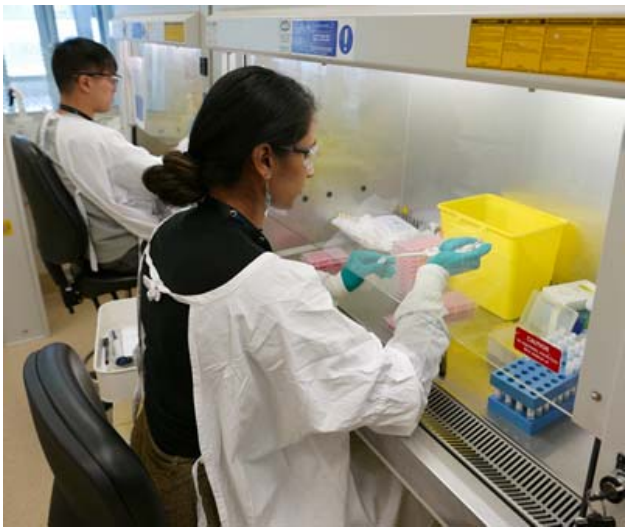
Highlights and developments 2019: *In vitro* studies have shown that nitazoxanide and oseltamivir have an additive to synergistic interaction. In a ferret model, combination treatment of the nitazoxanide and oseltamivir was more effective at preventing influenza virus infection and lower respiratory tract replication compared to oseltamivir treatment alone. In 2019 the results of these studies were accepted for publication in Antiviral Research.

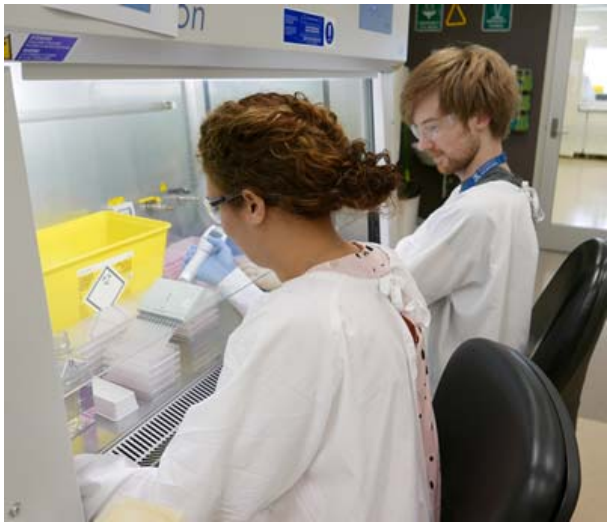
Cooperative Research and Development Agreement with Shionogi TechnoAdvance Research (2018-2020)

Centre staff: Leo Lee, Edin Mifsud, Paulina Koszalka, Aeron Hurt

Overview: The Centre is evaluating the effectiveness of the influenza antiviral baloxavir in preventing the transmission of influenza virus.

Highlights and developments 2019: We found that treating influenza-infected ferrets with baloxavir significantly reduced viral shedding and reduced the risk of virus transmission to untreated ferrets. Our results provide significant evidence supporting the role of baloxavir to control the spread of influenza from infected patients to household contacts without prophylaxis. We also developed a rapid pyrosequencing assay to detect amino acid substitutions (eg. I38X) in the PA gene which would reduce the susceptibility of viruses to baloxavir. We are also investigating the fitness costs of the PA/I38T substitution emerging in baloxavir-treated patients using competitive fitness experiments in ferrets.





Research Students

PhD Candidates

Ms Rubaiyea Farrukee, a PhD candidate from the University of Melbourne, completed her PhD project project titled "Assessing replication, transmission and fitness of antiviral resistant influenza viruses" in August 2019. She was supervised by **Aeron Hurt** and **Patrick Reading**.

Ms Paulina Koszalka, a PhD candidate from Monash University, continued her PhD project titled "Efficacy, resistance and drug interactions for influenza antivirals in clinical development", under the supervision of **Aeron Hurt**, **Kanta Subbarao** and Vijaykrishna Dhanasekaran (Monash University).

Ms Annika Suttie, a PhD candidate from Federation University, completed her PhD project titled "Molecular epidemiology of influenza virus in Cambodia" in August 2019. She was supervised by Andrew Greenhill (Federation University), **Yi-Mo Deng**, Jenny Mosse (Federation University) and Paul Horwood (Institut Pasteur du Cambodge).

Research Funding and Awards

Centre staff members are Chief Investigators in grants

Australian Government's Agricultural Competitiveness White Paper: *Enhancing avian influenza serology capability in Australia – ascertaining maintenance of HA specific neutralizing antibodies in shorebirds*

$33,000 awarded for the period 1 February – 31 December 2019. **Michelle Wille** and **Aeron Hurt** were investigators. Co-investigators are Marcel Klaassen (Deakin University), Andrew Breed (Australian Government Department of Agriculture and Water Resources) and Frank Wong (AAHL). The project was administered by Deakin University and the work was undertaken at primarily at the Centre, with support from Deakin University, the Australian Government Department of Agriculture, and the Australian Animal Health Laboratory.

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?*

USD $4.2 million awarded for the period 3 July 2019 – 30 June 2024. **Sheena Sullivan**, **Annette Fox** and Adam Kucharski (London School of Hygiene and Tropical Medicine) are Principal Investigators and Project Directors. **Kanta Subbarao** is a Co-Investigator. 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.

Australian Research Council (ARC) Discovery Early Career Researcher Award (DECRA)

$419,016 awarded to **Michelle Wille** for the period July 2020 – June 2023. The award will enable Dr Wille to continue her research which focuses on investigating host factors associated with the diversity, evolution and dynamics of viruses in Australian in wild birds using state-of-the-art metatranscriptomics. The DECRA will be administered by the University of Sydney.

ARC Discovery Project Grant: *Harnessing innate immunity to mitigate bovine respiratory disease.*

$583,049 awarded for the period 1 January 2020 – 31 December 2022. **Patrick Reading** is Partner Investigator, Professor David Jackson (University of Melbourne) is Chief Investigator. The project will be administered at the University of Melbourne, where the work will be undertaken.

Contract with the US Centers for Disease Control: *Understanding Heterogeneity in Influenza Vaccine Responses*

USD$587,200 awarded for the period 1 July 2019 – 31 December 2020. Sheena Sullivan is lead investigator. The project is administered by the University of Melbourne and the work will be undertaken at the Centre.

Melbourne Health Kearton Travel Award

$4,000 awarded to **Vivian Leung**. She used the award to attend the Options for the Control of Influenza X conference, held in Singapore, 28 August – 1 September 2019.

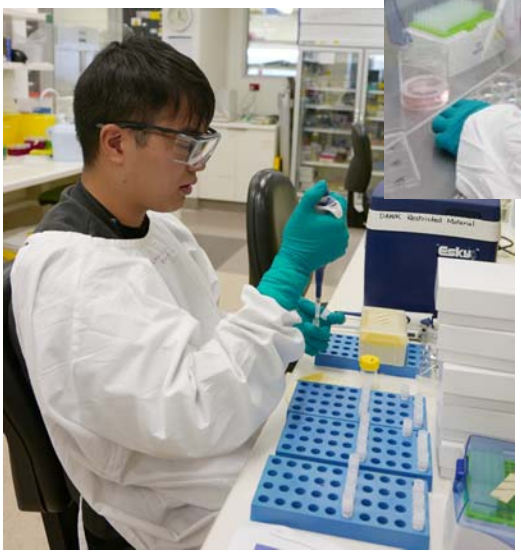
Royal Melbourne Hospital Grant in Aid: *Can rapid point of care testing improve influenza infection outcomes for residents in aged care?*

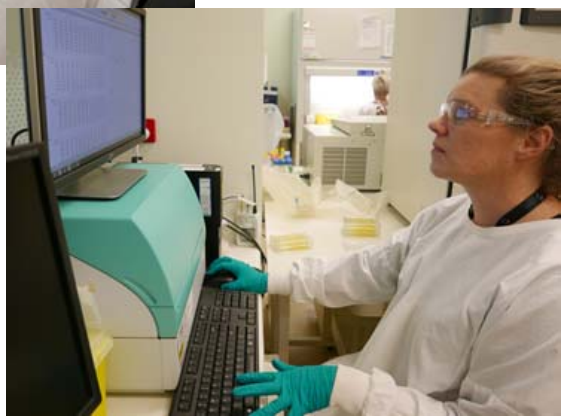
$25,000 awarded for the period 1 February 2020-31 January 2021. Sheena Sullivan is the lead investigator. The grant will be administered by the Royal Melbourne Hospital and the work will be undertaken at the Centre.

Australian Government Department of Agriculture (National Avian Influenza Wild Bird Surveillance Special Project Proposal): *Placing Australia in the global avian influenza phylogeography*

$69,132 for the period November 2019 – December 2020. **Michelle Wille** and Frank Wong (Australian Animal Health Laboratory, AAHL) are lead investigators. Co-investigators are Marcel Klaassen (Deakin University) and Edward Holmes (University of Sydney). The project will be administered by the Royal Melbourne Hospital and AAHL, and the work will be undertaken at the Centre, AAHL and the University of Sydney.

Around the labs....





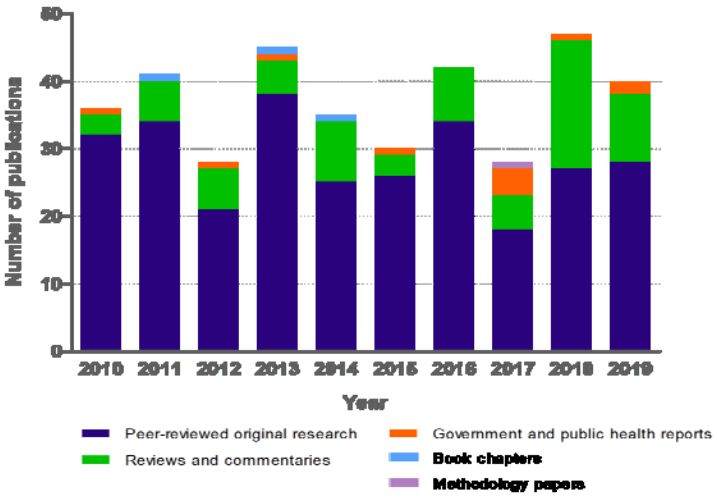
Communications and Advisory Activities

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 40 original research papers, reviews and reports in 2019 (Figure 20).

Figure 20. Centre publications 2010–2019.



Centre Publications 2019

1. Auladell M, Jia X, Hensen L, Chua B, **Fox A**, Nguyen THO, Doherty PC and Kedzierska K. Recalling the future: immunological memory toward unpredictable influenza viruses. Front Immunol, 2019. 10: 1400.

2. Auladell M, Nguyen TH, Garcillan B, Mackay F, Kedzierska K and **Fox A**. Distinguishing naive- from memory-derived human B cells during acute responses. Clin Transl Immunology, 2019. 8 (11): e01090.

3. **Barr IG**, **Deng YM**, Grau ML, Han AX, Gilmour R, Irwin M, Markey P, Freeman K, Higgins G, Turra M, **Komadina N**, **Peck H**, Booy R, Maurer-Stroh S, Dhanasekaran V and **Sullivan S**. Intense interseasonal influenza outbreaks, Australia, 2018/19. Euro Surveill, 2019. 24(33).

4. Beale DJ, **Oh DY**, Karpe AV, **Tai C**, Dunn MS, **Tilmanis D**, Palombo EA and **Hurt AC**. Untargeted metabolomics analysis of the upper respiratory tract of ferrets following influenza A virus infection and oseltamivir treatment. Metabolomics, 2019. 15(3): 33.

5. Bedford JG, O'Keeffe M, **Reading PC** and Wakim LM. Rapid interferon independent expression of IFITM3 following T cell activation protects cells from influenza virus infection. PLoS One, 2019. 14(1): e0210132.

6. Chang WS, Pettersson JH, Le Lay C, Shi M, Lo N, **Wille M**, Eden JS and Holmes EC. Novel hepatitis D-like agents in vertebrates and invertebrates. Virus Evol, 2019. 5(2): vez021.

7. Cowling BJ and **Sullivan SG**. The value of neuraminidase inhibition antibody titers in influenza seroepidemiology. J Infect Dis, 2019. 219(3): 341-343.

8. Hay JA, **Laurie K**, White M and Riley S. Characterising antibody kinetics from multiple influenza infection and vaccination events in ferrets. PLoS Comput Biol, 2019. 15(8): e1007294.

9. Horwood PF, Karlsson EA, Horm SV, Ly S, Heng S, Chin S, Darapheak C, Saunders D, Chanthap L, Rith S, Y P, Chea KL, Sar B, Parry A, Ieng V, Tsuyouka R, **Deng YM**, **Hurt AC**, **Barr IG**, **Komadina N**, Buchy P and Dussart P. Circulation and characterization of seasonal influenza viruses in Cambodia, 2012-2015. Influenza Other Respir Viruses, 2019. 13(5): 465-476.

10. **Hurt AC**. Antiviral therapy for the next influenza pandemic. Trop Med Infect Dis, 2019. 4 (2).

11. **Hurt AC**, **Tai CM** and **Oh DY**. Evaluation of the open-access video tracking programme Swarm Sight to evaluate lethargy in a ferret model of influenza infection. *J Anim Health Behav Sci*, 2019. 3

Centre Publications (continued)

12. Jennings LC and **Barr IG**. Future pandemic influenza virus detection relies on the existing influenza surveillance systems: a perspective from Australia and New Zealand. Trop Med Infect Dis, 2019. 4(4).

13. Kode SS, Pawar SD, Tare DS, Keng SS, **Hurt AC** and Mullick J. A novel I117T substitution in neuraminidase of highly pathogenic avian influenza H5N1 virus conferring reduced susceptibility to oseltamivir and zanamivir. Vet Microbiol, 2019. 235: 21-24.

14. **Komadina N**, **Sullivan SG**, Kedzierska K, Quiñones-Parra S, Leder K and McVernon J. Prior exposure to immunogenic peptides found in human influenza A viruses may influence the age distribution of cases with avian influenza H5N1 and H7N9 virus infections. Epidemiol Infect, 2019. 147: e213.

15. Koutsakos M, Illing PT, Nguyen THO, Mifsud NA, Crawford JC, Rizzetto S, Eltahla AA, Clemens EB, Sant S, Chua BY, Wong CY, Allen EK, Teng D, Dash P, Boyd DF, Grzelak L, Zeng W, **Hurt AC**, **Barr I**, Rockman S, Jackson DC, Kotsimbos TC, Cheng AC, Richards M, Westall GP, Loudovaris T, Mannering SI, Elliott M, Tangye SG, Wakim LM, Rossjohn J, Vijaykrishna D, Luciani F, Thomas PG, Gras S, Purcell AW and Kedzierska K. Human $CD8^+$ T cell cross-reactivity across influenza A, B and C viruses. Nat Immunol, 2019. 20(5): 613-625.

16. Koutsakos M, Kedzierska K and **Subbarao K**. Immune responses to avian influenza viruses. J Immunol, 2019. 202(2): 382-391.

17. Koutsakos M, McWilliam HEG, Aktepe TE, Fritzlar S, Illing PT, Mifsud NA, Purcell AW, Rockman S, **Reading PC**, Vivian JP, Rossjohn J, Brooks AG, Mackenzie JM, Mintern JD, Villadangos JA, Nguyen THO and Kedzierska K. Downregulation of MHC class I expression by influenza A and B viruses. Front Immunol, 2019. 10: 1158.

18. **Lau H**, **Deng YM**, Xu X, Sessions W and **Barr IG**. Rapid detection of new B/Victoria-lineage haemagglutinin variants of influenza B viruses by pyrosequencing. Diagn Microbiol Infect Dis, 2019. 93(4): 311-317.

19. Lee JM, Eguia R, Zost SJ, Choudhary S, Wilson PC, Bedford T, Stevens-Ayers T, Boeckh M, **Hurt AC**, Lakdawala SS, Hensley SE and Bloom JD. Mapping person-to-person variation in viral mutations that escape polyclonal serum targeting influenza hemagglutinin. Elife, 2019. 8.

20. **Leung VK**, **Deng YM**, **Kaye M**, **Buettner I**, **Lau H**, **Leang SK**, **Gillespie L** and **Chow MK**. Annual report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza in 2016. Commun Dis Intell (2018), 2019. 43.

21. Liu Y, Tan HX, Koutsakos M, Jegaskanda S, Esterbauer R, **Tilmanis D**, **Aban M**, Kedzierska K, **Hurt AC**, Kent SJ and Wheatley AK. Cross-lineage protection by human antibodies binding the influenza B hemagglutinin. Nat Commun, 2019. 10(1): 324.

22. Marcelino VR, **Wille M**, **Hurt AC**, Gonzalez-Acuna D, Klaassen M, Schlub TE, Eden JS, Shi M, Iredell JR, Sorrell TC and Holmes EC. Meta-transcriptomics reveals a diverse antibiotic resistance gene pool in avian microbiomes. BMC Biol, 2019. 17(1): 31.

23. **Price OH**, Carville KS and **Sullivan SG**. Right sizing for vaccine effectiveness studies: how many is enough for reliable estimation? Commun Dis Intell (2018), 2019. 43.

24. **Roe M**, **Kaye M**, **Iannello P**, **Lau H**, **Buettner I**, **Tolosa MX**, **Zakis T**, **Leung VK** and **Chow MK**. Report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza in 2017. Commun Dis Intell (2018), 2019. 43.

25. Smith DW, **Barr IG**, Loh R, Levy A, Tempone S, O'Dea M, Watson J, Wong FYK and Effler PV. Respiratory illness in a piggery associated with the first identified outbreak of swine influenza in Australia: assessing the risk to human health and zoonotic potential. Trop Med Infect Dis, 2019. 4(2).

Centre Publications (continued)

26. **Subbarao K**. The critical interspecies transmission barrier at the animal-human interface. Trop Med Infect Dis, 2019. 4(2).

27. **Subbarao K** and **Barr I**. A tale of two mutations: beginning to understand the problems with egg-based influenza vaccines? Cell Host Microbe, 2019. 25(6): 773-775.

28. **Sullivan SG**, Arriola CS, Bocacao J, Burgos P, Bustos P, Carville KS, Cheng AC, Chilver MB, Cohen C, **Deng YM**, El Omeiri N, Fasce RA, Hellferscee O, Huang QS, Gonzalez C, Jelley L, **Leung VK**, Lopez L, McAnerney JM, McNeill A, Olivares MF, Peck H, Sotomayor V, Tempia S, Vergara N, von Gottberg A, Walaza S and Wood T. Heterogeneity in influenza seasonality and vaccine effectiveness in Australia, Chile, New Zealand and South Africa: early estimates of the 2019 influenza season. Euro Surveill, 2019. 24(45).

29. **Sullivan SG** and Cowling BJ. Reconciling estimates of the global influenza burden. Lancet Respir Med, 2019. 7(1): 8-9.

30. **Sullivan SG**, **Price OH** and Regan AK. Burden, effectiveness and safety of influenza vaccines in elderly, paediatric and pregnant populations. Ther Adv Vaccines Immunother, 2019. 7: 2515135519826481.

31. **Suttie A**, **Deng YM**, Greenhill AR, Dussart P, Horwood PF and Karlsson EA. Inventory of molecular markers affecting biological characteristics of avian influenza A viruses. Virus Genes, 2019. 55(6): 739-768.

32. **Suttie A**, Tok S, Yann S, Keo P, Horm SV, **Roe M**, **Kaye M**, Sorn S, Holl D, Tum S, Buchy P, **Barr I**, **Hurt A**, Greenhill AR, Karlsson EA, Vijaykrishna D, **Deng YM**, Dussart P and Horwood PF. Diversity of A(H5N1) clade 2.3.2.1c avian influenza viruses with evidence of reassortment in Cambodia, 2014-2016. PLoS One, 2019. 14(12): e0226108.

33. Tan HX, Jegaskanda S, Juno JA, Esterbauer R, Wong J, Kelly HG, Liu Y, **Tilmanis D**, **Hurt AC**, Yewdell JW, Kent SJ and Wheatley AK. Subdominance and poor intrinsic immunogenicity limit humoral immunity targeting influenza HA stem. J Clin Invest, 2019. 129(2): 850-862.

34. Tare DS, Kode SS, **Hurt AC** and Pawar SD. Assessing the susceptibility of highly pathogenic avian influenza H5N1 viruses to oseltamivir using embryonated chicken eggs. *Indian J Med Res*, 2019. 150(5): 486-491.

35. Villalón-Letelier F and **Reading PC**. Unraveling the role of the MOV10 RNA helicase during influenza A virus infection. Biochem J, 2019. 476(6): 1005-1008.

36. Wang H, FitzPatrick M, Wilson NJ, Anthony D, **Reading PC**, Satzke C, Dunne EM, Licciardi PV, Seow HJ, Nichol K, Adcock IM, Chung KF, Anderson GP, Vlahos R, Wark P and Bozinovski S. CSF3R/CD114 mediates infection-dependent transition to severe asthma. J Allergy Clin Immunol, 2019. 143(2): 785-788 e6.

37. **Wille M**, **Aban M**, Wang J, Moore N, Shan S, Marshall J, Gonzalez-Acuna D, Vijaykrishna D, Butler J, Wang J, Hall RJ, Williams DT and **Hurt AC**. Antarctic penguins as reservoirs of diversity for avian avulaviruses. J Virol, 2019. 93(11).

38. **Wille M**, Lisovski S, Risely A, Ferenczi M, Roshier D, Wong FYK, Breed AC, Klaassen M and **Hurt AC**. Serologic evidence of exposure to highly pathogenic avian influenza H5 viruses in migratory shorebirds, Australia. Emerg Infect Dis, 2019. 25(10): 1903-1910.

39. **Wille M**, Shi M, Klaassen M, **Hurt AC** and Holmes EC. Virome heterogeneity and connectivity in waterfowl and shorebird communities. ISME J, 2019. 13(10): 2603-2616.

40. Young B, Sadarangani S, Haur SY, Yung CF, **Barr I**, Connolly J, Chen M and Wilder-Smith A. Semiannual versus annual influenza vaccination in older adults in the tropics: an observer-blind, active-comparator-controlled, randomized superiority trial. Clin Infect Dis, 2019. 69(1): 121-129.

Presentations

Centre staff members presented talks and posters at numerous events during 2019, including national and international conferences, WHO meetings, educational lectures and research seminars.

ORAL PRESENTATIONS

Event/Institute; Location, date	SPEAKER, Title(s)
Immunisation Coalition Annual Scientific Meeting; Melbourne, 3-4 February	AERON HURT: New influenza antivirals. IAN BARR: Review of 2018 influenza season in Australia and what to expect in 2019. KANTA SUBBARAO: Vaccine options for seasonal influenza.
Victorian Biodiversity Conference; Melbourne, 7-8 February	MICHELLE WILLE: Factors affecting RNA virus diversity in wild birds.
Vaccines in the 21st Century; Melbourne, 14 February	KANTA SUBBARAO: Influenza vaccine: current options.
Pathology Update: The Power of Personalised Pathology; Melbourne, 22-24 February	ANNETTE FOX: Update on influenza monitoring and vaccine development.
Doherty Institute Respiratory Research Seminars; Melbourne, 25 March	RUBAIYEA FARRUKEE: Assessing the replication, transmission and fitness of antiviral resistant influenza viruses.
Immunological assays and correlates of protection for next generation influenza vaccines; Siena, Italy, 31 March - 2 April	KANTA SUBBARAO: Prime boosting studies.
WHO Meeting to Launch Phase-2 of the RSV Surveillance Pilot based on the GISRS; Kathmandu, Nepal, 10-12 April	IAN BARR: RSV typing protocols and EQAP.
Pathogen discovery: from genomics to disease recognition and response; Perth, 2 -3 May	KANTA SUBBARAO: Animal models for influenza pathogenicity assessment.
Visit to University Malaya; Kuala Lumpur, Malaysia, 13-24 May	PATRICK READING: Virus isolation external quality assessment programme in WPR/SEAR, 2016.; Cell culture and influenza virus isolation in cell culture.; Test methods for influenza: clinical specimens and virus isolates.; The importance of influenza virus isolates – virus sharing and GISRS.; Final recommendations.
Visit to the Institute for Medical Research; Kuala Lumpur, Malaysia, 13-24 May	PATRICK READING: Virus isolation external quality assessment programme in WPR/SEAR, 2016.; Cell culture and influenza virus isolation in cell culture.; Test methods for influenza: clinical specimens and virus isolates.; The importance of influenza virus isolates – virus sharing and GISRS.; Final recommendations.
Laboratory research meeting, Catherine Satzke's group, Royal Children's Hospital; Melbourne, 21 May	EDIN MIFSUD: Influenza and the pneumococcus.
You Never Forget the Flu - what you need to know about this year's flu season; Melbourne, 27 May	KANTA SUBBARAO: Influenza: the illness and the vaccine.

ORAL PRESENTATIONS (continued)

Event/Institute; Location, date	SPEAKER, Title(s)
2009 Influenza Pandemic in Victoria 10th Anniversary; Melbourne, 28 May	IAN BARR: Reflections on the 2009 H1N1 pandemic and the virus that is still with us.
PhD Completion Seminar, Department of Microbiology and Immunology, The University of Melbourne; Melbourne, 31 May	RUBAIYEA FARRUKEE: Assessing the transmission, replication and fitness of antiviral resistant influenza viruses.
Ecosystem Change and Population Health Symposium; Brisbane, 3-4 June	IAN BARR: Influenza pandemics and epidemics in Australia.
Veterinary BioSciences Seminar Series, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne; Melbourne, 6 June	MICHELLE WILLE: Factors affecting RNA virus diversity in wild birds.
Pacific Public Health Surveillance Network (PPHSN) Regional Meeting; Nadi, Fiji, 6 June	PATRICK READING: Influenza virus – current global overview.
PhD Completion Seminar, Federation University; Phnom Penh, Cambodia, 11 June	ANNIKA SUTTIE: Molecular epidemiology of influenza virus in Cambodia.
I-MOVE (Influenza Monitoring Vaccine Effectiveness) meeting; Annecy, France, 17-20 June	SHEENA SULLIVAN: High interseasonal activity 2018–19 Australia.
Influenza Data Management and Basic Analysis workshop; Bandung, Indonesia, 17-22 June	VIVIAN LEUNG: Importance of data management; Constructing a data management system; Identifying problem sources at data entry and collection
Visit to National Institute of Health Research and Development (NIHRD); Jakarta, Indonesia, 17-27 June	PATRICK READING: Virus isolation external quality assessment programme in WPR/SEAR, 2016.; Cell culture and influenza virus isolation in cell culture.; Test methods for influenza: clinical specimens and virus isolates.; The importance of influenza virus isolates – virus sharing and GISRS.; Final recommendations
WHO Influenza Burden of Disease (BOD) Policy Planning meeting; Geneva, Switzerland, 25-27 June	SHEENA SULLIVAN: The burden of influenza in Australia and its implications for vaccination policy.
Australasian Ornithological Conference; Darwin, 3-5 July	MICHELLE WILLE: Factors affecting RNA virus diversity in wild birds.
Australian Society for Microbiology Annual Scientific Meeting 2019; Melbourne, 5-8 July	HEIDI PECK: Characterisation of influenza viruses circulating in Australia during a high inter-seasonal period in 2018-9.
Victorian Infectious Diseases Service (VIDS) seminar; Melbourne, 7 July	KANTA SUBBARAO: Influenza update 2019.
Doherty Institute Work in Progress seminar; Melbourne, 9 July	RUBAIYEA FARRUKEE: Assessing the transmission, replication and fitness of antiviral resistant influenza viruses.
GI-CoRE: 7th Meeting of the Consortium for the Control of Zoonoses; Sapporo, Japan, 11-12 July	EDIN MIFSUD: Utilizing the ferret model to investigate antiviral compounds for respiratory infections.

ORAL PRESENTATIONS (continued)

Event/Institute; Location, date	SPEAKER, Title(s)
Visit to Institute Pasteur; Ho Chi Minh City, Vietnam, 15-26 July	PATRICK READING: Virus isolation external quality assessment programme in WPR/SEAR, 2016.; Cell culture and influenza virus isolation in cell culture.; Test methods for influenza: clinical specimens and virus isolates.; The importance of influenza virus isolates - virus sharing and GISRS.; Final recommendations.
ASEAN Health Cluster 2 Regional Consultative Meeting on Strengthening Influenza Laboratory Surveillance; Nusa Dua, Indonesia, 16-18 July	KANTA SUBBARAO: Detection and characterization of influenza viruses.; Antiviral treatment and susceptibility
Doherty Institute Special Postgraduate Association for Students of Immunology and Microbiology (SPASIM) student retreat; Melbourne, 8-9 August	PAULINA KOZSALKA: Baloxavir marboxil: Understanding the selection of resistance to a new influenza antiviral drug. *(Awarded the prize for the best talk)*
Research seminar, School of Life Sciences, La Trobe University; Melbourne, 12 August	PATRICK READING: Influenza: supporting WHO regional surveillance Networks and insights into innate immunity.
13th Biregional Meeting of National Influenza Centres and Influenza Surveillance in the Western Pacific and South-East Asia Regions; Ulaanbaatar, Mongolia, 20-23 August	IAN BARR: Data integration - combining data from the laboratory and meta data.
WHO GISRS Influenza Bioinformatics workshop; Singapore, 26-27 August	NAOMI KOMADINA: The GISAID Initiative: real time global communication in disease prevention.; Searching and downloading data using GISAID EpiFlu YI-MO DENG: WHO Guidance for implementing NGS for genetic analysis of influenza viruses in NICs.
Options for the Control of Influenza X; Singapore, 28 August - 1 September	ANNETTE FOX: Immune responses to repeated vaccination. (*invited presentation)* EDIN MIFSUD: Evaluating the window of susceptibility to secondary bacterial infections post-influenza infection in ferrets. KANTA SUBBARAO: A bird's eye view of influenza research. (*Keynote session)* RUBAIYEA FARRUKEE: Predicting evolutionary pathways to 'fit' oseltamivir resistant influenza viruses. SHEENA SULLIVAN: Explaining differences in vaccine effectiveness and virus circulation in the southern hemisphere, 2019. (*invited presentation)* VIVIAN LEUNG: Antibody response and influenza-like illness among healthcare workers after influenza vaccination.
The University of Newcastle School of Biomedical Sciences & Pharmacy Seminar Series; Newcastle, NSW, 6 September	KANTA SUBBARAO: Seasonal and pandemic influenza.
Doherty Institute 5 year anniversary event: Patient Zero; Melbourne, 10 September	HEIDI PECK: Characterisation of influenza viruses circulating in Australia during an intense inter-seasonal period in 2018-19.

ORAL PRESENTATIONS (continued)

Event/Institute; Location, date	SPEAKER, Title(s)
13th Vaccine Congress; Bangkok, Thailand, 15-18 September	KANTA SUBBARAO: Seasonal and pandemic influenza vaccines: principles and challenges.
India Vaccinology Course (INDVAC); Vellore, India, 19-21 September	KANTA SUBBARAO: Seasonal influenza vaccines.; pandemic influenza vaccines.
Australian Influenza Vaccine Committee; Canberra, 9 October	KANTA SUBBARAO: Update on influenza viruses of pandemic potential.
Australian Academy of Health and Medical Sciences General and Scientific Annual Meeting 2019; Perth, 9-11 October	KANTA SUBBARAO: A hundred years of influenza pandemics.
Doherty Institute Respiratory Research Seminar; Melbourne, 21 October	SHEENA SULLIVAN: Establishing immune correlates of protection for influenza vaccines.
Australasian Epidemiological Association Annual Scientific Meeting; Brisbane, 23-25 October	SHEENA SULLIVAN: Sparse data bias in influenza vaccine effectiveness estimates for the elderly.; The forgotten art of standardisation when generalising effect estimates from a sample.
Australian Influenza Sympsosium; Brisbane, 28-29 October	EDIN MIFSUD: Evaluating the window of susceptibility to secondary bacterial infections post-influenza infection in ferrets. IAN BARR: Intense interseasonal influenza outbreaks, Australia, 2018/19. YI-MO DENG: Human infection of a locally acquired swine-origin influenza A(H3N2) variant in Australia.
University of Queensland, Molecular Biosciences Seminar Series; Brisbane, 30 October	MICHELLE WILLE: Viruses on the wing: Factors affecting the RNA virus diversity in wild birds.
PHAA Communicable Disease Conference; Canberra, 19-20 November	SHEENA SULLIVAN: Influenza vaccine effectiveness when the season doesn't end.
Australasian Virology Society meeting; Queenstown, New Zealand, 2-5 December	KANTA SUBBARAO: What do post-vaccination antibody kinetics suggest about the timing of the seasonal influenza vaccine?

POSTER PRESENTATIONS

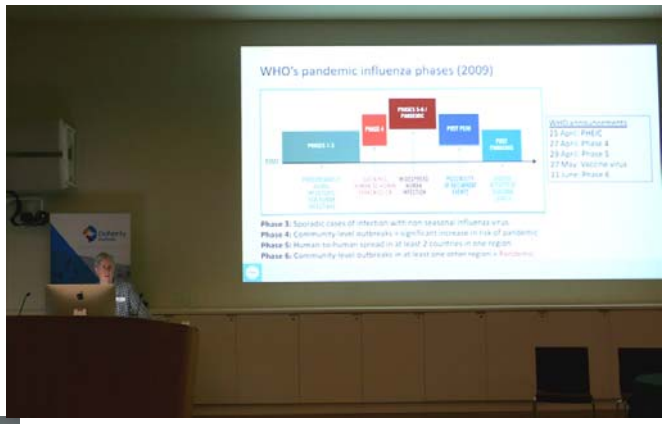
Event; Location, date	Title and authors *(Centre authors are marked in bold, presenting author is underlined)*
Immunological assays and correlates of protection for next generation influenza vaccines; Siena, Italy, 31 March - 2 April	Influenza vaccine immune responses and effectiveness are influenced by exposure history. **Fox A**, **Sullivan S**, Auladell M, **Carolan L**, Phuong HVM, **Leung V**, Thanh LT, Hằng NLK, Anh DD, Thai PQ, Duong TN, **Barr I**, **Subbarao K**, van Doorn R and Mai LQ
WHO Meeting to Launch Phase-2 of the RSV Surveillance Pilot based on the GISRS; Kathmandu, Nepal, 10-12 April	Leveraging GISRS for RSV Surveillance in Australia. Pratt J, McMinn A, **Todd A**, **Deng YM**, **Sullivan S**, **Subbarao K**, Crawford N and **Barr IG**
Options for the Control of Influenza X; Singapore, 28 August - 1 September	Combination therapy with nitazoxanide and oseltamivir reduces the impact of influenza virus infection *in vitro* and *in vivo*. **Mifsud E**, **Tilmanis D**, **Oh DY**, **Tai CM**, Nuessing S, Rossignol JF, Kedzierska K and **Hurt AC** Intense interseasonal influenza outbreaks, Australia, 2018/19. **Peck H**, **Leung V**, **Deng YM**, **Sullivan S** and **Barr I** Baloxavir reduces transmission of influenza virus by direct contact in ferrets. **Lee LY**, **Mifsud E**, **Koszalka P**, Noda T, Baba K, Ando Y, Sato K, Ishikawa Y, Shishido T and **Hurt AC** Prioritizing influenza vaccine allocation during a pandemic - a review of pandemic plans. Fielding J, Beard F, Dawson A, Macartney K, McVernon J, Massey P, Moss R, **Subbarao K** and **Sullivan S**. Who should get priority access to pandemic influenza vaccine? An ethics response. Williams J, Fielding J, Massey P, McVernon J, Moss R, **Sullivan S** and Dawson A Estimating the burden of influenza to inform vaccination policy in Australia. **Leung VKY**, Wong JY, Barnes R, Cowling BJ, Kelso J, Milne G, Moore HC and **Sullivan SG** The first human case of zoonotic influenza swine H3N2 variant in Australia and its association with local swine influenza viruses. **Deng Y-M**, Wong F, **Spirason N**, **Kaye M**, Beazley R, **Grau ML**, **Sullivan S**, **Barr IG** and Vijaykrishna D Antibody titres elicited by the 2018 seasonal inactivated influenza vaccine persist for at least 6 months. Mordant F, Rudraraju R, **Price O**, Slavin M, Marshall C, Worth L, **Peck H**, **Barr I**, **Sullivan S** and **Subbarao K**
Australasian Virology Society meeting; Queenstown, New Zealand, 2-5 December	Development of a cell-based antiviral assay to determine the susceptibility of HPAI viruses to influenza antivirals. **Tilmanis D**, **Koszalka P**, **Oh DY**, Rossignol JF and **Hurt AC** Egg adaptions of A(H3N2) influenza viruses and potential effects on influenza vaccine effectiveness. **Lau H** and **Barr IG** The first human case of zoonotic influenza swine H3N2 variant in Australia and its association with local swine influenza viruses. **Deng YM**, Wong F, **Spirason N**, **Kaye M**, Beazley R, **Grau ML**, **Sullivan S**, **Barr IG** and Vijaykrishna D Hollow fibre infection model to study the selection of resistance to baloxavir - a new influenza antiviral. **Koszalka P**, **Tilmanis D**, Vijaykrishna D, **Subbarao K** and **Hurt AC** *(Awarded the Monash Biomedicine Discovery Institute poster prize)*

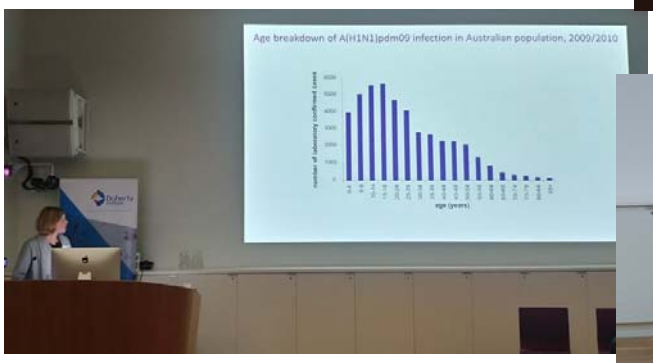
Special seminar: retrospective on the 2009 influenza pandemic in Victoria

In May 2019, the Centre organised and hosted a half-day seminar to commemorate 10 years since the 2009 influenza pandemic in Victoria. As this was a significant event in the history of the Centre and for many of the organisations that it works and collaborates with, this retrospective seminar provided an opportunity to reflect on the 2009 pandemic and lessons learned.

Talks were presented by people who worked at the forefront of the pandemic response in 2009, including WHO influenza surveillance laboratories, the Victorian and Australian Government Departments of Health, hospitals, vaccine manufacturers and research groups. A panel discussion considered the question of whether we are in better position to manage an influenza pandemic now compared to 2009. Some themes that were raised through the presentations and discussion included:

- the importance of collaboration and communication between different organisations - highlighted by the effectiveness with which the Australian influenza community worked together in 2009, and the importance of maintaining those networks between emergencies
- challenges in the surge capacity of resources and personnel to keep up with clinical, diagnostic surveillance and data management needs during the pandemic
- recognising that different geographical areas within the country may experience different levels of infection at any given time, and the national plan might require inter-jurisdiction flexibility
- establishing and maintaining collaborative relationships, protocols and policies as much as possible in advance of a pandemic or emergency situation will help when such an event occurs
- disease severity and public response to pandemics may provide unpredictable challenges
- flexibility in ethical review can enable timely research to inform pandemic control strategies





Australian Influenza Symposium

The 13th Australian Influenza Symposium was held at the Queensland University of Technology (QUT) on 28-29 October 2019. The Symposium was attended by approximately 200 people, including representatives from the biomedical, clinical research, public health, government and industry sectors from Australia and other countries including the United States, China and the Republic of the Congo.

Delegates enjoyed of talks presented by national and international speakers, including:

Benjamin Cowling, The University of Hong Kong, Hong Kong SAR
Janet Englund, Fred Hutchinson Cancer Research Center, Seattle WA, USA
Belinda Herring, WHO Regional Office, Brazzaville, Republic of the Congo
Jonathan Temte, University of Wisconsin, Madison WI, USA
Hui-Ling Yen, The University of Hong Kong, Hong Kong SAR

A wide variety of topics related to influenza were discussed, including influenza surveillance and community studies in different parts of the world; epidemiological studies involving mapping, modelling and forecasting the incidence of influenza and effects of vaccination; the unusual 2019 influenza season in Australia; developments in methods of influenza detection and vaccination and potential policy impacts; and recent developments in fundamental influenza research and commercial industry. A roundtable discussion also considered how influenza surveillance in Australia could be improved. The Symposium was also followed by a special joint session on Respiratory Syncytial Virus (RSV) with the Australian Respiratory Virology Meeting.

For the first time Symposium was reported on Twitter (#AIS2019Bris). The organising committee for the Symposium was Ian Barr, Jayde Simpson, Kirsten Spann (QUT) and Anne-Marie Lacaze (QUT). Most staff members from the Centre attended the Symposium. In addition, Ian Barr, Yi-Mo Deng and Edin Mifsud presented talks. Kanta Subbarao chaired a plenary session and Sheena Sullivan chaired the roundtable discussion.

Committees and Advisory Groups

Centre staff members served on the following governing boards, committees and advisory groups during 2019.

Ian Barr:
Australasian Vaccine & Immunotherapeutics Development Group, Organising Committee
Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Centre of Excellence for Influenza Research and Surveillance) program at St Judes Children's Research Hospital, Scientific Advisory Committee
Doherty Institute Shared PC3 Laboratory Advisory Committee, Member
Influenza Research and Treatment, Editorial Board
Influenza and other respiratory viruses, Editorial Board
Public Health Laboratory Network (Department of Health)

Michelle Chow
Doherty Institute Communications Working Group, Member

Yi-Mo Deng
WHO Working Group for GISRS PCR detection for influenza surveillance, Member

Aeron Hurt (*until July 2019*)**:**
Antiviral Research, Editorial Board
Avian Influenza in Wild Birds, Australian Wildlife Health Network, Steering Committee
Doherty Institute Safety Committee, Member
Frontiers in Microbiology, Associate editor
Infection, Ecology and Epidemiology – The One Health Journal, Editorial Advisory Board
Influenza Specialist Group, Scientific Committee
International Society for Influenza and other Respiratory Virus Diseases, Board of Trustees
Neuraminidase Inhibitor Susceptibility Network Meeting/Committee of Antiviral Special Interest Group of the International Society for Influenza and other Respiratory Virus Diseases, Committee member
Victorian Infection and Immunity Network, Executive Committee member
WHO Working Group for influenza antiviral resistance, Committee member

Naomi Komadina
Global Initiative on Sharing All Influenza Data (GISAID), GISAID Database Technical Committee (Chair)

Katie Milne
Medical Laboratory Quality Network
Victorian Infectious Disease Reference Laboratory NATA Action Group, Member

Patrick Reading
Australian Respiratory Virology Meeting, Organising committee
Influenza and Other Respiratory Viruses, Editorial board
Doherty Institute, Discipline leader, Education and Professional Development

Committees and Advisory Groups (continued)

Kanta Subbarao
National Influenza Surveillance Committee (Department of Health)
Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Doherty Institute Leadership Group, Member
Doherty Institute Operational Management Committee, Member
Doherty Institute, Discipline leader, Global Health
Scientific Advisory Board for the Universal Influenza Vaccine Project at Mount Sinai School of Medicine, New York City NY, USA Member
External Advisory Board, FLUCOP consortium, Member
Scientific Advisory Board for the Gates Center for Structure Guided Design of Next Generation Vaccine Immunogens at The Scripps Research Institute, La Jolla, CA, USA. Member,
Scientific Advisory Working Group for the Vaccine Research Center, NIAID, National Institutes of Health, Bethesda, MD, USA
PLoS Pathogens, Section Editor
mBio, Editorial board

Sheena Sullivan
BMC Public Health, Associate Editor
National Influenza Surveillance Committee (Department of Health), Observer
Doherty Institute, Equity and Diversity in Science Committee
Australasian Epidemiology Association, Secretary
WHO SAGE Working Group on Influenza Vaccines, Member

Angela Todd
Victorian Infectious Diseases Reference Laboratory Safety Committee, Member

Michelle Wille
National Avian Influenza Wild Bird Surveillance, Steering Committee
PLoS One, Editor
Wildlife Health Australia, Member
Victorian Wader Study Group, Member

Visitors to the Centre

The Centre was pleased to host the following visitors during 2019.

Date	VISITOR and affiliation
16 May	DR DANIEL LAYTON; Australian Animal Health Laboratory, CSIRO, Geelong, Victoria; Visiting scientist, presented a research seminar
29 May	DELEGATION FROM SK BIOSCIENCE: HUN KIM, OHSEOK JEONG AND YUNHEE KIM; SK Bioscience, Andong, South Korea
20 June	DR G. ARUNKUMAR: Manipal Institute of Virology, Manipal, India; Visiting scientist
18 October	DR NIHAL ALTAN-BONNET; National Institutes of Health, Bethesda MD, USA; Visiting scientist
31 October	DR HUI-LING YEN; University of Hong Kong, Hong Kong SAR, China; Visiting scientist
31 October	DR MARK TOMPKINS; University of Georgia, Athens GA, USA; Visiting scientist
31 October	PROF JOHNATHAN TEMTE; University of Wisconsin, Madison WI, USA; Visiting scientist
5 November -6 December	MS HUIYIN CHUA; The University of Hong Kong, Hong Kong SAR, China; Visiting PhD student

Engagement in WHO activities

Event; Location, Date	Centre staff involved
WHO Consultation on the Composition of Influenza Virus Vaccines for Use in the 2019-20 Northern Hemisphere Influenza Season; Beijing, China, 18-20 February	Ian Barr and Kanta Subbarao participated. Aeron Hurt attended.
SEARO Regional Workshop of International Health Regulations (IHR) National Focal Points; New Delhi, India, 25-29 March	Ian Barr and Olivia Price attended.
WHO Mission, Hanoi, Vietnam on 29 July-2 August	Sheena Sullivan participated by providing technical advice and support to the Ministry of Health (MOH) of Vietnam and its partners on its influenza surveillance activities. The mission conducted a review of Influenza-like Illness (ILI) sentinel surveillance.
13th Biregional Meeting of National Influenza Centres and Influenza Surveillance in the Western Pacific and South-East Asia Regions; Ulaanbaatar, Mongolia, 20-23 August	Heidi Peck, Kanta Subbarao, Patrick Reading, Sheena Sullivan and Yi-Mo Deng attended. Ian Barr presented a talk.
WHO PIP Partnership Contribution Independent Technical Expert Mechanism meeting; Geneva, Switzerland, 1-2 October	Kanta Subbarao attended.

Other Conference Participation and Professional Engagement

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

Event; Location, date	Centre staff involvement
2009 Influenza Pandemic in Victoria 10th Anniversary; Melbourne, 28 May	Kanta Subbarao chaired a panel discussion. Yi-Mo Deng was a panel discussion participant.
12th Annual CEIRS Network Meeting; Baltimore MD, USA, 23-28 June	Ian Barr attended.
Options for the Control of Influenza X; Singapore, 28 August - 1 September	Ian Barr and Naomi Komadina attended. Kanta Subbarao was a member of the International Organising Committee and chaired a session.
Australian Respiratory Virology Meeting; Brisbane, 30 October	Danielle Tilmanis, Edin Mifsud, James Barnes, Kanta Subbarao and Paul Whitney attended. Patrick Reading was a member of the organising committee and chaired a session.
Australasian Virology Society meeting; Queenstown, New Zealand, 2-5 December	Malet Aban 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 the year.

Ian Barr

- Participated in an Interview with RN Breakfast for the segment "Summer flu spike", published 4 February 2019; https://www.abc.net.au/radionational/programs/breakfast/summer-flu-spike/10776210
- Participated in an interview with on the radio program PM, ABC News for the segment "Could Australia be headed for its worst flu season on record?" on 8 April 2019; https://www.abc.net.au/radio/programs/pm/could-australia-be-headed-for-its-worst-flu-season-on-record/10982296
- Participated in an interview for the ABC news article "Flu vaccine program brought forward as surge in cases surprises health authorities", published 8 April 2019; https://www.abc.net.au/news/2019-04-08/flu-vaccine-program-brought-forward-as-numbers-spike/10980370
- Wrote an article "When's the best time to get your flu shot?" for The Conversation, published 15 April 2019; https://theconversation.com/whens-the-best-time-to-get-your-flu-shot-114978
- Participated in an interview for the Triple J Hack article "Here's why getting the flu shot will never give you the flu", published 15 May 2019; https://www.abc.net.au/triplej/programs/hack/heres-why-getting-the-flu-shot-will-never-give-you-the-flu/11115552
- Participated in an interview with The Age for the article "Three children among 26 dead in Victoria's start to flu season", published 21 May 2019; https://www.theage.com.au/national/victoria/three-children-among-26-dead-in-victoria-s-start-to-flu-season-20190521-p51pij.html
- Participated in an interview with 9 News for the segment "Victoria's deadly flu season starts earlier than expected", published 21 May 2019; https://www.9news.com.au/videos/victorias-deadly-flu-season-starts-earlier-than-expected/cjvxjt4we006f0gph6lrtxret
- Participated in an interview with ABC news for the article "Influenza cases just the 'tip of the iceberg', worse than reported figures, researcher says", published 2 June 2019; https://www.abc.net.au/news/2019-06-02/influenza-cases-tip-of-iceberg-may-be-ten-times-what-we-thought/11125192
- Reviewed the article "Flu season is here. This is all you need to know about symptoms, complications and vaccines" for ABC Life, published 14 June 2019; https://www.abc.net.au/life/flu-symptoms-complications-and-vaccines/11110294

Aeron Hurt

- Participated in an interview with The Wire radio for the segment "Is Australia prepared for the flu season?", published 2 May 2019; http://thewire.org.au/story/is-australia-prepared-for-the-flu-season/
- Participated in an interview with The Age for the article "Does wearing a face mask prevent the flu?", published 21 May 2019; https://www.theage.com.au/national/victoria/does-wearing-a-face-mask-prevent-the-flu-20190521-p51pm3.html
- Participated in an interview with Now To Love for the article "Flu vaccine explainer: Everything you need to know about Australia's horror flu epidemic", published 21 May 2019; https://www.nowtolove.com.au/health/body/flu-vaccine-side-effects-55885
- Participated in an interview with 3AW Breakfast for a segment on whether wearing face masks are effective in preventing influenza, published 22 May 2019.

Community Engagement (continued)

Heidi Peck

- Participated in an interview on ABC AM for the segment "April flu cases six times higher than worst April on record", published 21 May 2019; https://www.abc.net.au/radio/programs/am/april-flu-cases-six-times-higher-than-worst-april-on-record/11132584
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Patrick Reading

- Was Master of Ceremonies and speaker at The University of Melbourne Faculty of Medicine, Dentistry and Health Sciences (MDHS) Early Career Research Network event “Alternative Careers in Science”, held April 11, 2019

Kanta Subbarao

- Participated in a special International Women's Day 2019 event: 'My Brilliant Career', at the University of Melbourne on 7 March 2019; https://biomedicalsciences.unimelb.edu.au/news-and-events/international-womens-day-2019-my-brilliant-career
- Participated in an interview for The Age article "Flu shot orders at record levels as 'summer' outbreak claims 13 lives", published 11 April 2019; https://www.theage.com.au/national/victoria/flu-shot-orders-at-record-levels-as-summer-outbreak-claims-13-lives-20190411-p51d5p.html
- Participated in an interview with Medical Journal of Australia InSight for the article "Flu season: data mount but path remains a mystery", published 29 April 2019; https://insightplus.mja.com.au/2019/16/flu-season-data-mount-but-its-path-remains-a-mystery/
- Participated with an interview for the Courier Mail article "Flu rate rockets as WA health industry struggles to cope ", published 26 May 2019; https://www.perthnow.com.au/news/wa/flu-rate-rockets-as-wa-health-industry-struggles-to-cope-ng-b881210401z
- Participated in a special public seminar “You Never Forget the Flu - what you need to know about this year’s flu season”, held at the Doherty Institute on 27 May 2019.
- Participated with an interview with The Quicky podcast on MamaMia for the article "Mark was strong, fit and bubbly. Two weeks ago he caught the flu. He died last Monday.", published 28 May 2019; https://www.mamamia.com.au/should-i-get-the-flu-shot/
- Participated in an interview with ABC news for the article "Mother of five-year-old boy with influenza-A 'didn't think flu could be this serious'", published 29 May 2019; https://www.abc.net.au/news/2019-05-29/didnt-think-it-could-be-this-serious-says-mother-of-boy-with-flu/11157578
- Participated in an interview with The Age for the article "'Like wearing a seatbelt': Experts say flu vaccine is not foolproof", published 28 June 2019; https://www.theage.com.au/national/victoria/like-wearing-a-seatbelt-experts-say-flu-vaccine-is-not-foolproof-20190628-p5226r.html?cspt=1563250491|2c20474d237591780c32b4138a97db50
- Participated in an interview with The Guardian for the article "'It isn't too late': why you should still get the flu vaccine", published 30 June 2019; https://www.theguardian.com/australia-news/2019/jun/30/it-isnt-too-late-why-you-should-still-get-the-flu-vaccine

Community Engagement (continued)

Sheena Sullivan

- Co-wrote an article "We can't predict how bad this year's flu season will be but here's what we know so far" for The Conversation, published 18 April 2019; https://theconversation.com/we-cant-predict-how-bad-this-years-flu-season-will-be-but-heres-what-we-know-so-far-115303
- Spoke to The Signal Podcast to provide background for the segment "Are we due for a super flu?", published 23 April 2019; https://www.abc.net.au/radio/programs/the-signal/super-flu/11093762
- Participated in an interview on ABC AM for the segment "April flu cases six times higher than worst April on record", published 21 May 2019; https://www.abc.net.au/radio/programs/am/april-flu-cases-six-times-higher-than-worst-april-on-record/11132584

Michelle Wille

- Wille et al. Viruses. 2018 Dec 17;10(12) was featured on This Week in Virology (TWiV) podcast on 5 May 2019; http://hwcdn.libsyn.com/p/9/7/a/97a71bc1222f040e/TWiV546.mp3?c_id=41663228&cs_id=41663228&expiration=1557206886&hwt=03347742187e87ac41c69ed03175d5f9
- Co-authored a blog post for BioMedCentral titled "The canary in a superbug mine" for the On Biology blog, published 9 April 2019; http://blogs.biomedcentral.com/on-biology/2019/04/09/canary-superbug-mine/
- Wrote a blog post for the Nature Microbiology community titled "A duck is not a duck is not a duck: Virome heterogeneity and connectivity in waterfowl and shorebird communities", published 26 June 2019; https://naturemicrobiologycommunity.nature.com/users/264656-michelle-wille/posts/50305-a-duck-isn-t-a-duck-isn-t-a-duck-virome-heterogeneity-and-connectivity-in-waterfowl-and-shorebird-communities
- wrote an article for the University of Melbourne news site Pursuit, titled "How our 'avian athletes' could spread influenza", published 12 September 2019; https://pursuit.unimelb.edu.au/articles/how-our-avian-athletes-could-spread-influenza

Website and social media

The Centre website was maintained and updated throughout the year. During 2019, the website was viewed by 9,759 unique users from 132 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 2019. The Centre's Twitter profile gained 305 followers during the year, with a total of 418 followers by 31 December 2019.

Management and staff

Director
Prof Kanta Subbarao

Deputy Director
Prof Ian Barr

Administrative officer
Jayde Simpson

Quality & Regulatory Officer
Katie Milne

Educator/Research scientist
Prof Patrick Reading

Laboratory Manager/ Senior Medical Scientist
Dr Paul Whitney

Communications officer
Dr Michelle Chow

Surveillance

ANTIGENIC ANALYSIS
Head: Heidi Peck
Medical scientists:
Iwona Buettner
Joelle Dharmakumara
Leah Gillespie
Anthony Leggieri*
Angela Todd
Megan Triantafilou*
Tasoula Zakis
Laboratory Assistant:
Violeta Stojceva

GENETIC ANALYSIS
Head: Dr Yi-Mo Deng
Medical scientists:
Pina Iannello
Hilda Lau
Jean Moselen*
Natalie Spirason
Angela Todd
Bioinformatician
Miguel Grau Lopez*

ANTIVIRAL DRUG SENSITIVITY
Head: A/Prof Aeron Hurt
Medical scientist: Sook Kwan Brown

BIOINFORMATICS
Manager: Naomi Komadina

Vaccine development

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

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

Research

IMMUNE RESPONSES TO INFLUENZA
Senior research scientist:
Dr Annette Fox
Research assistant
Louise Carolan

ANTIVIRALS
Senior research scientist:
A/Prof Aeron Hurt
Post-doctoral researchers
Dr Edin Mifsud
Dr Danielle Tilmanis
Research assistants
Ankita George*
Leo Lee

ANIMAL INFLUENZAS
Post-doctoral researcher:
Dr Michelle Wille

EVOLUTION OF INFLUENZA
Research assistants:
Malet Aban
Manisha Patel

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

PhD Students
Rubaiyea Farrukee
Paulina Kozsalka
Annika Suttie

Epidemiology

Head
A/Prof Sheena Sullivan
Epidemiologists
Vivian Leung
Olivia Price
Research assistant
Arseniy Khvorov*

**New staff members in 2019*