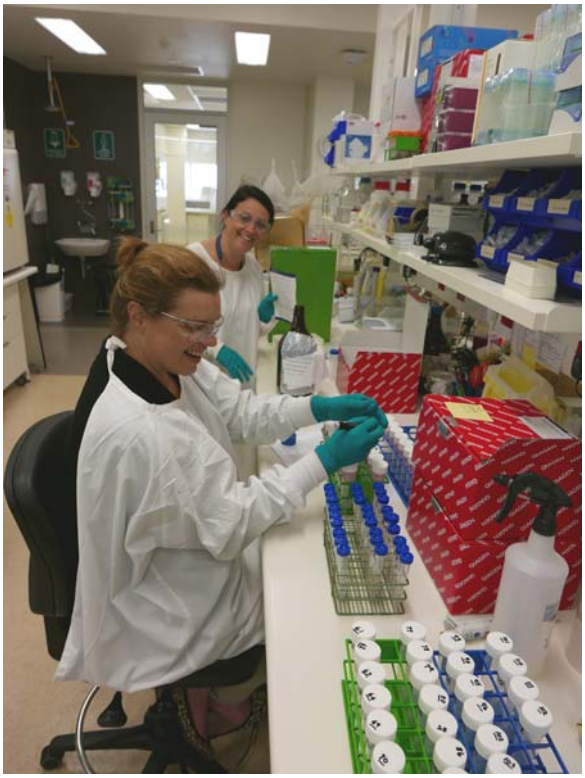

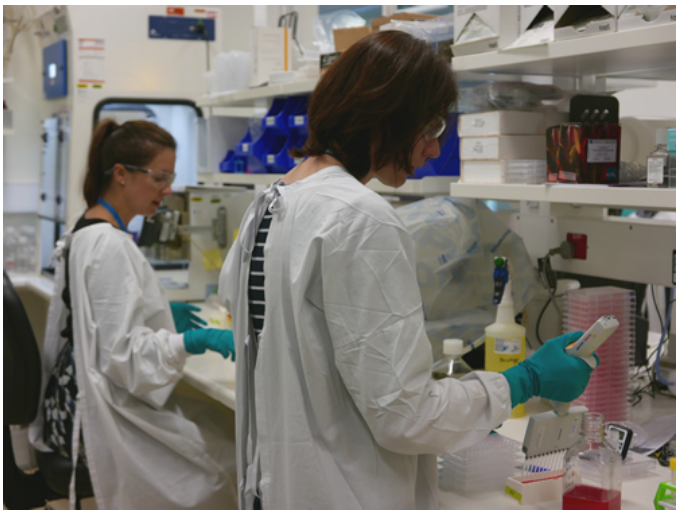

Annual Report 2016

WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL

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 2015-2019) are:

1. To obtain, isolate and preserve representative viruses from outbreaks and sporadic cases of influenza, and characterise their antigenic and other relevant properties, including resistance to anti-influenza drugs;
2. To exchange information and new antigenic variants of influenza viruses with other WHO Collaborating Centres for Reference and Research on Influenza and with Essential Regulatory Laboratories;
3. To assist WHO in developing recommendations on viruses to be included in influenza vaccines;
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 collect epidemiological information on the prevalence of influenza, especially in countries and areas in the Region;
6. To undertake research to improve the detection, prevention and treatment of influenza;
7. To assist WHO and national health authorities in developing and implementing plans for responding to pandemic influenza; and
8. To comply with the Terms of Reference for WHO Collaborating Centres for Influenza related to work with Pandemic Influenza Preparedness biological materials as specified in Annex 5 of the Pandemic Influenza Preparedness 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

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Highlights of 2016

SURVEILLANCE

The Centre received and processed over 4,200 influenza samples during 2016, of which 98% were tested. Of the samples analysed, the largest proportion were A(H3N2) viruses (44%), followed by A(H1N1)pdm09 (27%).

CENTRE LEADERSHIP

The Centre welcomed Dr Kanta Subbarao as the new Director in November 2016.

PUBLICATIONS

Centre staff contributed to a total of 42 original research papers, reviews and reports in 2016, including a paper that was featured on the cover of the Journal of Virology.

TECHNOLOGY

The integration of Next Generation Sequencing (NGS) techniques into routine surveillance activities has resulted in the largest annual number of viruses analysed by full genome sequencing at the Centre in 2016.

The Centre also worked towards performing the Focus Reduction Assay on a routine basis to detect antigenic change in A(H3N2) viruses.

REGIONAL DEVELOPMENT

The Centre undertook the development and delivery of an External Quality Assessment (EQA) for virus isolation involving 21 National Influenza Centres in the WHO Western Pacific and South-East Asia Regions.

Director's report

It is a pleasure to present the 2016 Annual Report of the WHO Collaborating Centre for Reference and Research on Influenza as the newly appointed Director. The Centre has continued to actively fulfil its commitments to the WHO, National Influenza Centres in the region, and the Australian Commonwealth and to participate in training and research activities.

The Centre received and processed over 4000 influenza samples from Australia and 13 other countries in the region during 2016. The largest proportion of the samples analysed were A(H3N2) viruses and while there has been considerable genetic diversification of the H3 HA gene, they are increasingly difficult to characterise in the laboratory. We normally rely on the hemagglutination inhibition (HI) assay for antigenic analysis of influenza viruses but this is not possible with a large proportion of A(H3N2) viruses, especially the 3C.2a and subclade 3C.2a1 viruses. About 50% of the A(H3N2) viruses that we received could not be tested in HI assays because they did not yield sufficiently high haemagglutination titres to be tested, even when oseltamivir carboxylate was added to circumvent neuraminidase-mediated agglutination or the viruses could not be recovered. Therefore, the Centre has incorporated the Focus Reduction Assay for detection of antigenic changes in A(H3N2) viruses into its routine surveillance activities.

The integration of Next Generation Sequencing (NGS) techniques into routine surveillance activities has resulted in the largest annual number of viruses undergoing full genome sequencing in 2016. With the increasing difficulty in isolating influenza A(H3N2) viruses and to support Vaccine Effectiveness studies analysing viral subpopulations, we have turned to genetic characterisation by NGS. In 2016, the Centre sequenced 141 full genomes and 728 partial genomes (HA and NA genes plus M gene for influenza A viruses).

In conjunction with Regional Offices from WHO WPR and SEAR, the Centre developed and executed an External Quality Assurance Project (EQAP) for virus isolation and identification in 21 National Influenza Centres in the WHO Western Pacific and South-East Asia Regions. The EQAP revealed that NICs in the Asia Pacific Region were proficient in human influenza virus isolation and characterisation. As part of our commitment to education and outreach, the Centre continued to provide training and hosted visiting scientists from Cambodia and Turkey for training and provided in-country training at the Pasteur Institute in Ho Chi Minh City, Vietnam and the Fiji Center for Communicable Disease Control in Suva, Fiji.

During 2016 the Centre provided various egg-isolated viruses for vaccine production by companies as listed in the WHO approved Candidate Vaccine Viruses for the Southern Hemisphere 2016 and Northern Hemisphere 2016-7 seasons. The Centre also continued to monitor potential pandemic influenza viruses and seeks to obtain new viruses as they are detected (such as A(H5Nx) viruses), to check reagents and prepare virus and RNA stocks.

Centre staff contributed to a total of 42 original research papers, reviews and reports in 2016. One highlight was a paper published by Aeron Hurt and colleagues within and outside the Centre, in the Journal of Virology about the introduction, reassortment, and persistence of diverse influenza A viruses in Antarctica. This paper was accompanied by a cover illustration.

I would like to express sincere thanks to Dr Ian Barr for his excellent leadership as Acting Director of the Centre in the interim between Professor Anne Kelso's departure in April 2015 and my appointment in November 2016. We are very grateful to Dr Mike Catton, Director of VIDRL, and many other members of VIDRL staff, especially Dr Bill Maskill, Jane Brewster, Anna Ayres and Dallas Wilson, for their support of the Centre's work at every level during 2016. 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 2016. It is a privilege to be associated with the Centre and I look forward to working with the Centre staff and all of our partners in 2017 and onwards.

Dr Kanta Subbarao
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.

Receipt of Influenza Viruses

During 2016 the Centre received 4267 clinical specimens and/or virus isolates from 34 laboratories in 14 countries (Figures 1 and 2, Table 1). A total of 464 samples came from Australian general practictioner based surveillance systems (Table 2). Amongst samples received by the Centre for which the age of the patient was known, the largest portion were from subjects aged under 5 years (Figure 3).

A total of 4167 samples (98%) were cultured and analysed by haemagglutination inhibition (HI) assay and/or real-time reverse-transcription polymerase chain reaction (RT-PCR). 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, 27% were identified as A(H1N1)pdm09, 44% were A(H3N2) viruses, 8.5% were B/Victoria and 8% were B/Yamagata viruses (Table 3).

Isolation 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.

Figure 1. Samples received by the Centre, 2011-2016.

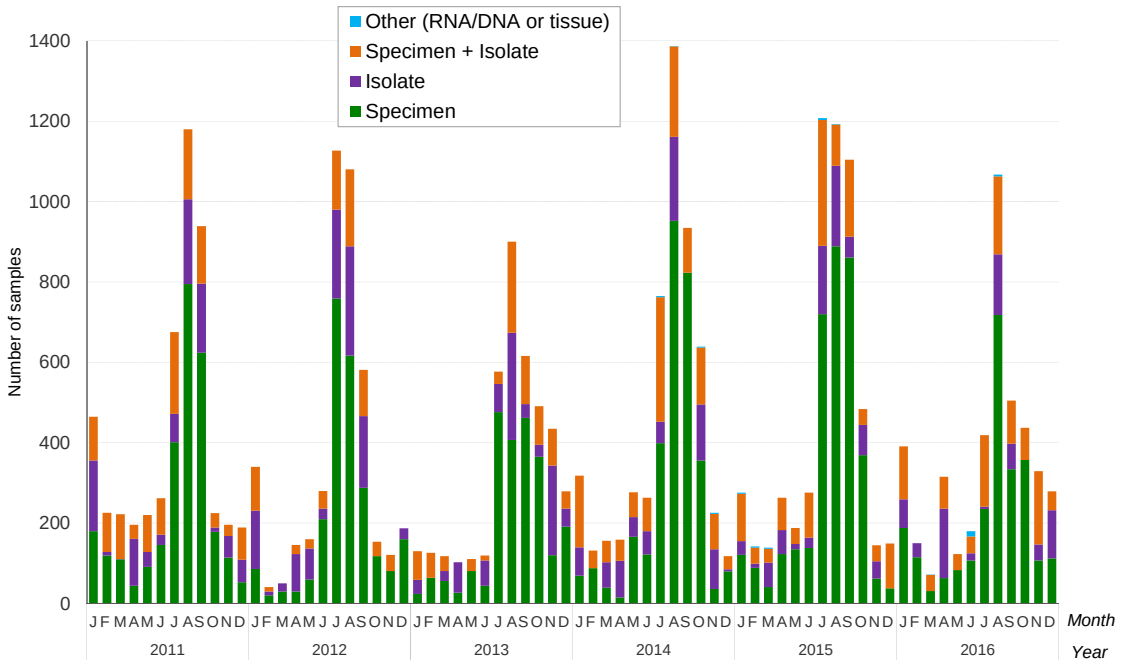


Table 1. Samples received by the Centre in 2016, by country.

Country	Samples received				% Samples tested
	Specimens	Isolates	Specimen + Isolate	Other (eg. RNA/ DNA/tissue)	
AUSTRALASIA					
Australia	1821	248	615	18	97%
New Zealand	34	218	32	0	100%
SOUTH PACIFIC					
Fiji	219	0	0	0	96%
New Caledonia	142	0	0	0	100%
Solomon Islands	25	0	0	0	100%
SOUTH EAST ASIA					
Cambodia	68	45	0	0	100%
Malaysia	0	247	0	0	100%
Philippines	16	0	20	0	100%
Singapore	3	6	255	0	100%
Thailand	9	31	0	0	100%
Vietnam	19	0	34	0	100%
EAST ASIA					
Macau SAR	0	25	0	0	100%
SOUTH ASIA					
Sri Lanka	90	0	0	0	100%
AFRICA					
South Africa	4	0	23	0	100%
TOTAL	**2450**	**820**	**979**	**18**	**98%**

Figure 2. Geographic spread of influenza laboratories sending viruses to the Centre during 2016.

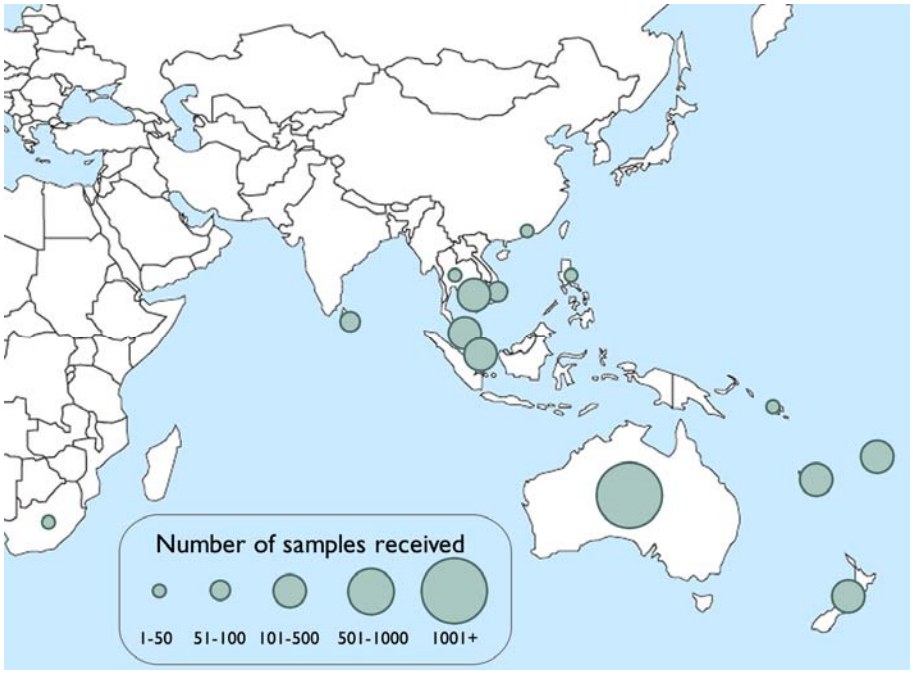


Table 2. Samples received from general practitioner based surveillance systems in Australia, 2016.

	No. samples received	No. isolates recovered*	Viruses analysed by HI assay
Australian Sentinel Practices Research Network *(ASPREN)*	258	64	57
Victorian Sentinel Practices Influenza Network *(VicSPIN)*	158	72	69
Sentinel Practices Network of Western Australia *(SPN(WA))*	48	20	19

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

Figure 3. Age distribution of subjects from whom samples were received at the Centre in 2016 and the age is known.

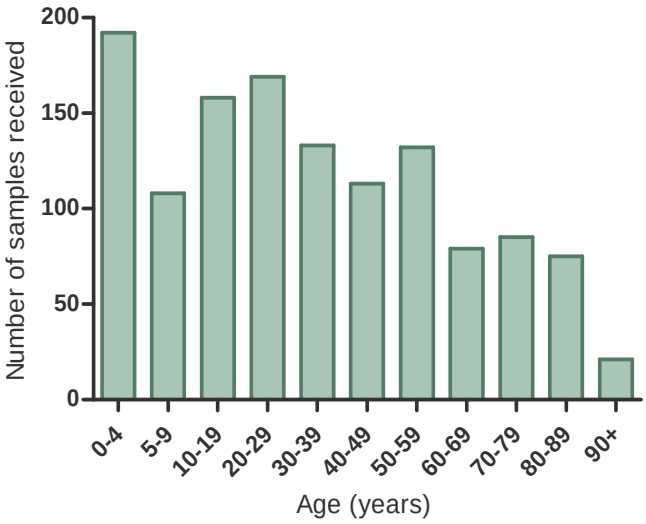


Table 3. Samples tested by HI and/or RT-PCR assay at the Centre in 2016, by country.

Country	Samples tested by HI and/or RT-PCR assay							
	A(H1N1) pdm09	A(H3N2)	A (mixed)	A (unsub-typed)	B/ Victoria	B/ Yamagata	B lineage undetermined	Untyped
AUSTRALASIA								
Australia	714	1309	2	263	76	110	97	39
New Zealand	52	191	0	0	12	29	0	0
SOUTH PACIFIC								
Fiji	35	76	1	0	32	59	7	1
New Caledonia	28	31	0	0	62	9	12	0
Solomon Islands	17	0	0	0	0	0	0	8
SOUTH EAST ASIA								
Cambodia	54	21	0	0	13	25	0	0
Malaysia	60	31	1	21	78	35	21	0
Philippines	15	13	0	2	2	2	2	0
Singapore	82	84	0	1	48	49	0	0
Thailand	8	12	0	2	8	9	1	0
Vietnam	15	31	0	0	2	3	2	0
EAST ASIA								
Macau SAR	20	1	0	0	4	0	0	0
SOUTH ASIA								
Sri Lanka	21	23	0	8	6	2	30	0
AFRICA								
South Africa	4	10	0	0	13	0	0	0
TOTAL	**1125**	**1833**	**4**	**297**	**356**	**332**	**172**	**48**

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. Subtypes are based on analysis of the HA and in some cases are confirmed by genetic analysis of the NA.

Antigenic analyses 2016

A total of 4153 isolates that were received at the Centre in 2016 were cultured and isolated in MDCK cells, of which 3375 (81.3%) produced a positive result. The largest proportion of viruses were A(H3N2) (49.2%), followed by A(H1N1)pdm09 viruses (30.6%) (Figure 4). The relative proportions of different subtypes and lineages of samples received varied between different world regions (Figure 5).

Figure 4. Influenza sub/types and lineages of samples received in 2016 and characterised by antigenic analysis.

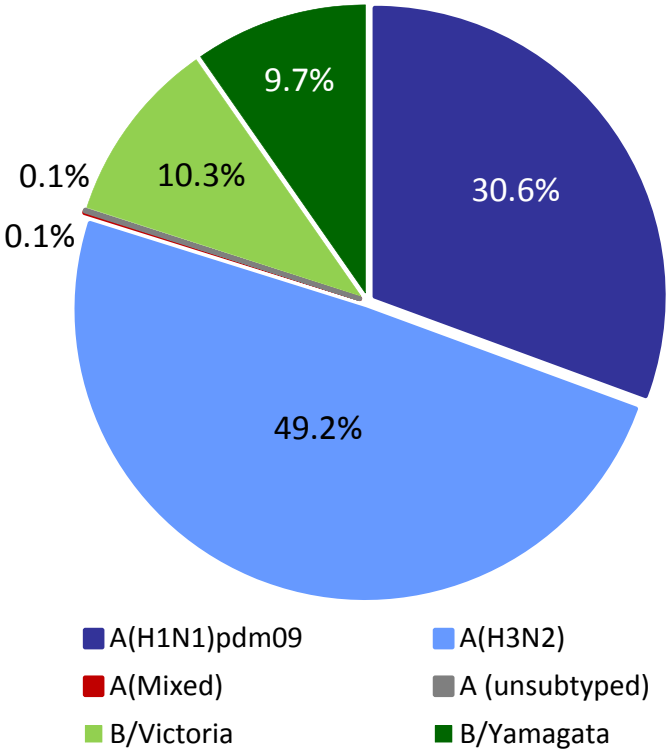
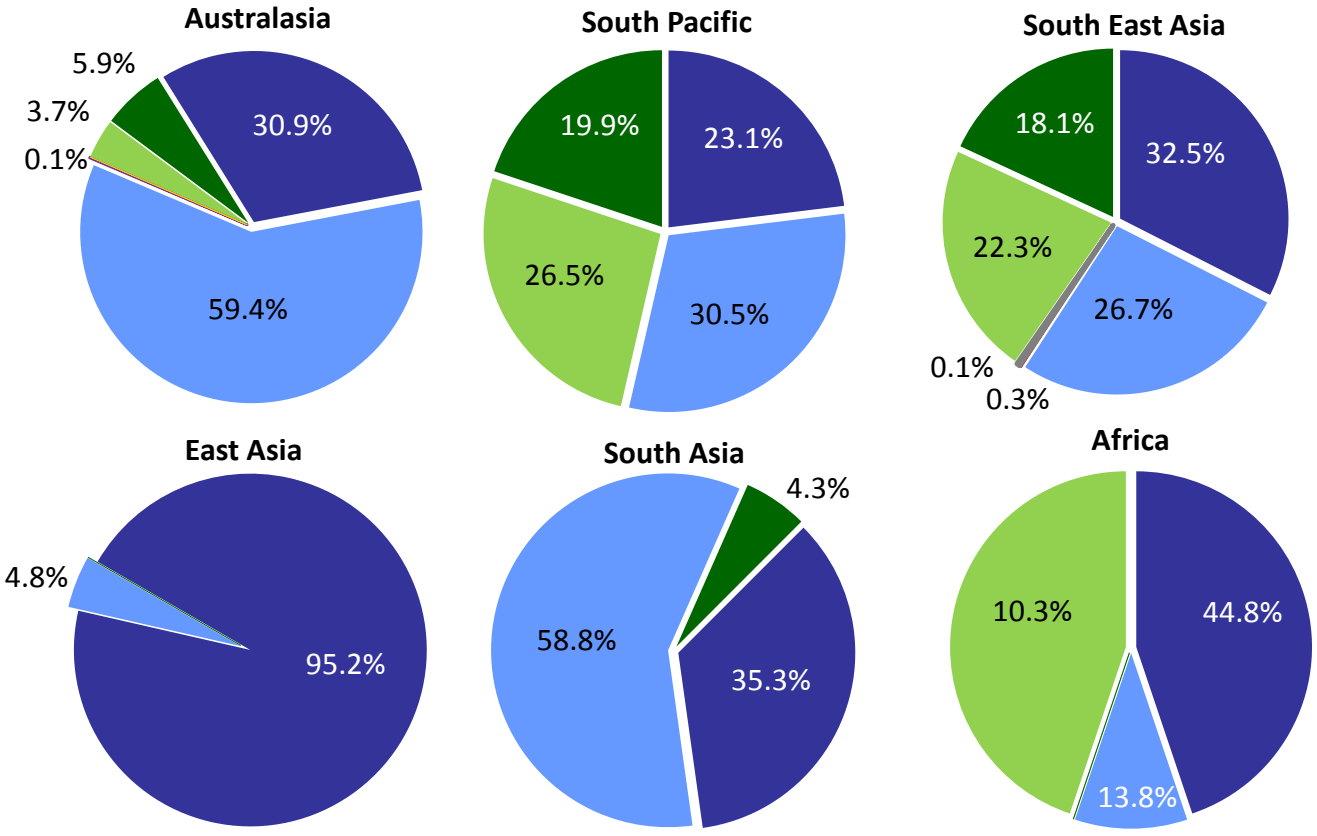


Figure 5. Influenza sub/types and lineages of isolates received from different world regions during 2016 as determined by antigenic analysis.



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 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.

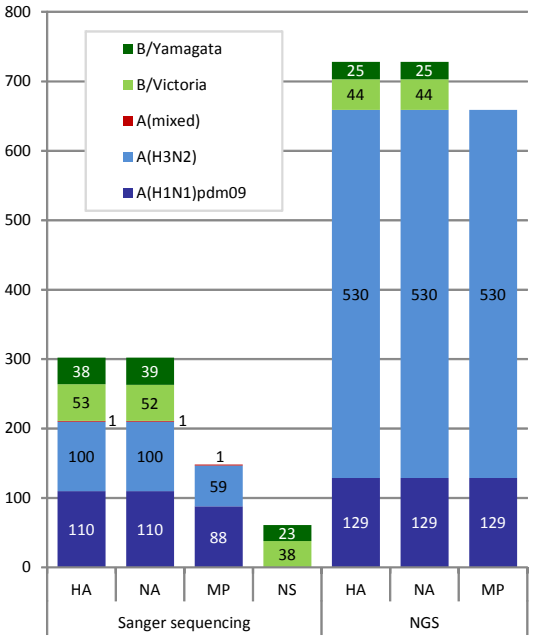
Since the acquisition of an IonTorrent PGM™ system in 2014, next generation sequencing (NGS) techniques have been increasingly employed at the Centre for efficient and cost-effective sequencing of whole genomes of viruses, and/or selected influenza virus genes. This has become particularly evident during 2016, when the number of viral genes undergoing NGS analysis at the Centre has far surpassed the number of genes analysed by traditional Sanger sequencing (Figures 6 and 8). This has also resulted in the largest number of viruses with their full genome sequenced in 2016 (Figure 8) .

Sequencing 2016

In 2016, 302 HA, 302 NA, 148 MP and 61 NS genes from 304 human viruses received at the Centre were analysed by Sanger sequencing (Figure 6). In addition, the HA, NA and MP genes of 659 influenza A and 69 influenza B (HA and NA only) viruses were sequenced by NGS techniques (Figures 6 and 7).

Full genome sequencing was performed on 144 viruses, mostly using NGS techniques (Figures 8 and 9). 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 6. Sanger and NGS sequence analysis of samples received at the Centre in 2016.



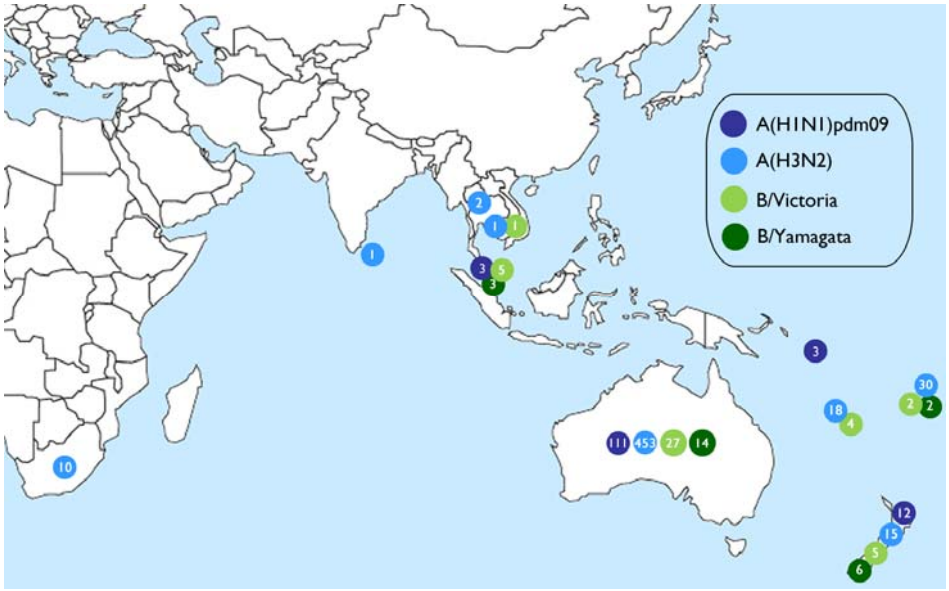


Figure 7. Geographic spread of submitting laboratories and numbers of viruses with HA, NA and MP (Influenza A only) genes sequenced using NGS at the Centre in 2016.

Figure 8. Number of viruses analysed by full genome sequencing 2010-2016 using Sanger sequencing and NGS techniques.

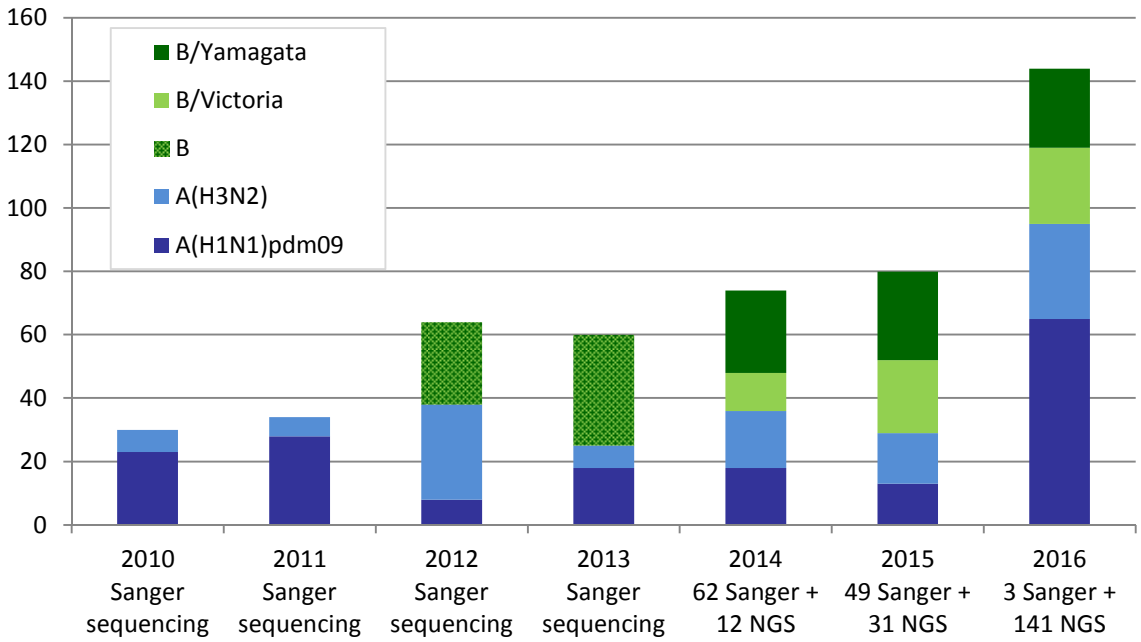
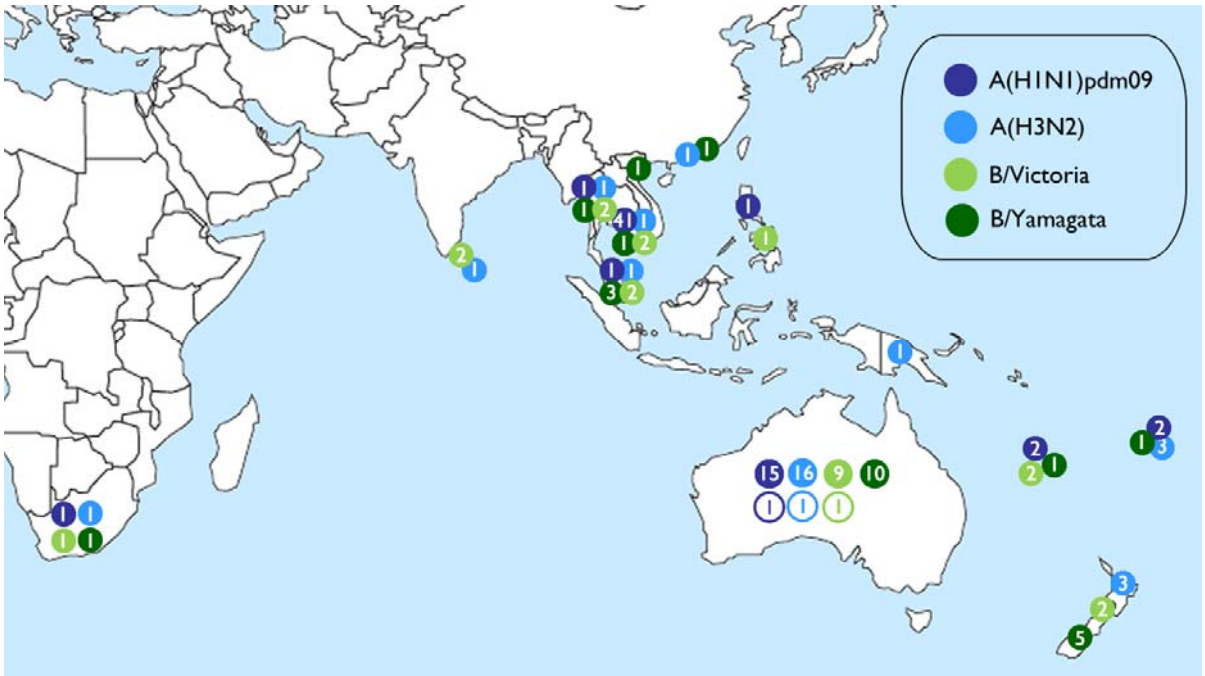


Figure 9. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing at the Centre in 2016. Solid markers indicate viruses sequenced using NGS techniques, outlined markers indicate viruses sequenced using Sanger sequencing.



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).

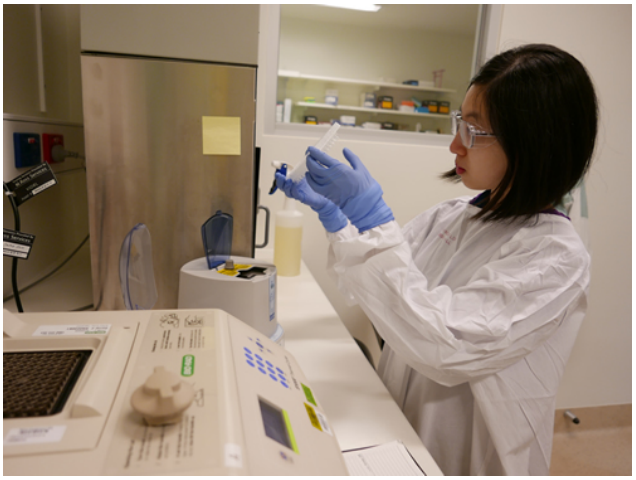
Sequences submitted in 2016

A total of 3558 gene sequences from 1160 viruses were deposited with GISAID in 2016 (Table 4). The largest number of these sequences were of HA and NA genes, followed by MP and NS genes. Full genomes of 81 influenza viruses were also represented in the Centre's submissions (data not shown).

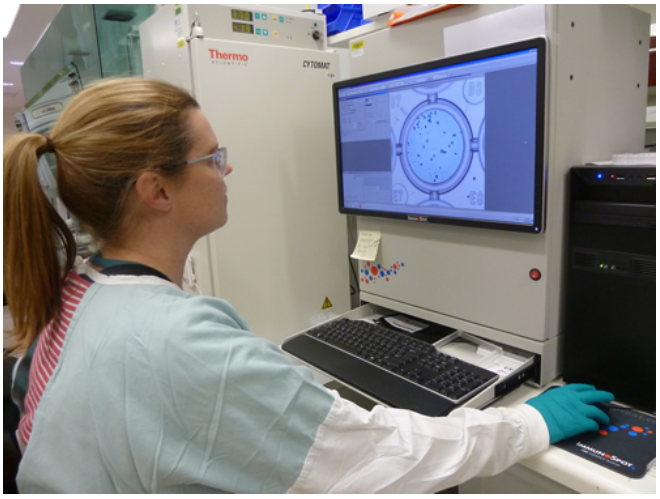
Table 4. Genetic sequences submitted to GISAID by the Centre in 2016.*

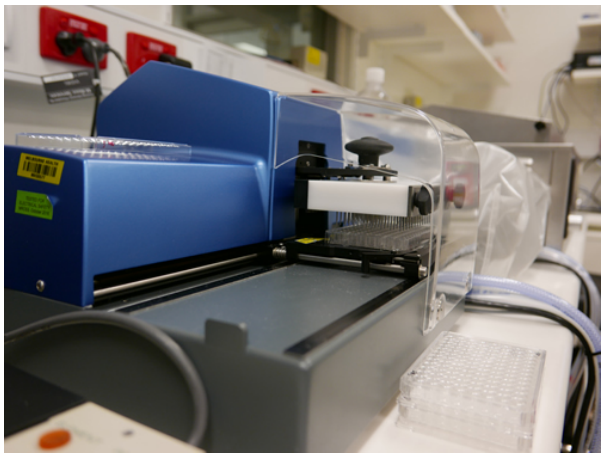
Gene / Type/ Subtype/Lineage	HA	NA	MP	NS	PB1	PB2	PA	NP	HE	**Total**
A(H1N1)pdm09	274	276	210	50	54	50	54	57	0	**1025**
A(H3N2)	664	662	485	16	18	18	18	18	0	**1899**
B/Victoria	102	102	12	51	12	12	12	12	0	**315**
B/Yamagata	90	90	12	46	12	12	12	12	0	**286**
C	0	0	0	0	0	0	0	0	22	**22**
A / H11	1	0	1	1	0	0	0	0	0	**3**
A(H5N5)	1	1	1	1	1	1	1	1	0	**8**
Total	**1132**	**1131**	**721**	**165**	**97**	**93**	**97**	**100**	**22**	**3558**

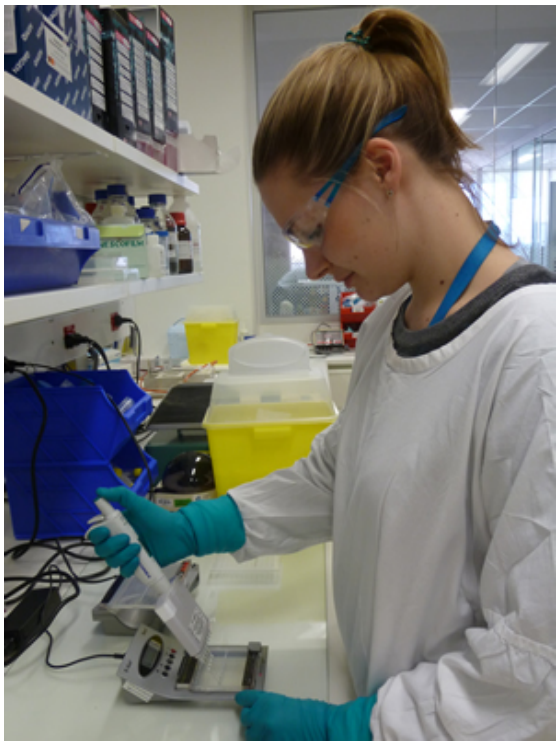
** Counts include all sequences submitted to GISAID during 2016, which includes viruses received in previous years and viruses sequenced for research purposes.*



Around the laboratories...







Surveillance Results by Influenza Subtype

Viruses were analysed by comparison with reference viruses recommended by WHO for the 2016 Southern Hemisphere and 2016-2017 Northern 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 999 A(H1N1)pdm09 isolates were analysed by HI assay in 2016. The majority (95.5%) of these viruses displayed similar antigenic properties to the vaccine reference strain A/California/7/2009 (Figure 10, Table 5).

Whilst HI assays using post-infection ferret sera indicated that recently circulating A(H1N1)pdm09 viruses were antigenically similar to the vaccine strain A/California/7/2009, genetic analysis of recent viruses indicated that a number of changes have occurred in the HA protein. Furthermore, analyses by WHO Collaborating Centres indicated a reduced response in an age-related subset of post-vaccination human sera to such recent viruses. As a result of the collected antigenic and genetic data from the five Collaborating Centres, a change in the A(H1N1)pdm09 vaccine virus was recommended for the southern hemisphere 2017 influenza vaccines.

Haemagglutinin gene sequencing

Sequencing was performed on a total of 630 HA genes. Phylogenetic analysis showed that circulating A(H1N1) pdm09 viruses sent to the Centre during 2016 contained some genetic changes compared to the vaccine reference strain A/California/7/2009, with the emergence of two distinct subclades (Figure 11). The majority of HA genes sequenced belong to subclade 6B.1, which contains the new vaccine strain A/Michigan/45/2015.

Figure 10. Summary of fold differences in HI titres of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/California/7/2009 reference virus.

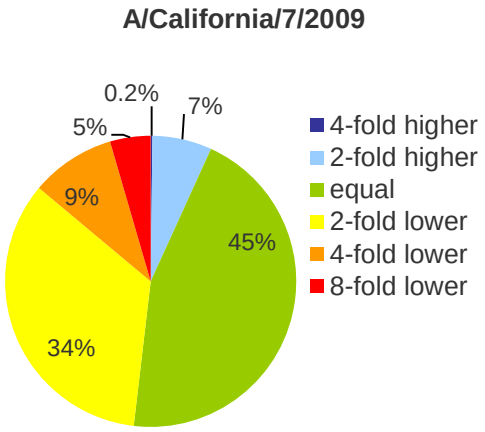


Table 5. Antigenic characterisation of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/California/7/2009 reference virus.

	A(H1N1)pdm09 reference strain: A/California/7/2009	
Region	**Like**	**Low reactor (%)**
Australasia	643	34 (5.0%)
Pacific	74	0
South East Asia	202	11 (5.2%)
East Asia	20	0
South Asia	12	0
Africa	3	0
Total	**954**	**45 (4.5%)**

Figure 11. Phylogenetic tree of representative HA genes of A(H1N1)pdm09 viruses received by the Centre during 2016.

Legend

2016 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses

} Brackets indicate clades

Influenza A(H3N2)

Antigenic analysis

Of 829 A(H3N2) subtype isolates analysed by HI assay, the majority were antigenically similar to the cell-grown reference strain A/Hong Kong/4801/2014 (Figure 12, Table 6). In addition, a small number of viruses which had been collected in 2014 but received at the Centre during 2016 were analysed in comparison to the previous vaccine strain A/Switzerland/9715293/2013 (data not shown).

Evolutionary changes in A(H3N2) viruses in recent years 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 the Focus Reduction Assay (FRA), a form of virus neutralisation assay, are required to test the antigenic characteristics of these viruses. During 2017 FRAs were performed on a regular basis and continue to be integrated into the Centre's routine surveillance activities.

Haemagglutinin gene sequencing

A total of 630 HA genes from A(H3N2) viruses were sequenced. Phylogenetic analysis indicate that the predominant proportion of these viruses had undergone genetic change compared to the previous vaccine strain A/Switzerland/9715293/2013 (sub-clade 3C.3a), and were genetically similar to the A/Hong Kong/4801/2014 reference strain (sub-clade 3C.2a), which was recommended by WHO for inclusion in Southern Hemisphere vaccine in 2016 (Figure 13).

Figure 12. Summary of fold differences in HI titres of A(H3N2) viruses analysed at the Centre compared to the A/Hong Kong/4801/2014 reference virus.

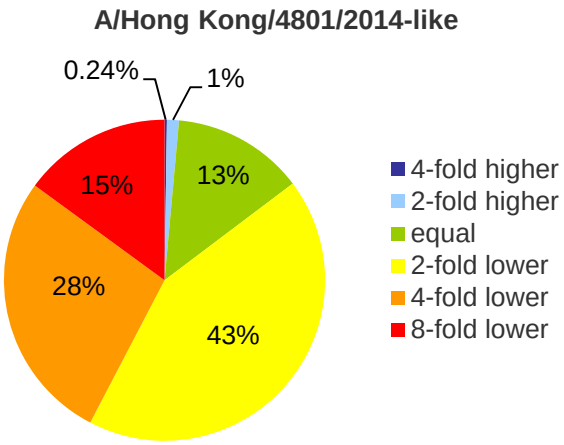
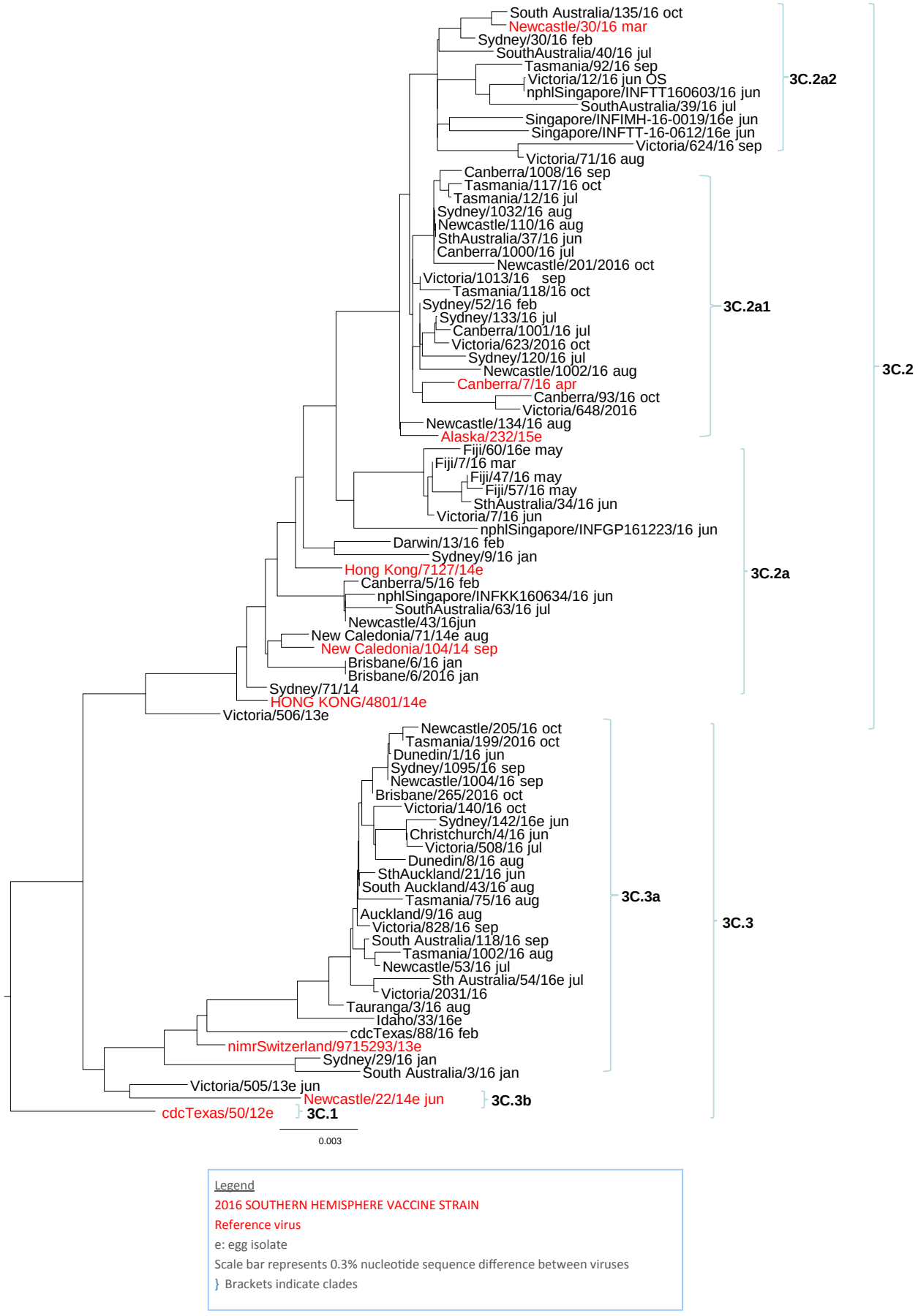


Table 6. Antigenic characterisation of A(H3N2) viruses analysed at the Centre compared to the A/Hong Kong/4801/2014 reference virus.

	A(H3N2) reference strain: A/Hong Kong/4801/2014	
Region	**Like**	**Low reactor (%)**
Australasia	587	119 (16.9%)
Pacific	7	1 (12.5%)
South East Asia	94	4 (4.1%)
East Asia	0	0
South Asia	10	0
Africa	7	0
Total	**705**	**124 (15.0%)**

Figure 13. Phylogenetic tree of representative HA genes of A(H3N2) viruses received by the Centre during 2016.



Legend

2016 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses

} Brackets indicate clades

Influenza B

Antigenic analysis

There are currently two antigenically and genetically distinct lineages of influenza B virus in circulation, the B/Victoria/2/87 lineage (represented by the 2016 vaccine strain B/Brisbane/60/2008) and the B/Yamagata/16/88 lineage (represented by the southern hemisphere 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 2016 the Centre received roughly equal proportions of B/Victoria and B/Yamagata lineage viruses. All of the 340 B/Victoria viruses that were analysed antigenically were similar to B/Brisbane/60/2008 (Figure 14, Table 7). Similarly, a total of 315 B/Yamagata viruses were analysed by HI assay and all were antigenically similar to B/Phuket/3073/2013. (Figure 15, Table 7).

Haemagglutinin gene sequencing

A total of 97 HA genes from B/Victoria and 63 B/Yamagata viruses were sequenced. All of the viruses of B/Victoria lineage belonged to the same genetic clade as the B/Brisbane/60/2008 reference virus (Figure 16). Almost all of the B/Yamagata lineage viruses belonged to the clade represented by B/Phuket/3073/2013 (Figure 17).

Figure 14. Summary of fold differences in HI titres of B/Victoria viruses analysed at the Centre compared to the B/Brisbane/60/2008 reference virus.

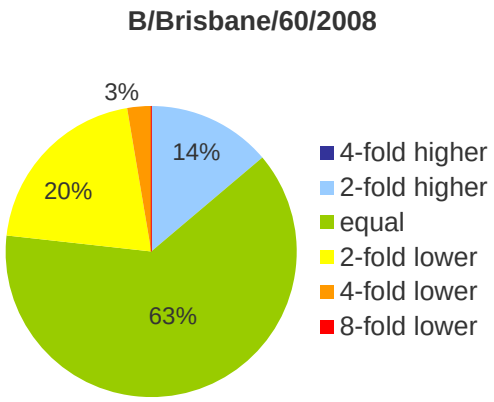


Figure 15. Summary of fold differences in HI titres of B/Yamagata viruses analysed at the Centre compared to the B/Phuket/3073/2013 reference virus.

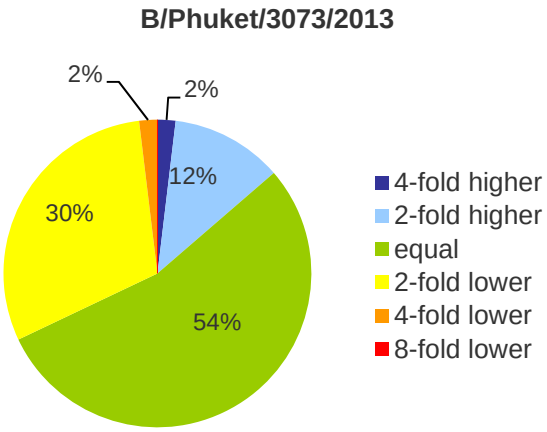
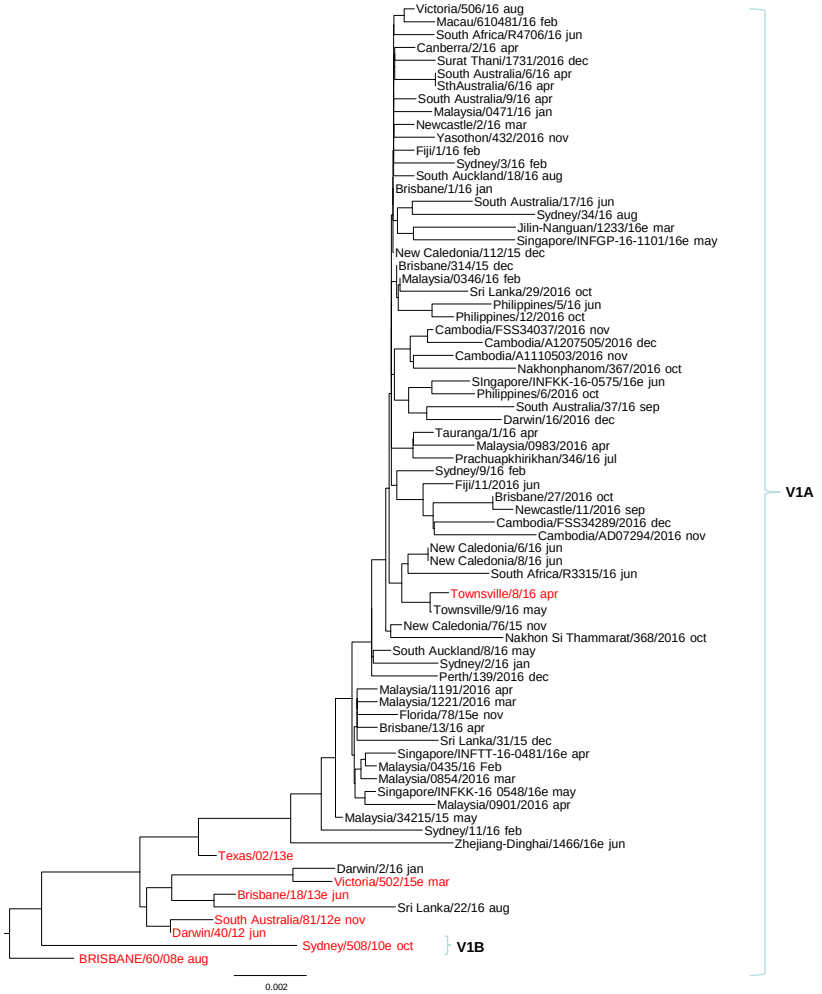


Table 7. Antigenic characterisation of B viruses received at the Centre during 2016 compared to the B/Brisbane/60/2008 and B/Phuket/3073/2013 reference viruses.

	B/Victoria reference strain: B/Brisbane/60/2008		B/Yamagata reference strain: B/Phuket/3073/2013	
Region	**Like**	**Low reactor (%)**	**Like**	**Low reactor (%)**
Australasia	76	0	124	0
Pacific	92	0	67	0
South East Asia	149	0	122	0
East Asia	4	0	0	0
South Asia	6	0	2	0
Africa	13	0	0	0
Total	**340**	**0**	**315**	**0**

Figure 16. Phylogenetic tree of representative HA genes of B/Victoria viruses received by the Centre during 2016.



Legend

2016 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.2% nucleotide sequence difference between viruses

} Brackets indicate clades

Figure 17. Phylogenetic tree of representative HA genes of B/Yamagata viruses received by the Centre during 2016.

Legend

2015 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.4% 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 are in use 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. The application of the Tecan EVO 200 liquid handling robot to NAI assays since 2014 has enabled routine automation of screening of all viruses.

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.

Antiviral resistance analyses 2016

NAI assays were used to analyse 3275 viruses for reduced inhibition by the NAIs (Tables 8 and 9). Viruses showing highly reduced inhibition to one or more NAIs underwent further analysis to determine the presence of amino acid substitutions in the NA protein associated with the reduction of inhibition by NAIs. In total, six A (H1N1)pdm09 viruses (from Australia, Vietnam, Malaysia and Singapore) that had highly reduced inhibition by oseltamivir and peramivir were found to contain the histidine to tyrosine mutiation at position 275 (H275Y) of the neuraminidase protein that is associated with reduced inhibition by oseltamivir. Two B/Victoria viruses from Malaysia had highly reduced inhibition by all four NAIs; one of these viruses contained a G104E mutation, and the other was found to contain an H431Y mutation.

Table 8. Viruses received by the Centre in 2016 and tested by NAI assay, by country.

Country \ Type/subtype/lineage	A(H1N1) pdm09	A(H3N2)	A(mixed)	B/Victoria	B/Yamagata	TOTAL
Australasia						
Australia	634	1123	2	64	95	**1918**
New Zealand	52	175	-	12	28	**267**
South Pacific						
Fiji	32	73	-	30	58	**193**
New Caledonia	28	31	-	62	9	**130**
Solomon Islands	17	-	-	-	-	**17**
South East Asia						
Cambodia	42	21	-	12	25	**100**
Malaysia	60	31	1	78	35	**205**
Philippines	12	4	-	2	2	**20**
Singapore	79	87	-	47	48	**261**
Thailand	7	11	-	8	9	**35**
Vietnam	13	22	-	2	3	**40**
East Asia						
Macau	20	1	-	4	-	**25**
South Asia						
Sri Lanka	12	18	-	6	2	**38**
Africa						
South Africa	3	10	-	13	-	**26**
TOTAL	**1011**	**1607**	**3**	**340**	**314**	**3275**

Table 9. Neuraminidase inhibitor sensitivity of viruses received by the Centre in 2016.*

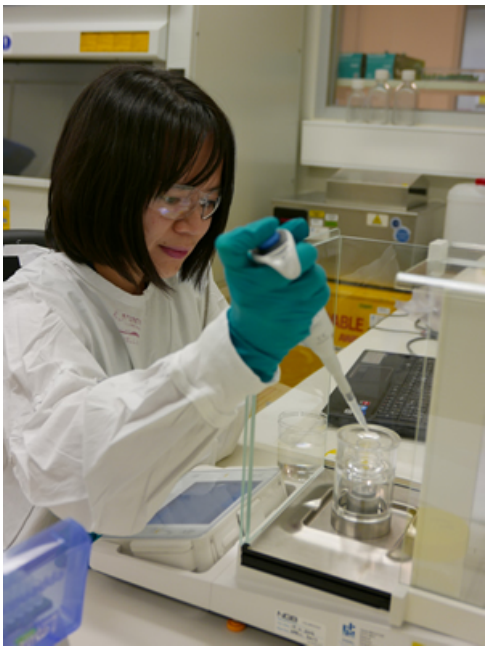
Type/Subtype	No. tested	Oseltamivir		Peramivir		Laninamivir		Zanamivir	
		Reduced inhibition	Highly reduced inhibition	Reduced inhibition	Highly reduced inhibition	Reduced inhibition	Highly reduced inhibition	Reduced inhibition	Highly reduced inhibition
A(H1N1)pdm09	1011	0	6 (0.59%)	0	6 (0.59%)	0	0	0	0
A(H3N2)	1607	0	0	0	0	0	0	0	0
A(mixed)	3	0	0	0	0	0	0	0	0
B/Victoria	340	0	2 (0.59%)	2 (0.59%)	2 (0.59%)	0	2 (0.59%)	1 (0.29%)	2 (0.59%)
B/Yamagata	314	0	0	2 (0.59%)	0	0	0	0	0
TOTAL	**3275**	**0**	**8 (0.24%)**	**4 (0.12%)**	**8 (0.24%)**	**0**	**2 (0.06%)**	**1 (0.03%)**	**2 (0.06%)**

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 2014-2015. **Reduced inhibition** = 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** = IC_{50} values are greater than 100 fold above the median value of viruses with normal inhibition (above 50 fold for influenza B viruses).*

Resistance to Adamantanes

Background

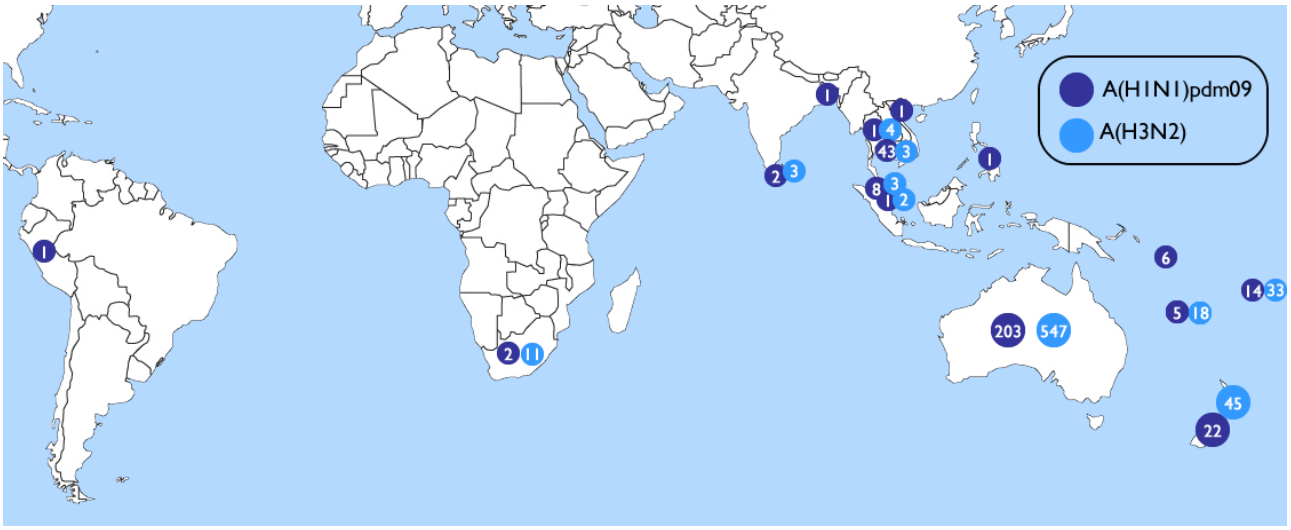
The adamantane class of antiviral drugs (amantadine and rimantadine) were previously used to treat cases of influenza A, but are no longer recommended due to the almost universal adamantane resistance amongst circulating influenza A strains in recent years. All five WHO Collaborating Centres continue 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 2016

Real-time PCR or sequencing was used to analyse 980 influenza A viruses, which were representative of those submitted to the Centre during 2016 (Figure 18). Based on S31N analysis, one H3 virus from Australia was found to be sensitive to the adamantanes.

Figure 18. Geographic spread of viruses received at the Centre during 2016 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 hen's 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 2016

In 2016, 41 viruses were successfully isolated in eggs at the Centre, representing an overall isolation rate of 36.9% (Tables 10 and 11).

Table 10. Virus isolation in eggs at the Centre in 2016.

Type/subtype	Isolates attempted	Isolates obtained	Success rate (%)
A(H1N1)pdm09	49	22	44.9%
A(H3N2)	48	11	22.9%
B/Victoria	5	4	80.0%
B/Yamagata	9	4	44.4%
Total	**111**	**41**	**36.9%**

Table 11. Potential candidate vaccine strains isolated in eggs at the Centre in 2016.

A(H1N1)pdm09		
A/Brisbane/196/2015	A/Victoria/900/2016	A/Brisbane/104/2016
A/Singapore/KK967/2015	A/Canberra/1/2016	A/Brisbane/105/2016
A/Singapore/GP1911/2015	A/Fiji/17/2015	A/South Auckland/2/2016
A/Newcastle/65/2015	A/Darwin/20/2015	A/Victoria/501/2016
A/Victoria/2159/2015	A/Fiji/2/2016	A/Singapore/16-0059/2016
A/Singapore/KK915/2015	A/Fiji/3/2016	A/Singapore/16-0063/2016
A/Singapore/GP1908/2015	A/Fiji/8/2016	**B/Victoria**
A/Singapore/TT1307/2015	A/Brisbane/103/2016	B/Singapore/INFGP-16-1101/2016
A(H3N2)		B/Singapore/INFTT-16-0481/2016
A/Singapore/GP2050/2015	A/Fiji/60/2016	B/Singapore/INFKK-16-0548/2016
A/Alaska/232/2015	A/Sydney/142/2016	B/Singapore/INFKK-16-0575/2016
A/Alaska/240/2015	A/South Australia/54/2016	**B/Yamagata**
A/Nebraska/19/2015	A/Singapore/INFMH-16-0019/2016	B/Singapore/INFTT-16-0610/2016
A/Sri Lanka/61/2015	A/Singapore/INFTT-16-0612/2016	B/Singapore/INFGP-16-1190/2016
A/Brisbane/183/2015		B/Singapore/INFGP-16-1079/2016
		B/Singapore/INFKK-16-0569/2016

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 2016, 36 candidate vaccine viruses, including seven avian influenza viruses, were received from other WHO Collaborating Centres and laboratories and then passaged in eggs at the Centre (Table 12).

Selected egg-isolated candidate vaccine strains are made available to the three laboratories that undertake virus reassortment for WHO — bioCSL (Australia), 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 12. Potential candidate vaccine viruses received from other WHO Collaborating Centres during 2016.

A(H1N1)pdm09	A(H3N2)
A/Shanghai-Putuo/SWL1860/2015	NYMC X-269 (hy A/Brisbane/47/2015)
A/Michigan/45/2015	A/Saitama/103/2014
A/Yokohama/50/2015	NYMC X-273 (hy A/Norway/2178/2014)
A/Slovenia/2903/2015	NYMC X-281 (hy A/Victoria/503/2015)
A/Scotland/P2/2015	A/Jiangsu-Sucheng1309/2016
A/Lisboa/32/2015	A/Idaho/33/2016
A/Yokohama/94/2015	**B/Victoria**
NYMC X-275 (hy A/Michigan/45/2015)	BX-61 (hy B/Indiana/25/2015)
NYMC X-277 (hy A/Iowa53/2015)	B/Jilin-Nanguan/1223/2016
A/Guangdong-Luohu/SWL1294/2016	B/Zhejiang-Dinghai/1466/2016
A/Shanghai-Jiading/SWL1970/2015	B/Florida/78/2015
A/Iowa53/2015	**B/Yamagata**
NYMC X-283A (A/Lisboa/32/2015)	NYMC BX-59A (hy B/California/12/2015)
NYMC X-285 (A/Scotland/P2/2015)	NYMC BX-59B (hy B/California/12/2015)
	B/Anhui-Dangtu/1427/2016
	B/Sichuan-Dongqu/1371/2016
	B/Arizona/10/2015
Avian influenza viruses	
A(H5N6): A/Sichuan/26221/2014 (PR8-IDCDC-RG42A)	
A(H5N1): A/Chicken/Vietnam/NCVD-03/2008 (PR8-IDCDC-RG25A)	
A(H5N1): A/Egypt/N03072/2010 (PR8-IDCDC-RG29)	
A(H5N1): A/Guizhou/1/2013 (PR8-IDCDC-RG35)	
A(H5N8): A/Gyrfalcon/Wash41088-6/2014 (PR8-IDCDC-RG43A)	
A(H1N1)v: A/Hunan/42443/2015	
A(H1N1)v: (CNIN-1601) A/Hunan/42443/2015	

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

Serum panel analyses in February 2016

In February the Centre analysed serum panels from recipients of seasonal trivalent influenza vaccines in Australia, China and USA. The combined data from all WHO Collaborating Centres and ERLs showed that for the majority of panels tested, geometric mean HI titres (GMT) of anti-HA antibodies against some but not all recent representative A(H1N1)pdm09 viruses were significantly lower compared to GMTs against the vaccine strain A/California/7/2009. GMTs of antibodies against representative recent A(H3N2) lineage viruses were significantly reduced compared to A/Switzerland/9715293/2013 vaccine virus grown in eggs, but not compared to cell-propagated vaccine virus. These findings were also confirmed by geometric mean titres determined using microneutralisation assays (GMNT). Serum panel analyses of influenza B viruses showed that GMT of antibodies against recent representative B/Victoria/2/87 lineage viruses and B/Yamagata/16/88 lineage viruses were similar to HI titres to the B/Brisbane/60/2008 and B/Phuket/3073/2013 vaccine strains respectively.

Table 13. Representative and vaccine candidate strains used for serological analyses during 2016. All viruses are egg grown unless indicated otherwise.

FEBRUARY	
A(H1N1)pdm09	**A(H3N2)**
A/California/7/2009*	A/Switzerland/9715293/2013
A/Singapore/GP1911/2015	A/Hong Kong/4801/2015*
A/Michigan/45/2015	A/Switzerland/9715293/2013
A/Shanghai-Putuo/WL1806/2015	A/Brisbane/183/2016 (C)
A/Minnesota/32/2015 (C)	
A/Darwin/17/2015 (C)	
B/Victoria	**B/Yamagata**
B/Brisbane/60/2008*	B/Phuket/3073/2013^
B/Brisbane/46/2015	B/California/12/2015 (C)
B/Sapporo/23/2015 (C)	B/Victoria/845/2015
B/Victoria/502/2015	
SEPTEMBER	
A(H1N1)pdm09	**A(H3N2)**
A/California/07/2009*	A/Sydney/142/2016 (O)
A/Victoria/501/2016	A/Alaska/232/2015 (O)
A/Singapore/GP1911/2015	A/Hong Kong/4801/2014 (O)*
A/Shanghai-Jiading/SW1970/2015	A/Hong Kong/4801/2014 (C,O)
A/Michigan/45/2015 (C)	A/Texas/88/2016 (C,O)
A/Singapore/GP1908/2015 (C)	A/Newcastle/30/2016 (C,O)
A/Shanghai-Jiading/SW1970/2015	A/Canberra/7/2016 (C,O)
A/South Australia/28/2016	A/Sydney/142/2016 (C,O)
A/Victoria/501/2016 (C)	
B/Victoria	**B/Yamagata**
B/Jilin-Nanguan/1223/2016	B/Phuket/3073/2013^
B/Zhejang-Dinghai/1466/2016	B/Arizona/10/2015
B/Florida/78/2015	B/Anhui-Dangtu/1427/2016
B/Brisbane/60/2008*	B/Arizona/10/2015 (C)
B/South Australia/17/2016	B/South Auckland/14/2016 (C)
B/Townsville/7/2016 (C)	B/Sydney/5/2016 (C)

**Trivalent vaccine strain ^ Quadrivalent vaccine strain*

(C): Cell-grown virus

(O): HI assays performed in the presence of oseltamivir

Serum panel analyses in September 2016

In September, the Centre analysed serum panels from Australia and USA. The combined data from all ERLs and WHO Collaborating Centres showed that GMTs of antibodies against the almost all recent A(H1N1)pdm09 viruses were not significantly reduced compared to the A/California/7/2009 vaccine when tested in HI assays using ferret antisera. However, representative recent viruses were found to react poorly with sera from people who had been vaccinated with the A/California/7/2009 strain. It was found that GMTs of antibodies against recent A(H3N2) viruses were slightly lower compared to the cell-grown vaccine virus A/Hong Kong/4801/2015, however GMNTs against recent representative A(H3N2) viruses were similar to those of the same cell-grown virus. Comparison of GMTs of antibodies against some representative recent B/Victoria/2/87 lineage viruses were reduced compared to titres against the cell-grown vaccine virus B/Brisbane/60/2008. Similarly, GMTs of antibodies against some representative recent B/Yamagata/16/88 lineage viruses were lower compared to titres against the egg-grown B/Phuket/3073/2013 quadrivalent vaccine virus.

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 at both Consultations in 2016, by teleconference in February and in person 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 2016 WHO made the recommendations reported here.

WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2016–2017, Geneva, Switzerland, 22–24 February 2016

It is recommended that vaccines for use in the 2016–2017 influenza season (northern hemisphere winter) contain the following:

- an A/California/7/2009 (H1N1)-like virus;
- an A/Hong Kong/4801/2014 (H3N2)-like virus;
- a B/Brisbane/60/2008*-like virus.

It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Phuket/3073/2013*-like virus.

WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2017, Geneva, Switzerland, 22–24 September 2016

It is recommended that vaccines for use in the 2017 influenza season (southern hemisphere winter) contain the following:

- an A/Michigan/45/2015 (H1N1)-like virus;
- an A/Hong Kong/4801/2014 (H3N2)-like virus;
- a B/Brisbane/60/2008*-like virus.

It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Phuket/3073/2013*-like virus.

* *These viruses were originally isolated at the WHO Collaborating Centre in Melbourne.*

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.

At its meeting on 13 October AIVC accepted the September WHO recommendation and decided that the Australian influenza vaccine for 2017 should contain the following:

- A(H1N1): an A/Michigan/45/2015 (H1N1)-like virus
- A(H3N2): an A/Hong Kong/4801/2014 (H3N2)-like virus
- B: a B/Brisbane/60/2008-like virus

Quadrivalent vaccines should contain viruses listed above, plus the additional B virus: B/Phuket/3073/2013-like virus.

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 2016, 44 kits were sent to 22 laboratories in 15 countries. Each kit contained 10 mL each of the reference antigens A/California/7/2009, A/Hong Kong /4801/2015, B/Phuket/3073/2013 and B/Brisbane/60/2008, and homologous antisera.

Recipients of the 2016 Kit

AUSTRALIA: SA Pathology Adelaide, South Australia; Queensland Health Scientific Services, Brisbane, Queensland; Vaxxas, Brisbane, Queensland; Westmead Hospital, Sydney, New South Wales; Australian Institute for Bioengineering and Nanotechnology, Brisbane , Queensland

CAMBODIA: Institut Pasteur du Cambodge, Phnom Penh

HONG KONG SAR: University of Hong Kong

INDIA: Manipal University, Karnataka; VP Chest Institute, New Delhi;

KENYA: Center for Virus Research, Kenya Medical Research Institute, Nairobi

MACAU SAR: Public Health Laboratory

MALAYSIA: Institute for Medical Research, Kuala Lumpur; International Islamic University, Kuala Lumpur

NEW ZEALAND: Canterbury Health Services, Christchurch, New Zealand

PHILIPPINES: Research Institute for Tropical Medicine, Mutinlupa City

SINGAPORE: Singapore General Hospital; Duke-NUS Graduate Medical School

SOUTH AFRICA: National Institute for Communicable Diseases, Johannesburg

SRI LANKA: Medical Research Institute, Colombo

TAIWAN: National Cheng Kung University, Tainan

THAILAND: National Institute of Health, Bangkok

VIETNAM: National Hospital of Tropical Diseases, Hanoi

Virus Panels for Analysis of Resistance to Antiviral Drugs

The Centre produces and distributes a panel of reference viruses on request to laboratories conducting NAI assays on behalf of the International Society for Influenza and other Respiratory Virus Diseases (isirv) Antiviral Group. In 2016 panel kits were sent to Westmead Hospital, Sydney; Universite de Sherbrooke, Quebec, Canada; Utah Public Health Laboratory, Salt Lake City UT, USA; and Genomics Research Center, Academia Sinica, Taipei, Taiwan. Kits were composed of 2 vials (250 µL) of each of the reference viruses listed in the table below.

Viruses in the 2016 NAI assay panel

Reference virus	Inhibition by antiviral drugs			
	Oseltamivir	**Laninamivir**	**Peramivir**	**Zanamivir**
(Former seasonal A(H1N1); A/New Caledonia/20/99-like)				
A/Mississippi/3/01 (H1N1) wild-type	Normal	Normal	Normal	Normal
A/Mississippi/3/01 (H1N1) variant **(H275Y)**	Highly reduced	Normal	Highly reduced	Normal
(A(H3N2); A/Fujian/411/2002-like)				
A/Fukui/20/04 (H3N2) wild-type	Normal	Normal	Normal	Normal
A/Fukui/45/04 (H3N2) variant **(E119V)**	Highly reduced	Normal	Normal	Normal
(B; B/Sichuan/379/1999-like)				
B/Perth/211/2009 wild-type	Normal	Normal	Normal	Normal
B/Perth/211/2009 variant **(D197E)**	Highly reduced	Normal	Highly reduced	Normal
(A(H1N1)pdm09; A/California/7/2009-like)				
A/Perth/265/2009 (H1N1)pdm09 wild-type	Normal	Normal	Normal	Normal
A/Perth/261/2009 (H1N1)pdm09 variant **(H275Y)**	Highly reduced	Normal	Highly reduced	Normal

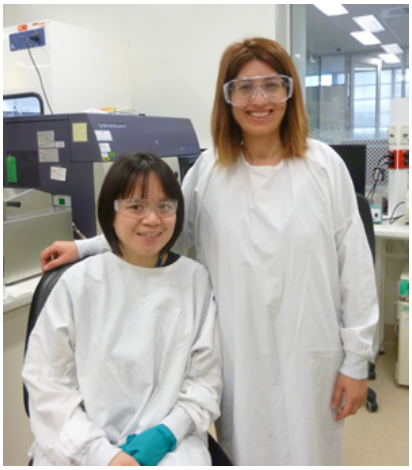
Training

Training and Support of National Influenza Centres

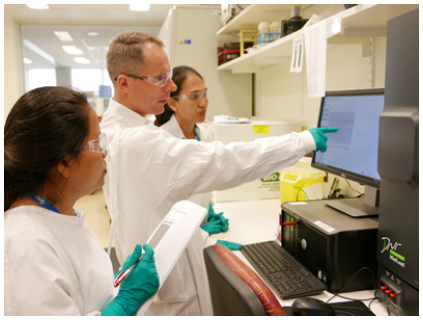
The Centre provides support in the form of training and advice to WHO National Influenza Centres (NICs) and other diagnostic laboratories, especially in the Asia-Pacific region. Strengthening technical capabilities and infrastructure for surveillance work in regional laboratories increases their capacity 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.

In-house Training

Dr Sevim Mese (*below right)*, from the National Influenza Reference Laboratory at the Public Health Institution of Turkey (PHIT), Ankara, Turkey, visited the Centre 21 June – 8 July for training in antiviral resistance testing.



Ms Sokhoun Yann (*right)* and Ms Kimlay Chea (*centre)* from the Institut Pasteur, Phnom Penh, Cambodia, and Ms Phally Vy (*left)*, from the National Public Health Laboratory, Phnom Penh, Cambodia; visited the Centre from 21 November to 2 December. They undertook training in various techniques related to detection and characterisation of seasonal influenza viruses, including sequencing of influenza genes and phylogenetic analysis, viral isolation in MDCK cells and serological analysis of isolates, egg inoculation and harvest for growth of influenza viruses, and analysis and management of surveillance data.



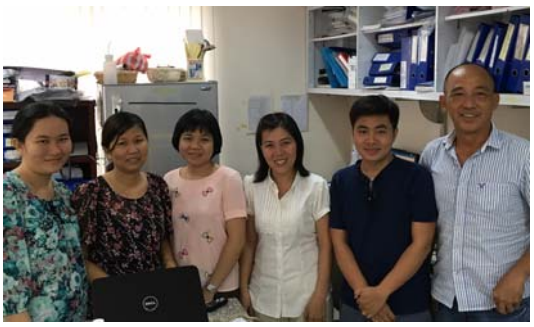
External Quality Assurance Project for Virus Isolation

Under the leadership of Patrick Reading, the Centre developed an External Quality Assessment (EQA) to test virus isolation capacity of National Influenza Centres (NICs) in the WHO Western Pacific Region (WPR) and South-East Asia Region (SEAR). The Centre prepared a panel of 16 samples to send to NICs in WPR/SEAR to test capacity for virus isolation. Samples were prepared and validated by real-time RT-PCR and isolation at the Centre, then sent to Collaborating Centres in Japan China for validation. Test panels each containing 16 test samples were sent to 14 laboratories in WPR and 7 laboratories in SEAR in September and October and results were received from all participating NICs by the end of the year. Results from this EQAP will form the basis of a workshop coordinated by the Centre (and supported by the Regional Office for WPR, WPRO) that is planned for May/June 2017. This workshop will bring together approximately 20 scientists from WPR countries to train them in techniques relating to cell culture and isolation of influenza viruses.

Training Programs and Visits to Regional Laboratories

Aeron Hurt participated as an instructor at the isirv School of Influenza, held in Siena, Italy on 11–15 April. He presented two talks and assisted as a workshop co-ordinator.

Sheena Sullivan co-taught a WPRO Burden of Disease training workshop held in Hanoi, Vietnam on 16–20 May. The workshop was attended by 20 field epidemiology training programme trainees from Vietnam. **Vivian Leung** helped to prepare data and slides for the presentations.



Patrick Reading visited the Pasteur Institute, Ho Chi Minh City, Vietnam, on 6–17 June. He worked with scientists and laboratory technicians at the Institute to perform TCID50 and microneutralisation and assays to detect antibodies to A(H7N9) and A(H1N1)pdm09 viruses. Additional strategies to improve virus isolation capacity were also implemented (*right*).

Naomi Komadina was a training session facilitator at the WHO-GISAID-ISIRV Workshop on Genetic Analyses of Influenza Viruses, Chicago IL, USA, on 29 August (*right*).

Patrick Reading visited the National Influenza Centre at the Fiji Centre for Communicable Disease Control, Suva, Fiji on 14–21 November to facilitate discussions regarding the implementation of cell culture and virus isolation. Plans were developed to modify existing laboratories and obtain appropriate equipment and reagents, with the aim of establishing cell culture and virus isolation techniques in 2017. Practical training in real-time RT-PCR was also provided to new staff members at the National Influenza Centre.

Patrick Reading contributed to planning and presented two lectures at the Influenza-like illness Surveillance Workshop, held in Goroka, Papua New Guinea on 24–26 October (*below*). The workshop was attended by approximately 20 participants with representatives from a number of regions in Papua New Guinea to plan implementing enhanced surveillance for human and avian influenza.

Patrick Reading worked with Dr Linda Oskam (DATOS) as coordinators and instructors at the Introduction to Laboratory Quality Management and Good Laboratory Practice workshop, held in Jakarta, Indonesia, on 21–25 November (*below*). The workshop was attended by laboratory managers and technicians from laboratories across different provinces in Indonesia that are involved in laboratory testing for influenza virus. There were 17 attendees from the provinces, plus several staff members from the National Institute of Health Research and Development.

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

Aeron Hurt, Ding Yuan Thomas Oh, Danielle Tilmanis, Sook Kwan Leah Brown, Rubaiyea Farrukee, Celeste Tai, Paulina Koszalka

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 a project funded by the National Health and Medical Research Council (NHMRC) and the Agency for Science, Technology and Research (A*STAR, Singapore), we have developed a ferret antiviral treatment model to determine the clinical effectiveness of antiviral treatment of variant viruses with reduced *in vitro* sensitivity.

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 was initiated in 2016 to investigate *in vitro* and *in vivo* aspects of the repurposed drug Nitazoxanide for its effectiveness against human and potentially pandemic avian influenza viruses.

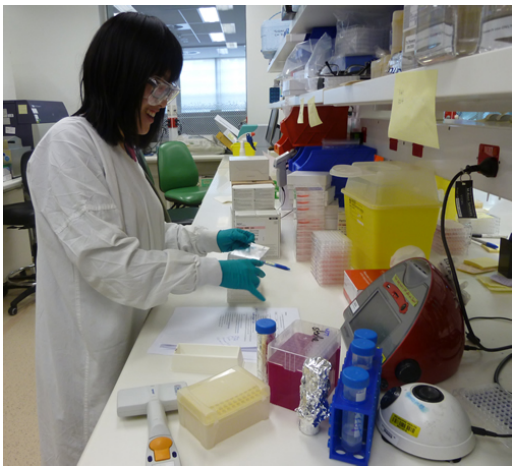
Highlights and developments 2016

The NHMRC/A*STAR-funded project has made substantial progress since establishing and optimising the ferret antiviral treatment model. Our findings demonstrated that oseltamivir had a reduced clinical effect against particular variant viruses, where *in vivo* clinical data was not previously known. Further studies to assess other variant viruses with reduced NAI sensitivity are being conducted to better understand the correlation between *in vitro* NAI sensitivity and *in vivo* effectiveness.

Two NA mutations have been detected at higher than normal levels in influenza B viruses in recent years. These viruses, which have reduced antiviral sensitivity, are being characterized *in vitro* for NA enzymatic function and replication and also *in vivo* in the ferret to determine whether the mutations result in reduced replication or transmission.

Collaborators

Sebastian Maurer-Stroh (A*STAR, Singapore); Gary Lau (Duke-NUS Graduate Medical School, Singapore); Carl Kirkpatrick (Monash University, Victoria); Veronika von Messling (Paul-Ehrlich-Institut, Langen, Germany); Jean-Francois Rossignol (Romark Laboratories, USA)



Evolution and Modelling of Influenza Viruses

Centre staff

Ian Barr, Aeron Hurt, Malet Aban, Yi-Mo Deng, Natalie Spirason, Sheena Sullivan

Research overview

We are pursuing several projects in collaboration with international and local groups to investigate various aspects of influenza virus evolution and the immune responses to influenza viruses and vaccines. One of these projects is an ongoing study on the evolution of A(H1N1)pdm09 viruses since the 2009 pandemic with colleagues at Duke-NUS Medical School in Singapore.

Work also continues on studying the evolution of influenza B viruses in a collaboration with Edward Holmes (University of Sydney, NSW), Vijaykrishna Dhanasekaran (Monash University) and the J. Craig Venter Institute. Over 700 B/Yamagata lineage viruses collected from Australia, New Zealand and Singapore during 2012-2014 have been sequenced, with the full genome sequencing of these viruses completed in 2016. These data, together with previous sequence data from our earlier studies, will be analysed to determine evolutionary patterns in these viruses as they circulated in this region.

A project funded by the US Department of Health and Human services via the Biomedical Advanced Research and Development Authority (BARDA) with collaborators at Cambridge University, UK and other experts is also continuing. This five-year program aims to develop and clinically test novel vaccines for current and potentially future A(H5N1) viruses, based on antigenic mapping and predictive algorithms for the evolution of viruses.

Highlights and developments 2016

All of the sequencing data have been collated for the study of influenza B viruses, and we are now in the process of analysing the data and preparing a manuscript.

Steady progress has also been made on the BARDA-funded A(H5N1) vaccine project with animal testing of the first of the modified A(H5N1) viruses completed. These viruses will also be used to make the advanced vaccines that will be tested first in animals and if successful will move into testing in human subjects. Further funding has been provided through the Centers of Excellence in Influenza Research and Sureillance (CEIRS) network to replicate this work for seasonal influenza viruses. A further extension of the work to understand serological responses to influenza infection and vaccination will be conducted using a cohort in North Vietnam, in collaboration with Annette Fox (University of Melbourne). This work has been funded by the NHMRC for the period 2016 to 2019, and the Centre's participation in this project will begin in 2017.

Collaborators

Annette Fox, (University of Melbourne); Derek Smith and Colin Russell (Cambridge University, UK); Yoshihiro Kawaoka (The University of Wisconsin, Madison, WI, USA and The University of Tokyo, Japan); Vijaykrishna Dhanasekaran (Monash University); Gavin Smith and Yvonne Su (Duke-NUS Graduate Medical School, Singapore); Trevor Bedford (Fred Hutchinson Cancer Research Center, Seattle WA, USA); Ron Fouchier (Erasmus University, Rotterdam, The Netherlands); Ed Bolongia (Marshfield Clinic Research Foundation, Marshfield WI, USA); Alan Durbin, Rebecca Halpin and Das Suman (JCVI; J. Craig Venter Institute, Rockville and San Diego, USA); Edward Holmes (University of Sydney, NSW)

Epidemiology

Centre staff

Sheena Sullivan, Vivian Leung

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, including the Australian Sentinel Practices Research Network (ASPREN), the Sentinel Practices Network of WA (SPNWA), the Victorian General Practice Sentinel Surveillance (GPSS) network, and the Influenza Complications Alert Network (FluCAN) to estimate influenza vaccine effectiveness in the community. We are evaluating the validity of the studies used to estimate vaccine effectiveness and working with groups internationally to improve the utility of vaccine effectiveness estimates for influenza vaccine strain selection.

A poorly understood consequence of repeated vaccination is that it may attenuate the vaccine's effectiveness. This may occur when residual antibodies stimulated by one year's vaccine may negatively interfere with a subsequent year's vaccination. Thus, protection afforded by the vaccine in target groups for vaccination, namely hospital workers, may be compromised by repeated annual vaccination. We have established cohorts at two hospitals in Melbourne to examine whether serological responses to vaccination differ among frequently and infrequently vaccinated staff. We have also been investigating the validity of methods used to estimate antibody responses to vaccination and infection.

Highlights and developments 2016

In 2016, we continued to receive all influenza-positive samples from Australian sentinel practices networks enabling calculation of vaccine effectiveness at the molecular level and the comparison of surveillance samples with samples routinely submitted to the Centre. Together with these networks, we published two manuscripts, one demonstrating that pooling Australian data to estimate vaccine effectiveness is valid, and the other reporting estimates for 2015. We also published several papers exploring the validity of the test-negative method for estimation of vaccine effectiveness.

In addition, we also recruited our second cohort of hospital workers to examine the immunological effects of repeated vaccination. We observed poorer antibody responses among the most highly-vaccinated hospital workers and observed surprisingly high antibody titres among A(H3N2)-infected workers.

Collaborators

James Fielding, Kylie Carville, and Kristina Grant (Epidemiology Unit, VIDRL); Avram Levy, David Smith (PathWest Laboratory Medicine, Western Australia); Paul Effler, Annette Regan, Gary Dowse (Department of Health, Western Australia); Nigel Stocks, Monique Chilver (Australian Sentinel Practices Research Network); Allen Cheng (Influenza Complications Alert Network); Ben Cowling, Shuo Feng, Helen Bond (University of Hong Kong); Caroline Marshall (Royal Melbourne Hospital); Annette Fox (Department of Microbiology and Immunology, University of Melbourne); Monica Slavin, Leon Worth, Susan Harper, Ben Teh (Department of Infectious Diseases/ Infection Prevention, Peter MacCallum Cancer Centre); Eric Tchetgen Tchetgen (Harvard University); Sander Greenland (University of California, Los Angeles)

Animal Influenza Viruses

Centre staff

Aeron Hurt, Michelle Wille, Malet Aban, Chantal Baas, Yi-Mo Deng, Heidi Peck, Natalie Spirason

Research overview

Animal influenza viruses can pose a threat to humans via direct infection from an animal 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 and sent to the Centre for analysis during 2016.

Swine influenza viruses collected from pig farms in Western Australia and Queensland are also being assessed by the Centre to determine the risk that these viruses pose to humans. Using the ferret model we are assessing the infectivity of the viruses and whether the viruses transmit between ferrets by either contact and/or aerosol transmission.

Collaborators

Marcel Klaassen, Bethany Hoye (Deakin University, Victoria); Simone Warner (Department of Primary Industries, Victoria); Eddie Holmes (University of Sydney, New South Wales); Daniel González-Acuña (University of Concepción, Chile)

Highlights and developments 2016

In 2016, we collected and screened 659 samples from wild birds in Victoria, South Australia and Tasmania, with 15 influenza A virus detections. 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. Additionally, from birds sampled for active infection, we have also screened paired serum samples for anti-influenza NP antibodies using a commercial NP-ELISA assay.

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. Furthermore, we started screening archived serum samples collected from Australian wild birds for anti-influenza antibodies to better understand changes in exposure across the last 5 years.

The swine influenza viruses collected from pig farms were shown to readily infect ferrets at a range of virus dilutions and could easily transmit between ferrets housed within the same cage. Studies are ongoing to determine if the viruses are able to transmit via aerosol transmission i.e. between ferrets housed in different cages but separated by a mesh screen. Should the viruses transmit via aerosol transmission then they would be considered a potential risk to public health.

Novel Inhalation Delivery of a DNA-Based Influenza Vaccine

Centre staff

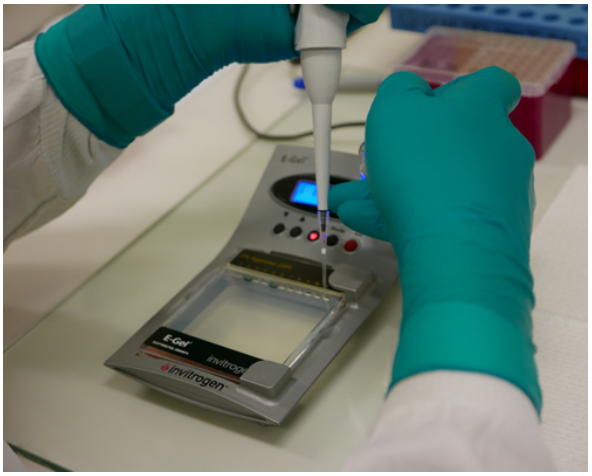
Aeron Hurt, Leonard Izzard

Research overview

Our research, funded by an NHMRC development grant, investigates the feasibility of using a novel ultrasonic nebuliser device to deliver DNA vaccines in an *in vivo* model. To this end we use an influenza A model of infection in ferrets. Historically vaccines consist of either inactivated or live attenuated viruses. The presence of these foreign proteins stimulate the host's immune system to mount an immune response against the virus and help to protect it against a genuine viral challenge. Our model differs in that we are delivering DNA plasmids that program the host's own cells to produce the foreign viral proteins. The route of administration is via the respiratory tract, which is facilitated by nebulisation of the plasmid mix using a novel ultrasonic nebuliser. This system will result in efficient delivery of the plasmid deep into the lungs including alveoli, allowing the production of viral proteins in this clinically relevant location. This influx of foreign viral proteins will effectively 'kick start' the immune response in a similar way to the traditional whole protein vaccines.

Highlights and developments 2016

The project commenced in late 2016. To date we have designed and optimised expression vectors that encode the HA protein of influenza A(H1N1)pdm09. This viral protein was chosen as it is the key target of the host immune system in context of generating neutralising antibodies, the main adaptive immune response against influenza A.



Collaborators

Leslie Yeo (RMIT University) and David Piedrafita (Federation University)

Early Recognition and Response to Influenza Infection

Centre staff

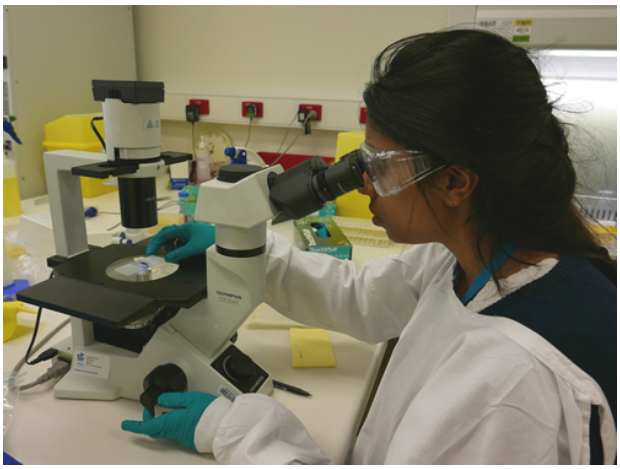
Patrick Reading

Research overview

Our research, which is undertaken 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) identification of cell-surface receptors used by influenza and other respiratory viruses to gain entry into host cells, (ii) how different cell types in the respiratory tract sense and respond to influenza virus infection, and (iii) 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.

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 2016

During 2016, we identified specific cell-surface receptors that function as attachment and/or entry receptors for influenza virus and characterized the functional domains of these receptors required to facilitate infection. In addition, we identified cell-surface receptors used by human metapneumovirus (HMPV), but not by related paramyxoviruses such as respiratory syncytial virus (RSV) or parainfluenza virus (PIV)-3, to infect host cells. We also used a range of experimental approaches, including proteomics, to identify and characterise soluble neutralising inhibitors present in airway fluids derived from naïve ferrets and examined their ability to modulate influenza disease in the ferret model.

Overall, our research contributed to seven peer-reviewed publications during 2016, including senior author publications in journals such as The Journal of Virology, Scientific Reports and Virology. Dr Reading presented several research talks at conferences and institutes during the year, and was an invited speaker at the 6th Infection and Immunity Conference, Lorne, Victoria and the 4th National Respiratory Leadership Summit, Melbourne, Victoria. In July, Dr Reading was awarded an honorary appointment as Professorial Fellow within the Department of Microbiology and Immunology at the University of Melbourne. In 2016, his research group consisted of one post-doctoral scientist, one Ph.D. student and one Master of Biomedical Science student. Dr Reading is co-supervisor of an additional three Ph.D. students enrolled at the University of Melbourne.

Understanding the Interplay between the Immune Response and Influenza Viruses

Centre staff

Karen Laurie, Kok Fei (Jimmy) Chan, Louise Carolan, Patrick Reading

Research overview

Viral interference is a phenomenon whereby primary virus infection of the host will lower the host susceptibility to subsequent infection by another virus in a short time interval. In a previous study, we established evidence for viral interference between antigenically related and unrelated influenza A viruses and showed that the ability of different influenza viruses to induce interference in ferrets can be hierarchically ranked. We have extended the study to understand viral interference between different influenza B lineages and showed that the ability of a particular virus lineage in inducing interference was well correlated with its dominance in the circulating influenza B lineages.

We have also established a collaboration with Dr Daniil Korenkov and Professor Larisa Rudenko from the Institute of Experimental Medicine, Saint Petersburg, Russia to assess the efficacy of six new live attenuated influenza vaccine (LAIV) strains in conferring protection to ferrets from influenza virus infections. This study was aimed to determine: (1) the safety of using LAIVs in ferrets, (2) whether lung infection in ferrets can be prevented by vaccination, and (3) if the vaccine can protect against viruses that are more distantly matched with the LAIV strains.

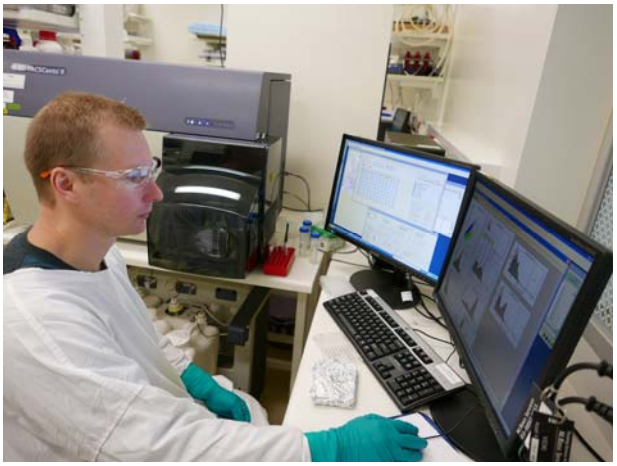
Highlights and developments 2016

We have made good progress in finalising our viral interference study between different influenza B lineages. This study has filled in an important gap in understanding the role of viral interference and cross-reactive protective immunity between influenza B lineages. A manuscript has been drafted and submitted for publication.

During 2016 we welcomed Dr Korenkov as a visiting scientist to the Centre. Together we made substantial progress in assessing the efficacy of six new LAIV strains. We found that these LAIV strains were effective at protecting ferrets from homologous influenza virus infection. The LAIV vaccinated ferrets shed significantly lower amount of challenge virus in the upper respiratory tract when compared to the mock-vaccinated ferrets. We are now working closely with Dr Korenkov and Prof Rudenko to finalise this study.

Collaborators

Daniil Korenkov, Larisa Rudenko (Institute of Experimental Medicine, Saint Petersburg, Russia); Daniel Layton, Andrew Bean (Australian Animal Health Laboratory, CSIRO, Victoria); Vijay Dhanasekaran (Department of Microbiology, Monash University, Victoria); James McCaw (Melbourne School of Population and Global Health, The University of Melbourne); Lorena Brown, Katherine Kedzierska, Oanh Nguyen (Department of Microbiology and Immunology, The University of Melbourne)



A Ferret Model for Human Respiratory Syncytial Virus (RSV) Infection and Disease

Centre staff

Karen Laurie, Kok Fei (Jimmy) Chan, Louise Carolan, Patrick Reading

Research overview

We have developed a ferret model for RSV to further understand the disease pathogenesis, induction of humoral immunity and contact-dependent transmission during the course of an RSV infection. Globally, RSV is a major cause of severe respiratory infections in children and older adults, with an estimated up to 200,000 children aged under 5 years old dying from RSV-associated acute lower respiratory infection annually[1]. It is therefore important to study the disease pathogenesis, transmissibility, sites of infection in the respiratory tract and immune response elicitation in the host, and to find ways to control and prevent RSV infections.

Our RSV ferret model involves: (1) studying the growth kinetics of different RSV strains in the upper and lower respiratory tracts (URT and LRT), (2) investigating the transmissibility of RSV between co-housed donor and recipient ferrets, (3) understanding the induction of different cytokine, chemokine and immune mediator expressions in ferret URT, (4) observing clinical signs after infection, (5) determining seroconversion and anti-RSV antibody kinetics in ferrets after primary and secondary infections, and (6) understanding the cross-reactivity of antibody and cellular immune responses against different RSV strains.

Our RSV ferret model is also used to investigate viral interference between different RSV strains and influenza A(H1N1)pdm09 virus. We are investigating if prior infection with RSV could also limit further influenza virus infection in ferrets and vice versa. Understanding the factors which contribute to viral interference may assist us in developing novel therapeutic strategies to control and prevent influenza viruses and RSV infections.

[1] *http://dx.doi.org/10.7488/ds/1491*

Highlights and developments 2016

We have successfully developed a ferret model for human RSV infection and disease. We found that ferrets infected with different RSV strains transmitted the disease to healthy co-housed recipient ferrets. RSV shedding in ferret URT peaked between 3 to 7 day post infection (dpi). We also detected RSV shedding in ferret LRT at 5 dpi but by 9 dpi most LRT sections were cleared of viral RNA. Concurrently, we isolated a few recently circulating RSV strains from human clinical specimens, and tested these in our RSV ferret model. In general, we observed minimal clinical signs in RSV-infected ferrets, and found that ferrets infected with certain RSV strains were less susceptible to subsequent re-infection by the same virus strain.

We have also successfully developed the RSV Infectious ViroSpot and RSV ViroSpot Microneutralisation assays to detect infectious RSV titre and neutralising antibodies against RSV in ferrets. We showed that infected ferrets had developed antibodies to RSV by the end of the experiment, and elicited cellular immune responses (by ELISpot IFN-γ assay) when the harvested lymph node cells were stimulated *in vitro* with RSV.

Our viral interference studies showed that primary infection with A(H1N1)pdm09 influenza virus can prevent or delay RSV shedding in ferret URT when challenged with RSV at 3 or 7 dpi. Interestingly, when the primary and challenge viruses were reversed in order, shedding of A(H1N1)pdm09 influenza virus was detected in ferret URT regardless of the time interval between primary and secondary infections. A similar outcome was observed when a ten-fold higher infectious dose of RSV was used to infect ferrets prior to challenge virus infection, indicating a hierarchical ranking among different respiratory viruses to induce interference. We are currently planning co-infection experiments to further delineate this phenomenon in ferrets.

Collaborators

Christopher McMillan, Daniel Watterson, Paul Young (University of Queensland); Julian Druce, Thomas Tran (VIDRL, Melbourne); Lorena Brown, Katherine Kedzierska, Oanh Nguyen (Department of Microbiology and Immunology, The University of Melbourne); Lien Anh Ha Do (Murdoch Childrens Research Institute, Melbourne)

Collaborative Agreements

The Centre is party to three ongoing collaborative research and development agreements with industry bodies. As with all potential collaborations with the commercial sector, these agreements have undergone review by the Australian Government 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) (2015-2016)

Centre staff: Hilda Lau, Robert Shaw, Ian Barr, Chantal Baas, 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 2016: A total of 41 egg isolates were obtained from 111 inoculations with original clinical specimens from various geographical locations. Isolation rates varied from 23% to 80% 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 production (2015-2016)

Centre staff: Heidi Peck, Joelle Dharmakumara, Ian Barr, Cleve Rynehart

Project overview: The suitability of a proprietary Seqirus cell line for isolating and growing influenza viruses as a basis for cell-based vaccine manufacture is being evaluated. A number of original clinical specimens are used to isolate viruses directly into the MDCK33016PF cell line in parallel with egg isolation. The resultant isolates undergo analysis of their growth, antigenic and other properties.

Highlights and developments 2016: During 2016, 144 clinical specimens were cultured in MDCK 33016PF cells, of which 124(86%) produced isolates. As in previous years, this was much higher than the rate of isolation in eggs. The isolates, which comprised A(H1N1)pdm09, A(H3N2) and B viruses, were sent to Seqirus in Holly Springs NC, USA, for further evaluation as potential vaccine candidates produced by cell culture. A number of important milestones were achieved in 2016. Approval was given by the US Food and Drug Administration (FDA) to use cell based isolates to produce influenza vaccines. This resulted in the first ever listing of two influenza B cell-culture derived candidate vaccine viruses for use in a influenza vaccine by WHO in September 2016. These viruses were first isolated and characterised at the Centre. Furthermore, an A(H3N2) cell-derived candidate isolated and characterised at the Centre will become the first ever cell-derived seed to enter manufacturing for the 2017-2018 Northern Hemisphere influenza vaccine.

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

Centre staff: Ding Yuan Thomas Oh, 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 2016: A new nitazoxanide susceptibility assay was developed utilizing a cell culture-based focus-reduction assay, and over 200 circulating viruses were shown to be susceptible to the antiviral. Experiments involving serial passage of viruses in increasing levels of nitazoxanide failed to select for resistance. A ferret model of influenza infection and nitazoxanide treatment was also established, showing good tolerability of the drug and effectiveness in preventing infection.

Research Students

PhD Candidates

Ms Rubaiyea Farrukee, a PhD candidate from the University of Melbourne, commenced her PhD project titled: "Assessing replication, transmission and fitness of antiviral resistant influenza viruses", under the supervision for **Aeron Hurt** and **Patrick Reading**.

Ms Annika Suttie, a PhD candidate from Federation University, commenced her PhD project titled "Molecular epidemiology of influenza virus in Cambodia", under the supervision of Andrew Greenhill (Federation University), **Yi-Mo Deng**, Jenny Mosse (Federation University) and Paul Horwood (James Cook University).

MSc Candidate

Ms Chantal Baas submitted her MSc thesis entitled "Use of the ferret model to investigate the transmission of swine and human influenza viruses" in July. Chantal was supervised by **Aeron Hurt**, **Ian Barr** and Ms Jenny Mosse (Federation University, Gippsland). Her thesis was passed in February 2017.

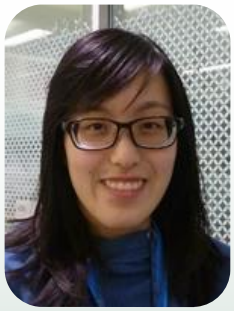
Other training

Ms Golnoosh Torabian, a PhD student from the University of Sydney, visited the Centre 9–13 May to undertake training in the plaque reduction assay. The Centre also hosted several undergraduate and secondary school students for work experience placements during the year: Ms Caitlyn Fitzgerald (Ava Maria College) on 22 June, Ms Nicole Baxter (Deakin University) on 23 June, Mr Stefan Piantella (La Trobe University) on 24 November and Ms Lauren Akers (Trafalgar High School) on 1 December.

Honours students

Ms Paulina Koszalka, a BSc (Honours) student enrolled through the University of Melbourne, completed her Honours project under the supervision of **Aeron Hurt** and **Danielle Tilmanis**. Her project, titled "Phenotypic and genotypic characterisation of influenza viruses selected *in vitro* to Nitazoxanide, a new influenza antiviral", studied the propensity for circulating influenza viruses to develop resistance to nitazoxanide (NTZ). By serially passaging influenza viruses in increasing concentrations of tizoxanide (the metabolically active form of NTZ), her results indicated that influenza viruses are unlikely to develop resistance to NTZ, and furthermore, the use of NTZ in combination therapy with oseltamivir may reduce the likelihood of influenza viruses developing resistance to oseltamivir. Paulina was awarded First-Class Honours.

Ms Celete Tai, a BSc(Honours) student enrolled through the University of Melbourne, completed her Honours project under the supervision of **Aeron Hurt** and **Ding Yuan Thomas Oh**. Her project titled, "The clinical effectiveness of oseltamivir in the ferret model of influenza infection", investigated the effectiveness of oseltamivir treatment in a ferret model of infection with influenza variant viruses containing the H275Y mutation in the neuraminidase protein. Her results showed that oseltamivir was not able to prevent infection or reduce viral shedding in ferrets infected with influenza A(H1N1) or A(H1N1)pdm09 variants harbouring the H275Y mutation, compared with their respective wild-type viruses. In addition, oseltamivir treatment was unable to improve the clinical symptoms of ferrets infected with the H275Y variant A(H1N1) and A(H1N1)pdm09 viruses compared to placebo treatment. Celeste was awarded First-Class Honours.



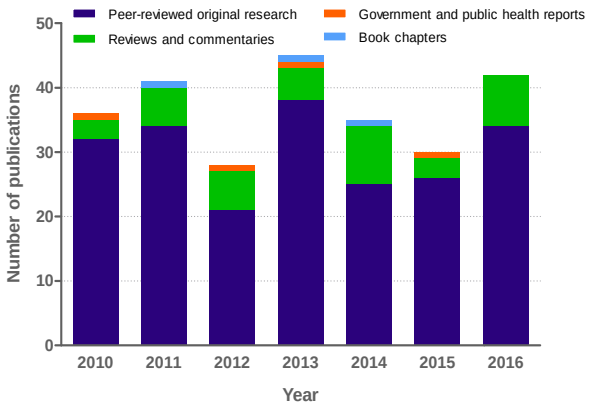
Communications and Advisory Activities

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. The Australian Influenza Symposium was not held in 2016 due to the similar timing of two major international meetings in August (Options IX for the Control of Influenza and the 16th International Congress of Immunology).

Publications and Reports

The Centre continued to build its research and surveillance profile with the publication of 42 original research papers, reviews and reports in 2016 (Figure 19). Amongst these publications was a paper that was featured on the cover on the Journal of Virology (*shown at right)*.

Figure 19. Centre publications 2010–2016.



Centre Publications 2016

1. Ana-Sosa-Batiz F, Vanderven H, Jegaskanda S, Johnston A, Rockman S, **Laurie K**, **Barr I**, **Reading P**, Lichtfuss M and Kent SJ. Influenza-specific antibody-dependent phagocytosis. PLoS One, 2016. 11(4): e0154461.
2. **Barr IG**, Vijaykrishna D and **Sullivan SG**. Differential age susceptibility to influenza B/Victoria lineage viruses in the 2015 Australian influenza season. Euro Surveill, 2016. 21(4).
3. **Barr IG** and Wong FY. Avian influenza: why the concern? Microbiology Australia, 2016. November.
4. Beale DJ, Jones OA, Karpe AV, Dayalan S, **Oh DY**, Kouremenos KA, Ahmed W and Palombo EA. A review of analytical techniques and their application in disease diagnosis in breathomics and salivaomics research. Int J Mol Sci, 2016. 18(1).
5. Bond HS, **Sullivan SG** and Cowling BJ. Regression approaches in the test-negative study design for assessment of influenza vaccine effectiveness. Epidemiol Infect, 2016. 144(8): 1601-11.
6. **Carolan LA**, Rockman S, **Borg K**, **Guarnaccia T**, **Reading P**, Mosse J, **Kelso A**, **Barr I** and **Laurie KL**. Characterization of the localized immune response in the respiratory tract of ferrets following infection with influenza A and B viruses. J Virol, 2016. 90(6): 2838-48.
7. Dalziel AE, **Peck HA**, **Hurt AC**, Cooke J and Cassey P. Proposed surveillance for influenza A in feral pigs. Ecohealth, 2016. 13 (2): 410-4.
8. Edenborough KM, Lowther S, **Laurie K**, Yamada M, Long F, Bingham J, Payne J, Harper J, Haining J, Arkinstall R, Gilbertson B, Middleton D and Brown LE. Predicting disease severity and viral spread of H5N1 influenza virus in ferrets in the context of natural exposure routes. J Virol, 2016. 90(4): 1888-97.
9. Eichelberger MC, Couzens L, Gao Y, Levine M, Katz J, Wagner R, Thompson CI, Hoschler K, **Laurie K**, Bai T, Engelhardt OG and Wood J. Comparability of neuraminidase inhibition antibody titers measured by enzyme-linked lectin assay (ELLA) for the analysis of influenza vaccine immunogenicity. Vaccine, 2016. 34(4): 458-65.
10. Feng S, Cowling BJ and **Sullivan SG**. Influenza vaccine effectiveness by test-negative design - Comparison of inpatient and outpatient settings. Vaccine, 2016. 34(14): 1672-9.
11. Fielding JE, Levy A, Chilver MB, Deng YM, Regan AK, Grant KA, Stocks NP and **Sullivan SG**. Effectiveness of seasonal influenza vaccine in Australia, 2015: An epidemiological, antigenic and phylogenetic assessment. Vaccine, 2016. 34(41): 4905-12.
12. Fielding JE, Regan AK, Dalton CB, Chilver MB and **Sullivan SG**. How severe was the 2015 influenza season in Australia? Med J Aust, 2016. 204(2): 60-1.
13. FitzPatrick M, Royce SG, Langenbach S, McQualter J, **Reading PC**, Wijburg O, Anderson GP, Stewart A, Bourke J and Bozinovski S. Neonatal pneumococcal colonisation caused by influenza A infection alters lung function in adult mice. Sci Rep, 2016. 6: 22751.

Centre Publications (continued)

14. Gillespie L, Gerstenberg K, Ana-Sosa-Batiz F, Parsons MS, **Farrukee R**, Krabbe M, Spann K, Brooks AG, Londrigan SL and **Reading PC**. DC-SIGN and L-SIGN are attachment factors that promote infection of target cells by human metapneumovirus in the presence or absence of cellular glycosaminoglycans. J Virol, 2016. 90(17): 7848-63.
15. Gillespie L, Roosendahl P, Ng WC, Brooks AG, **Reading PC** and Londrigan SL. Endocytic function is critical for influenza A virus infection via DC-SIGN and L-SIGN. Sci Rep, 2016. 6: 19428.
16. Gordon DL, Sajkov D, Honda-Okubo Y, Wilks SH, **Aban M**, **Barr IG** and Petrovsky N. Human Phase 1 trial of low-dose inactivated seasonal influenza vaccine formulated with Advax delta inulin adjuvant. Vaccine, 2016. 34(33): 3780-6.
17. **Hurt AC**, Besselaar TG, Daniels RS, Ermetal B, Fry A, Gubareva L, Huang W, Lackenby A, Lee RT, Lo J, Maurer-Stroh S, Nguyen HT, Pereyaslov D, Rebelo-de-Andrade H, Siqueira MM, Takashita E, Tashiro M, **Tilmanis D**, Wang D, Zhang W and Meijer A. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014-2015. Antiviral Res, 2016. 132: 178-185.
18. **Hurt AC** and Kelly H. Debate regarding oseltamivir use for seasonal and pandemic influenza. Emerg Infect Dis, 2016. 22(6): 949-55.
19. **Hurt AC**, Su YC, **Aban M**, **Peck H**, **Lau H**, **Baas C**, **Deng YM**, **Spirason N**, Ellstrom P, Hernandez J, Olsen B, **Barr IG**, Vijaykrishna D and Gonzalez-Acuna D. Evidence for the introduction, reassortment, and persistence of diverse influenza A viruses in Antarctica. J Virol, 2016. 90(21): 9674-9682.
20. **Jelley L**, Levy A, **Deng YM**, **Spirason N**, Lang J, **Buettner I**, Druce J, Blyth C, Effler P, Smith D and **Barr IG**. Influenza C infections in Western Australia and Victoria from 2008 to 2014. Influenza Other Respir Viruses, 2016. 10(6): 455-461.
21. Job ER, Pizzolla A, Nebl T, Short KR, **Deng YM**, **Carolan L**, **Laurie KL**, Brooks AG and **Reading PC**. Neutralizing inhibitors in the airways of naive ferrets do not play a major role in modulating the virulence of H3 subtype influenza A viruses. Virology, 2016. 494: 143-57.
22. **Komadina N**, Quiñones-Parra SM, Kedzierska K, McCaw JM, **Kelso A**, Leder K and McVernon J. High conservation level of $CD8^+$ T cell immunogenic regions within an unusual H1N2 human influenza variant. J Med Virol, 2016. 88(10): 1725-32.
23. Kotecha RS, Wadia UD, Jacoby P, Ryan AL, Blyth CC, Keil AD, Gottardo NG, Cole CH, **Barr IG** and Richmond PC. Immunogenicity and clinical effectiveness of the trivalent inactivated influenza vaccine in immunocompromised children undergoing treatment for cancer. Cancer Med, 2016. 5(2): 285-93.
24. Kristensen AB, Lay WN, Ana-Sosa-Batiz F, Vanderven HA, Madhavi V, **Laurie KL**, **Carolan L**, Wines BD, Hogarth M, Wheatley AK and Kent SJ. Antibody responses with Fc-mediated functions after vaccination of HIV-infected subjects with trivalent influenza vaccine. J Virol, 2016. 90(12): 5724-34
25. **Lau H** and **Hurt AC**. Assessment of the RNASound RNA sampling card for the preservation of influenza virus RNA. Front Microbiol, 2016. 7: 1736.
26. **Leung VK**, Cowling BJ, Feng S and **Sullivan SG**. Concordance of interim and final estimates of influenza vaccine effectiveness: a systematic review. Euro Surveill, 2016. 21(16).
27. Li C, Hatta M, Burke DF, Ping J, Zhang Y, Ozawa M, Taft AS, Das SC, Hanson AP, Song J, Imai M, Wilker PR, Watanabe T, Watanabe S, Ito M, Iwatsuki-Horimoto K, Russell CA, James SL, Skepner E, Maher EA, Neumann G, Klimov AI, **Kelso A,** McCauley J, Wang D, Shu Y, Odagiri T, Tashiro M, Xu X, Wentworth DE, Katz JM, Cox NJ, Smith DJ and Kawaoka Y. Selection of antigenically advanced variants of seasonal influenza viruses. Nat Microbiol, 2016. 1(6): 16058.
28. McBride WJ, Abhayaratna WP, **Barr I**, Booy R, Carapetis J, Carson S, De Looze F, Ellis-Pegler R, Heron L, Karrasch J, Marshall H, McVernon J, Nolan T, Rawlinson W, Reid J, Richmond P, Shakib S, Basser RL, Hartel GF, Lai MH, Rockman S and Greenberg ME. Efficacy of a trivalent influenza vaccine against seasonal strains and against 2009 pandemic H1N1: A randomized, placebo-controlled trial. Vaccine, 2016. 34(41): 4991-7.
29. Mifsud EJ, Tan AC, **Reading PC** and Jackson DC. Mapping the pulmonary environment of animals protected from virulent H1N1 influenza infection using the TLR-2 agonist Pam(2)Cys. Immunol Cell Biol, 2016. 94(2): 169-76.
30. Milne GJ, Halder N, Kelso JK, **Barr IG**, Moyes J, Kahn K, Twine R and Cohen C. Trivalent and quadrivalent influenza vaccination effectiveness in Australia and South Africa: results from a modelling study. Influenza Other Respir Viruses, 2016. 10(4): 324-32.
31. Moss R, McCaw JM, Cheng AC, **Hurt AC** and McVernon J. Reducing disease burden in an influenza pandemic by targeted delivery of neuraminidase inhibitors: mathematical models in the Australian context. BMC Infect Dis, 2016. 16(1): 552.
32. Ng WC, Londrigan SL, Nasr N, Cunningham AL, Turville S, Brooks AG and **Reading PC**. The C-type lectin langerin functions as a receptor for attachment and infectious entry of influenza A virus. J Virol, 2016. 90(1): 206-21.
33. Nguyen ML, Hatton L, Li J, Olshansky M, **Kelso A**, Russ BE and Turner SJ. Dynamic regulation of permissive histone modifications and GATA3 binding underpin acquisition of granzyme A expression by virus-specific $CD8^+$ T cells. Eur J Immunol, 2016. 46(2): 307-18.
34. Pennington K, Bareja C, **Sullivan SG**, Franklin LJ and Raupach J. Influenza surveillance in Australia. Commun Dis Intell Q Rep, 2016. 40(3): E315-E316.

Centre Publications (continued)

35. **Oh DY** and **Hurt AC**. Using the ferret as an animal model for investigating influenza antiviral effectiveness. Front Microbiol, 2016. 7: 80.
36. Ren B, McKinstry WJ, Pham T, Newman J, Layton DS, Bean AG, Chen Z, Laurie KL, Borg K, **Barr IG** and Adams TE. Structural and functional characterisation of ferret interleukin-2. Dev Comp Immunol, 2016. 55: 32-8.
37. **Sullivan SG**, Carville KS, Chilver M, Fielding JE, Grant KA, Kelly H, Levy A, Stocks NP, Tempone SS and Regan AK. Pooled influenza vaccine effectiveness estimates for Australia, 2012-2014. Epidemiol Infect, 2016. 144(11): 2317-28.
38. **Sullivan SG**, Cowling BJ and Greenland S. Frailty and influenza vaccine effectiveness. Vaccine, 2016. 34(39): 4645-6.
39. **Sullivan SG**, Raupach J, Franklin LJ, Pennington K, Bareja C, de Kluyver R and and the National Influenza Surveillance Committee ftCDNA. A brief overview of influenza surveillance systems in Australia, 2015. Commun Dis Intell Q Rep, 2016. 40 (3): E351-E355.
40. **Sullivan SG**, Tchetgen Tchetgen EJ and Cowling BJ. Theoretical basis of the test-negative study design for assessment of influenza vaccine effectiveness. Am J Epidemiol, 2016. 184(5): 345-53.
41. Tang FS, Van Ly D, Spann K, **Reading PC**, Burgess JK, Hartl D, Baines KJ and Oliver BG. Differential neutrophil activation in viral infections: Enhanced TLR-7/8-mediated CXCL8 release in asthma. Respirology, 2016. 21(1): 172-9.
42. Vanderven HA, Ana-Sosa-Batiz F, Jegaskanda S, Rockman S, **Laurie K**, **Barr I**, Chen W, Wines B, Hogarth PM, Lambe T, Gilbert SC, Parsons MS and Kent SJ. What lies beneath: antibody dependent natural killer cell activation by antibodies to internal influenza virus proteins. EBioMedicine, 2016. 8: 277-90.

Presentations

Centre staff members presented talks and posters at numerous events during 2016, including national and international conferences, WHO meetings, educational lectures and research seminars.

ORAL PRESENTATIONS

Event/Institute; Location, date	Speaker, *Title(s)*
Incidence, Severity and Impact of Influenza 2016; Paris, France, 21–22 January	Sheena Sullivan: *Appropriate assessment of seasonal severity - media interpretation of notifications data versus surveillance.*
Influenza Specialist Group Annual Scientific Meeting; Melbourne, 31 January – 1 February	Ian Barr: *An update on the 2015 and 2015-6 influenza seasons.* Aeron Hurt: *Uncomplicated community illness vs hospitalized patients with severe disease.* Aeron Hurt: *Pandemics.*
Lorne Infection and Immunity Conference; Lorne, Victoria, 17–19 February 2016	Patrick Reading: *Sensing and responding to influenza virus infection.* (Invited speaker)
4th Annual National Respiratory Leadership Summit; Melbourne, 27 February	Patrick Reading: *Sensing and responding to respiratory virus infection.*
3rd WHO Meeting on Seasonal Influenza Vaccine Composition/3rd REVELAC-i Network Meeting; Santiago, Chile, 15–17 March	Ian Barr: *Evolution of the influenza virus in the tropics: which vaccine formulation to use?* Ian Barr: *The use of trivalent versus quadrivalent influenza vaccines: rationale, considerations and some modelling.*
Paul-Ehrlich-Insitut; Langen, Germany, 5 April	Aeron Hurt: *Influenza antiviral resistance - use of the ferret model to investigate viral fitness and drug effectiveness.*
University of Siena - ISIRV School of Influenza; Siena, Italy, 11–15 April	Aeron Hurt: *The role of neuraminidase; Mechanism of action and resistance.*
Departmental seminar, School of Public Health and Preventative Medicine, Alfred Centre, Monash University; Melbourne, 19 April	Naomi Komadina: *Understanding the likely population impact of new and improved influenza vaccines.*

ORAL PRESENTATIONS (continued)

Event/Institute; Location, date	Speaker, *Title(s)*
6th Australasian Vaccines & Immunotherapeutics Development Meeting; Brisbane, 20–22 April	Louise Carolan: *Characterisation of the localised immune response in the respiratory tract of ferrets following infection with influenza A and B viruses.* Kok Fei Jimmy Chan: *The structural basis underpinning polyclonal T-cell receptor recognition of an unusually long 13-mer peptide from tumor antigen NY-ESO-1 presented by HLA-B*0702 and its implication in cancer vaccine design.* Heidi Peck: *Evaluation of influenza B viruses for use in cell-based quadrivalent influenza vaccines.*
Hong Kong University; Hong Kong, 16 May	Sheena Sullivan: *Noncollapsibility of the odds ratio.*
Lecture to 3rd year students, University Breadth Subject "Global health, security and sustainability", University of Melbourne; Melbourne, 18 May	Aeron Hurt: *Influenza*.
15th National Immunisation Conference; Brisbane, 7–9 June	Vivian Leung: *Healthcare worker antibody response to influenza vaccination at an Australian centre.* Sheena Sullivan: *Are sentinel surveillance samples sufficiently diverse to inform influenza vaccine strain selection?*
Monitoring Vaccine Effectiveness During Seasonal Influenza in the European Union; Veyrier du Lac, France, 22–24 June	Sheena Sullivan: *Influenza vaccine effectiveness in Australia and New Zealand, 2015; The bias we introduce when we try to control bias.*
Australian Society for Microbiology Annual Scientific Meeting; Perth, 3–6 July	Aeron Hurt: *Influenza virus antivirals and resistance.*
2016 ANZCCART conference; Melbourne, 19–21 July	Aeron Hurt: *Influenza and other viruses in Antarctica - who knew that penguins 'flu?* Ding Yuan Thomas Oh: *A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity.*
10th Bi-Regional Meeting of WHO NIC's from WPRO and SEARO; Bangkok, Thailand, 26–29 July	Aeron Hurt: *Recent advances in influenza laboratory diagnostics.* Aeron Hurt: *What should be considered when establishing influenza viral testing.* Ian Barr: *Influenza activity in the Southern Hemisphere.*
ASM and Doherty Institute Public Health Night; Melbourne, 10 August	Aeron Hurt: *Antiviral resistance in influenza viruses.*
16th International Congress of Immunology; Melbourne, 21–26 August	Kok Fei Jimmy Chan: *An unusually long 13-mer tumor antigenic peptide "molded" around distinct T-cell receptors when presented by HLA-B*0702 and provided the basis for polyclonal T-cell receptor recognition.*

ORAL PRESENTATIONS (continued)	
Event/Institute; Location, date	Speaker, *Title(s)*
Options IX for the Control of Influenza; Chicago IL, USA, 24–28 August	Aeron Hurt: *Detection of antiviral resistance. (Featured speaker)* Sheena Sullivan: *Healthcare worker antibody response to influenza vaccination at an Australian centre* Sheena Sullivan: *Contemporary approaches to estimation of influenza vaccine effectiveness.*
Doherty Virology Symposium; Melbourne, 30 August	Ian Barr: *So what about influenza, can it be eradicated?*
Workshop on Strengthening Influenza Surveillance; New Delhi, India, 4–6 October (*lecture delivered via Skype*)	Patrick Reading: *Using cell culture to isolate influenza viruses – why and how?*
University of Sydney seminar; 19 October 2016	Patrick Reading: *Influenza: supporting regional surveillance networks and insights into innate immunity*.
Influenza-like Illness Surveillance Workshop; Goroka, Papua New Guinea, 24–26 October	Patrick Reading: *Global influenza surveillance network and regional perspectives; Specimen collection and testing in National Influenza Centres and regional laboratories.*
NHMRC "Limiting the Impact of Influenza" Program retreat; Melbourne, 25–26 October	Ian Barr: *The A, B, C and D's of Influenza.* Kok Fei Jimmy Chan: *Development of a ferret model of human Respiratory Syncytial Virus (RSV) infection and disease.*
3rd New Zealand Influenza Symposium; Wellington, New Zealand, 2 November	Aeron Hurt: *Use of antivirals for seasonal and pandemic flu.*
Visit to Institute Of Environmental Science And Research; Wellington, New Zealand, 3 November	Aeron Hurt: *The search for influenza viruses in Antarctica.*
Introduction to Laboratory Quality Management and Good Laboratory Practice workshop; Jakarta, Indonesia, 21 –25 November	Patrick Reading: *Introduction to Good Laboratory Practice.* Patrick Reading: *Laboratory tests for detection and characterisation of influenza viruses.* Patrick Reading: *GLP in a laboratory testing for influenza virus in clinical specimens – some practical guidelines.* Patrick Reading: *Specimen collection, testing and reporting – documentation and quality assurance.*
Victorian Infectious Diseases Service; Melbourne, 28 November	Aeron Hurt: *Debate regarding oseltamivir use for seasonal and pandemic influenza.*
The Challenges and Rewards of Working in Global Health: Doherty Global Health Cross-Cutting Discipline Half-Day Symposium; Melbourne, 14 December	Patrick Reading: *Strengthening laboratory-based surveillance of influenza virus in the Asia/Pacific region.*

POSTER PRESENTATIONS

Event; Location, date: Title and authors *(presentations are posters unless otherwise indicated, Centre authors are marked in bold, presenting author is underlined)*

WHO Consultation on National, Regional and Global Estimates of the Burden of Influenza Disease; Geneva, Switzerland, 12–13 July: Preliminary estimates of seasonal influenza burden, Cambodia 2012-2014. Kheng S, Tek B, Seng H, Machingaidze C, Kab V, Ieng V, Hamid S, Breakwell L, **Leung V**, Ly S and Tsuyuoka R

16th International Congress of Immunology; Melbourne, 21–26 August: The time interval between infections and viral hierarchies are determinants of viral interference following influenza virus infection in a ferret model. **Laurie KL**, **Guarnaccia T**, **Carolan L**, Horman W, Yan A, **Aban M**, Petrie S, Cao P, Heffernan J, McVernon J, Mosse J, **Kelso A**, McCaw J and **Barr I**.

Options IX for the Control of Influenza; ChicagoIL, USA, 24–28 August:

In vitro and in vivo assessment of influenza A and B variants selected under zanamivir pressure. **Oh DY**, **Panozzo J**, **Vitesnik S**, Piedrafita D, Mosse J and **Hurt A**.

Restriction of seasonal influenza A virus replication in macrophages at a late stage in the virus life cycle is circumvented by highly pathogenic strains. Londrigan S, Short K, Ma J, Gillespie L, Rockman S, Brooks A and **Reading P**.

Transcriptional regulation in immune and epithelial cell types during influenza A infection Ma J, Brooks A and **Reading P**.

The differential burden of influenza B viruses. Giele C, Regan A, **Sullivan S**, Levy A, Lang J, Dowse G and David Smith

Are sentinel surveillance samples sufficiently diverse to inform influenza vaccine strain selection. **Sullivan SG**, **Deng YM**, Fielding J, Chilver M, Regan A, Levy A and **Barr IG.**

Sanger and Next Generation full genome sequencing of influenza viruses. **Deng YM**, **Spirason N**, **Iannello P**, **Lau H** and **Barr IG**.

Barwon Health and Deakin University Research Week; Geelong, Victoria, 14–18 November: Methods to screen for coronaviruses in wild birds. Nelson TM, Hodge J, **Hurt A**, Klaasen M and Alexandersen S

Second Regional Forum of WHO Collaborating Centres in the Western Pacific; Manila, Philippines, 28–29 November: WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia. **Barr IG** and **Chow MK**

Engagement in WHO Activities

Event; Location, Date	Centre staff involved
WHO Consultation on the Composition of Influenza Vaccines for the northern hemisphere 2016-2017; Geneva, Switzerland, 22–24 February	Ian Barr and Aeron Hurt participated. Sheena Sullivan presented the Global Influenza Vaccine Effectiveness report by teleconference.
3rd WHO Meeting on Seasonal Influenza Vaccine Composition/3rd REVELAC-i Network Meeting; Santiago, Chile, 15–17 March	Ian Barr presented a talk.
Launch of the Tool for Influenza Pandemic Risk Assessment (TIPRA); Geneva, Switzerland, 4–5 May	Ian Barr participated.
Visit to the General Department of Preventive Medicine, Ministry of Health; Hanoi, Vietnam, 16 May	Sheena Sullivan met with officials and WHO representatives to discuss influenza data submission to FluID.
WHO Technical Meeting on Piloting RSV Surveillance based on GISRS; Geneva, Switzerland, 28–30 June	Patrick Reading participated.
WHO Modelling Workshop; Princeton NJ, USA, 6-8 July	Ian Barr participated.
WHO Consultation on National, Regional and Global Estimates of the Burden of Influenza Disease; Geneva, Switzerland, 12–13 July	Vivian Leung participated.
22nd meeting between WHO Essential Regulatory Laboratories, Influenza Collaborating Centres and influenza vaccine manufacturers; London, UK, 19–20 July	Ian Barr participated.
Influenza vaccine response during the start of a pandemic, informal consultation; Geneva, Switzerland, 21–26 July	Ian Barr participated.
10th Bi-Regional Meeting of WHO NICs from WPRO and SEARO; Bangkok, Thailand, 26–29 July	Ian Barr was a meeting co-chair and presented a talk. Aeron Hurt and Patrick Reading presented talks. Vivian Leung and Yi-Mo Deng attended.
WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2017; Geneva, Switzerland, 26–29 September	Ian Barr, Aeron Hurt and Sheena Sullivan attended.
GISRS, WHO headquarters Geneva, Switzerland, 18–28 October	Aeron Hurt filled in as team leader.
Second Regional Forum of WHO Collaborating Centres in the Western Pacific; Manila, Philippines, 28–29 November	Ian Barr attended and presented a poster.
WHO Consultation on updating the WHO research agenda for influenza; Geneva, Switzerland, 5–9 December	Aeron Hurt and Ian Barr participated.

Delegates at the 10th Bi-Regional Meeting of WHO NICs from WPRO and SEARO.

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
4th Consortium for Influenza Sero-Epidemiology (CONSISE) expert meeting; Paris, France, 20 January	Sheena Sullivan attended.
National Institute for Biological Standards and Control's (NIBSC) 21st Meeting between WHO ERLs, CCs and influenza vaccine manufacturers; London, UK, 26–27 January	Ian Barr attended.
6th Australasian Vaccines & Immunotherapeutics Development Meeting; Brisbane, 20–22 April	Ian Barr chaired a session.
16th International Congress of Immunology; Melbourne, 21–26 August	Ian Barr was a member of the Organising Committee member. Kok Fei Jimmy Chan received an ASI Travel Bursary to attend. Louise Carolan attended.
Options IX for the Control of Influenza; Chicago IL, USA, 24–28 August	Aeron Hurt was a featured speaker, a member of a workgroup committee and co-moderated a symposium session. Patrick Reading co-moderated a symposium session. Sheena Sullivan chaired an oral abstract session. Naomi Komadina attended.
National Avian Influenza Wild Bird Surveillance program - Annual Meeting; Melbourne, 14–15 September	Aeron Hurt attended.
Influenza–Repeat Vaccination Symposium (I-ReV); Vancouver, Canada, 13–14 October	Sheena Sullivan was part of the organizing committee, moderated two sessions, and took notes for one session.
VIIN Young Investigator Symposium 2016; Melbourne, 14 October	Leonard Izzard attended.
NHMRC "Limiting the Impact of Influenza" Program retreat; Melbourne, 25–26 October 2016	Naomi Komadina attended.
Ask Industry Anything forum; Melbourne, 29 November	Leonard Izzard attended.
One Health EcoHealth 2016 Congress; Melbourne, 3-7 December	Michelle Wille attended.

Delegates at the Influenza–Repeat Vaccination Symposium (I-ReV)

Committees and Advisory Groups

Centre staff members served on the following governing boards, committees and advisory groups during 2016.

Chantal Baas Doherty Institute, *Shared PC3 Laboratory Advisory Committee (until Sept 2016)*

Ian Barr 16th International Congress of Immunology, Melbourne 2016, *Organising Committee*
Australasian Vaccine & Immunotherapeutics Development (AVID) Group, Organising Committee
Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Doherty Institute, *Shared PC3 Laboratory Advisory Committee, Operational Management Committee*
Influenza Research and Treatment, *Editorial Board*
Influenza and Other Respiratory Viruses, *Associate Editor*
National Influenza Surveillance Committee (Department of Health) *(until Nov 2016)*
Public Health Laboratory Network (Department of Health)
WHO/OIE/FAO H5N1 Evolution Working Group *(until Nov 2016)*

Michelle Chow Doherty Institute, *Communications Working Group*

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

Chris Durrant Victorian Infectious Diseases Reference Laboratory, *Safety Committee*

Aeron Hurt Antiviral Research, *Editorial Board*
Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Avian Influenza in Wild Birds, Australian Wildlife Health Network, *Steering Committee*
Frontiers in Microbiology, *Associate Editor*
Group of the International Society for Influenza and other Respiratory Virus Diseases, *Committee member*
Infection, Ecology and Epidemiology – The One Health Journal, *Editorial Advisory Board*
Influenza Specialist Group, *Scientific Committee*
Neuraminidase Inhibitor Susceptibility Network Meeting/Committee of Antiviral Special Interest
Virology Journal, *Associate Editor*
WHO Working Group for influenza antiviral resistance, *Committee member*
Options for the Control of Influenza IX Conference, Chicago IL, USA, *Virology and pathogenesis scientific committee*

Matthew Kaye Doherty Institute, *Shared PC3 Laboratory Advisory Committee (from Sept 2016)*
Victorian Infectious Diseases Reference Laboratory, *Chemical Safety Officer*
Victorian Infectious Diseases Reference Laboratory, *Safety Committee*

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

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

Karen Laurie BMC Infectious Diseases, *Associate Editor*
Consortium for the Standardization of Influenza Seroepidemiology (CONSISE), *Steering Committee*
Doherty Institute, *Bioresources Facility Executive Committee; Operational Health and Safety and Environment Advisory Committee*
Global Influenza Seroepidemiology Standardisation Working Group

Ding Yuan Thomas Oh Frontiers in Microbiology, *Review Editor*
Doherty Institute, *Communications Working Group (from Nov 2016)*

Patrick Reading Australian Respiratory Virus Meeting, *Organising Committee*
Doherty Institute, *Discipline leader, Education and Professional Development*
Influenza and Other Respiratory Viruses, *Editorial Board*

Kanta Subbarao National Influenza Surveillance Committee (Department of Health) *(from Nov 2016)*

Sheena Sullivan Doherty Institute, *Equity and Diversity Committee*
National Influenza Surveillance Committee (Department of Health), *Proxy*

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 for The Age article "Now is the time to be washing your hands of the flu threat", published on 21 April. http://www.smh.com.au/national/health/now-is-the-time-to-be-washing-your-hands-of-the-flu-threat-20160421-gobrgy.html
- Participated in an interview for the The Age article "Flu cases on the rise in Victoria with season to peak later this month" published 18 August. http://www.theage.com.au/victoria/flu-cases-on-the-rise-in-victoria-with-season-to-peak-later-this-month-20160818-gqvexq.html
- Participated in an interview for the The Age article "Victoria 'dodged a bullet' this flu season", published 11 September.
  http://www.theage.com.au/victoria/victoria-dodged-a-bullet-this-flu-season-20160910-grdm7m.html

Aeron Hurt:

- Participated in an interview on Drive with Louise Saunders, 936 ABC Hobart, to talk about avian influenza viruses in Antarctic penguins, on 20 September
- Participated in an interview with Australian Associated Press, on avian influenza in Antarctic penguins, with the story appearing in several media outlets on 20 September.
  https://cosmosmagazine.com/biology/antarctic-penguins-catch-new-strain-of-bird-flu;
  http://www.news.com.au/national/breaking-news/more-bird-flu-found-in-antarctic-penguins/news-story/efcc21a65f607bc490772b6649a75847
  http://www.bbc.com/news/science-environment-37440164
  http://www.seeker.com/new-type-of-bird-flu-found-in-antarctic-penguins-2012653362.html

Leonard Izzard

- Participated as industry panel member in the "Science Career Conversations - Discover where studying biology can take you…" careers event run by the Faculty of Science at the University of Melbourne, on 6 October.

Website

The Centre website was maintained and updated throughout the year. During 2016, the website was viewed by 9,814 unique visitors from 142 different countries. The majority of visits to the website came from Australia, followed by the USA.

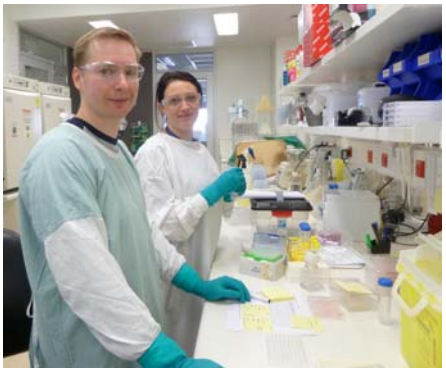
Visitors to the Centre

The Centre was pleased to host the following visitors during 2016:

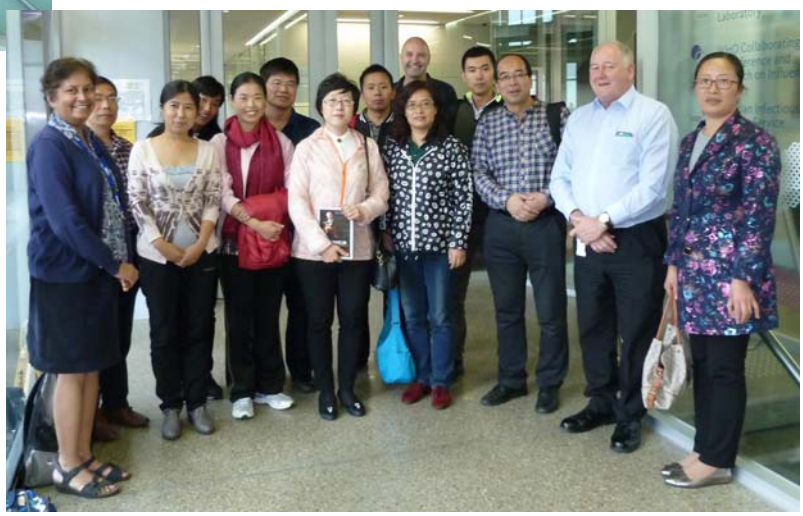
Date	Visitor and affiliation
2 February	Dr Mark Miller, Director, Division of International Epidemiology and Population Studies, Fogarty International Center, National Institutes of Health, Bethesda MD, USA
19 February	Prof Larisa Rudenko, Head, Virology Department, Institute of Experimental Medicine, St Petersburg, Russia
5 April	Dr Yazid Abdad, Institute of Medical Research, Papua New Guinea, Goroka, Papua New Guinea
18 July	Master of Veterinary Public Health (Emergency Animal Diseases) students, accompanied by Dr Simon Firestone, The University of Melbourne, Melbourne
18 August	Prof Jen-Ren Wang, National Cheng Kung University, Tainan, Taiwan
24 August	Dr Diana Roen and Dr Tameem Ansari, CTL Analyzers, Shaker Heights OH, USA
24 August	Dr Kristian Waalen, Department of Influenza, Norwegian Institute of Public Health, Oslo, Norway
6 September	Dr TuckWeng Kok, Chief Virologist, University of Adelaide, Adelaide
8 September	Dr Charles Guest, Victorian Chief Medical Officer, Victorian Government Department of Health and Human Services, Melbourne, Victoria
September 2016 to March 2017	Dr Daniil Korenkov, Institute of Experimental Medicine, St Petersburg, Russia, *Research collaborator*
21 October	Prof Larisa Rudenko, Head, Virology Department, Institute of Experimental Medicine, St Petersburg, Russia
24–25 October	Ms Monique Chilver, Project Manager, ASPREN, The University of Adelaide, Adelaide
15 November	Prof Edward Holmes, Australia Fellow, Sydney Institute of Emerging Infectious Diseases and Biosecurity, Sydney Medical School, The University of Sydney, Sydney
1 December	Delegation of veterinarians and research scientists from Shandong province, China:
1–2 December	Dr Josef Järhult, Uppsala University Hospital, Uppsala, Sweden

L to R: Ian Barr, Aeron Hurt, Jen-Ren Wang, Sheena Sullivan, Yi-Mo Deng

Yi-Mo Deng (L) and TuckWeng Kok (R)

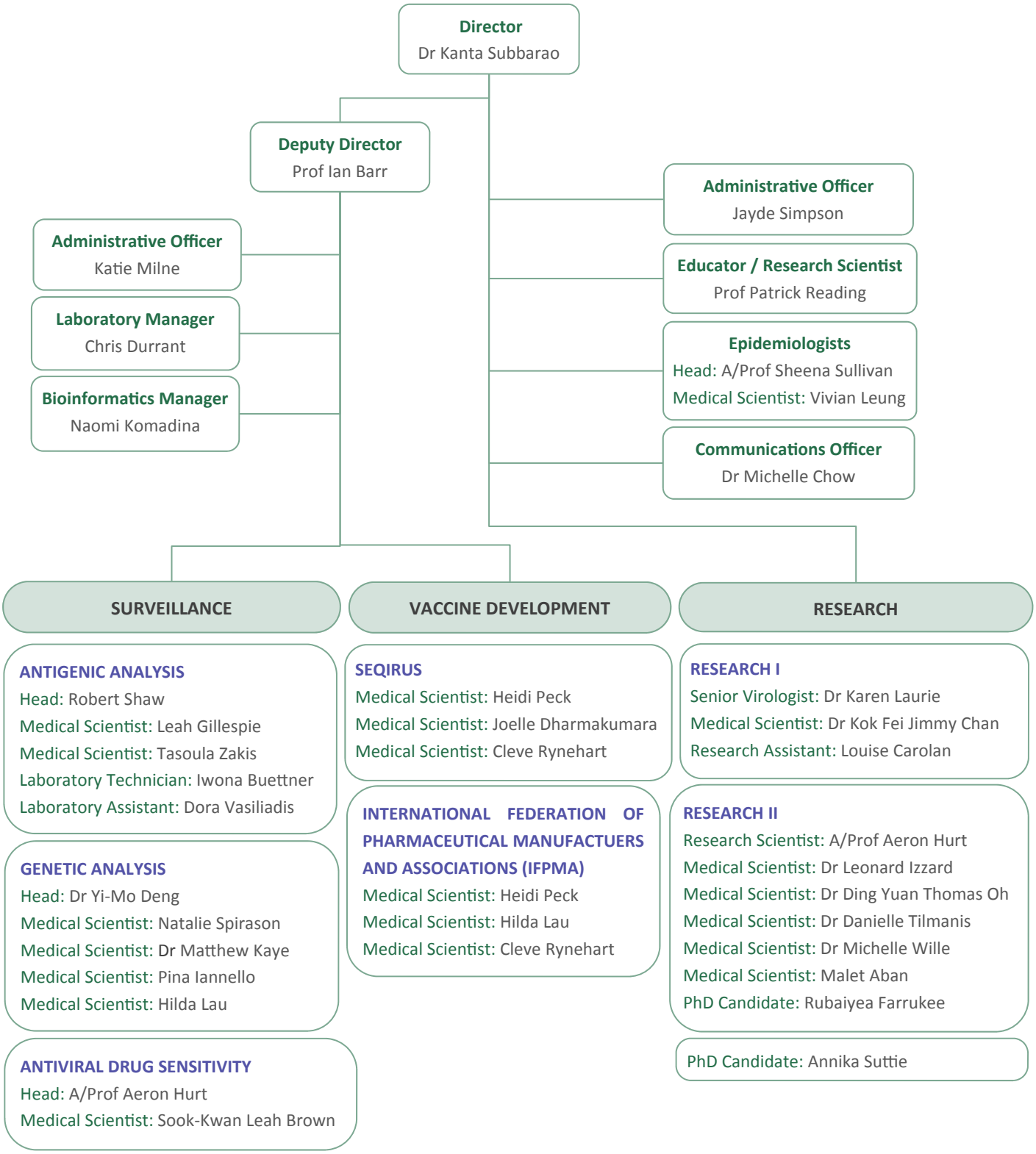


Daniil Korenkov (front) and Louise Carolan (back)



Delegation from Shandong Province with Kanta Subbarao, Ian Barr and Aeron Hurt

MANAGEMENT AND STAFF



Staff Changes 2016

Ms Leah Gillespie joined the Centre in January in the Antigenic Analysis group.
Dr Kok Fei Jimmy Chan was appointed as a post-doctoral researcher in March.
Dr Leonard Izzard joined the Centre as a post-doctoral researcher in August.
Dr Matthew Kaye joined the Centre in the Genetic Analysis group in September.
Dr Michelle Wille was appointed as a post-doctoral researcher in November.
Professor Kanta Subbarao commenced as Director of the Centre in November.
Mr Cleve Rynehart joined the Centre in December in the Seqirus and IFPMA projects, to replace Ms Chantal Baas who left the Centre in September.