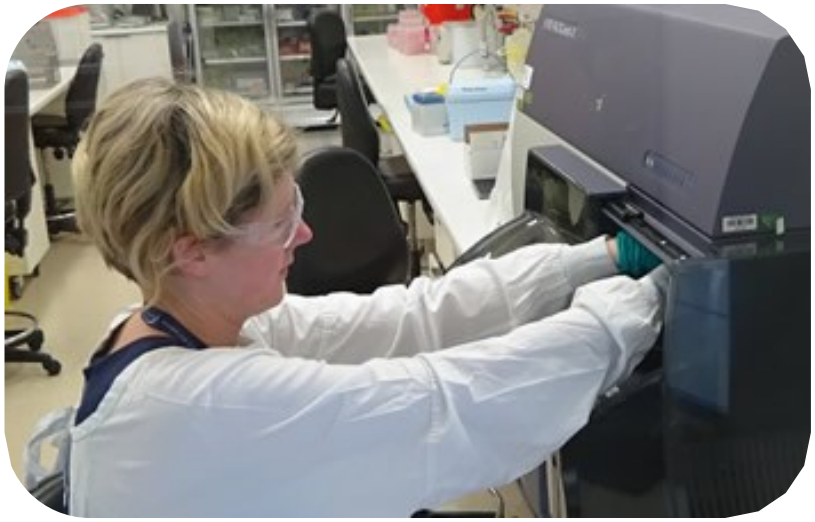

2015
Annual Report

WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL

Doherty Institute
A joint venture between the University of Melbourne and the Royal Melbourne Hospital

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 2015

Surveillance

The Centre received 5573 samples during 2015, the highest number since the pandemic in 2009. Overall, influenza B viruses predominated over each of the influenza A subtypes, making up 51.8% of all samples tested. Influenza B/Victoria and B/Yamagata lineage viruses were received in similar proportions overall, however the relative predominance of the two lineages changed in the later part of the year.

Technology

Following the acquisition of an IonTorrent PGM™ system in 2014, Next Generation Sequencing (NGS) techniques have been integrated into the Centre's repertoire of routine analytical techniques. This has facilitated a dramatic increase in the number of viruses sequenced and has enhanced our surveillance efforts, especially in the context of ongoing challenges in the serological analysis of A(H3N2) viruses.

Research

The Centre continued to expand and develop its research interests during 2015, including continuing investigation of the phenomenon of viral interference, the evolution of influenza viruses over time and geography, development of animal models for antiviral treatments and the antibody response to vaccination in health care workers.

Publications

Centre staff were authors on a total of 30 original research papers, reviews and reports, including a paper in *Nature*. Several other papers were published in high impact journals, including PloS Pathogens, Nature Communications and eLife.

Centre staff retreat, March 2015

Director's report

The Centre experienced a year of change in 2015. After more than 8 years as Director of the Centre Anne Kelso left in April to take on a new challenge as CEO of the NHMRC, Australia's main funding body for medical research. Anne was the second Director of the Centre following Ian Gust who was the inaugural Director in 1992, and we wish her well in her new position. Since April, I have been the Acting Director of the Centre and am very grateful for the opportunity to represent the Centre in this role during this period.

At the end of 2015 we have been residing at the Doherty Institute for nearly two years. Like any move it has taken us time to grow into our new home but we now feel part of the Institute and have worked out most of the intricacies of this very complex building. During 2015 the Centre has become more integrated into the Doherty enterprise, which has given us the opportunity to further our collaborations with groups working on influenza within the building and also engage with other groups that are interested in technical advancements such as Next Generation Sequencing (NGS). The Centre and the Doherty Institute are well placed to respond to any further global threats including another influenza pandemic.

During 2015 the Centre received a large number of influenza samples (over 5000) from Australia and from 13 other countries and territories. Interestingly Australia and New Zealand experienced a predominantly influenza B season, with the highest proportion of seasonal influenza viruses analysed being of influenza B type. This is a relatively unusual event which historically has only occurred approximately once every ten years. What was of further interest was the similarity of the 2015 season to the previous B-dominant year in 2008 where in both years there was a switch in the relative predominance of the two influenza B lineages mid-season. Early in both years, B/Yamagata-lineage viruses predominated but by mid-season B/Victoria-lineage viruses had significantly increased and were by far the predominant lineage by the end of the season. The reasons for these dynamic changes are not well understood but probably reflect subtle changes in the virus evolution and the susceptibility of the population to the different B-lineages. During 2015 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 2015 and Northern Hemisphere 2015-6 seasons. The Centre also continued to monitor potential pandemic influenza viruses and seeks to obtain new viruses as they were detected (such as A(H5NX) and A(H7N9) viruses), to check reagents and prepare virus and RNA stocks.

This year also saw the introduction of NGS into our laboratory to enhance the throughput of virus gene sequencing and to increase our capacity for full genome sequencing. The IonTorrent PGM™ platform that we purchased in 2014 performed extremely well and we have had multiple runs with 96 samples being sequenced simultaneously. The main issue then is in handling the extremely large amounts of data that are produced by these instruments. Fortunately we were assisted by a group of bioinformatics experts at Duke-NUS Medical School in Singapore led by Dr October Sessions and Uma Sangumathi Kamaraj who constructd a pipeline for us to rapidly process our data. This led to us sequencing many hundreds of A(H3N2) viruses to support our antigenic analysis and to also allow clade-specific vaccine effectiveness (VE) values to be determined by our epidemiology group based on data from the three sentinel surveillance systems in place in Australia.

Publications during 2015 were also a highlight for the Centre with articles in several high impact journals such as Nature, Nature Communications, eLife, PLoS Pathogens, Journal of Infectious Diseases and Journal of Virology. A total of 30 publications were achieved by Centre staff and collaborators during the year, an excellent outcome for a small group. We also had the pleasure of welcoming a number of visitors to the Centre including overseas scientists, trainees, students and lots of people interested in the Centre's activities. The Centre hosted the 11th Australian Influenza Symposium on 12–13 October at Deakin University's Waterfront Campus at Geelong. The meeting was co-organised with Sue Lowther from the Australian Animal Health Laboratory (AAHL, CSIRO), and financially supported by AAHL, the Therapeutic Goods Administration (Canberra) and the Commonwealth Department of Health. Almost 200 delegates registered and attended the event which covered a wide range of areas in both human and animal influenza.

As always we are grateful to Dr Mike Catton, Director of VIDRL, and other members of VIDRL staff, especially Dr Bill Maskill, Geoff Leek, Julio Vallencia, Jane Brewster, Anna Ayres and Dallas Wilson, for their support of the Centre's work at every level during 2015 and to our host organisation, the Royal Melbourne Hospital. The continuing support and counsel of the Office of Health Protection in the Australian Government Department of Health are also deeply appreciated. Finally, I would like to express my sincere thanks to the staff of the Centre for their sustained efforts in making the Centre a premier site for surveillance and research on the ever changing influenza virus family. Your dedication and professionalism continue to make a significant contribution to the global response in combating human and animal influenza.

Dr Ian Barr
Acting Director

Surveillance

Introduction

The WHO Collaborating Centre at VIDRL in Melbourne is one of five Collaborating Centres in the world that conduct human influenza surveillance for WHO by analysing samples submitted by WHO National Influenza Centres and other laboratories. 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, 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 originally of avian origin, with various combinations of 16 antigenically different HA variants and 9 NA variants. Although influenza B viruses are not classified into subtypes, there are two co-circulating lineages, B/Victoria and B/Yamagata. Currently there are three families of influenza viruses circulating in the human population — influenza A(H1N1), influenza A(H3N2) and influenza B. Since the emergence of the pandemic A(H1N1) strain in 2009 [A(H1N1)pdm09], circulation of the former seasonal A(H1N1) virus has ceased.

Receipt of Influenza Viruses

During 2015 the Centre received 5573 clinical specimens and/or virus isolates from 34 laboratories in 14 countries (Figures 1 and 2, Table 1). A total of 859 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 4985 samples (89%) 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, 9.3% were identified as A(H1N1)pdm09, 30.8% were A(H3N2) viruses, 19.4% were B/Victoria and 21.7% were B/Yamagata viruses (Table 3). The relative proportions of circulating influenza B viruses changed during the later part of the year, with predominance shifting from B/Yamagata to B/Victoria lineage viruses from August onwards (Figure 4).

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 direct isolation into eggs as 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, 2010-2015.

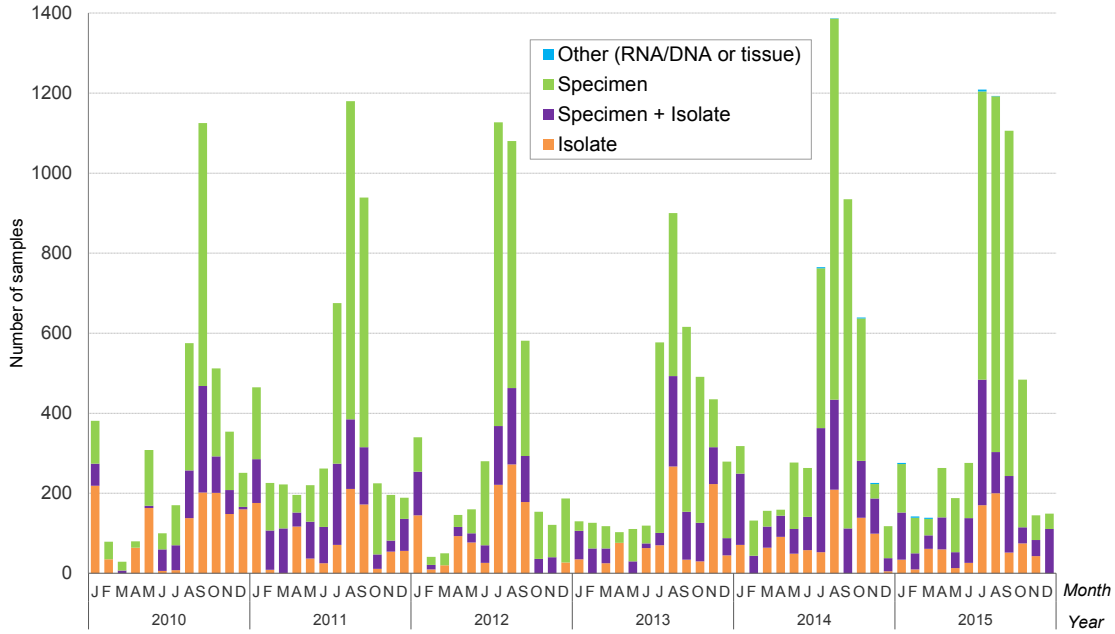


Table 1. Samples received by the Centre in 2015 by country.

Country	Samples received				Samples tested
	Specimens	Isolates	Specimen + Isolate	Other (eg. RNA/ DNA/tissue)	
AUSTRALASIA					
Australia	3177	382	865	12	87%
New Zealand	104	34	173	0	100%
SOUTH PACIFIC					
Fiji	73	0	0	0	100%
New Caledonia	54	0	0	0	100%
Papua New Guinea	7	0	34	0	100%
SOUTH EAST ASIA					
Cambodia	57	38	0	0	100%
Malaysia	0	59	0	0	100%
Philippines	17	0	16	0	100%
Singapore	0	9	221	0	100%
Thailand	7	13	0	0	100%
Vietnam	20	0	0	0	100%
EAST ASIA					
Macau SAR	0	50	30	0	100%
SOUTH ASIA					
Sri Lanka	90	0	0	3	100%
AFRICA					
South Africa	2	0	26	0	96%
TOTAL	**3608**	**585**	**1365**	**15**	**89%**

Figure 2. Geographic spread of influenza laboratories sending viruses to the Centre during 2015.

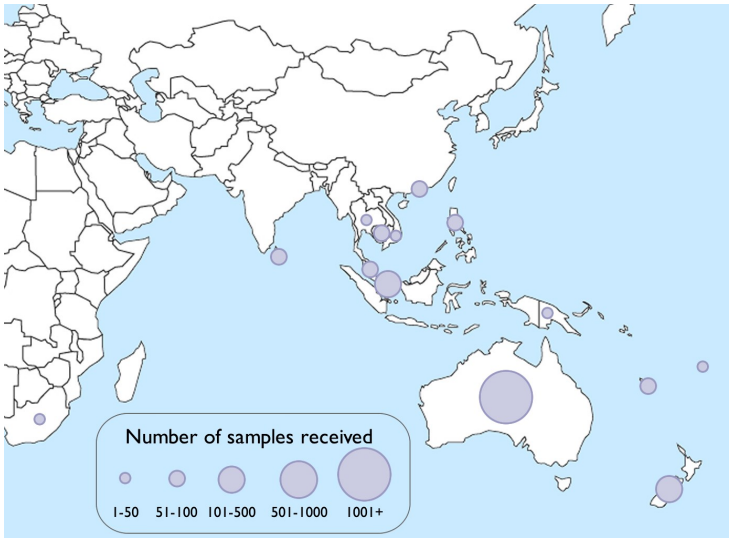


Table 2. Samples received from general practitioner based surveillance systems in Australia, 2015.

	No. samples received	No, samples isolated	Samples analysed by HI Assay
Australian Sentinel Practices Research Network *(ASPREN)*	409	152	106
Victorian Sentinel Practices Influenza Network *(VicSPIN)*	286	76	61
Sentinel Practices Network of Western Australia *(SPN(WA))*	164	135	106

Table 3. Samples tested by HI and/or RT-PCR assay at the Centre in 2015, by country.

Country	Samples tested by HI and/or RT-PCR assay								
	A(H1N1) pdm09	A(H3N2)	A (unsub-typed)	Mixed	B/ Victoria	B/ Yamagata	B lineage undetermined	C	Un-typed
AUSTRALASIA									
Australia	253	1133	306	5	859	788	460	31	17
New Zealand	8	80	1	0	83	131	8	0	0
SOUTH PACIFIC									
Fiji	17	16	1	2	0	9	4	0	24
New Caledonia	1	2	0	0	12	38	1	0	0
Papua New Guinea	1	18	1	0	0	6	15	0	0
SOUTH EAST ASIA									
Cambodia	15	75	0	0	0	5	0	0	0
Malaysia	16	15	3	0	0	13	12	0	0
Philippines	10	10	0	1	0	9	3	0	0
Singapore	62	100	0	0	9	56	0	0	0
Thailand	5	7	0	0	3	3	2	0	0
Vietnam	7	0	0	0	1	4	8	0	0
EAST ASIA									
Macau SAR	15	47	0	0	0	18	0	0	0
SOUTH ASIA									
Sri Lanka	42	25	7	0	0	2	17	0	0
AFRICA									
South Africa	13	8	2	0	0	0	4	0	0
TOTAL	**465**	**1536**	**321**	**8**	**967**	**1082**	**534**	**31**	**41**

Figure 3. Age distribution of subjects from whom samples were received at the Centre in 2015 and the age is known.

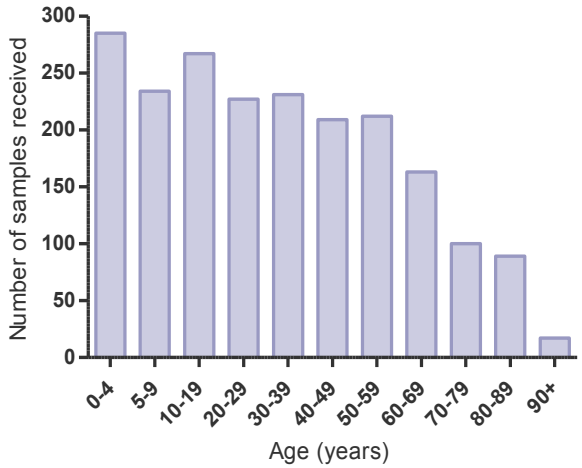
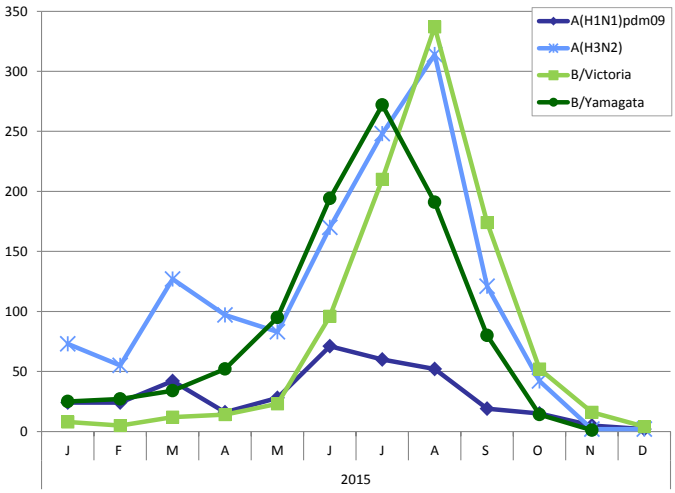


Figure 4. Sample date for viruses collected and received during 2015 with type and subtype or lineage confirmed by HI and/or RT-PCR assay.



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 an in some cases are confirmed by genetic analysis of NA.

Antigenic analyses 2015

Since the acquisition of a Tecan EVO 200 liquid handling robot by the Centre during 2014, an increasing number of HI assays have been automated, with increased efficiency and reproducibility. A total of 4928 isolates that were received at the Centre in 2015 were cultured and isolated in MDCK cells, of which 3613 (73.3%) produced a positive result. Whilst the largest proportion of viruses were A(H3N2) (38.6%), the overall proportion of influenza B viruses predominated over both influenza A subtypes (Figure 5). The relative proportions of different subtypes and lineages of samples received varied between different world regions (Figure 6).

Figure 5. Influenza sub/types and lineages of samples received in 2015 and analysed by HI assay.

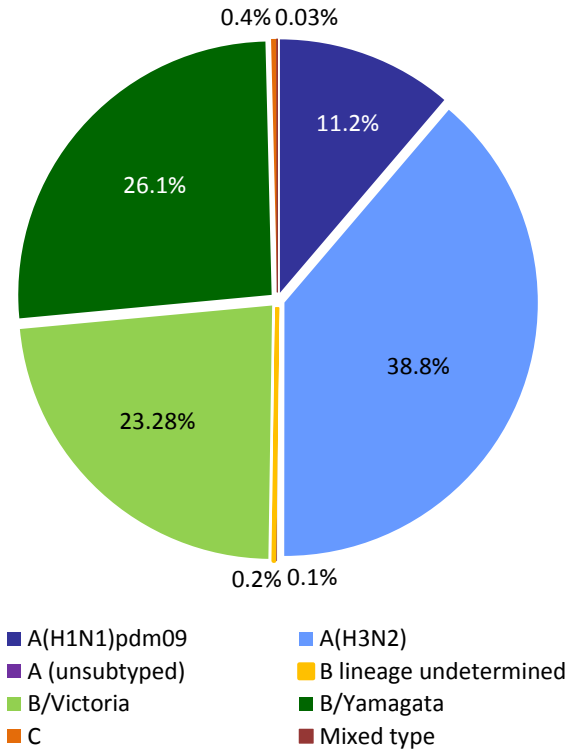
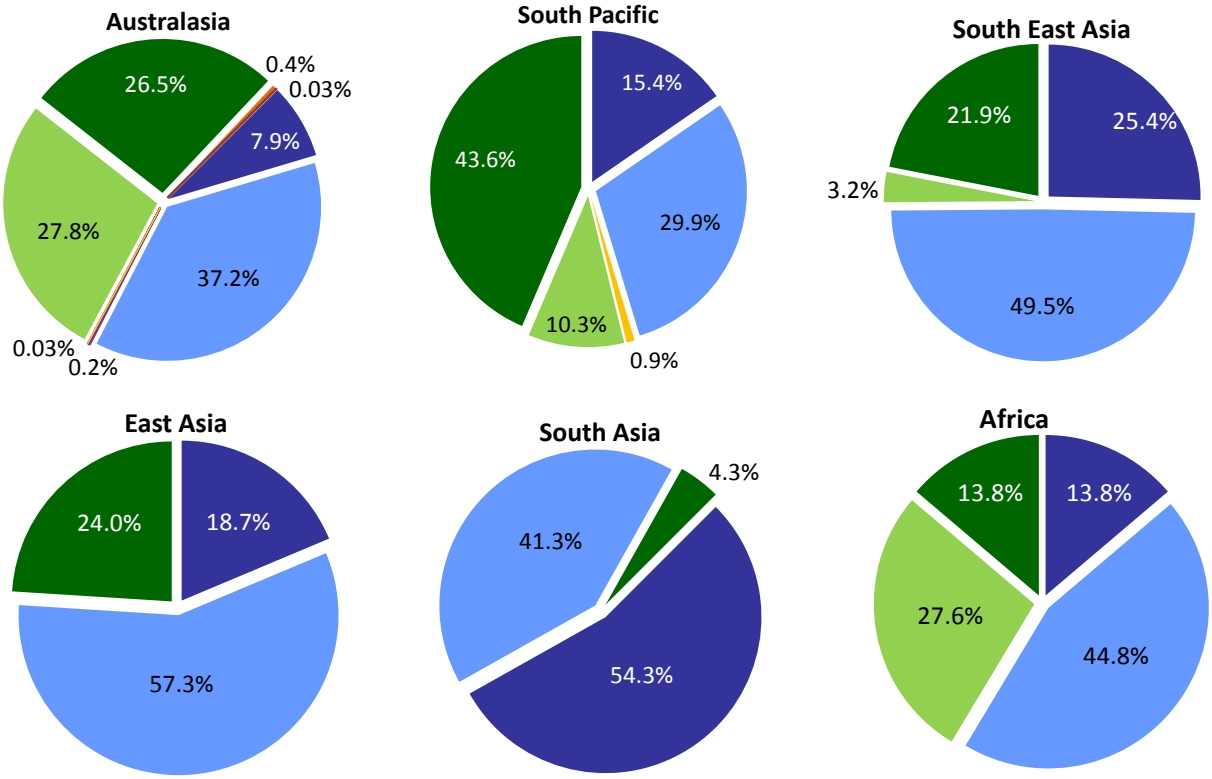


Figure 6. Influenza sub/types and lineages of isolates received from different world regions during 2015 as determined by antigenic analysis.



Genetic Analysis of Influenza Viruses

Background

A subset of all influenza viruses analysed at the Centre undergoes genetic analysis by sequencing of viral RNA 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 isolation. Sequence data are used to compare viruses from different parts of the world and help to inform the selection of vaccine strains.

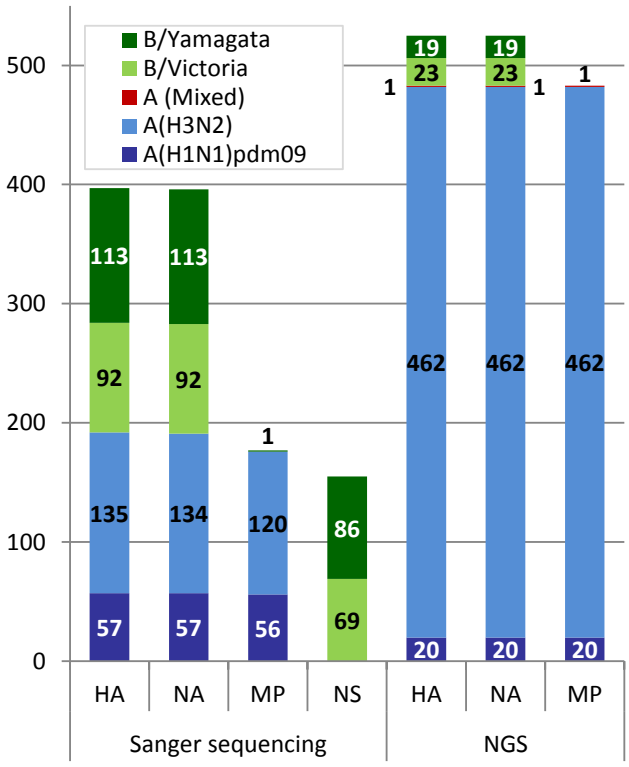
The acquisition of an IonTorrent PGM™ system in 2014 has also enabled next generation sequencing (NGS) techniques to be employed at the Centre for efficient and cost-effective sequencing of whole genomes of viruses, and/or selected influenza virus genes without prior knowledge of type or subtype.

Sequencing 2015

In 2015, 397 HA, 396 NA, 177 MP and 155 NS genes from 400 human viruses received at the Centre were analysed by Sanger sequencing (Figure 7). In addition, the HA, NA and MP genes of 483 influenza A and 43 influenza B (HA and NA only) viruses were sequenced by NGS techniques (Figures 7 and 8).

Full genome sequencing was performed on 49 viruses using traditional Sanger sequencing (Figure 9), while the full genomes of a further 31 influenza B viruses were analysed by NGS techniques (Figure 10). 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 7. Sanger and NGS sequence analysis of samples received at the Centre in 2015.



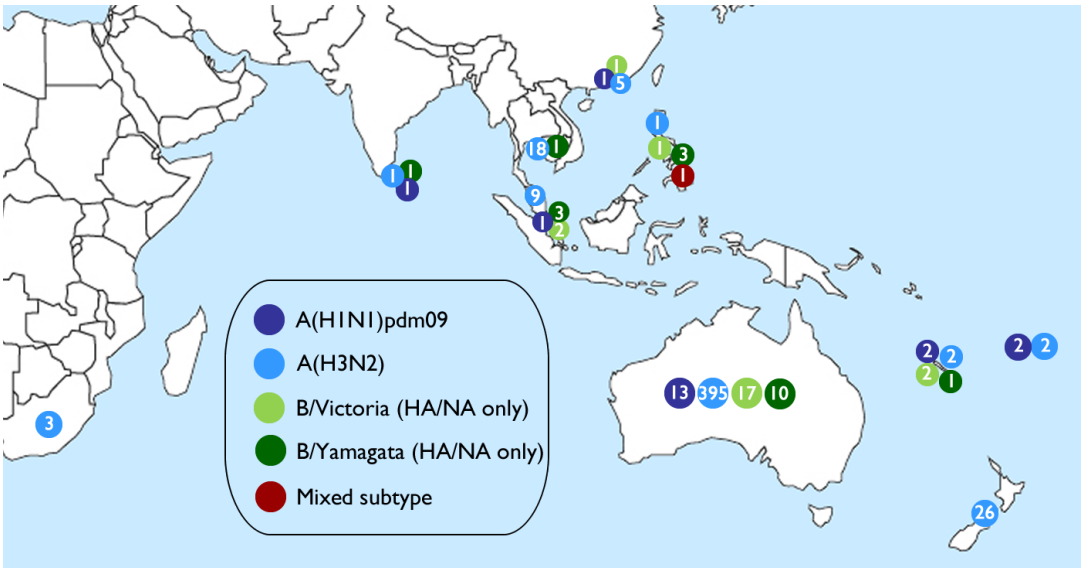


Figure 8. Geographic spread of submitting laboratories and numbers of viruses with HA, NA and MP genes sequenced using NGS at the Centre in 2015.

Figure 9. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing using Sanger sequencing at the Centre in 2015.

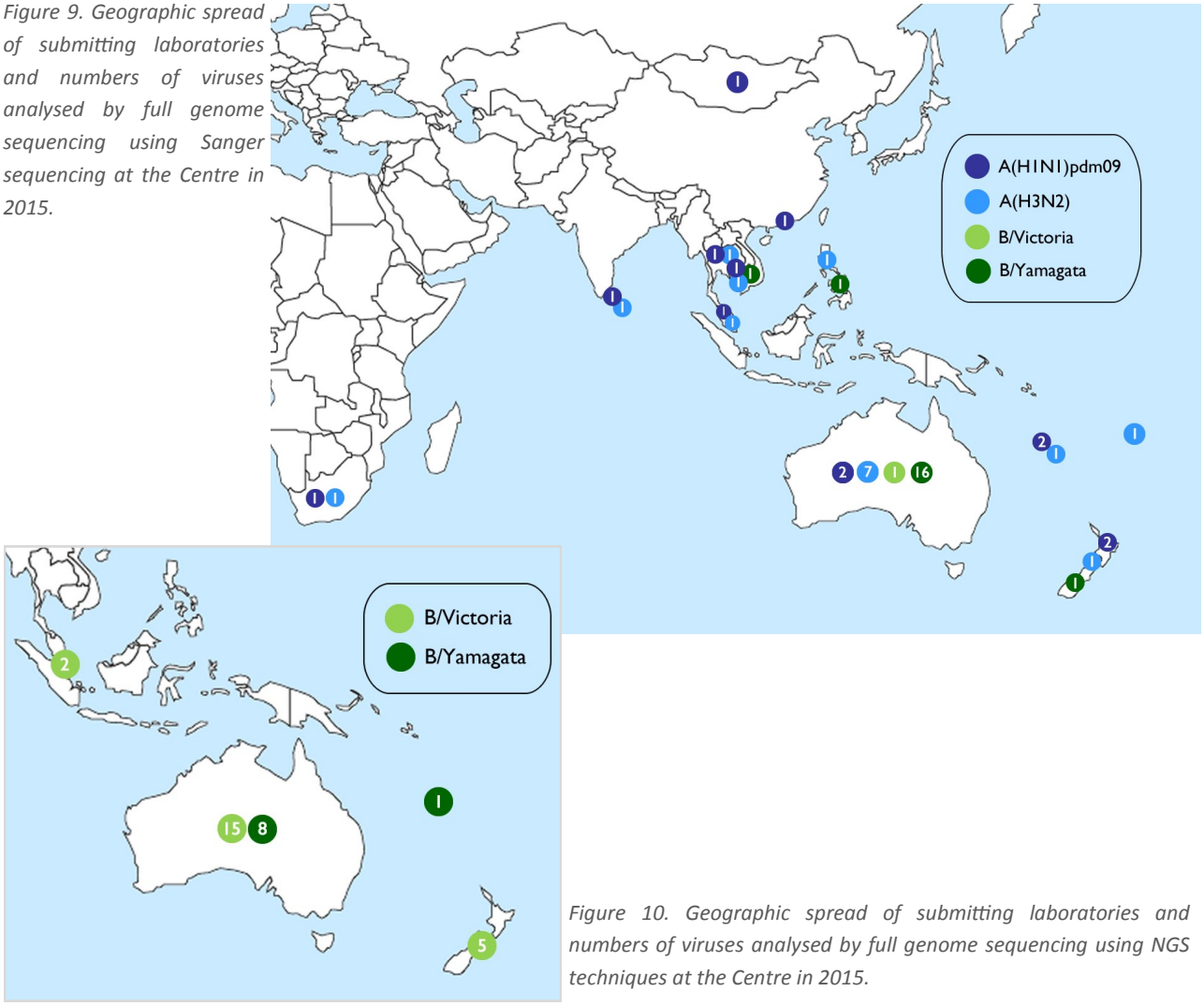


Figure 10. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing using NGS techniques at the Centre in 2015.

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 2015

A total of 2767 gene sequences from 960 viruses were deposited with GISAID in 2015 (Table 4). The largest number of these sequences were of HA and NA genes, followed by MP and NS genes. Full genomes of 13 influenza viruses were also represented in the Centre's submissions (data not shown).

Table 4. Genetic sequences submitted to GISAID by the Centre in 2015.*

Type/ Subtype/ Lineage / Gene	A(H1N1)pdm09	A(H3N2)	B/Victoria	B/Yamagata	A(H1N1) seasonal	A(H1N2)	Total
HA	96	656	93	108	4	0	**957**
NA	84	650	93	108	4	0	**939**
MP	72	563	1	3	4	2	**645**
PB2	2	4	1	3	4	2	**16**
PB1	2	4	1	3	4	2	**16**
PA	2	4	1	3	4	2	**16**
NP	2	4	1	3	4	2	**16**
NS	2	4	62	88	4	2	**162**
Total	**262**	**1889**	**253**	**319**	**32**	**12**	**2767**

** Counts include all sequences submitted to GISAID during 2015, which includes viruses received in previous years and viruses sequenced for research purposes.*

Surveillance Results by Influenza Subtype

Viruses were analysed by comparison with reference viruses recommended by WHO for the 2015 Southern Hemisphere and 2015-2016 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

Influenza A(H1N1)pdm09

Antigenic analysis

A total of 401 A(H1N1)pdm09 isolates were available for analysis by HI assay in 2015. The majority (98.5%) of these viruses displayed similar antigenic properties to the vaccine reference strain A/California/7/2009 (Figure 11, Table 5).

Haemagglutinin gene sequencing

Sequencing was performed on a total of HA genes from 77 viruses. Phylogenetic analysis showed that circulating A(H1N1)pdm09 viruses sent to the Centre during 2015 contained some genetic changes compared to the vaccine reference strain A/California/7/2009, with the emergence of two distinct subclades (Figure 12). However, these changes did not affect the antigenic behaviour of the viruses .

Figure 11. 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.

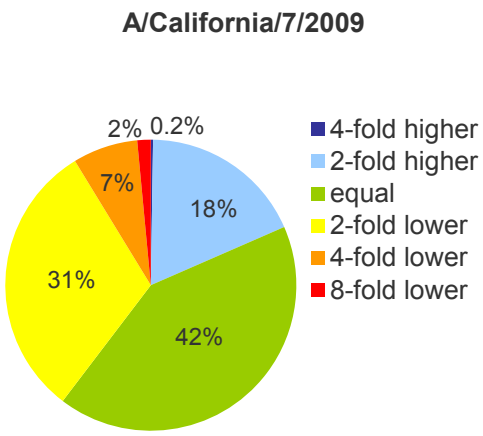
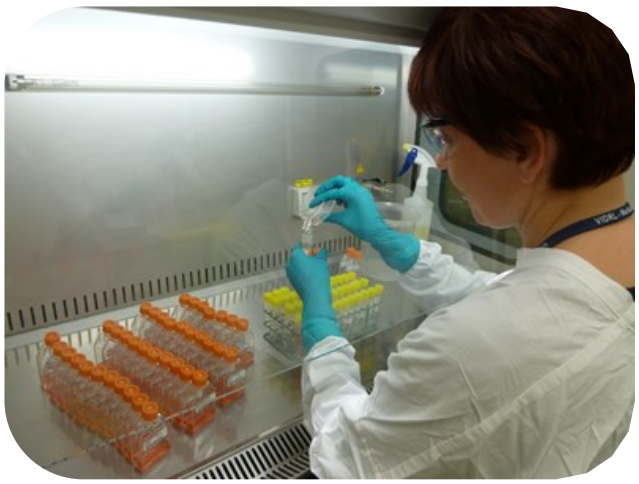


Table 5. Antigenic characterisation of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/California/7/2009 reference virus.

	A(H1N1)pdm09 reference strain: A/California/7/2009	
Region	**Like**	**Low reactor (%)**
Australasia	227	4 (1.7%)
Pacific	18	0
South East Asia	102	1 (1.0%)
East Asia	14	0
South Asia	25	0
Africa	9	1 (10%)
Total	**395**	**6 (1.5%)**



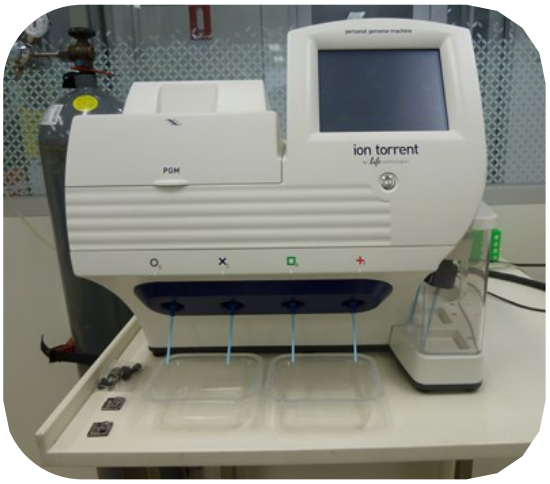


Figure 12. Phylogenetic tree of representative HA genes of A(H1N1)pdm09 viruses received by the Centre during 2015.

Legend
2016 SOUTHERN HEMISPHERE VACCINE STRAIN
Reference virus
e: egg isolate
Scale bar represents 0.2% nucleotide sequence difference between viruses
} Brackets indicate clades

Influenza A(H3N2)

Antigenic analysis

Of 647 A(H3N2) subtype isolates that were available for analysis by HI assay, very few low reactors to the cell-grown reference strain A/Switzerland/9715293/2013 were detected (Figure 13, Table 6). In recent years evolutionary changes in A(H3N2) viruses have made it difficult to detect antigenic change using conventional HI assays. To avoid binding of the neuraminidase protein to red blood cells, it has been necessary to add oseltamivir carboxylate to the assay. However, in the presence of oseltamivir, approximately 50% of current A(H3N2) isolates have insufficient haemagglutination titre to conduct the HI assay. Hence only a proportion of A(H3N2) virus isolates are successfully cultured could be analysed by HI assay. Other assays such as the Focus Reduction Assay, a form of virus neutralisation assay, are required to test the antigenic characteristics of these viruses and are under development to be performed more routinely.

Haemagglutinin gene sequencing

A total of 597 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 14).

Figure 13. Summary of fold differences in HI titres of A(H3N2) viruses analysed at the Centre compared to the A/Switzerland/9715293/2013 reference virus.

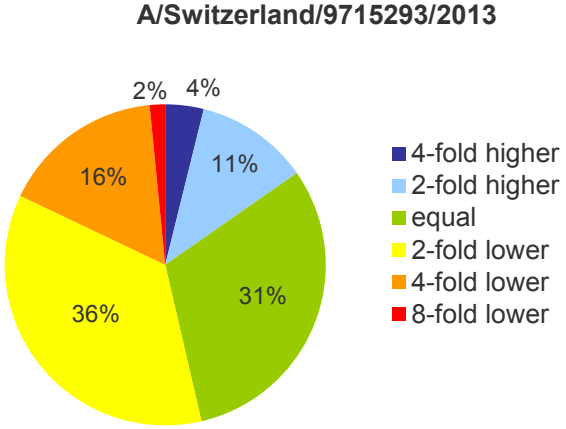


Table 6. Antigenic characterisation of A(H3N2) viruses analysed at the Centre compared to the A/Switzerland/9715293/2013 reference virus.

	A(H3N2) reference strain: A/Switzerland/9715293/2013	
Region	**Like**	**Low reactor (%)**
Australasia	456	8 (1.7%)
Pacific	7	0
South East Asia	133	2 (1.5%)
East Asia	26	0
South Asia	12	0
Africa	3	0
Total	**637**	**10 (1.5%)**

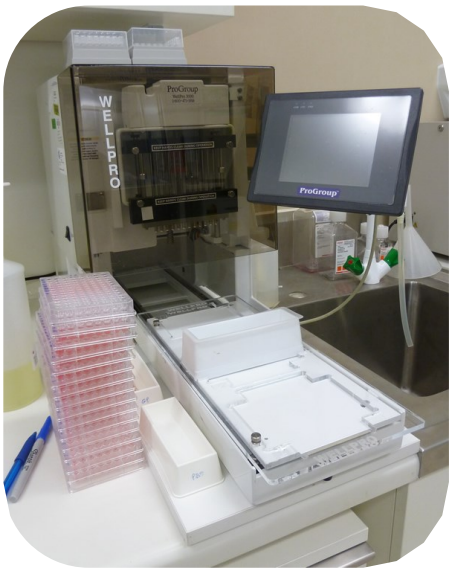
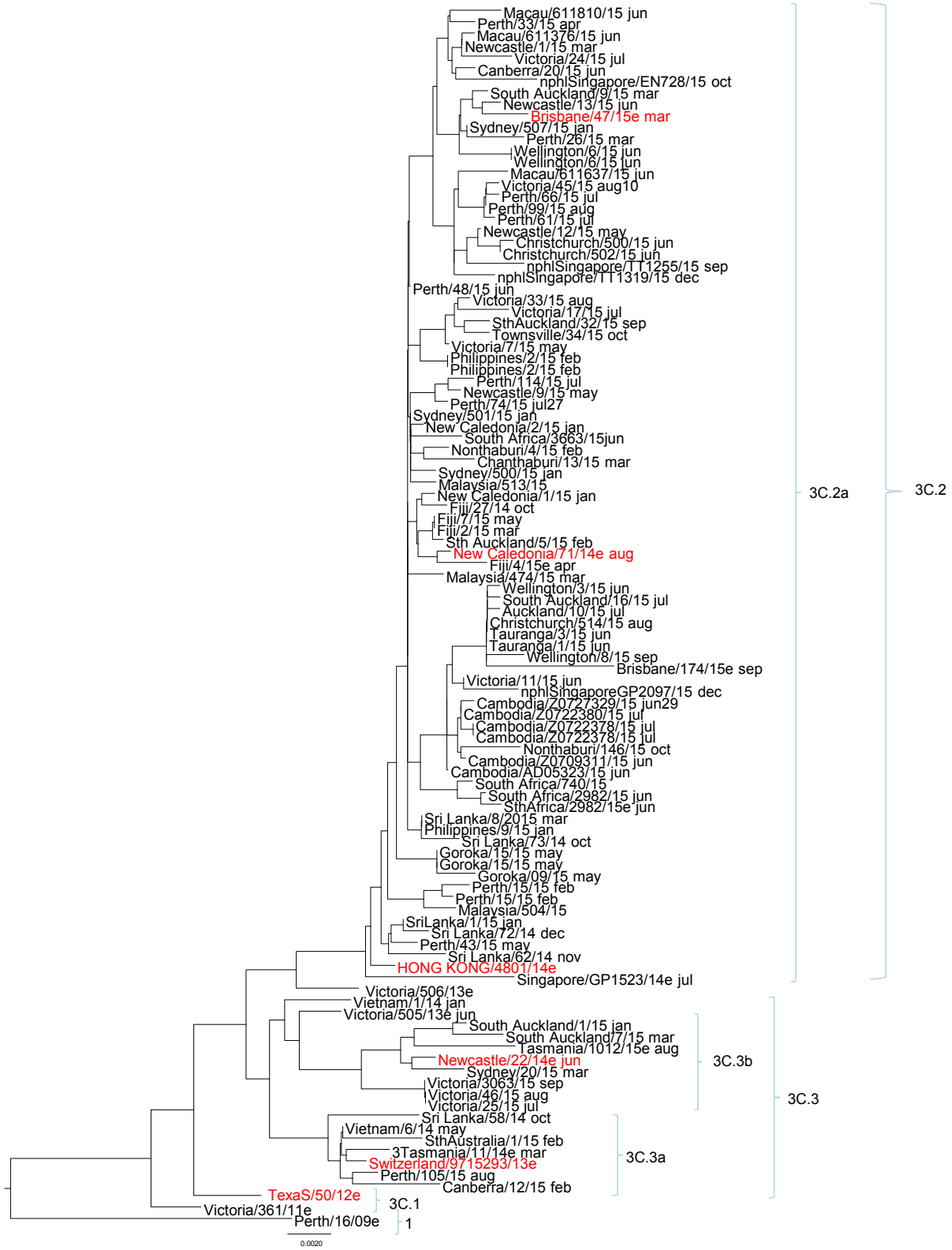


Figure 14. Phylogenetic tree of representative HA genes of A(H3N2) viruses received by the Centre during 2015



Legend

2016 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.2% 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 2015 the Centre received roughly equal proportions of B/Victoria and B/Yamagata lineage viruses. A total of 837 B/Victoria viruses were analysed antigenically, of which almost all were similar to B/Brisbane/60/2008 (Figure 15, Table 7). Similarly, the vast majority of the 933 B/Yamagata viruses that were analysed by HI assay were antigenically similar to B/Phuket/3073/2013. (Figure 16, Table 7).

Haemagglutinin gene sequencing

A total of 115 HA genes from B/Victoria and 132 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 17). Similarly, almost all of the B/Yamagata lineage viruses belonged to the clade represented by B/Phuket/3073/2013 (Figure 18).

Figure 15. Summary of fold differences in HI titres of B/Victoria viruses analysed at the Centre compared to the B/Brisbane/60/2008 reference virus.

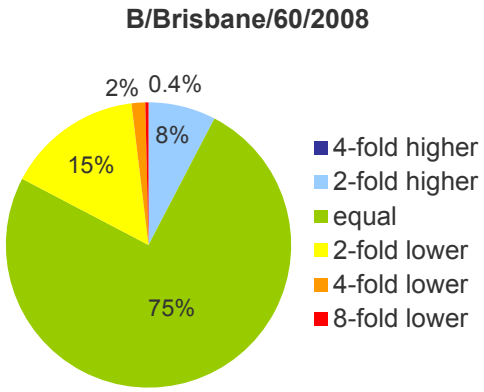


Figure 16. Summary of fold differences in HI titres of B/Yamagata viruses analysed at the Centre compared to the B/Phuket/3073/2013 reference virus.

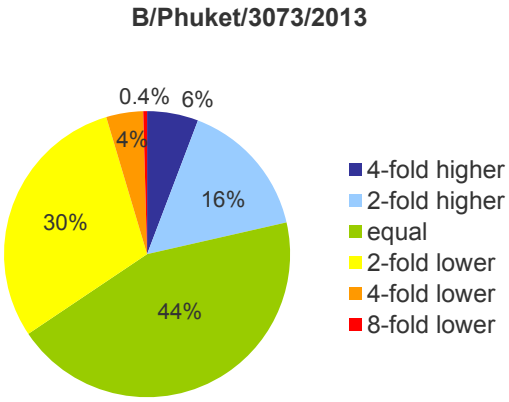
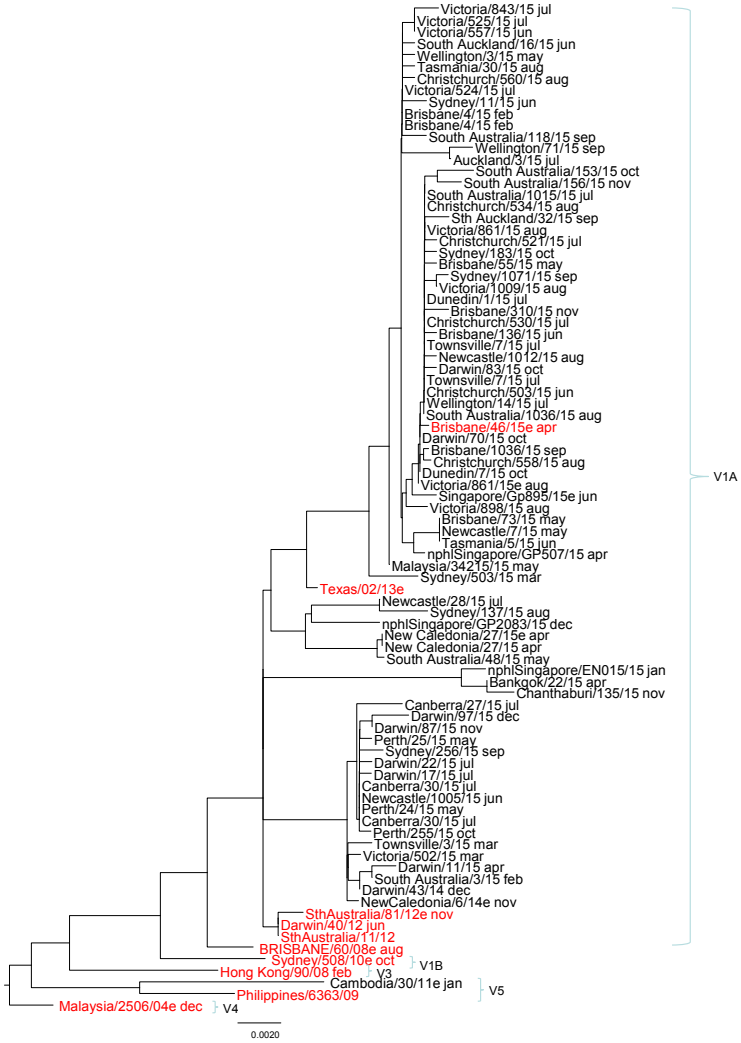


Table 7. Antigenic characterisation of B viruses received at the Centre during 2015 compared to the B/Brisbane/60/2008 and B/Phuket/3073/2013 reference viruses.

	B/Victoria reference strain: B/Brisbane/60/2008		B/Yamagata reference strain: B/Phuket/3073/2013	
Region	**Like**	**Low reactor (%)**	**Like**	**Low reactor (%)**
Australasia	809	3	767	2 (0.3%)
Pacific	12	0	51	0
South East Asia	13	0	87	2 (2.2%)
East Asia	0	0	18	0
South Asia	0	0	2	0
Africa	0	0	4	0
Total	**834**	**3 (0.4%)**	**929**	**4 (0.4%)**

Figure 17. Phylogenetic tree of representative HA genes of B/Victoria viruses received by the Centre during 2015.



Legend

2016 SOUTHERN HEMISPHERE VACCINE STRAIN

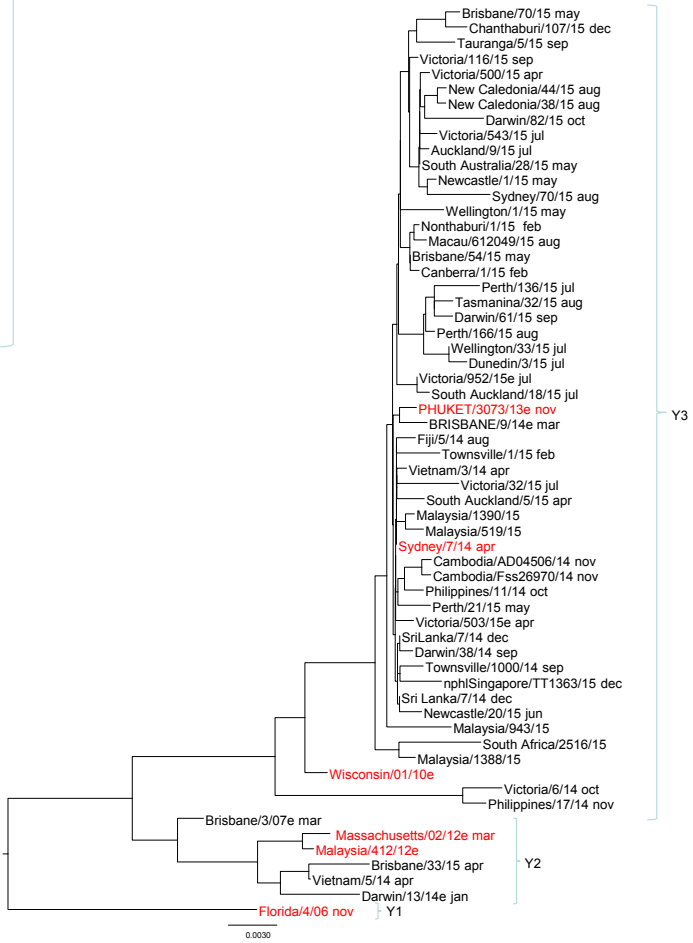
Reference virus

e: egg isolate

Scale bar represents 0.2% nucleotide sequence difference between viruses

} Brackets indicate clades

Figure 18. Phylogenetic tree of representative HA genes of B/Yamagata viruses received by the Centre during 2015.



Legend

2015 SOUTHERN HEMISPHERE VACCINE STRAIN

Reference virus

e: egg isolate

Scale bar represents 0.3% nucleotide sequence difference between viruses

} Brackets indicate clades

Antiviral Drug Resistance Testing

Sensitivity to neuraminidase inhibitors (NAIs)

Background

As influenza viruses continually undergo genetic change, their potential to develop resistance to antiviral drugs is an ongoing concern. To detect the emergence of drug-resistant influenza strains that could present future treatment challenges, viruses are tested for their sensitivity to the currently used neuraminidase inhibitors oseltamivir (Tamiflu), zanamivir (Relenza), laninamivir and peramivir using the neuraminidase inhibition assay. 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 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 2015

NAI assays were used to analyse 3572 viruses for reduced inhibition by the NAIs (Tables 8 and 9). In total, 4 viruses (1 A(H1N1)pdm09, 3 B/Yamagata) were found to have highly reduced inhibition by one or more of the NAIs. These viruses underwent further analysis to determine the presence of amino acid substitutions in the NA protein that associated with the reduction of inhibition by NAIs, for example histidine to tyrosine at position 275 (H275Y) of the neuraminidase protein of A(H1N1)pdm09 viruses, which reduces inhibition by oseltamivir, or the equivalent H273Y mutation in B viruses (Table 10).

Table 8. Viruses received by the Centre in 2015 and tested by NAI assay, by country.

Country \ Type/subtype/lineage	A(H1N1) pdm09	A(H3N2)	A(unsub-typed)	B/ Victoria	B/ Yamagata	Mixed type	TOTAL
Australasia							
Australia	213	1015	6	722	639	1	**2596**
New Zealand	8	79	0	83	131	0	**301**
South Pacific							
Fiji	18	15	0	0	7	0	**40**
New Caledonia	1	2	0	12	38	0	**53**
Papua New Guinea	1	17	0	0	6	0	**24**
South East Asia							
Cambodia	14	72	0	0	5	0	**91**
Malaysia	15	14	0	0	13	0	**42**
Philippines	6	3	0	0	9	0	**18**
Singapore	62	100	0	9	55	0	**226**
Thailand	5	4	0	3	3	0	**15**
Vietnam	0	7	0	1	4	0	**12**
East Asia							
Macau	14	49	0	0	18	0	**81**
South Asia							
Sri Lanka	25	21	0	0	2	0	**48**
Africa							
South Africa	13	8	0	0	4	0	**25**
TOTAL	**395**	**1406**	**6**	**830**	**934**	**1**	**3572**

Table 9. Neuraminidase inhibitor sensitivity of viruses received by the Centre in 2015.*

Type/Subtype	No. tested	Oseltamivir		Peramivir		Laninamivir		Zanamivir	
		Reduced inhibition	Highly reduced	Reduced inhibition	Highly reduced	Reduced inhibition	Highly reduced	Reduced inhibition	Highly reduced
A(H1N1)pdm09	395	1 (0.25%)	1 (0.25%)	0	1 (0.25%)	0	0	0	0
A(H3N2)	1406	1 (0.07%)	0	0	0	0	0	0	0
A(unsubtyped)	6	0	0	0	0	0	0	0	0
B/Victoria	830	1 (0.12%)	0	2 (0.24%)	0	0	0	0	0
B/Yamagata	934	5 (0.54%)	0	3 (0.32%)	3 (0.32%)	0	0	0	0
Mixed	1	0	0	0	0	0	0	0	0
TOTAL	**3572**	**8 (0.22%)**	**1 (0.03%)**	**5 (0.14%)**	**4 (0.11%)**	**0**	**0**	**0**	**0**

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.* ***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).*

Table 10. Characteristics of viruses received by the Centre during 2015 with highly reduced inhibition by NAIs.

Type/Subtype/ Lineage	Country/city of submitting laboratory	NAI(s) with highly reduced inhibition (marked with •)				Mutation detected
		Oseltamivir	Peramivir	Laninamivir	Zanamivir	
A(H1N1)pdm09	Sydney	•	•			H275Y
B/Yamagata	Brisbane		•			T146I
	South Australia		•			H273Y
	Sydney		•			H273Y

Resistance to Adamantanes

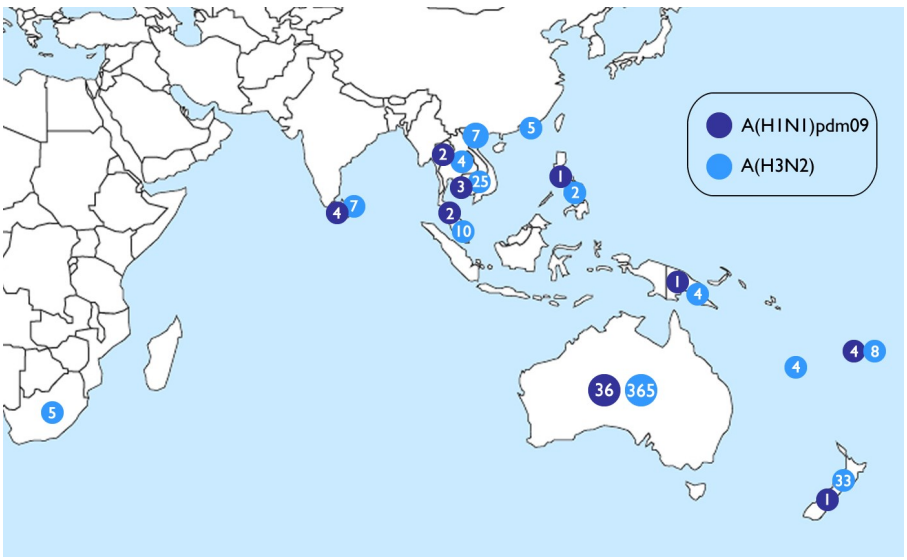
Background

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 2015

Real-time PCR or sequencing was used to analyse 533 influenza A viruses, which were selected as representative of those submitted to the Centre during 2015 (Figure 19). Based on S31N analysis, almost all tested viruses were resistant to the adamantanes. One virus, from Papua New Guinea, was found to contain a S31L substitution — the sensitivity of this virus to adamantanes is undetermined.

Figure 19. Geographic spread of viruses received at the Centre during 2014 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 2015

In 2015, 45 viruses were successfully isolated in eggs at the Centre, representing an overall isolation rate of 38% (Tables 11 and 12).

Table 11. Virus isolation in eggs at the Centre in 2015.

Type/subtype	Isolates attempted	Isolates obtained	Success rate (%)
A(H1N1)pdm09	23	9	39%
A(H3N2)	54	21	39%
B/Victoria	26	8	31%
B/Yamagata	15	7	47%
Total	**118**	**45**	**38%**

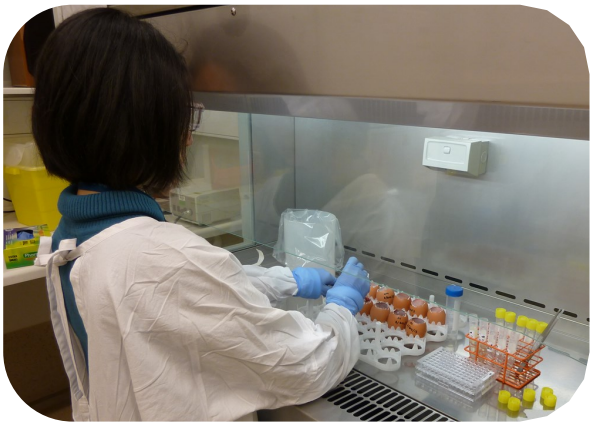


Table 12. Potential candidate vaccine strains isolated in eggs at the Centre in 2015.

A(H1N1)pdm09	A(H3N2)
A/Darwin/2/2015	A/Victoria/265/2014
A/Townsville/3/2015	A/Victoria/673/2014
A/South Australia/22/2015	A/Victoria/5006/2014
A/Brisbane/23/2015	A/Brisbane/341/2014
A/Singapore/GP1085/2015	A/Singapore/KK943/2014
A/Singapore/KK263/2015	A/South Australia/9/2015
A/Singapore/GP483/2015	A/South Australia/21/2015
A/Singapore/GP915/2015	A/Victoria/6001/2015
A/Brisbane/137/2015	A/Victoria/503/2015
B/Victoria	A/Brisbane/47/2015
B/Brisbane/63/2014	A/Fiji/2/2015
B/New Caledonia/6/2014	A/Fiji/4/2015
B/Victoria/502/2015	A/Brisbane/71/2015
B/Brisbane/46/2015	A/Brisbane/82/2015
B/New Caledonia/27/2015	A/Victoria/511/2015
B/Singapore/GP895/2015	A/Victoria/1000/2015
B/Victoria/861/2015	A/Tasmania/1012/2015
B/Victoria/849/2015	A/Fiji/7/2015
B/Yamagata	A/South Africa/2982/2015
B/Sydney/39/2014	A/Brisbane/174/2015
B/New Caledonia/4/2014	A/Auckland/10/2015
B/Victoria/503/2015	
B/Victoria/845/2015	
B/Victoria/519/2015	
B/Christchurch/502/2015	
B/Victoria/952/2015	

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 2015

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 recent representative A(H1N1)pdm09 viruses were similar to GMTs against the vaccine strain A/California/7/2009. In contrast, GMTs of antibodies against representative recent A(H3N2) and B/Yamagata/16/88 lineage viruses were significantly reduced as compared to titres to the vaccine viruses A/Texas/50/2012 (both egg- and cell-grown) and B/Massachusetts/2/2012, respectively. Antibodies raised in serum panels from recipients of trivalent vaccines not containing a B/Victoria/2/87 lineage antigen also had reduced GMTs to B/Victoria/2/87 lineage viruses.

In September, the Centre analysed serum panels from Australia. The combined data from all ERLs and WHO Collaborating Centres showed that GMTs of antibodies against the majority of representative recent A(H1N1) pdm09 and B/Yamagata/16/88 lineage viruses were not significantly reduced as compared to titres to the vaccine viruses A/California/7/2009 and B/Phuket/3073/2013 respectively. Antibodies raised in serum panels from recipients of trivalent vaccines not containing a B/Victoria/2/87 lineage antigen had reduced GMTs compared to B/Victoria/2/87 lineage viruses. Titres against recent A(H3N2) viruses from phylogenetic clade 3C.2a that were grown in cells were significantly lower than HI titres to egg-propagated A/Switzerland/9715293/2013 vaccine virus (clade 3C.3a), but not when compared to cell-propagated A/Switzerland/9715293/2013 virus. When analysed by microneutralisation assay, geometric mean microneutralisation titres (GMNT) of antibodies against two out of the three cell-propagated 3C.2a viruses tested were significantly reduced compared to GMNTs against cell-propagated A/Switzerland/9715293/2013 virus.

Table 13. Representative and vaccine candidate strains used for serological analyses during 2015. All viruses are egg grown unless indicated otherwise.

A(H1N1)pdm09	
February	September
A/California/07/2009 *	A/California/7/2009
A/New Caledonia/58/2014	A/Brisbane/23/2015
A/Kanagawa/163/2014 (C)	A/South Australia/22/2015
	A/Brisbane/35/2015 (C)
A(H3N2)	
February	September
A/Texas/50/2012	A/Switzerland/9715293/2013* (+/- O)
A/New Caledonia/71/2014	A/Switzerland/9715293/2013 (C, +/-
A/New Caledonia/71/2014 (C)	A/New Caledonia/71/2014 (+/- O)
A/Switzerland/9715293/2013*	A/New Caledonia/71/2014 (C, +/- O)
A/Switzerland/9715293/2013	A/Brisbane/47/2015 (+/- O)
A/Victoria/5060/2014	A/Victoria/511/2015 (+O)
A/Canberra/82/2014	A/Victoria/511/2015 (C, +O)
	A/South Australia/55/2014 (+O)
	A/South Australia/21/2015 (+O)
	A/Brisbane/82/2015 (+O)
	A/Hong Kong/4801/2014 (+O)
B/Victoria	
February	September
B/Brisbane/60/2008^	B/Brisbane/60/2008
	B/Texas/02/2013
	B/Brisbane/46/2015
B/Yamagata	
February	September
B/Massachusetts/2/2012	B/Phuket/3073/2013*
B/Phuket/3073/2013*	B/Phuket/3073/2013 (C)
B/Sydney/39/2014 (C)	B/Darwin/13/2014
	B/Victoria/503/2015
	B/Victoria/503/2015 (C)

**Trivalent vaccine strain ^ Quadrivalent vaccine strain*
(+/- O): indicates if HI assays performed in the presence or absence of oseltamivir, only relevant to A(H3N2) viruses in September 2015.
(C): Cell-grown 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). 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 from time to time. In 2015, WHO made the recommendations reported here.

WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2015–2016, Geneva, Switzerland, 23–25 February 2015

It is recommended that vaccines for use in the 2015–2016 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 2016, Memphis TN, USA, 22–24 September 2015

It is recommended that vaccines for use in the 2016 influenza season (southern 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.

* *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 relevant authority is the Therapeutic Goods Administration which 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 8 October AIVC accepted the September WHO recommendation and decided that the Australian influenza vaccine for 2016 should contain the following:

- A(H1N1): an A/California/7/2009 (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 2015, 40 kits were sent to 21 laboratories in 15 countries. Each kit contained 10 mL each of the reference antigens A/Switzerland/9715293/2013, A/California/7/2009, B/Phuket/3073/2013 and B/Brisbane/60/2008, and homologous antisera.

Recipients of the 2015 Kit

AUSTRALIA: SA Pathology Adelaide, South Australia; Queensland Health Scientific Services, Brisbane, Queensland; Westmead Hospital, Sydney, New South Wales;

CAMBODIA: Institut Pasteur du Cambodge, Phnom Penh

HONG KONG SAR: University of Hong Kong

INDIA: Manipal University, Karnataka; University of Delhi, New Delhi; Christian Medical College, Vellore

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

MACAU SAR: Public Health Laboratory

MALAYSIA: Institute for Medical Research, Kuala Lumpur

NEW ZEALAND: Institute of Environmental Science and Research, Wellington; Auckland City Hospital, Auckland

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

USA: Food and Drug Administration/Center for Biologics Evaluation and Research/OBRR/DHRR/LPD, Silver Spring MD

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 2015 panel kits were sent to InDevR, Boulder CO, USA and College of Veterinary Medicine, Cornell University, Ithaca NY, USA. Kits were composed of 2 vials (250 µL) of each of the reference viruses listed in the table below.

Viruses in the 2015 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/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

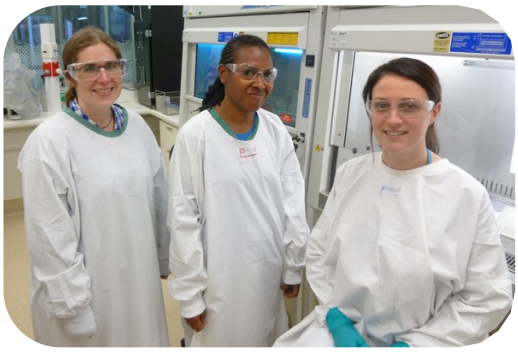
Training

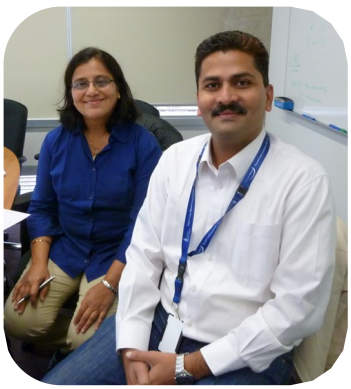
Training and Support of National Influenza Centres

The Centre regularly provides 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. We host scientists for training at the Centre for in-house training, participate in regional workshops and visit laboratories to provide direct assistance in strengthening surveillance capabilities.

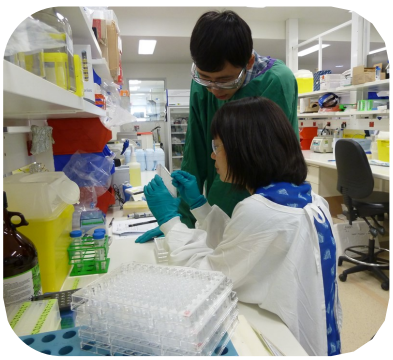
In-house Training

Dr Amanda Lang (*above right*) and Ms Debbie Kisa (*above left*), from the Institute of Medical Research, Goroka, Papua New Guinea, visited the Centre 15–24 June 2015. They undertook training in a range of techniques related to detection and characterisation of influenza viruses. These included molecular detection, cell culture, virus isolation and assays to detect the susceptibility of influenza viruses to neuraminidase inhibitors.



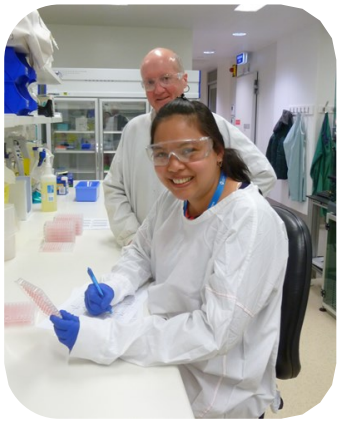


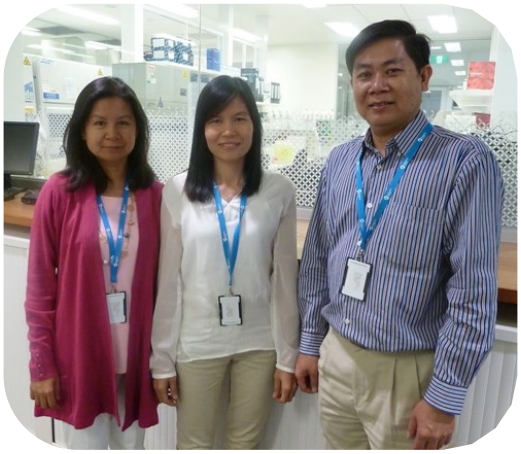
Ms Sadhana Kode (*left*) and Dr Shailesh Pawar (*right*), from the National Institute of Virology, Pune, India, visited the Centre 13–24 July for training in antiviral resistance testing methods.



Dr Pan Yang, from the Chinese Center For Disease Control And Prevention, Beijing, China, visited the Centre 21 September – 13 November. During his visit Dr Yang undertook training in various techniques, including serology, molecular biology, antiviral resistance testing, data entry and egg culture of viruses.

Mrs Julie Ann Ira, from Canterbury Health Services, Christchurch, New Zealand, visited the Centre 5–9 October for training in serology techniques and HI assays.





Dr Horm Srey Viseth (*left*) and Ms Y Phalla (*centre*), from the Institut Pasteur, Phnom Penh, Cambodia, and Dr Chin Savuth (*right*), from the National Public Health Laboratory, Phnom Penh, Cambodia visited the Centre from 4–13 November for training in sequencing of influenza genes and sequence analysis, egg inoculation and harvest for growth of influenza viruses, and influenza virus isolation in MDCK cells.

Visits to Regional Laboratories

Patrick Reading visited the National Institute of Health Research and Development (NIHRD) in Jakarta, Indonesia from 20 April – 1 May 2015. During this visit Dr Reading worked with scientists and laboratory technicians to establish and validate TCID50 and microneutralisation assays to detect antibodies in human serum samples to avian influenza A(H7N9).

Patrick Reading visited the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam on 3 July for discussions regarding influenza surveillance in Vietnam.

Patrick Reading visited the Fiji Center for Communicable Disease Control, Suva, Fiji on 23–24 July. He assisted the laboratory in molecular detection techniques and discussed issues with influenza surveillance in Fiji with staff at the Center.

Patrick Reading visited the Institut Pasteur in Ho Chi Minh City (Vietnam NIC), Vietnam, 29 June – 2 July 2015. He provided assistance to the influenza laboratory with a particular focus on improving cell culture and virus isolation techniques. He also discussed the development of collaborative projects between the Centre and the Institut Pasteur.

Patrick Reading visited the National Institute of Health Research and Development (NIHRD) in Jakarta, Indonesia from 21 September – 2 October. He worked with scientists and laboratory technicians at NIHRD to perform microneutralisation and HI assays to detect antibodies to A(H7N9) and A(H5N1) viruses using over 500 human serum samples obtained from workers in the live bird markets of Indonesia.

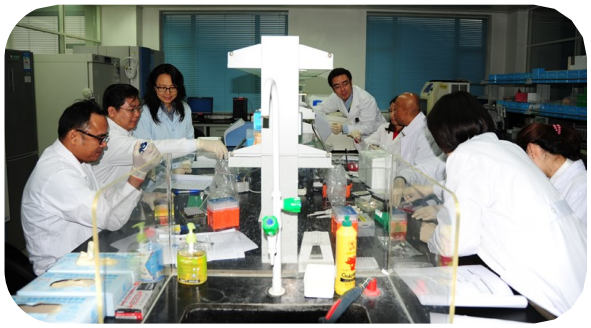
Training workshops

Patrick Reading participated as a facilitator and influenza specialist in the Pacific Public Health and Surveillance Network (PPHSN) Regional LabNet/EpiNet Meeting held in Nadi, Fiji on 20–22 July.

Vivian Leung (*pictured below, front row, 4th from left*) was a co-facilitator at the WPRO Burden of Disease training workshop held in Kampong Cham, Cambodia, on 11–14 August.

Naomi Komadina ran the Sequencing and the GISAID EpiFlu™ Database workshop, in Phnom Penh, Cambodia on 20 August.

Yi-Mo Deng (*pictured below, back left*) gave a talk and participated as a trainer in a workshop on Influenza Laboratory Surveillance Techniques in Beijing, China on 18–20 November. The workshop was attended by representatives from several National Influenza Centres.



Vivian Leung (*pictured below, standing*) participated in the Estimating Burden of Influenza Disease in Lao PDR workshop, Vientiane, Laos on 27–31 July as a lecturer and facilitator. The objective of the workshop was to teach participants how to obtain reliable disease burden estimates and to provide a better understanding of the impact of influenza in vulnerable communities or subpopulations. A total of 41 participants from the National Center for Laboratory and Epidemiology attended, including health office workers, laboratory technicians, medical practitioners and nurses.

Naomi Komadina was a training session facilitator at the GISAID-ISIRV Workshop on Genetic Analyses of Influenza Viruses, Hong Kong SAR, China on 21–22 November. This workshop was attended by 39 representatives from various National Influenza Centres (*pictured below*).

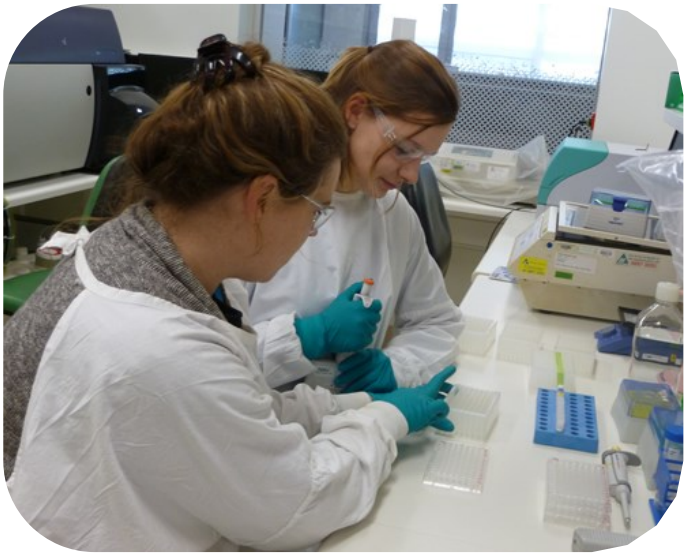
Staff Development

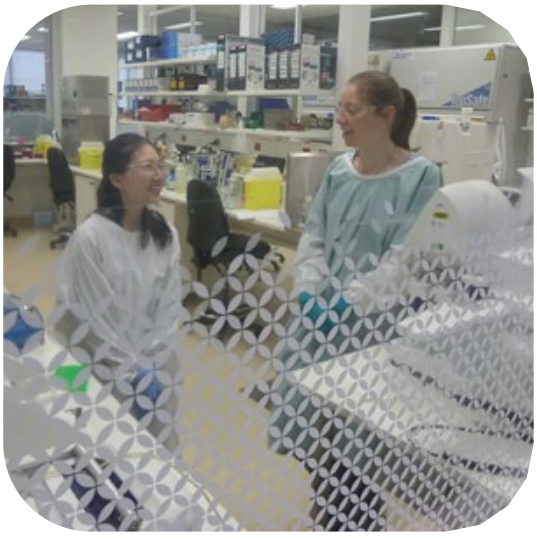
Aeron Hurt attended the Research Leader Development Program, run by Marlow Hampshire, Melbourne, 13–14 April and 25–26 May.

Louise Carolan attended a RNA-Seq wetlab workshop run by Integrated Sciences, in which she undertook training in preparation of purified RNA sample to be run on a multiplex sequencer, on 21–22 July.

Vivian Leung attended the “Modern Demographic Methods in Epidemiology with R” workshop run by the Melbourne School of Population Health on 23 November.

Around the laboratories...





Research

The Centre's research interests continue to expand and develop with a broad range of projects, both within the Centre and with external collaborators.

Evolution and Modelling of Influenza Viruses

Centre staff

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

Research overview

The Centre is 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. These have included a collaboration with scientists at Duke-NUS Medical School in Singapore on the A(H1N1)pdm09 virus to examine how this virus had changed since it emerged in 2009, while another study with scientists at University of Sydney examined the origin of influenza viruses during non-epidemic periods in Australia from 2009–14. A further study was conducted with scientists at both Duke -NUS Medical School and the University of Sydney on the evolution of influenza B lineages from Eastern Australia and New Zealand, which revealed some fundamental differences between the two lineages.

We were also part of a multi-centre study headed by Trevor Bedford (Fred Hutchinson Cancer Research Center, Seattle WA, USA) and Colin Russell (University of Cambridge, UK) that performed a large study on the genetic and antigenic characteristics of human influenza viruses and how they circulate globally. This study found distinct differences in the way genetic variants of A(H3N2) viruses behave compared to other human influenza viruses. A(H3N2) viruses did not persist locally between epidemics and were reseeded from East and Southeast Asia, whereas variants of A(H1N1) and B viruses persisted across several seasons and exhibited complex global dynamics with East and Southeast Asia playing only a limited role in disseminating new variants globally.

A long standing collaboration of the Centre with Derek Smith and colleagues at Cambridge University, UK, has been expanded to include leading international scientists Yoshihiro Kawaoka (The University of Wisconsin, USA and The University of Tokyo, Japan), Ron Fouchier (Erasmus University, The Netherlands) and Ed Belongia (Marshfield Clinic Research Foundation, USA) through funding from the US Department of Health and Human services via the Biomedical Advanced Research and Development Authority (BARDA). The funding was announced in early October 2015 with work to commence shortly after. This funding has been provided to develop the groups methods of antigenic mapping to allow it to forecast how influenza viruses may change over time and to develop and test vaccines that would protect people against future A(H5N1) viruses as part of the US pandemic preparedness program.

Highlights and developments 2015

These projects were published in several high impact journals during 2015, with the paper entitled "Global circulation patterns of seasonal influenza viruses vary with antigenic drift" appearing in the July edition of Nature and other papers from the work described above being published in eLife, Nature Communications and PLoS Pathogens. The BARDA funding of USD$8 million (with an option to increase this funding) will enable the project to not only develop new vaccines but will allow testing in humans to be performed and detailed serological testing to be undertaken, some of which will take place at the Centre's laboratories.

Collaborators

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 and Gavin Smith (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); Rebecca Halpin and Das Suman (J. Craig Venter Institute, Rockville MD, 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 studying 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.

An outcome of this research has been the observation that vaccine effectiveness appears to attenuate with repeated administration. Serological studies suggest that the vaccine-induced antibody response generated also wanes with repeated administration. Thus, we are interested in understanding whether the protection afforded by the vaccine in target groups for vaccination, namely hospital workers, may be compromised by repeated annual vaccination.

Influenza vaccines can only be expected to be highly effective when the vaccine is closely matched to the circulating strains. Thus, it is important to understand how vaccine strains are selected and whether the viruses used to inform strain selection are representative of the viruses circulating during a season. We are thus trying to understand whether there is any bias in how samples are submitted, whether there are differences in successful isolation of particular strains, and whether there is any bias in the selection of samples for antigenic and genetic analysis. To this end, we have been attempting to compare the viruses submitted to the laboratory with viruses obtained as part of sentinel surveillance systems around the country. In turn, detailed antigenic and genetic information for these samples has been fed back into estimating vaccine effectiveness

Highlights and developments 2015

In 2015, we ran a serological survey at the Peter MacCallum Cancer Centre to measure the vaccine response in a highly vaccinated population. We observed poorer antibody responses among the most highly-vaccinated hospital workers. We have obtained funding from the Royal Melbourne Hospital to conduct a similar larger study, which will include follow-up of the hospital's staff to enumerate episodes of influenza and measure whether an attenuated antibody response results in increased susceptibility to infection.

To enhance monitoring of influenza vaccine effectiveness, the Centre worked with influenza surveillance networks around Australia to obtain virus samples for antigenic and genetic analysis. This included working with those surveillance networks to estimate the effectiveness at the genetic level. A complementary benefit of obtaining so many these surveillance samples may be sufficiently informative for the Centre's virological surveillance.

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)

Antivirals and Viral Fitness

Centre staff

Aeron Hurt, Ding Yuan Thomas Oh, Sook Kwan Leah Brown, Danielle Tilmanis

Research overview

Our research focuses on improving our understanding of the effectiveness of influenza antivirals 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 use a ferret model to investigate the effect of oseltamivir treatment on influenza A and B infection, with the final goal being to understand whether certain mutations result in clinical resistance to the drug.

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.

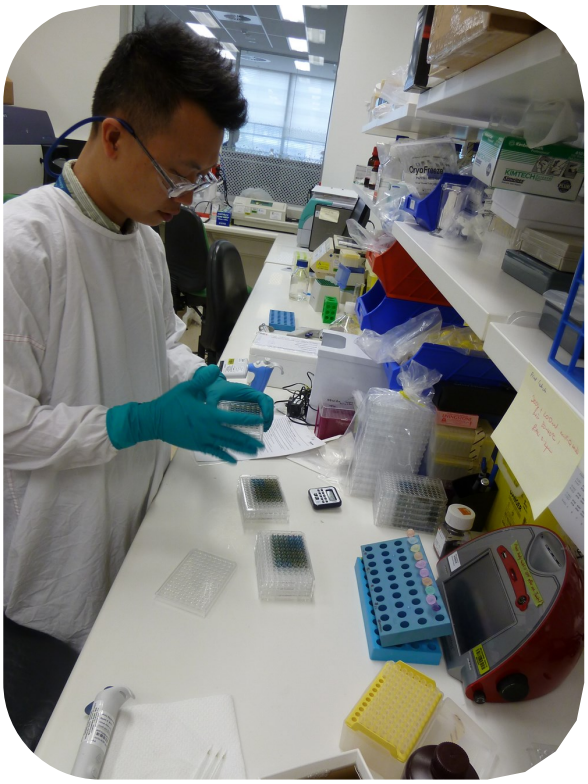
Highlights and developments 2015

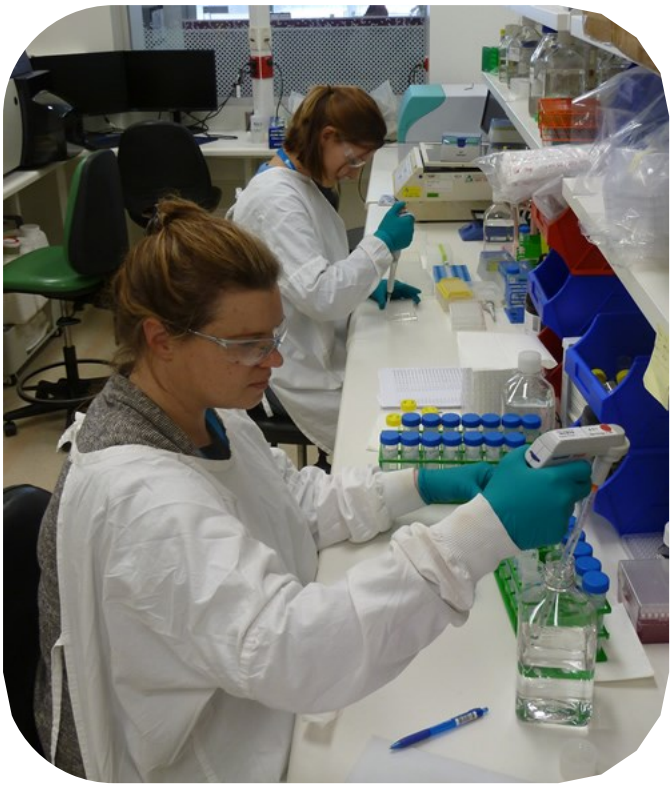
The NHMRC/A*STAR-funded project has made substantial progress in establishing and optimising the ferret antiviral treatment model. This has included the measurement of addition variables such as infiltrating cell numbers and protein levels to assess inflammatory responses. We also developed a novel video tracking method to assess ferret activity (wellness) and published a manuscript describing this technique. In addition, we have completed a pharmacokinetic/dynamic analysis of oseltamivir in the ferret. The model is now fully optimised and will allow the assessment of different variant viruses in 2016.

We have assessed a number of viruses with different neuraminidase mutations to determine if the mutations resulted in compromised viral function. This was completed for a number of influenza A and B viruses detected via our surveillance testing at the Centre.

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)





Animal Influenza Viruses

Centre staff

Aeron Hurt, 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. Avian species are routinely sampled by our collaborators in Australia to determine the types of avian influenza viruses circulating in either resident ducks or migratory waders in Australia. The Centre is involved with the characterisation of viruses sampled from birds in Australia, including culture, sequencing and phylogenetic analysis. 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 2015.

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.

Highlights and developments 2015

A large number of avian influenza viruses from Australia were characterised and isolated in embryonated hens' eggs. These viruses included many different subtypes and 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. One avian influenza A(H5N5) virus was detected in the samples collected from Antarctica in 2015 and was successfully cultured in eggs.

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.

Collaborators

Marcel Klaassen, Bethany Hoye, Simeon Lisovski (Deakin University, Victoria); Simone Warner (Department of Primary Industries, Victoria); Daniel González-Acuña (University of Concepción, Chile)

Understanding the Interplay between the Immune Response and Influenza Viruses

Centre staff

Karen Laurie, Louise Carolan, William Horman (Honours student)

Research overview

Our research encompasses two areas — characterising immune responses to influenza infection and human seroepidemiological studies. Increasing our understanding of the immune response to influenza infection can lead to improved strategies for prevention or treatment of infection. To this end, we use a ferret model to investigate the immune response following influenza virus infection. We have developed real time PCR assays to measure changes in levels of mRNA encoding ferret cytokines and chemokines to enable characterisation of the early and late immune response and are developing additional complementary assays. By using these methods with various infection and challenge experiments, we can determine the contribution of different aspects of the immune response in controlling virus infections in the ferret model.

Following infection or vaccination with influenza viruses, most people develop antibodies that can be measured by serological assays. Assessing antibody titres provides insight into the likely impact of a novel influenza virus introduced to a population, and can help inform public health decisions. We are involved in various local and international serological studies, including investigating seroconversion following infection and vaccination with influenza virus in infants under the age of 1 year and the protective effect and persistence of maternal antibodies to influenza virus. In a separate study we are assessing the response to influenza vaccines in children undergoing cancer therapy. Centre staff are also part of the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE), which aims to improve standardisation and assay development for seroepidemiological studies around the world.

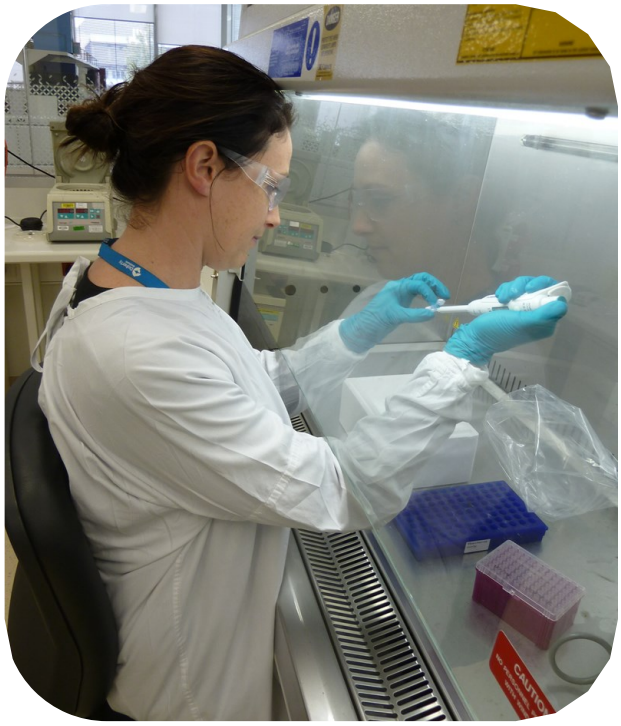
Highlights and developments 2015

We published a paper describing the characterisation of the localised immune response in the ferret respiratory tract following infection with different influenza viruses. Our data showed that the severity of disease and the cytokine and chemokine responses were similar, irrespective of the seasonal strain or the location of the infection in the respiratory tract. In collaboration with scientists at CSIRO we published a manuscript describing the generation, crystallisation and functional activity of recombinant ferret interleukin-2, and are now collaborating to validate an assay detecting interferon-gamma responses from immune cells in ferrets. Protocols that we previously developed for cytokine and chemokine mRNA real time PCR assays have also been used by other research groups.

We have continued to investigate viral interference, a phenomenon whereby following infection with an influenza virus the host experiences lower susceptibility to infection by any strain of influenza or other respiratory virus for a short period of time. We have found that viral interference can occur between antigenically related and unrelated viruses and that there is a hierarchy between the ability of different influenza viruses to induce interference. Our results were published in the Journal of Infectious Disease, with an accompanying editorial. Karen Laurie was also awarded best presentation at the 7th Orthomyxovirus Research Conference for her presentation based on these data. A complementary study using the data to develop mathematical models of the immune response was also published. We are now investigating viral interference between influenza B lineages and between different respiratory viruses. Work has also been initiated to ascertain the mechanism/s responsible for viral interference.

Highlights and developments 2015 (continued)

As part of CONSISE, we published two international comparison studies. In the first study, 13 laboratories compared a 2-day ELISA assay with a 3-day HA microneutralisation (MN) assay. In the second study, 35 laboratories tested an enzyme-linked lectin assay to measure antibodies to neuraminidase. A third international comparison study, co-ordinated by Centre staff, was started, in which at least 20 laboratories will compare the two main serological assays (HI assay and MN assay) used to detect antibodies to influenza viruses.



We have begun a new collaboration assessing seroconversion in kidney transplantation patients following receipt of the trivalent influenza vaccine. This is complemented by a study assessing seroconversion following vaccination in health care workers at Peter MacCallum Cancer Centre. We also assessed a cohort of paediatric oncology patients who have been followed since 2009 for seroconversion to the 2014 seasonal influenza vaccine.

Collaborators

Steve Rockman (bioCSL, Victoria); Jennifer Mosse (Federation University, Victoria); James McCaw, Stephen Petrie, Ada Yan, Pengxing Cao, Jodie McVernon (Melbourne School of Population and Global Health, The University of Melbourne); Cameron Simmons (Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam and the Department of Microbiology and Immunology, The University of Melbourne); Katie Anders (Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam); Ushma Wadia (Princess Margaret Hospital, Western Australia), Lorena Brown, Brad Gilbertson (Department of Microbiology and Immunology, The University of Melbourne), CONSISE, Timothy Adams, William McKinstry, Bin Ren, Tam Phan and Janet Newman (CSIRO, Victoria), Claire Dendle (Monash Medical Centre)

Early Recognition and Response to Influenza Infection

Centre staff

Patrick Reading (Centre Educator and Associate Professor, Department of Microbiology and Immunology, The University of Melbourne)

Research overview

Our research, which is undertaken at the University of Melbourne, investigates how the body first recognises and responds to infections with respiratory viruses. We employ *in vitro* studies using human proteins and cells, as well 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) the soluble proteins present in airway fluids that limit early replication and spread of influenza viruses, (iii) how different cell types in the respiratory tract sense and respond to influenza virus infection, and (iv) novel strategies aimed at manipulating innate immune responses to reduce the severity of respiratory disease.

Highlights and developments 2015

During 2015, our research focussed on how cells sense and respond to influenza and other respiratory viruses. We identified specific cell-surface receptors that function as attachment and/or entry receptors for influenza virus and characterised the specific endocytic pathways used when particular receptors are engaged by virus. In addition, we have characterised influenza virus infection of macrophages, as these cells represent an important component of the early immune system that limit disease severity. While influenza virus can readily infect macrophages, we demonstrated that virus replication is blocked at a late stage in the virus life cycle, just prior to virion assembly and release. Additional studies have commenced to compare how cells respond to other respiratory viruses, such as respiratory syncytial virus and human metapneumovirus, as these cells use different receptors and entry mechanisms to infect host cells.

Overall, our research contributed to nine peer-reviewed publications during 2015, including two senior author publications in The Journal of Virology. Dr Reading was on the organizing committee for the inaugural Australian Respiratory Virology Meeting in Canberra (December) and presented several research talks at conferences and institutes during the year, including at the Department of Pharmacology at Monash University (June) and at the Peter Doherty Institute for Infection and Immunity (November). In 2015, the group consisted of three post-doctoral scientists, one research assistant and a Ph.D. student. Wy Ching Ng completed her Ph.D. studies at the University of Melbourne under the supervision of Dr Reading and her thesis was passed in November 2015.

Collaborators

Erika Crouch (Washington University School of Medicine, St. Louis, MO, USA); Stuart Turville (Westmead Millennium Institute, New South Wales); Tony Cunningham (University of Sydney); Nigel McMillan & Suresh Mahalingham (Griffith University, Queensland); Paul Young (University of Queensland); Andrew Brooks, Justine Mintern, Stephen Kent, David Jackson, Lorena Brown, Carol Hartley and Joanne Devlin (The University of Melbourne)

NHMRC Program Grant: Limiting the Impact of Influenza (2015 - 2019)

Centre staff:

Patrick Reading, Karen Laurie

Research overview

The Centre is a participant in a National Health and Medical Research Council Program Grant which commenced on 1 January 2015. The Program has two broad goals:

- to understand fundamental mechanisms that establish maximum effective cellular immunity to influenza A viruses
- to build the foundations for clinical application of strategies to induce cellular immunity to these viruses.

These goals are being addressed through a range of collaborative projects between the chief investigators and team members at the Department of Microbiology and Immunology at the University of Melbourne (UM), La Trobe University, the School of Population and Global Health (UM), WHO Collaborating Centre for Reference and Research on Influenza and the CSIRO Australian Animal Health Laboratory.

Highlights and developments 2015

A Program retreat held on 26 October was attended by representatives from all of the research groups in the Program. **Karen Laurie** presented a talk and **Louise Carolan, Aeron Hurt and Patrick Reading** attended.

With the renewal of the NHMRC Program Grant on influenza administered through the University of Melbourne (2015 – 2019; CIA Stephen Turner; new title "Limiting the impact of influenza"), the Centre continues its collaboration with colleagues at the University of Melbourne and La Trobe University on a range of biomedical and clinical studies of influenza virus infection.

Chief Investigators

Stephen Turner, Peter Doherty, David Jackson, Lorena Brown, Katherine Kedzierska (The University of Melbourne) and Weisan Chen (La Trobe University).

Research Funding and Awards

Several staff members are Chief Investigators in grants awarded in 2015 for projects due to commence in 2016.

National Health and Medical Research Council (NHMRC) Development Grant: ***Low-cost portable inhalation therapy platform for needle-free DNA-based influenza vaccine.***

$599,644 awarded for the period 1 January 2016 – 31 December 2018. Chief investigators Leslie Yeo, **Aeron Hurt**, David Piedrafita and David Morton. The grant will be administered by RMIT University and the work will be undertaken at RMIT University and the Centre.

Australian Research Council (ARC) Discovery Project Grant: ***Revealing the evolutionary and ecological dynamics of avian influenza virus.***

$534,100 awarded for the period 1 June 2016 – 30 June 2019. Chief investigators Edward Holmes, Marcel Klassen and **Aeron Hurt**. The grant will be administered by the University of Sydney and the work will be undertaken at the University of Sydney, Deakin University and the Centre.

Royal Melbourne Hospital (RMH) Home Lottery Grant in Aid: ***A prospective sero-survey to investigate antibody responses and risk of infection after influenza vaccination among repeatedly vaccinated health care workers.***

$25,000 awarded for the period 11 January 2016 – 10 January 2017. Chief investigators **Sheena Sullivan**, Caroline Marshall, **Vivian Leung**, **Karen Laurie**, Monica Slavin and Julian Druce. The grant will be administered by the Royal Melbourne Hospital and the work will be undertaken at Royal Melbourne Hospital, the Centre and VIDRL.

Royal Melbourne Hospital Home Lottery Grant in Aid: ***Development of a ferret model of respiratory syncytial virus.***

$25,000 awarded for the period 1 February 2016 – 31 January 2017. Chief investigators **Karen Laurie**, **Patrick Reading** and Julian Druce. The grant will be administered by the Royal Melbourne Hospital and the work will be undertaken at the Centre and VIDRL.

Collaborative Agreements

The Centre is party to two 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) (2014-2015)

Centre staff: Chantal Baas, Hilda Lau, Robert Shaw, Anne Kelso, Ian Barr

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

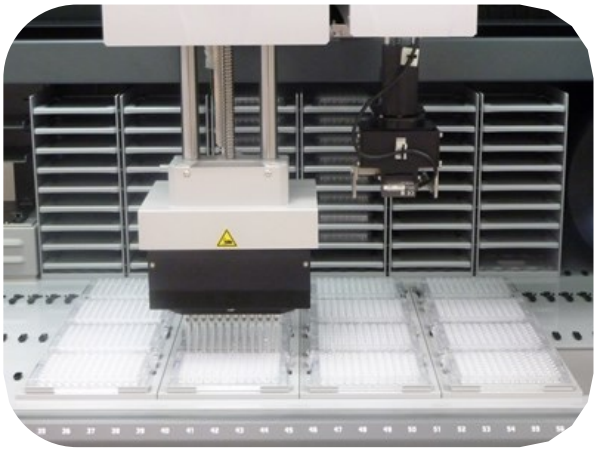
Highlights and developments 2015: A total of 45 egg isolates were obtained from 118 inoculations with original clinical specimens from various geographical locations. Isolation rates varied from 31% to 47% according to virus type/subtype and lineage. Suitable isolates were made available to other laboratories and industry for reassortment and assessment as vaccine candidates.

Cooperative Research and Development Agreement with Novartis Vaccines & Diagnostics/Seqirus: Development and provision of influenza virus strains isolated on MDCK 33016PF cells for vaccine production (2014–2015)

Centre staff: Heidi Peck, Joelle Dharmakumara, Danielle Tilmanis, Robert Shaw, Anne Kelso, Ian Barr

Project overview: The suitability of a proprietary Novartis/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 2015: Novartis Vaccines and Diagnostics was acquired by CSL Limited in July 2015 and now operates under the name of Seqirus. During 2015, 109 clinical specimens were cultured in MDCK 33016PF cells, of which 87 (79.8%) 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 Novartis in Holly Springs NC, USA, for further evaluation as potential vaccine candidates produced by cell culture. Heidi Peck and Ian Barr attended and presented talks at a workshop at Novartis in Holly Springs NC, USA, held in June, to discuss the implementation of cell vaccine candidate viruses in the production of cell-based influenza vaccines.

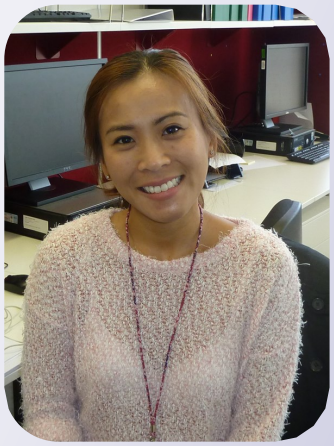


Research Students

MSc Candidate

Ms Chantal Baas, who commenced her MSc candidature part-time at the Centre in 2012, has continued her project entitled "Investigating the risk of non-human influenza viruses for public health by using a ferret model", under the supervision of **Aeron Hurt**, **Ian Barr** and Ms Jenny Mosse (Federation University, Gippsland).

Masters student



Ms Kanita Chong, a Master of Infectious Disease candidate from the University of Western Australia, completed a research project title "Understanding the role of genetic drift among influenza A(H3N2) virus isolated from vaccinated patients" at the Centre under the supervision of **Yi-Mo Deng** and **Sheena Sullivan**, 23 February – 15 June, 2015.

Honours student

Mr William Horman, a BSc(Honours) student enrolled through the University of Melbourne, completed his Honours project under the supervision of **Karen Laurie**. His project, titled "Investigating viral interference between influenza B lineages", used a ferret model of human influenza to explore whether infection with one influenza B lineage can limit subsequent infection by the other influenza B lineage, and vice versa. His results indicated that with a shorter interval (3 days) between a primary infection by one lineage and secondary challenge with the other lineage, a B/Victoria virus prevented subsequent infection with a B/Yamagata virus, whilst a primary infection with B/Yamagata virus delayed (but did not prevent) subsequent infection with B/Victoria virus. A longer time period (10 days) between infections resulted in viral interference between both influenza B lineages, irrespective of order of infection. Possible mechanisms of viral interference between influenza B lineages were also considered. William was awarded First-Class Honours.

Undergraduate students

Ms Cheryl Lee, a 3rd year Bachelor of Biomedicine student from the University of Melbourne undertook a student laboratory placement for 10 weeks from August to October under the supervision of **Karen Laurie**.

Ms Paulina Koszalka, a 3rd year Bachelor of Science student from the University of Melbourne undertook a student laboratory placement for 10 weeks from August to October under the supervision of **Aeron Hurt** and **Danielle Tilmanis**.

Secondary school students

The Centre hosted several secondary school students for work experience placements during the year: Jonathon Bradshaw on 2 April; Emily Kypriotis on 18 June, Madeleine Lemmo on 25 June, Siddhant Tandon (Melbourne High School) on 24 September, Maddison Ellul (Ave Maria College) on 1 October, Anjelica Corby (Canterbury Girls Secondary College) on 3 December and Cecilia Gu (MacRobertsons Girls School) on 10 December.

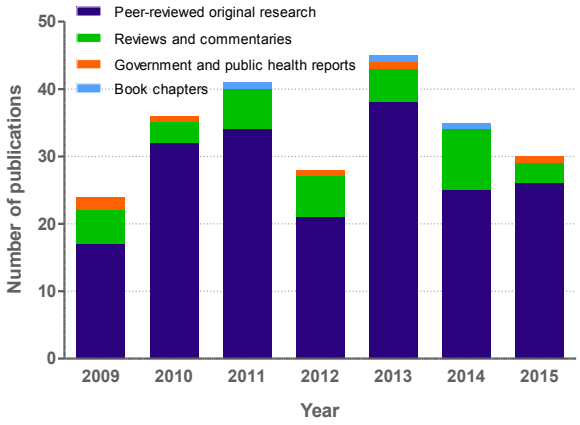
Communications and Advisory Activities

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 also co-organise the Australian Influenza Symposium, publish peer-reviewed journal papers and present numerous talks and posters.

Publications and Reports

The Centre continued to build its research and surveillance profile with the publication of 30 original research papers, reviews and reports in 2015 (Figure 20).

Figure 20. Centre publications 2009–2015.



Centre staff members were involved in a paper published in the July 2015 edition of *Nature* along with collaborators in the USA, United Kingdom, India, China, Japan, the Netherlands and Belgium. Sequence analysis of viruses from a 12-year period identified distinctive patterns in the global circulation and evolution of A(H3N2) viruses as compared to A(H1N1) pdm09 and B viruses. These patterns of circulation and antigenic drift could be related to patterns of human behaviour and travel.

*Bedford T, Riley S, **Barr IG**, Broor S, Chadha M, Cox NJ, Daniels RS, Gunasekaran CP, **Hurt AC**, **Kelso A**, Klimov A, Lewis NS, Li X, McCauley JW, Odagiri T, Potdar V, Rambaut A, Shu Y, Skepner E, Smith DJ, Suchard MA, Tashiro M, Wang D, Xu X, Lemey P, and Russell CA. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. Nature, 2015. 523(7559): 217-20.*

Centre Publications 2015

1. **Barr IG** and Kelly HA. Oseltamivir reduces clinical illness in households in Bangladesh. Lancet Infect Dis, 2015. 15(6): 617-9.
2. Bedford T, Riley S, **Barr IG**, Broor S, Chadha M, Cox NJ, Daniels RS, Gunasekaran CP, **Hurt AC**, **Kelso A**, Klimov A, Lewis NS, Li X, McCauley JW, Odagiri T, Potdar V, Rambaut A, Shu Y, Skepner E, Smith DJ, Suchard MA, Tashiro M, Wang D, Xu X, Lemey P and Russell CA. Global circulation patterns of seasonal influenza viruses vary with antigenic drift. Nature, 2015. 523 (7559): 217-20.
3. Bird NL, Olson MR, **Hurt AC**, Oshansky CM, **Oh DY**, **Reading PC**, Chua BY, Sun Y, Tang L, Handel A, Jackson DC, Turner SJ, Thomas PG and Kedzierska K. Oseltamivir prophylaxis reduces inflammation and facilitates establishment of cross-strain protective T cell memory to influenza viruses. PLoS One, 2015. 10(6): e0129768.
4. Cao P, Yan AW, Heffernan JM, Petrie S, Moss RG, **Carolan LA**, **Guarnaccia TA**, **Kelso A**, **Barr IG**, McVernon J, **Laurie KL** and McCaw JM. Innate immunity and the inter-exposure interval determine the dynamics of secondary influenza virus infection and explain observed viral hierarchies. PLoS Comput Biol, 2015. 11(8): e1004334.
5. Carville KS, Grant KA, **Sullivan SG**, Fielding JE, Lane CR, Franklin L, Druce J and Kelly HA. Understanding influenza vaccine protection in the community: An assessment of the 2013 influenza season in Victoria, Australia. Vaccine, 2015. 33(2): 341-5.
6. **Deng YM**, **Spirason N**, **Iannello P**, **Jelley L**, **Lau H** and **Barr IG**. A simplified Sanger sequencing method for full genome sequencing of multiple subtypes of human influenza A viruses. J Clin Virol, 2015. 68: 43-8.
7. **Farrukee R**, **Leang SK**, **Butler J**, Lee RT, Maurer-Stroh S, **Tilmanis D**, **Sullivan S**, Mosse J, **Barr IG** and **Hurt AC**. Influenza viruses with B/Yamagata- and B/Victoria-like neuraminidases are differentially affected by mutations that alter antiviral susceptibility. J Antimicrob Chemother, 2015. 70(7): 2004-12.
8. Foo SS, Chen W, Taylor A, Sheng KC, Yu X, Teng TS, **Reading PC**, Blanchard H, Garlanda C, Mantovani A, Ng LF, Herrero LJ and Mahalingam S. Role of pentraxin 3 in shaping arthritogenic alphaviral disease: from enhanced viral replication to immunomodulation. PLoS Pathog, 2015. 11(2): e1004649.
9. Fox A, Mai le Q, Thanh le T, Wolbers M, Le Khanh Hang N, Thai PQ, Thi Thu Yen N, Minh Hoa le N, Bryant JE, Duong TN, Thoang DD, **Barr IG**, Wertheim H, Farrar J, Hien NT and Horby P. Hemagglutination inhibiting antibodies and protection against seasonal and pandemic influenza infection. J Infect, 2015. 70(2): 187-96.

Centre Publications (continued)

10. Hsu AC, Starkey MR, Hanish I, Parsons K, Haw TJ, Howland LJ, **Barr I**, Mahony JB, Foster PS, Knight DA, Wark PA and Hansbro PM. Targeting PI3K-p110alpha suppresses influenza virus infection in chronic obstructive pulmonary disease. Am J Respir Crit Care Med, 2015. 191(9): 1012-23.
11. **Hurt AC**, Hui DS, Hay A and Hayden FG. Overview of the 3rd isirv-Antiviral Group Conference--advances in clinical management. Influenza Other Respir Viruses, 2015. 9(1): 20-31.
12. Infusini G, Smith JM, Yuan H, Pizzolla A, Ng WC, Londrigan SL, Haque A, **Reading PC**, Villadangos JA and Wakim LM. Respiratory DC use IFITM3 to avoid direct viral infection and safeguard virus-specific $CD8^+$ T cell priming. PLoS One, 2015. 10(11): e0143539.
13. Jiang L, Lee VJ, Lim WY, Chen MI, Chen Y, Tan L, Lin RT, Leo YS, **Barr I** and Cook AR. Performance of case definitions for influenza surveillance. Euro Surveill, 2015. 20(22).
14. **Laurie KL**, Engelhardt OG, Wood J, Heath A, Katz JM, Peiris M, Hoschler K, Hungnes O, Zhang W and Van Kerkhove MD. International laboratory comparison of influenza microneutralization assays for A(H1N1)pdm09, A(H3N2) and A(H5N1) influenza viruses by CONSISE. Clin Vaccine Immunol, 2015. 22(8): 957-64.
15. **Laurie KL**, **Guarnaccia TA**, **Carolan LA**, Yan AW, **Aban M**, Petrie S, Cao P, Heffernan JM, McVernon J, Mosse J, **Kelso A**, McCaw JM and **Barr IG**. Interval between infections and viral hierarchy are determinants of viral interference following influenza virus infection in a ferret model. J Infect Dis, 2015. 212(11): 1701-10.
16. Lee HK, Tang JW, Loh TP, **Hurt AC**, Oon LL and Koay ES. Molecular surveillance of antiviral drug resistance of influenza A/H3N2 virus in Singapore, 2009-2013. PLoS One, 2015. 10(1): e0117822.
17. **Little K**, **Leang SK**, **Butler J**, **Baas C**, Harrower B, Mosse J, **Barr IG** and **Hurt AC**. Zanamivir-resistant influenza viruses with Q136K or Q136R neuraminidase residue mutations can arise during MDCK cell culture creating challenges for antiviral susceptibility monitoring. Euro Surveill, 2015. 20(45).
18. Londrigan SL, Tate MD, Job ER, Moffat JM, Wakim LM, Gonelli CA, Purcell DF, Brooks AG, Villadangos JA, **Reading PC** and Mintern JD. Endogenous murine BST-2/Tetherin is not a major restriction factor of influenza A virus infection. PLoS One, 2015. 10(11): e0142925.
19. Mohr PG, **Deng YM** and McKimm-Breschkin JL. The neuraminidases of MDCK grown human influenza A(H3N2) viruses isolated since 1994 can demonstrate receptor binding. Virol J, 2015. 12: 67.
20. 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, 2015. 90(1): 206-21.
21. **Oh DY**, **Barr IG** and **Hurt AC**. A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity. PLoS One, 2015. 10(3): e0118780.
22. **Panozzo J**, **Oh DY**, Margo K, Morton DA, Piedrafita D, Mosse J and **Hurt AC**. Evaluation of a dry powder delivery system for laninamivir in a ferret model of influenza infection. Antiviral Res, 2015. 120: 66-71.
23. Patterson Ross Z, **Komadina N**, **Deng YM**, **Spirason N**, Kelly HA, **Sullivan SG**, **Barr IG** and Holmes EC. Inter-seasonal influenza is characterized by extended virus transmission and persistence. PLoS Pathog, 2015. 11(6): e1004991.
24. Proudfoot O, Esparon S, Tang CK, **Laurie K**, **Barr I** and Pietersz G. Mannan adjuvants intranasally administered inactivated influenza virus in mice rendering low doses inductive of strong serum IgG and IgA in the lung. BMC Infect Dis, 2015. 15(1): 101.
25. Su YC, Bahl J, Joseph U, Butt KM, **Peck HA**, Koay ES, Oon LL, **Barr IG**, Vijaykrishna D and Smith GJ. Phylodynamics of H1N1/2009 influenza reveals the transition from host adaptation to immune-driven selection. Nat Commun, 2015. 6: 7952.
26. **Sullivan SG**, **Chow MK**, **Barr IG** and **Kelso A**. Influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza annual report, 2014. Commun Dis Intell, 2015. 39(4): E854-E593.
27. **Sullivan SG** and Cowling BJ. "Crude Vaccine Effectiveness" is a misleading term in test-negative studies of influenza vaccine effectiveness. Epidemiology, 2015. 26(5): e60.
28. Takashita E, Meijer A, Lackenby A, Gubareva L, Rebelo-de-Andrade H, Besselaar T, Fry A, Gregory V, **Leang SK**, Huang W, Lo J, Pereyaslov D, Siqueira MM, Wang D, Mak GC, Zhang W, Daniels RS, **Hurt AC** and Tashiro M. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013-2014. Antiviral Res, 2015. 117: 27-38.
29. Vijaykrishna D, Holmes EC, Joseph U, Fourment M, Su YC, Halpin R, Lee RT, **Deng YM**, Gunalan V, Lin X, Stockwell TB, Fedorova NB, Zhou B, **Spirason N**, Kuhnert D, Boskova V, Stadler T, Costa AM, Dwyer DE, Huang QS, Jennings LC, Rawlinson W, **Sullivan SG**, **Hurt AC**, Maurer-Stroh S, Wentworth DE, Smith GJ and **Barr I**. The contrasting phylodynamics of human influenza B viruses. Elife, 2015. 4.
30. Wang J, Moore NE, **Deng YM**, Eccles DA and Hall RJ. MinION nanopore sequencing of an influenza genome. Front Microbiol, 2015. 6: 766.

Presentations

Centre staff members presented talks and posters at numerous events during 2015, including national and international conferences, WHO meetings, government advisory meetings, educational lectures and research seminars.

ORAL PRESENTATIONS

Event; Location, date	Speaker, *Title(s)*
Influenza Specialist Group Annual Scientific Meeting; Melbourne, 1–2 February 2015	Ian Barr: *An update on the 2014 and 2014–5 influenza seasons.*
	Sheena Sullivan: *Vaccine effectiveness – assessing pros & cons of the "case negative" approach.*
Lecture in Medical Microbiology to 3rd year Applied Science students, RMIT University; Melbourne, 7 April 2015	Patrick Reading: *Laboratory-based surveillance of influenza virus in the Western Pacific region.*
Lecture to 3rd year students, University of Melbourne Breadth Subject "Global health, security and sustainability"; Melbourne, 13 April	Anne Kelso: *Influenza.*
David Danks Seminar, Murdoch Childrens Research Institute; Melbourne, 14 April	Anne Kelso: *Influenza, a virus for our times.*
3rd International Symposium on Neglected Influenza Viruses; Athens GA, USA, 15–17 April	Karen Laurie: *The time-interval between infections and viral hierarchies are determinants of viral interference following influenza virus infection in a ferret model.*
Doherty Institute seminar; Melbourne, 16 April	Anne Kelso: *Reflections on influenza.*
St Jude Children's Research Hospital; Memphis TN, USA, 20–21 April	Karen Laurie: *Investigating viral interference using the ferret model of influenza.*
Viruses in May 2015; Katoomba, NSW, 30 April – 2 May	Aeron Hurt: *Influenza viruses in Antarctica.*
Student lecture, Specialist Certificate in Travel Medicine, The University of Melbourne; Melbourne, 6 May	Ian Barr: *Influenza vaccines for the traveler.*
isirv Workshop on Next Generation Sequencing of Viruses; Paris, France, 20–21 May	Aeron Hurt: *Use of Next Generation Sequencing for the detection of antiviral resistant influenza viruses.*
National Institute for Medical Research London, UK, 27–29 May	Iwona Buettner: *Influenza surveillance and research at the WHO Collaborating Centre.*
Innovations in Genetic Solutions World Tour symposium Melbourne, 1 June	Yi-Mo Deng: *Full genome sequencing of influenza A virus using Ion Torrent™ Personal Genome Machine.*
Seminar, Department of Pharmacology, Monash University; Melbourne, 1 June	Patrick Reading: *Understanding innate immunity to influenza and other respiratory viruses.*
2nd Asia-Pacific Influenza Summit; Hanoi, Vietnam, 10–11 June	Aeron Hurt: *Antiviral resistance surveillance: a global program.*
Novartis cell CRADA face-to-face meeting; Holly Springs NC, USA, 22–26 June	Ian Barr: *Generating a WHO approved influenza CVV (candidate virus vaccine).*
	Heidi Peck: *NVD-WHO CC Melbourne cell culture CRADA, Background and procedures.*

ORAL PRESENTATIONS (continued)

Event; Location, date	Speaker, *Title(s)*
8th WHO working group meeting on RT-PCR for the detection and subtyping of influenza viruses; Geneva, Switzerland, 23–24 June	Yi-Mo Deng: *Update of molecular surveillance work at the WHO CC Melbourne in 2014–2015.*
5th meeting of the WHO expert working group for GISRS on surveillance of antiviral susceptibility of influenza viruses; Geneva, Switzerland, 25–26 June	Aeron Hurt: *Update of the status of neuraminidase inhibition susceptibility of circulating viruses including A (H5N1) and A(H7N9): Oceania and Pacific; From antiviral susceptibility surveillance to policies, including stockpiling; GISRS surveillance capacity (2014 NIC survey).*
Institut Pasteur; Ho Chi Minh City, Vietnam, 29 June – 2 July	Patrick Reading: *Reference laboratories at VIDRL; Influenza surveillance and research at the WHO Collaborating Centre in Melbourne; Laboratory techniques for detection and characterization of influenza; Cell culture and virus isolation for influenza viruses.*
National Institute of Hygiene and Epidemiology; Hanoi, Vietnam, 3 July	Patrick Reading: *Influenza surveillance and research at the WHO Collaborating Centre in Melbourne.*
Australian Society for Microbiology Annual Scientific Meeting; Canberra, 12–15 July	Karen Laurie: *The time-interval between infections and viral hierarchies are determinants of viral interference following influenza virus infection in a ferret model.*
Pacific Public Health and Surveillance Network (PPHSN) Regional LabNet/EpiNet Meeting; Nadi, Fiji, 20–22 July	Patrick Reading: *The role of the WHO CC for Influenza in supporting Pacific Island Countries and Territories.*
Estimating Burden of Influenza Disease in Lao PDR workshop; Vientiane, Laos, 27–31 July	Vivian Leung: *Examining seasonal patterns of influenza part 2; Estimating burden of influenza in risk groups.*
Lecture to 3rd year Immunology students, Monash University; Melbourne, 31 July	Ian Barr: *Influenza; A disease worth controlling.*
Research group meeting in the Department of Microbiology and Immunology, The University of Melbourne; Melbourne, 11 August	Ding Yuan Thomas Oh: *Novel method to measure activity level of influenza infected ferrets.*
9th Bi-Regional Meeting of WHO National Influenza Centres of the Western Pacific and South East Asian Regions; Phnom Penh, Cambodia, 18–21 August	Ian Barr: *Influenza activity in the Southern Hemisphere in 2015.* Yi-Mo Deng: *Next generation sequencing for avian and zoonotic influenza viruses.*
1st International Meeting on Respiratory Pathogens; Singapore, 2–4 September	Aeron Hurt: *Influenza antivirals - resistance and new compounds.* (Invited speaker)
National Avian Influenza Wild Bird Steering Group meeting; Melbourne, 9–10 September	Heidi Peck: *Update on avian influenza virus detection in Antarctica.*
Parkville Community Leaders Breakfast; Melbourne, 15 September	Ian Barr: *Are we prepared for a pandemic? A perspective from the inside.*

ORAL PRESENTATIONS (continued)	
Event; Location, date	Speaker, *Title(s)*
7th Orthomyxovirus Research Conference; Toulouse, France, 16–18 September	Karen Laurie: *The time-interval between infections and viral hierarchies are determinants of viral interference following influenza virus infection in a ferret model.* (Received award for the best oral presentation)
11th Australian Influenza Symposium; Geelong, VIC, 12–13 October	Ding Yuan Thomas Oh: *Using the ferret as an animal model for investigating influenza antiviral effectiveness.*
NHMRC Program on "Limiting the Impact of Influenza" retreat; Melbourne, 26 October	Karen Laurie: *Standardising serological data across laboratories.*
Virology 2015: The Viral Horzon – Presenting Paths to the Future; Melbourne, 2 November	Patrick Reading: *Sensing and responding to respiratory virus infection.*
Association of Biosafety for Australia and New Zealand 5th Annual Conference; Canberra, 9–13 November	Aeron Hurt: *What flu virus will cause the next pandemic?* (Invited speaker)
University of Melbourne, 2nd year subject, Veterinary Microbiology; Melbourne, 9 November	Patrick Reading: *WHO and influenza surveillance.*
5th International Symposium on Infectious Disease and Signal Transduction; Tainan, Taiwan, 14–15 November	Ian Barr: *Influenza B - The Forgotten One.*
Centers for Disease Control, Taiwan; Taipei, Taiwan, 16 November	Ian Barr: *Influenza surveillance and research at the WHO Collaborating Centre in Melbourne.*
Training workshop on Influenza Laboratory Surveillance Techniques; Beijing, China, 16–20 November	Yi-Mo Deng: *Sequencing of influenza viruses.*
4th WHO Consultation on Improving Influenza Vaccine Virus Selection; Hong Kong SAR, China, 18–20 November	Ian Barr: *Overview of public health use of influenza virus genetic sequence information and role of NGS.* Sheena Sullivan: *Vaccine effectiveness and its potential contribution to vaccine virus selection.*
GISAID-ISIRV Workshop on Genetic Analyses of Influenza Viruses; Hong Kong SAR, China, 21–22 November	Naomi Komadina: *GISAID - sharing and analysis of sequence data; Searching and Analysis of Data GISAID EpiFlu™ Database.*
Ivanhoe U3A Community Group Annual forum; Melbourne, 23 November	Ian Barr: *Viruses and you.*
National Influenza Surveillance Committee meeting; Canberra, 24–25 November	Ian Barr: *WHO CCRRI Melbourne; 2015 report.*
Fiji National Influenza Surveillance Meeting; Suva, Fiji, 25–26 November	Patrick Reading: *Influenza viruses of the Pacific Region.*
Australian Respiratory Virus Meeting; Canberra, 4–5 December	Patrick Reading: *Approaches to defining receptors for respiratory virus infection of host cells.*

POSTER PRESENTATIONS

Event; Location, date	Title and authors *(presentations are posters unless otherwise indicated, Centre authors are marked in bold, presenting author is underlined)*
Fourth isirv Antiviral Group Conference - Novel Antiviral Therapies for Influenza and Other Respiratory Viruses: Bench to Bedside; Austin TX, USA, 2–4 June	A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity. **Oh DY**, **Barr IG** and **Hurt AC** Evaluation of dry powder insufflator as a delivery system for laninamivir in a ferret model of influenza infection. **Panozzo J**, **Oh DY**, Margo K, Morton DA, Piedrafita D, Mosse J and **Hurt AC**
10th Asia-Pacific Medical Virology Conference; Taipei, Taiwan, 15–18 October	Comparison of immunogenic peptides from an unusual H1N2 human variant against historical sequences. **Komadina N**, Quiñones-Parra S, Kedziereska K, McCaw J, Leder K and McVernon J
Inaugural MDHS ECR Network Symposium; Melbourne, 24 November	A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity. **Oh DY**, **Barr IG** and **Hurt AC**
Epidemics5 - Fifth International Conference on Infectious Disease Dynamics; Clearwater Beach FL, USA, 1–4 December	Characterizing antibody kinetics from multiple infection and vaccination events. Hay, JA, **Laurie KL** and Riley S
8th Australian Virology Society Meeting; Hunter Valley, NSW, 6 –9 December	Attachment factors, entry receptors and pathways for internalization of respiratory viruses. Gillespie L, Ng WC, Londrigan SL, Brooks AG and **Reading PC** Characteristics of currently circulating human A(H3N2) influenza viruses with low HA titres. **Lau H**, **Deng YM** and **Barr IG** Assessment of RNA*Sound* RNA card for the preservation of influenza virus RNA. **Lau H** and **Hurt AC**

Engagement in WHO Activities

Event; Location, Date	Centre staff involved
WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2015-2016; Geneva, Switzerland, 23–26 February	Anne Kelso and Ian Barr participated.
WHO project on Seasonal Influenza Vaccine Composition for the Tropics - experts meeting; Geneva, Switzerland, 23–24 April	Ian Barr participated.
WHO Regional Workshop on Implementation of the Pandemic Influenza Preparedness Framework in the South-East Asia Region; Jakarta, Indonesia, 27 April	Patrick Reading participated.
8th WHO working group meeting on RT-PCR for the detection and subtyping of influenza viruses; Geneva, Switzerland, 23–24 June	Yi-Mo Deng chaired the meeting and presented a talk.
5th meeting of the WHO expert working group for GISRS on surveillance of antiviral susceptibility of influenza viruses; Geneva, Switzerland, 25–26 June	Aeron Hurt presented three talks and chaired a session.
WHO informal consultation on influenza vaccine response during the start of a pandemic; Geneva, Switzerland, 29 June – 1 July	Ian Barr participated.
9th Bi-Regional Meeting of WHO National Influenza Centres of the Western Pacific and South East Asian Regions; Phnom Penh, Cambodia, 18–21 August	Sheena Sullivan chaired a breakout session and moderated a feedback session. Ian Barr and Yi-Mo Deng presented talks. Naomi Komadina, Robert Shaw and Vivian Leung attended.
WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2016; Memphis TN, USA, 21–23 September	Aeron Hurt and Ian Barr participated. Sheena Sullivan presented the Global Influenza Vaccine Effectiveness report by teleconference.
4th WHO Consultation on Improving Influenza Vaccine Virus Selection; Hong Kong SAR, China, 18–20 November	Ian Barr was a panel discussion participant and presented a talk. Sheena Sullivan presented a talk.
3rd WHO Technical Consultation on the Burden of Influenza Disease; Geneva, Switzerland, 8–10 December	Sheena Sullivan participated.

9th Bi-Regional Meeting of WHO National Influenza Centres of the Western Pacific and South East Asian Regions, photos courtesy of WPRO.

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
Influenza Specialist Group Annual Scientific Meeting; Melbourne, 1–2 February	Aeron Hurt attended.
Laboratory Working Group meeting with WHO, US CDC, US AID, FAO and other regional representatives; Jakarta, Indonesia, 21 April	Patrick Reading attended.
Viruses in May 2015; Katoomba, NSW, 30 April – 2 May	Louise Carolan attended.
2nd Asia-Pacific Antiviral Forum; Hanoi, Vietnam, 11 June	Aeron Hurt participated.
isirv Workshop on Next Generation Sequencing of Viruses; Paris, France, 20–21 May	Yi-Mo Deng attended.
Fourth isirv Antiviral Group Conference - Novel Antiviral Therapies for Influenza and Other Respiratory Viruses: Bench to Bedside; Austin TX, USA, 2–4 June	Aeron Hurt chaired a session.
I-MOVE Annual meeting; Annecy, France, 14–16 July	Sheena Sullivan led a discussion session: Proposal of criteria to include studies in the GIVE report.
Applied Genomics Symposium - Embracing the Genomic Revolution; Melbourne, 20 July	Karen Laurie attended.
1st International Meeting on Respiratory Pathogens; Singapore, 2–4 September	Ian Barr attended.
National Avian Influenza Wild Bird Steering Group meeting; Melbourne, 9–10 September	Aeron Hurt attended.
7th Orthomyxovirus Research Conference; Toulouse, France, 16–18 September	Karen Laurie co-chaired a session.
10th Asia-Pacific Medical Virology Conference; Taipei, Taiwan, 15–18 October	Naomi Komadina attended.
Fiji National Influenza Surveillance Meeting; Suva, Fiji, 25–26 November	Patrick Reading and Sheena Sullivan each facilitated a group discussion.
Australian Respiratory Virus Meeting; Canberra, 4–5 December	Patrick Reading was part of the organising committee and chaired two sessions.

Website

The Centre website was maintained and updated throughout the year. During 2015, the website was viewed by 12,384 unique visitors from 153 different countries. The majority of visits to the website came from Australia, followed by the USA. Most of the traffic to the website came from search engines.

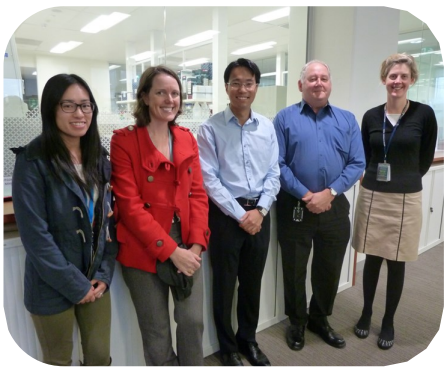
Visitors to the Centre

The Centre was pleased to host the following visitors during 2014:

Date	Visitor and affiliation
10 February	Prof Ian Gust, former Centre Director
11 February	Staff from Brawijaya University: Dr Sri Andarini (Incoming dean of the Faculty of Medicine), Professor Karyono Mintaroem (Dean of the Faculty of Medicine), Professor A.S. Noorhamdani (Head of the Department of Microbiology), Professor Sanarto (Department of Microbiology), Dr Retty Retinati (Head of the International Office of the Faculty of Medicine), Brawijaya University, Malang, Indonesia
13 February	Dr Heather Wilson, Microbiology registrar, Canberra Hospital, Canberra; *VIDRL visiting registrar program - overview of influenza surveillance processes*
16–20 March	Dr Thomas Rowe (Centers for Disease Control and Prevention, Bethesda MD, USA) and Dr Mark Tompkins (University of Georgia, Athens GA, USA)*; visit to investigate and learn more about the Tecan EVO 200 liquid handling robot for HI assays.*
28 April	Dr Vernon Lee, Head, Singapore Armed Forces' Biodefence Center, Singapore Armed Forces' Biodefence Center, National University of Singapore, Singapore
25–26 May	Dr Vijay Dhanasekaren, Program of Emerging Infectious Diseases, Duke-NUS Graduate Medical School, Singapore; *research collaborator*
7 July	Dr Jacqueline Katz, Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta GA, USA
21 July	Master of Veterinary Public Health (Emergency Animal Diseases) students, accompanied by Dr Simon Firestone, The University of Melbourne, Melbourne
29 July	Dr George Gao, Deputy Director-General, China CDC, Beijing, China
26 August	Mr Adam Bandt, Federal member for Melbourne, Melbourne
14–16 October	Dr Tristan Gibbs, Virology Fellowship clinician, PathWest Laboratory Medicine WA, Perth; *visit of laboratory and basic training in influenza surveillance techniques (serology, molecular analysis, antiviral resistance, egg culture, epidemiology)*
23 October	Prof Larisa Rudenko, Head, Virology Department, Institute of Experimental Medicine, St Petersburg, Russia; *research collaborator*
16–20 November	Mrs Amelia Buys, National Institute for Communicable Diseases, Johannesburg, South Africa; *overview of processes at the Centre and Tecan robot*
16–27 November	Dr Kirsty Short, University of Queensland, Brisbane; *research collaborator*

L to R: Ian Barr, Anne Kelso and Ian Gust

L to R: Robert Shaw, Thomas Rowe, Mark Tompkins and Iwona Buettner



L to R: Vivian Leung, Sheena Sullivan, Vernon Lee, Ian Barr and Karen Laurie

L to R: Louise Carolan, Aeron Hurt, Larisa Rudenko and Karen Laurie

Australian Influenza Symposium

The 11th Australian Influenza Symposium was held at Deakin University Geelong Waterfront Campus on 12–13 October 2015, and was attended by almost 200 delegates from Australia, China, Hong Kong, New Zealand, Singapore, Thailand, United Kingdom and the United States, including five invited international speakers:

Eduardo Azziz-Baumgartner, Centers for Disease Control and Prevention, Atlanta GA, USA
Philippe Buchy, GlaxoSmithKline, Singapore
Filip Claes, Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations (FAO) Regional Office for Asia and the Pacific, Bangkok, Thailand
David Salisbury, ex-Department of Health, London, UK
Erica Spackman, US National Poultry Research Center, Athens GA, USA

With a major theme of avian-animal influenza viruses and zoonotic infections, several talks were presented on the spread and investigation of avian and animal influenzas indifferent parts of the world. A roundtable discussion also considered the potential threat of zoonotic infections and the surveillance systems in place to detect potential pandemic viruses in birds and animals. A variety of other themes were also explored in other presentations, including surveillance in the American Tropics, vaccination policies, epidemiological studies, influenza biology, antiviral drugs and vaccines, computer models of infection, and surveillance systems.

The organising committee for the symposium was Ian Barr, Sue Lowther (Australian Animal Health Laboratory, CSIRO) and Jayde Simpson. Almost all staff members from the Centre attended the symposium. Aeron Hurt was a roundtable discussion panelist and Ding Yuan Thomas Oh presented a talk. Ian Barr and Sheena Sullivan each chaired a plenary session and Karen Laurie co-chaired a workshop.

Committees and Advisory Groups

Centre staff members served on the following governing boards, committees and advisory groups during 2015.

Chantal Baas
Doherty Institute, *Shared PC3 Laboratory Advisory Committee*

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 (from April 2015)*
Influenza Research and Treatment, *Editorial Board*
Influenza and Other Respiratory Viruses, *Associate Editor*
National Influenza Surveillance Committee (Department of Health) (from April 2015)
Public Health Laboratory Network (Department of Health)
WHO/OIE/FAO H5N1 Evolution Working Group (from April 2015)

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*

Anne Kelso
Australian Technical Advisory Group on Immunisation (Department of Health), *Influenza Working Party* (*until April 2015*)
BioMed Central Immunology, *Editorial Advisor*
Burnet Institute, *Research Advisory Committee* (*until March 2015)*
Doherty Institute, *Operational Management Committee, Leadership Group* (*until April 2015*)
Florey Institute of Neuroscience and Mental Health, Board, *Council of Governors and Nomination Committee* (*until March 2015*)
Influenza Pandemic Planning Steering Committee (Victorian Dept of Health) (*until April 2015*)
Influenza Surveillance Strategy Working Group/National Influenza Surveillance Committee (Department of Health) (*until April 2015*)
International Immunology, *Associate Editor (until March 2015*)
International Society for Influenza and other Respiratory Virus Diseases, *Board of Trustees*
National Health and Medical Research Council, *Council* (*until March 2015*)
Telethon Institute for Child Health Research, *Board* (*until March 2015*)
WHO/OIE/FAO H5N1 Evolution Working Group (*until April 2015*)

Katie Milne
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*

Committees and Advisory Groups (continued)

Patrick Reading	Australian Respiratory Virus Meeting*, Organising Committee*
	Influenza and Other Respiratory Viruses, *Editorial Board*
Jayde Simpson	Victorian Infectious Disease Reference Laboratory, *NATA Action Group (from Oct 2015)*
Sheena Sullivan	National Influenza Surveillance Committee (Department of Health)*, Observer*
Danielle Tilmanis	Victorian Infectious Disease Reference Laboratory, *NATA Action Group ((from Oct 2015)*

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

- Interview with Channel 10 news, 6 March
- "You can now protect against more strains of flu but it will cost you", http://www.news.com.au,2 April;
- http://www.news.com.au/lifestyle/health/you-can-now-protect-against-more-strains-of-flu-but-it-will-cost-you/story-fneuz9ev-1227289663302;
- International Day of Immunology and conducted tours of the Centre laboratories, 29 April
- Interview with Richard Stubbs on ABC Radio 774, 26 May
- "Winter, flu go hand-in-hand", Canberra Times (Ask Fuzzy section), 2 June
- "Why is EVERYBODY sick? Deadly flu strain H3N2 hits Australia with doctors predicting deaths and record numbers to fall ill this winter (but here's how you can beat it)", Daily Mail, 5 June; http://www.dailymail.co.uk/news/article-3111789/There-hospital-admissions-deaths-Deadly-flu-strain-H3N2-hits-Australia-doctors-predicting-record-numbers-fall-ill-winter.html#ixzz3cWqbwtkB
- "Flu cases tracking at record levels", The Age, published 5 June; http://www.theage.com.au/national/health/flu-cases-tracking-at-record-levels-20150605-ghhn9k.html
- "Fighting flu as it travels", Herald Sun, 9 June
- Daily Mail article, 8 July; http://www.dailymail.co.uk/news/article-3152988/More-14-000-Australians-diagnosed-flu-record-outbreak-doctors-urge-people-free-vaccine-shot.html
- "Gwynneville GP concerned about new flu strain", Illawarra Mercury, 8 July; http://www.illawarramercury.com.au/story/3198708/gwynneville-gp-concerned-about-new-flu-strain/
- "Universal flu vaccine on the horizon after successful animal tests", The World Today (ABC News) program segment, 25 August; http://www.abc.net.au/worldtoday/content/2015/s4299672.htm
- Presented a talk at the Ivanhoe U3A Community Group Annual forum, 23 November

Aeron Hurt

- ABC TV program Catalyst for a segment on Tamiflu, 3 March; http://www.abc.net.au/catalyst/stories/4190452.htm
- "The 2015 flu vaccine – what's new, who should get it and why", The Conversation, 25 March; https://theconversation.com/the-2015-flu-vaccine-whats-new-who-should-get-it-and-why-38500

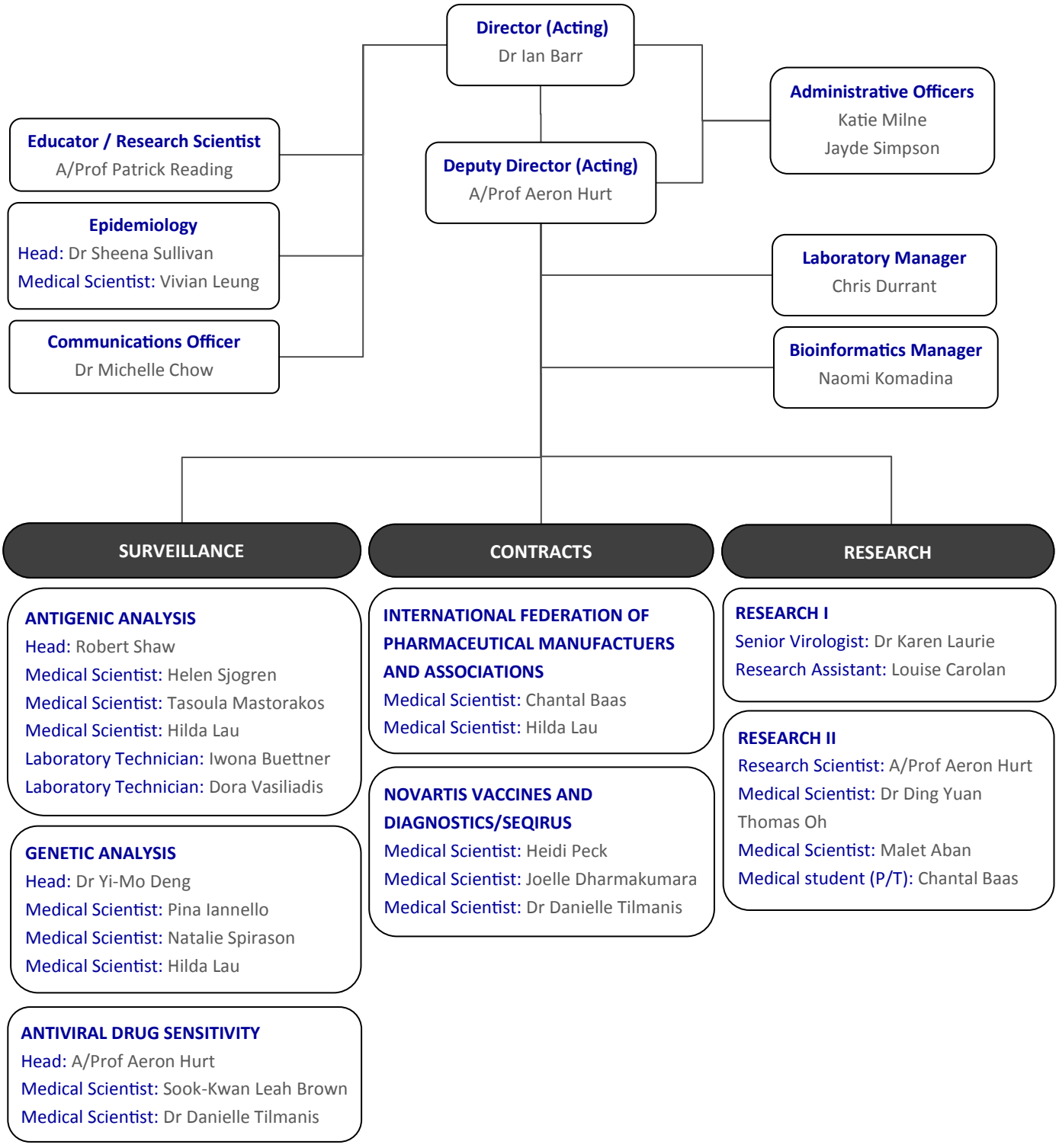
Anne Kelso

- "Flu fighters: WHO expert reveals story behind mismatched influenza vaccines in HK and world", South China Morning Post, 18 February; http://www.scmp.com/lifestyle/health/article/1717906/flu-fighters-who-expert-reveals-inside-story-mismatched-influenza
- "Calling the shots: how the WHO formulates vaccines for flu season", South China Morning Post, 19 February; http://www.scmp.com/lifestyle/health/article/1718817/calling-shots-how-who-formulates-vaccines-flu-season
- Walter and Eliza Hall Institute Discoveries for Humanity website, 25 March; http://discovery.wehi.edu.au/
- Interview with Franklin Women newsletter, April 2015 edition; http://franklinwomen.com.au/chat-professor-anne-kelso-nhmrc-ceo; https://franklinwomen.com.au/wp-content/uploads/2015/04/newsletter-april-2015.pdf;

Karen Laurie:

- Participated as a panellist in Experience Matters: Q&A Panel Biomedical Science Event (organised by Melbourne Careers Centre at the University of Melbourne), 21 August.

Management and Staff



Staff Changes 2015

Prof Anne Kelso resigned from her position as Centre Director in April 2015 — the process for appointing a new Director is under way. In the interim Dr Ian Barr is Acting Director of the Centre and A/Prof Aeron Hurt is Acting Deputy Director.

Mr Greg Waller left the Centre in April 2015.

Ms Dora Vasiliadis joined the Centre in July 2015 as a laboratory technician in the Antigenic Analysis group.