

WHO Collaborating Centre
for Reference and
Research on Influenza
VIDRL

Annual Report 2012

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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 2011-2015) are:

- i. 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;
- ii. 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;
- iii. to assist WHO in developing recommendations on viruses to be included in influenza vaccines;
- iv. 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;
- v. to collect epidemiological information on the prevalence of influenza, especially in countries and areas in the Region;
- vi. to undertake research to improve the detection, prevention and treatment of influenza; and
- vii. to assist WHO and national health authorities in developing and implementing plans for responding to pandemic influenza.

Governance

The Centre is supported by the Australian Government Department of Health and Ageing through a funding agreement between the Commonwealth and Melbourne Health, and reports directly to the Department as well as to WHO. An Australian Government Advisory Committee (AGAC) reviews the Centre's work program and progress, provides advice to assist the Centre and the Commonwealth with its objectives under the work program, and monitors and advises on the scientific performance and direction of the Centre.

AUSTRALIAN GOVERNMENT ADVISORY COMMITTEE 2012

Prof Chris Baggoley, Chair (Commonwealth Chief Medical Officer)

Dr Gary Lum AM, Deputy Chair (Assistant Secretary, Health Emergency Management Branch)

Prof Michael Richards (Director, Victorian Infectious Diseases Service, Royal Melbourne Hospital)

Prof Peter Doherty AC FAA FRS (Laureate Professor, Department of Microbiology and Immunology, The University of Melbourne)

Prof John Horvath AO (Principal Medical Consultant for the Department of Health and Ageing)

Prof John Mackenzie AO (Professor of Tropical Infectious Diseases, Curtin University of Technology)

Dr Greg Stewart (Director Operations, Ambulatory and Primary Health Care, South East Sydney Local Health District)

Dr Heather Wellington (Consultant, Health Law Team, DLA Piper Australia)

Dr Martyn Jeggo, observer (Director, Australian Animal Health Laboratory, CSIRO)

Dr Mike Catton (Director, Victorian Infectious Diseases Reference Laboratory)

Prof Anne Kelso AO (Director of the Centre)

Highlights of 2012

Twenty years as a WHO Collaborating Centre

2012 marks 20 years since the Centre was first designated as a WHO Collaborating Centre for Reference and Research on Influenza. To reflect on this anniversary, a special session was held during the 8th Australian Influenza Symposium, which was attended by all four present and former Directors and Deputy Directors of the Centre (*pictured at right*).



L to R: Ian Barr, Ian Gust, Anne Kelso, Alan Hampson

Surveillance

The Centre received and processed 4266 samples from 13 countries during 2012. A total of 4069 samples were analysed, of which 46% were subtyped as A(H3N2).



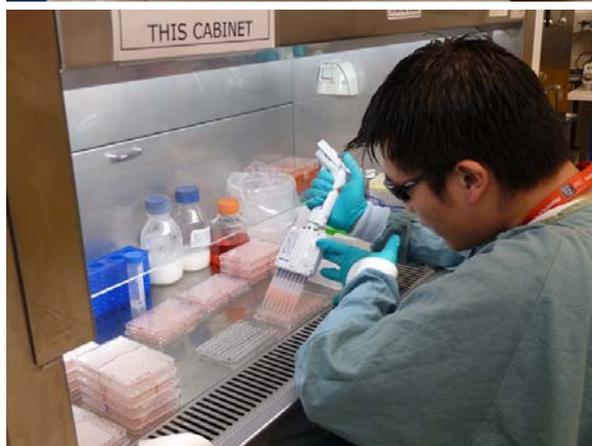
Research

The Centre's research interests continued to expand during 2012, with the initiation of several new collaborative projects, including participation in the Consortium for the Standardisation of Influenza Seroepidemiology (CONSISE) and large scale analysis of the influenza B genome. A Centre staff member was also recognised for his contribution to Life Sciences and Biological Sciences research in Australasia.



Publications

Centre staff members were authors on 28 publications in 2012, including 21 original research and surveillance papers.



Director's Report

It is a pleasure to present the 2012 Annual Report of the WHO Collaborating Centre for Reference and Research on Influenza at VIDRL. During 2012 we celebrated the 20th anniversary of the Centre's designation as a WHO Collaborating Centre for Reference and Research on Influenza. The occasion gave us an opportunity to reflect on the growth and progress of the Centre over the years, and a special retrospective session was held during the Australian Influenza Symposium in October to commemorate the anniversary.

The Centre's surveillance activities continued apace during 2012, with a similar number of samples received and analysed as in the previous two years following the pandemic of 2009. After three years in which the A(H1N1) pdm09 subtype was predominant, 2012 brought a sharp change in circulating viruses, with A(H3N2) viruses making up almost half of those characterised at the Centre with most of the remainder being type B viruses. The majority of viruses we analysed during 2012 were antigenically similar to those recommended by WHO for use in trivalent influenza vaccines in the 2012-2013 northern hemisphere winter and the 2013 southern hemisphere winter. We were also pleased to continue our support for international influenza surveillance activities by providing influenza typing reagents, training and advice to other laboratories and scientists around the world.

Research on influenza immunity, pathogenesis, antiviral drug susceptibility and analytical techniques is an integral part of the Centre's work, both drawing on and contributing to our core surveillance activities for WHO. During 2012, Centre staff engaged in several new national and international research collaborations, as well as continuing and reporting on projects from previous years. For example, Dr Karen Laurie is a founding member of the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE) established in 2012, a new international collaboration of immediate importance for analysing human population immunity to influenza viruses. Karen's participation in CONSISE builds on her involvement in seroprevalence studies in Australia during and after the 2009 pandemic and ensures that the Centre's work in this area is harmonised with that undertaken by our colleagues in other parts of the world.

We were very proud that Dr Aeron Hurt was runner-up in the Life Sciences and Biological Sciences category of the 2012 Scopus Young Researcher of the Year Award. These awards recognise outstanding young scientists and researchers in Australasia who have made significant contributions in their areas of research – in Aeron's case on understanding resistance of influenza viruses to the neuraminidase inhibitor class of antiviral drugs.

I would like to thank the many people who have supported the Centre and worked with us throughout 2012. Particular thanks are due to the WHO National Influenza Centres and other laboratories that submitted viruses to the Centre in 2012 and assisted us in other ways: they make a crucial contribution to WHO Global Influenza Surveillance and Response System and enable us to meet our commitments to the global monitoring of human influenza viruses and the updating of influenza vaccines. Our special thanks also go to the Commonwealth Chief Medical Officer Professor Chris Baggeley, other members of the Australian Government Advisory Committee, and our colleagues in the Office of Health Protection of the Australian Government Department of Health and Ageing for their counsel and support, and to Dr Mike Catton and colleagues at VIDRL and our many research collaborators for their interest and engagement with the Centre during 2012. Finally, once again I thank all of the staff and students of the Centre for their excellent work in 2012.

Professor Anne Kelso AO
Director



Celebrating 20 years of the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne

2012 marks the 20th anniversary of the designation of the Centre as a WHO Collaborating Centre for Reference and Research on Influenza.

The Centre was originally established in Melbourne at the Commonwealth Serum Laboratories (CSL), a Government body responsible for the production of human and animal health products for the Australian population. CSL was designated as a WHO Regional Influenza Centre in 1951. The Centre was initially directed by Dr W.J. O'Connor from 1951, then Mr Keith Harcourt from 1961, Dr Frank Warburton from 1964, Dr Brian Feery from 1974 and Mr Alan Hampson from 1978.

In 1952, to improve the detection and control of influenza epidemics and pandemics, the WHO Global Influenza Surveillance Network (now the Global Influenza Surveillance and Response System, or GISRS) was established to coordinate influenza surveillance activities between laboratories around the world. The US Centers for Disease Control and Prevention (CDC) in Atlanta and the World Influenza Centre at the National Institute for Medical Research (NIMR) in London were designated as WHO Collaborating Centres working on human influenza in 1956 and 1957, respectively.

In 1992, under the leadership of Professor Ian Gust as director and Alan Hampson as deputy director, the Centre at CSL was designated as a WHO Collaborating Centre for Reference and Research on Influenza, the third globally and the first in the southern hemisphere. The importance of Centre's role as a WHO Collaborating Centre became especially evident in 1998 when WHO started making formal recommendations on influenza vaccine composition for the Southern Hemisphere in addition to the Northern Hemisphere.

Centre staff on the last day located at the premises of CSL Limited in 2008.



Centre staff working in the laboratories at CSL Limited in 2001.

CSL continued to host the Centre after it was privatised as CSL Limited in 1994. In 2006, with additional Australian Government funding to establish the Centre with a new host organisation, responsibility for the Centre was passed to the Victorian Infectious Diseases Reference Laboratory (VIDRL), a department of Melbourne Health. Following Alan Hampson's retirement in September 2005, Dr Ian Barr was appointed as deputy director and then, with the transition to VIDRL, Ian Gust retired in 2006 and Professor Anne Kelso commenced as director in February 2007. In December 2008, the Centre left its premises at CSL Limited in Parkville and moved into purpose-built PC2 and PC3 (BSL2 and BSL3) facilities at VIDRL in North Melbourne, close to the Royal Melbourne Hospital and The University of Melbourne.

The past 20 years have brought many changes and advances at the Centre that have significantly increased its contribution to global efforts in influenza surveillance, updating of influenza vaccines and understanding of influenza virology and immunology. Routine surveillance



techniques have expanded from antigenic analysis alone to include genetic analysis since 1996 and screening of viruses for resistance to antiviral drugs since 2001. In keeping with global technological advances, the Centre has enjoyed significant equipment upgrades, automation of procedures and computerisation of data storage and analysis. This has enabled more reliable processing and documentation of significantly larger numbers of samples, as well as an increased repertoire of assays allowing more extensive analysis of circulating viruses. Technical developments have been complemented by substantial growth not only in the number of Centre staff (by approximately 5-fold in 20 years), but also in the breadth and depth of their skills.

In 2008 two collaborative agreements with industry partners were executed, one with the International Federation of Pharmaceutical Manufacturers and Associations and the other with Novartis Vaccines and Diagnostics. These agreements have enabled the Centre to engage additional scientists to contribute to global efforts to enhance the availability of candidate vaccine viruses isolated in eggs and mammalian cells for use in influenza vaccine manufacture.

One of the most challenging events in the Centre's recent history was the A(H1N1)pdm09 pandemic in 2009. The Centre was responsible for confirming some of the earliest cases of infection with the pandemic virus outside North America, submitted from New Zealand, as well as the first cases in Australia and some other countries in the Asia-Pacific region. In addition to handling a much larger number of samples than in previous years, in the early stages of the pandemic the Centre assisted other laboratories in the region by providing reagents and advice for detection of the new virus. Centre staff members also contributed to public health policy through government advisory committees and to community understanding of the pandemic through engagement with the media.

In addition to direct surveillance activities, the Centre has built its contribution to the global influenza surveillance and research community in other ways. The Centre has provided training and advice to scientists from WHO National Influenza Centres and other influenza laboratories for many years, and this capacity was greatly enhanced in 2008 by the appointment of a Centre Educator. In 2005, to honour Alan Hampson on his retirement as deputy director, the Centre and the Therapeutic Goods Administration of Australia co-hosted the first Australian Influenza Symposium. Now an annual event in its eighth year, the Symposium attracts a broad range of national and international delegates from surveillance, research, clinical, public health, government and industry sectors, fostering further connections and exchange of knowledge.

Besides bringing significant improvements to Centre facilities and capacity, the move to VIDRL in 2008 also placed the

Centre in closer proximity to other scientists working on influenza and infectious diseases at VIDRL and The University of Melbourne. This provided Centre staff with new opportunities for collaboration with researchers with expertise ranging from biomedicine to public health and clinical science. The strengthening of collaborative relationships since moving to VIDRL has contributed to the marked growth in the research profile of the Centre, as reflected in the increased number of Centre publications and presentations of Centre work at conferences and meetings. The number of staff involved in research and the diversity of research interests have increased, with two laboratory-based research groups and an epidemiologist at the Centre, and two other Centre staff having research laboratories in the Department of Microbiology and Immunology at The University of Melbourne.

From its beginnings in the 1950s, when its work was estimated to cost just £6,000, the Centre has received financial support from the Commonwealth Government. The stability that this long-term support has given the Centre over 60 years has played a crucial role in its continuing development and success, while the enhanced Commonwealth funding provided since 2005 underpinned the Centre's capacity to respond to the 2009 pandemic. Today, funding from the Australian Government Department of Health and Ageing not only enables the Centre to meet its core global and national functions but also leverages research funds from a variety of other national and international sources.

The next major event in the Centre's history will be its move in early 2014 to the newly established Peter Doherty Institute for Infection and Immunity, a partnership between Melbourne Health and The University of Melbourne. With access to a wider range of facilities and the new collaborative opportunities that will arise from co-location with other research, public health and clinical groups in the Institute, we look forward to further strengthening the Centre's contribution to global influenza surveillance and research for decades to come.



Centre staff at the staff retreat 2012.

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 come from the Asia-Pacific region. Twice a year (once each for the northern and southern hemispheres) WHO makes recommendations on suitable influenza strains to be included in the next seasonal vaccine based on data and advice from the five Collaborating Centres and other experts.

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, 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.

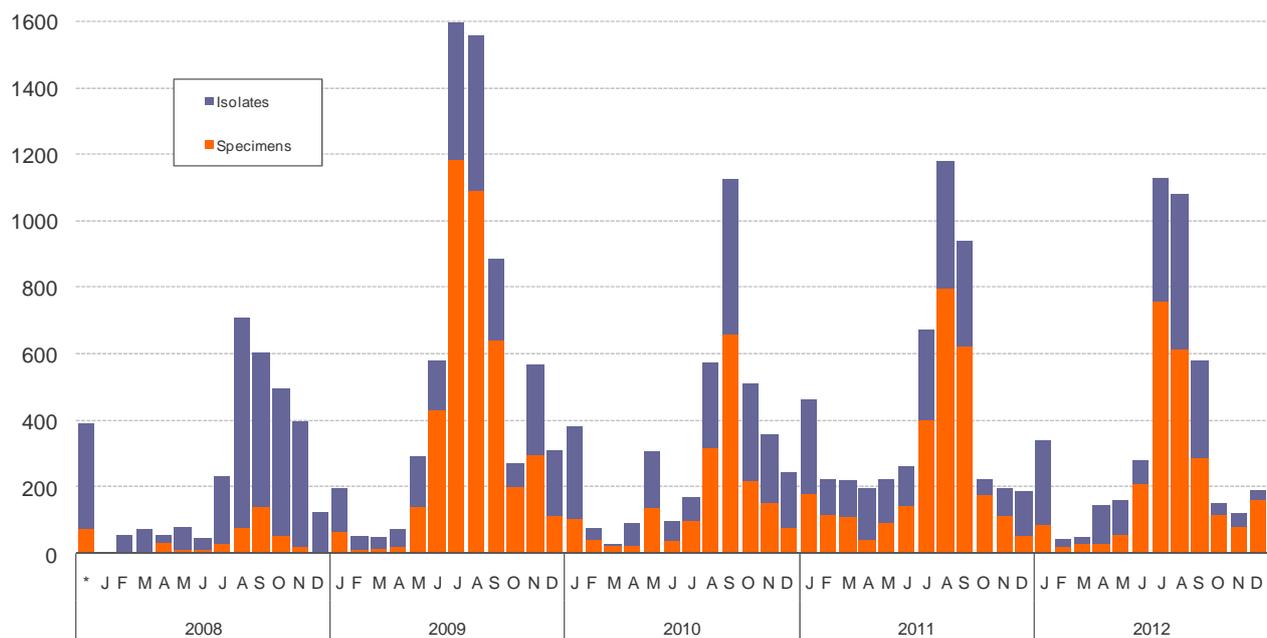
Receipt of Influenza Viruses

During 2012 the Centre received 4266 clinical specimens and virus isolates from 33 laboratories in 13 countries (Figures 1 and 2, Table 1). In total, 4069 (95%) samples were cultured and then analysed by haemagglutination inhibition (HI) assay and/or real-time reverse-transcription polymerase chain reaction (RT-PCR) reaction. For reporting purposes, subtypes and lineages are based on analysis of the HA and in some cases are confirmed by genetic analysis of NA. Of samples received by the Centre for which the age of the patient was known, most were taken from subjects younger than 5 years old (Figure 3).

Isolation of viruses

Original clinical specimens received by the Centre can be genetically analysed by sequencing or real-time RT-PCR and are also required for 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 and analysed at the Centre, 2008-2012.



* Samples received early in the indicated year but with sample dates from the preceding year. The method of recording receipt date was changed in 2009 to reflect more accurately the actual date that samples were received.

Figure 2. Geographic spread of influenza laboratories sending viruses to the Centre during 2012.

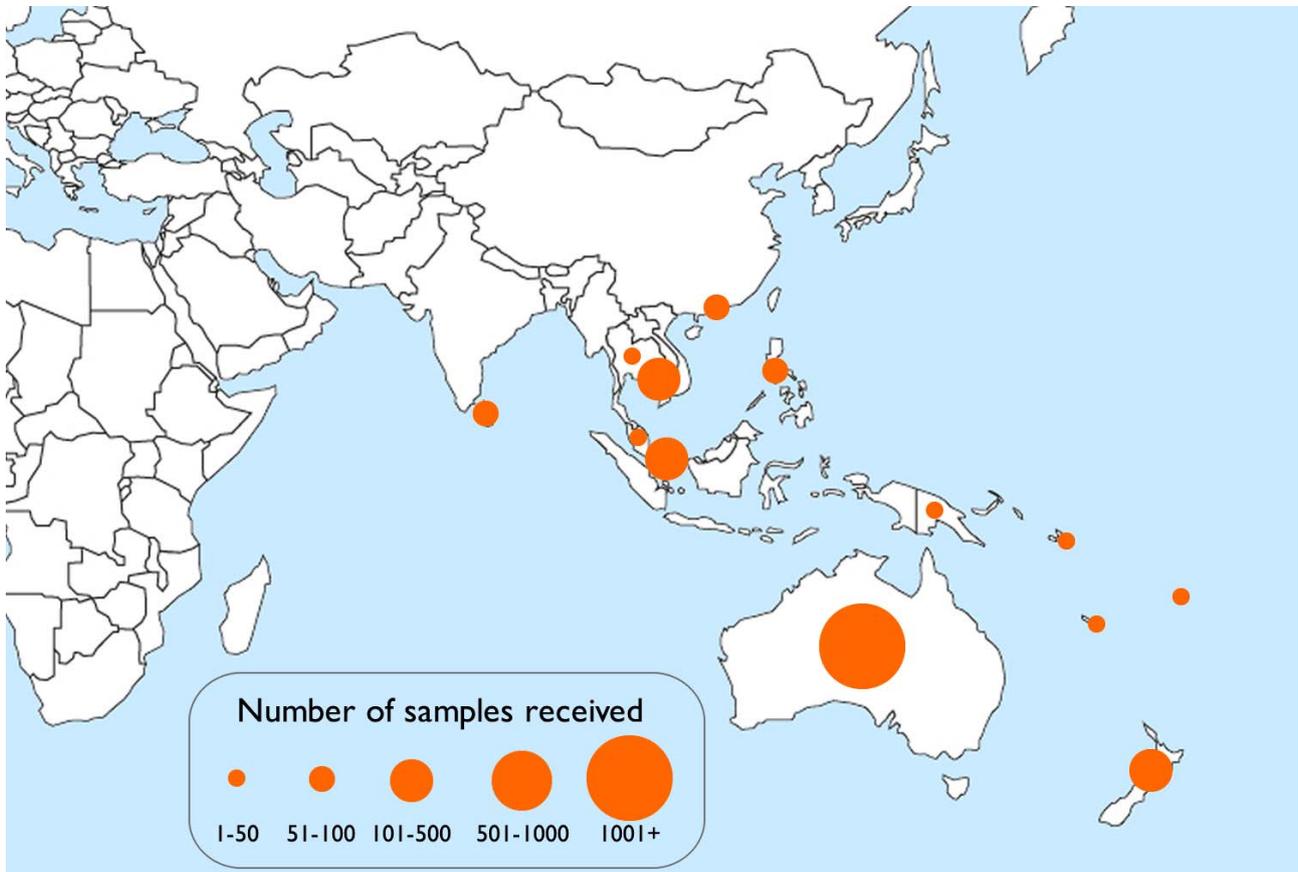


Figure 3. Age distribution of subjects from whom samples were received and the age is known at the Centre in 2012.

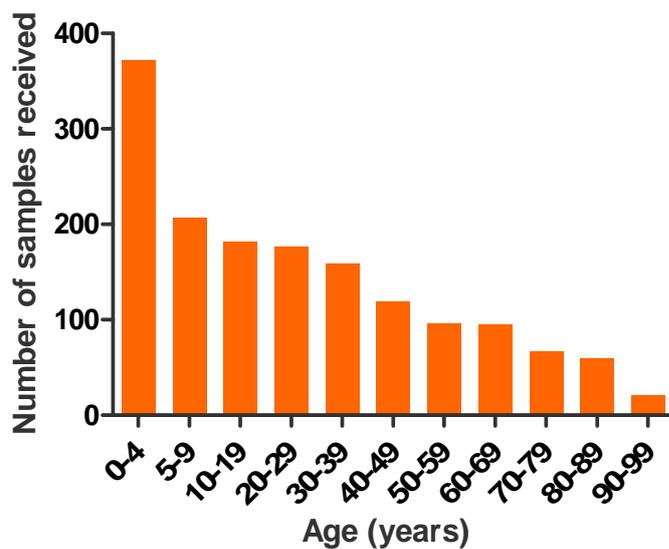


Table 1. Samples received at the Centre in 2012, by country, type and subtypelineage.

Country	Samples received		Samples tested	Samples tested by HI and/or RT-PCR assay						
	Specimens	Isolates		A(H1N1) pdm09	A(H3N2)	A(unsu- typed)	A mixed	B/ Victoria	B/ Yamagata	C
AUSTRALASIA	2226	1366	3396	186	1628	643	0	607	125	4
Australia	2166	1043	3013	99	1413	640	0	587	73	4
New Zealand	60	323	383	87	215	3	0	20	52	0
SOUTH PACIFIC	96	3	98	14	26	13	0	10	4	1
Fiji	29	3	32	6	8	2	0	8	1	1
New Caledonia	20	0	20	1	9	1	0	2	1	0
Papua New Guinea	29	0	29	7	9	9	0	0	1	0
Solomon Islands	18	0	17	0	0	1	0	0	1	0
SOUTH EAST ASIA	96	341	437	78	154	4	2	120	56	0
Cambodia	81	74	155	28	55	0	2	59	3	0
Malaysia	0	33	33	2	3	1	0	11	3	0
Philippines	2	74	76	4	41	3	0	5	23	0
Singapore	3	120	123	32	45	0	0	28	17	0
Thailand	10	40	50	12	10	0	0	17	10	0
EAST ASIA	0	70	70	0	27	0	0	30	12	0
Macau SAR	0	70	70	0	27	0	0	30	12	0
SOUTH ASIA	40	28	68	7	26	16	0	10	1	0
Sri Lanka	40	28	68	7	26	16	0	10	1	0
TOTAL	2458	1808	4069	285	1861	676	2	777	198	5

Antigenic Analysis of Influenza Isolates

Background

The antigenic properties of influenza viral isolates are analysed using the HI assay, in which viruses are tested for their ability to agglutinate red blood cells in the presence of ferret antisera previously raised against reference viruses. Subtypes are based on analysis of the HA and in some cases are confirmed by genetic analysis of NA.

Antigenic analyses 2012

A total of 3980 isolates that were received at the Centre in 2012 were cultured and isolated in MDCK cells, of which 2831 (71%) produced a positive result. The majority of viruses were A(H3N2) (59.1%), followed by B/Victoria lineage (27.1%) (Figure 4). The predominance of A(H3N2) and B/Victoria viruses was reflected in the distribution of isolates from all geographic regions (Figure 5).

Figure 4. Influenza sub/types and lineages of samples received in 2012 and analysed by HI assay.

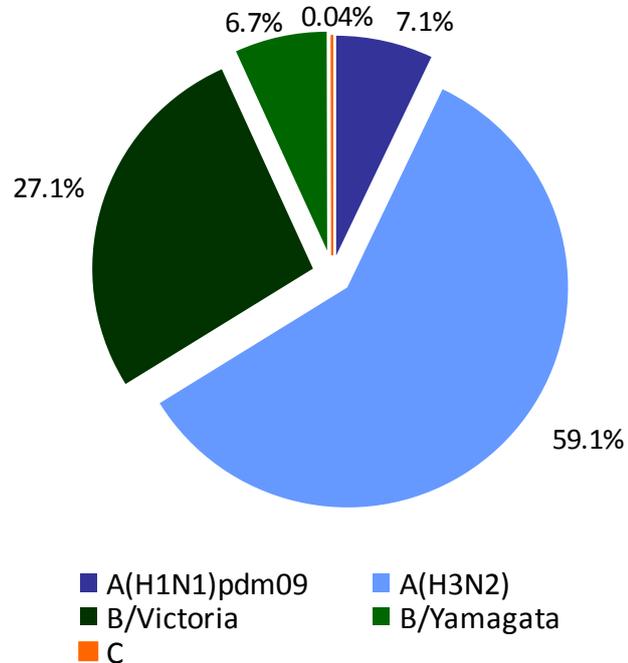
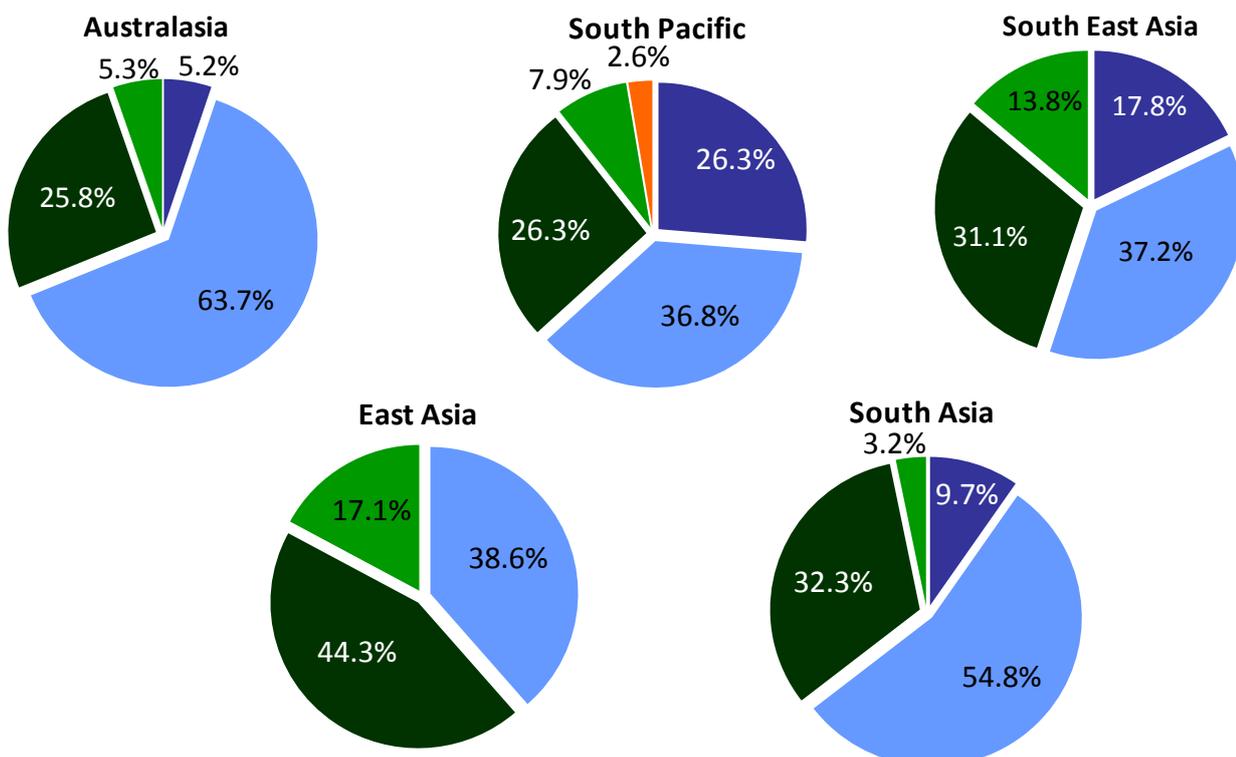


Figure 5. Influenza sub/types and lineages of isolates received from different world regions during 2012 as determined by antigenic analysis.



Genetic Analysis of Influenza Viruses

Background

A subset of all influenza viruses analysed at the Centre undergo genetic analysis by sequencing of viral RNA genes. Determining the amino acid sequence of antigenic regions of the HA and NA proteins provides a sensitive way 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.

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.

Sequencing 2012

In 2012, 410 HA, 409 NA, 253 MP and 214 NS genes of human viruses received at the Centre were sequenced (Figure 6). In addition, 64 influenza A viruses were analysed by full genome sequencing (Figure 7) and 39 viruses were analysed by pyrosequencing for evidence of reassortment (Figure 8). Viruses were selected for these analyses because they were representative of the viruses received and/or because they displayed unusual properties during antigenic analysis. The HE gene of one C virus was also sequenced (data not shown).

Figure 6. Sequence analysis of samples received at the Centre in 2012.

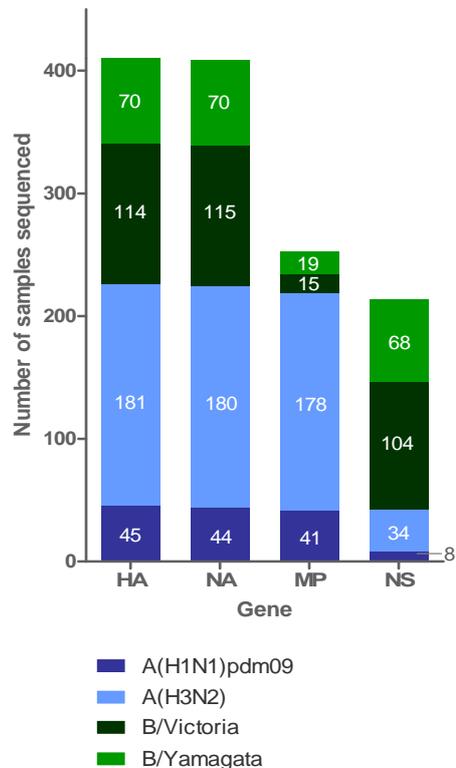


Figure 7. Geographic spread of submitting laboratories and numbers of viruses analysed by full genome sequencing at the Centre in 2012.

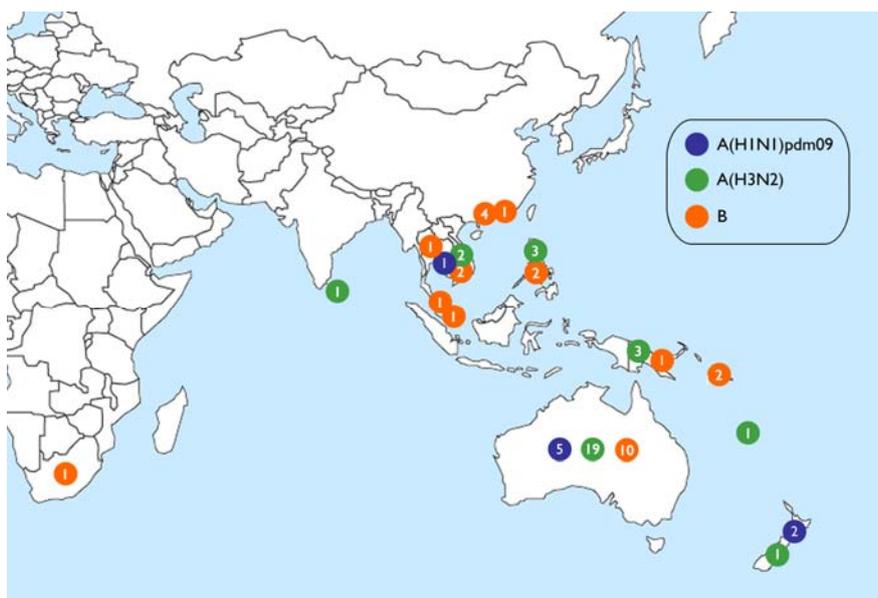
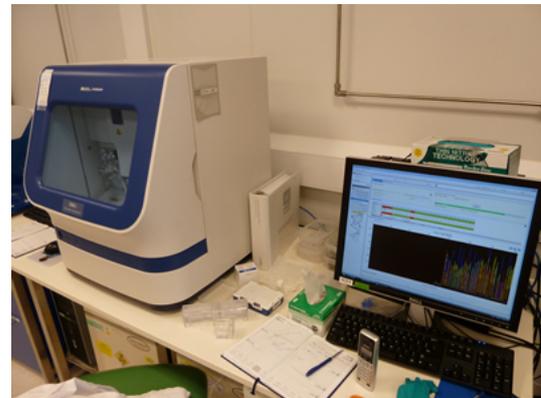
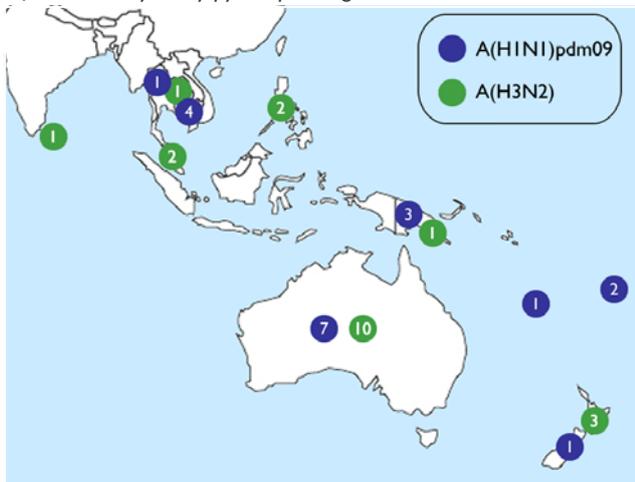


Figure 8. Geographic spread of submitting laboratories and numbers of viruses analysed by pyrosequencing at the Centre in 2012.



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 publically 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 2012

A total of 1437 gene sequences from 410 viruses were deposited with GISAID in 2012 (Table 2). The largest number of these sequences were of HA and NA genes, followed by MP and NS genes. Full genomes of 50 influenza viruses were also represented in the Centre's submissions (data not shown). Some of the sequences submitted to GISAID by the Centre were also submitted to GenBank, the genetic sequence database operated by the National Institutes of Health (NIH).

Table 2. Genetic sequences submitted to GISAID of samples received at the Centre in 2012.

Type/ Subtype / Lineage	Gene	HA	NA	MP	PB2	PB1	PA	NP	NS	HE	Total
A(H1N1)pdm09		119	104	98	16	16	16	16	16	0	401
A(H3N2)		140	139	129	25	25	25	25	25	0	533
B/Victoria		102	101	6	6	6	6	6	88	0	321
B/Yamagata		48	47	11	5	6	6	14	44	0	181
C		0	0	0	0	0	0	0	0	1	1
Total		409	391	244	52	53	53	61	173	1	1437

Surveillance Results by Influenza Subtype

Viruses were analysed by comparison with reference viruses recommended by WHO for the 2013 Southern Hemisphere and 2012-2013 Northern Hemisphere vaccines. Using the HI assay, viruses were identified as low-reactors if their titre

with the reference antiserum was at least 8-fold lower than the titre of the reference virus. Results of sequencing analysis of the HA region of the haemagglutinin gene are also described in the following sections.

Influenza A(H1N1)pdm09

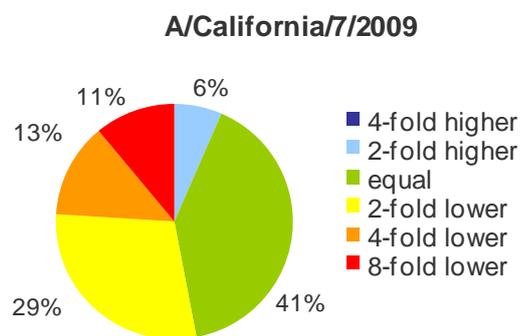
Antigenic analysis

A total of 200 A(H1N1)pdm09 isolates were available for analysis by HI assay in 2012. The majority (89.0%) of these viruses displayed similar antigenic properties to the vaccine reference strain A/California/7/2009 (Table 3, Figure 9).

Table 3. Antigenic characterisation of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/California.7/2009 reference virus.

Region	A(H1N1)pdm09 reference strain: A/California/7/2009	
	Like	Low reactor (%)
Australasia	107	13 (10.8%)
Pacific	9	1 (10.0%)
South East Asia	59	8 (11.9%)
East Asia	0	0
South Asia	3	0
Total	178	22 (11.0%)

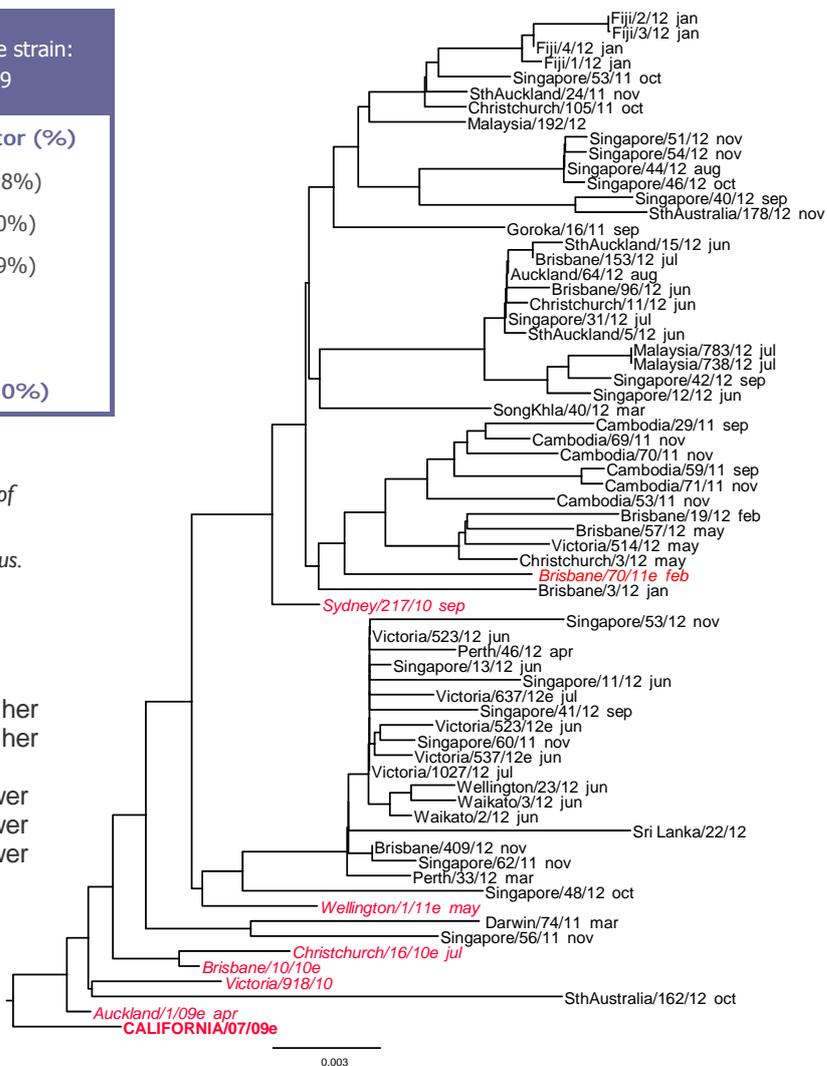
Figure 9. Summary of fold differences in HI titres of A(H1N1)pdm09 viruses analysed at the Centre compared to the A/California/7/2009 reference virus.



Haemagglutinin gene sequencing

Sequence analysis was performed on HA genes from 45 viruses indicated that circulating A(H1N1)pdm09 viruses sent to the Centre during 2012 were genetically similar to the vaccine reference strain A/California/7/2009 (Figure 10).

Figure 10. Phylogenetic tree of representative HA genes of A(H1N1)pdm09 viruses received by the Centre during 2012.



Legend
2013 SOUTHERN HEMISPHERE VACCINE STRAIN
Reference virus
 e: egg isolate
 Scale bar represents 0.3% nucleotide sequence difference between viruses

Influenza A(H3N2)

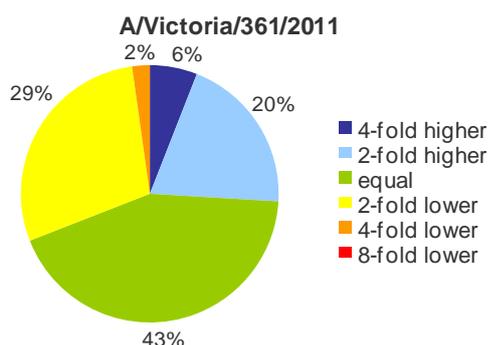
Antigenic analysis

A total of 1672 A(H3N2) subtype isolates were available for analysis by HI assay. A small number (119 viruses) were analysed in comparison to the previous vaccine strain A/Perth/16/2009 (Table 4). However, following a change in the WHO vaccine recommendations in February 2012, the majority (1553 viruses) were analysed in comparison to the new vaccine reference strain A/Victoria/361/2011 (Table 4, Figure 11). Amongst all A(H3N2) viruses analysed by HI assay, there were no low reactors to the reference viruses.

Table 4. Antigenic characterisation of A(H3N2) viruses analysed at the Centre compared to the A/Victoria/361/2011 and A/Perth/16/2009 reference viruses.

Region	A(H3N2) reference strain: A/Victoria/361/2011		A(H3N2) reference strain: A/Perth/16/2009	
	Like	Low reactor (%)	Like	Low reactor (%)
Australasia	1430	0	44	0
Pacific	14	0	0	0
South East Asia	77	0	63	0
East Asia	27	0	12	0
South Asia	5	0	0	0
Total	1553	0	119	0

Figure 11. Summary of fold differences in HI titres of A(H3N2) viruses analysed at the Centre compared to the A/Victoria/361/2011 reference virus.

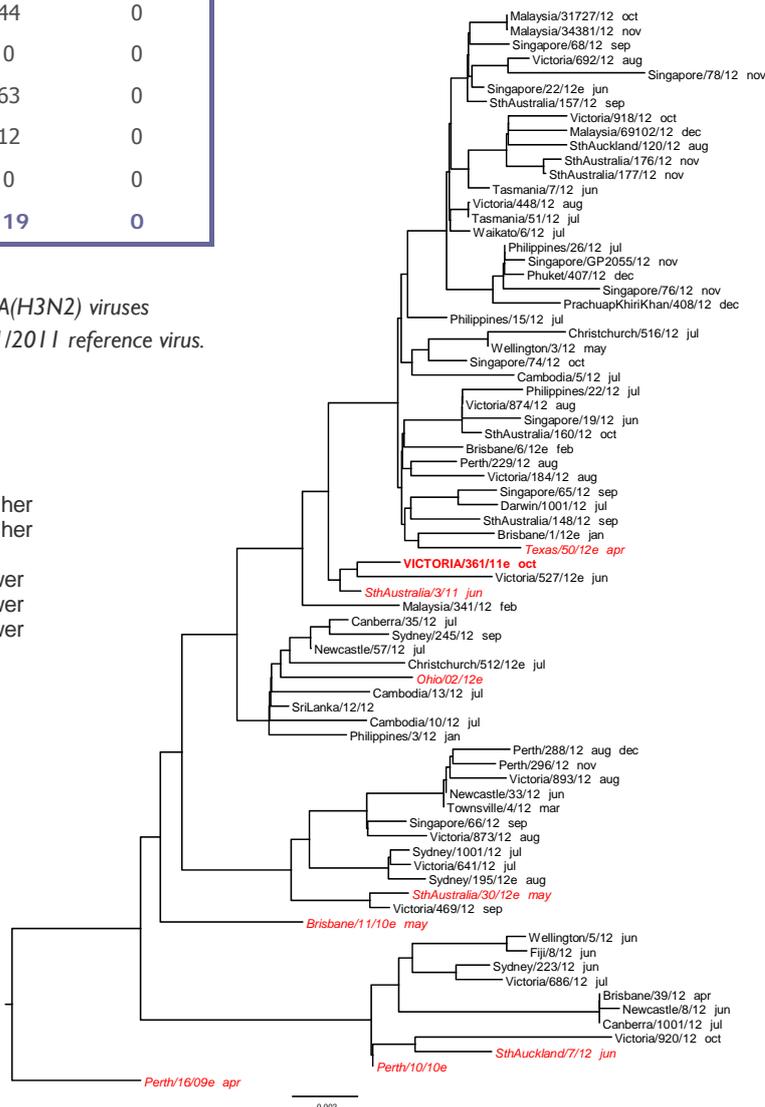


Haemagglutinin gene sequencing

Sequencing of HA genes from 181 A(H3N2) viruses showed that the majority of viruses were genetically similar to the 2013 Southern Hemisphere vaccine strain A/Victoria/361/2011 (Figure 12).

Figure 12. Phylogenetic tree of representative HA genes of A(H3N2) viruses received by the Centre during 2012.

Legend
2013 SOUTHERN HEMISPHERE VACCINE STRAIN
Reference virus
 e: egg isolate
 Scale bar represents 0.2% nucleotide sequence difference between viruses



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 2011 vaccine strain B/Brisbane/60/2008) and the B/Yamagata/16/88 lineage (represented by the 2012-2013 vaccine strain B/Wisconsin/1/2010). 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 2012 the B/Victoria lineage predominated amongst circulating influenza B viruses received at the Centre. Of the 956 type B viruses received and analysed antigenically at the Centre in 2012, the majority were similar to B/Brisbane/60/2008 (Table 5, Figure 13). Amongst B/Yamagata lineage viruses analysed antigenically, almost a quarter were low reactors to B/Wisconsin/1/2010 (Table 5, Figure 14).

Table 5. Antigenic characterisation of B viruses analysed at the Centre compared to the B/Brisbane/60/2008 and B/Wisconsin/1/2010 reference viruses.

Region	B/Victoria reference strain: B/Brisbane/60/2008*		B/Yamagata reference strain: B/Wisconsin/1/2010**	
	Like	Low reactor (%)	Like	Low reactor (%)
Australasia	456	142 (23.7%)	88	35 (28.5%)
Pacific	7	3 (30.0%)	3	1 (25%)
South East Asia	77	38 (33.0%)	49	3 (5.8%)
East Asia	15	16 (51.6%)	8	4 (33.3%)
South Asia	6	4 (40.0%)	1	0
Total	561	203 (26.6%)	149	43 (22.4%)

* B/Victoria lineage virus

** B/Yamagata lineage virus

Figure 13. Summary of fold differences in HI titres of B/Victoria viruses analysed at the Centre compared to the B/Brisbane/60/2008 reference virus.

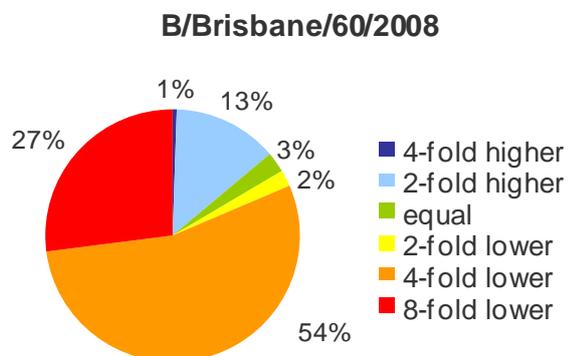
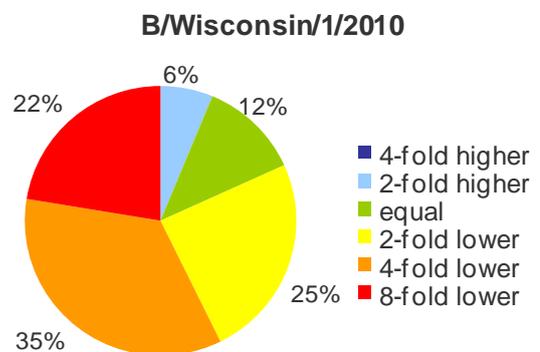


Figure 14. Summary of fold differences in HI titres of B/Yamagata viruses analysed at the Centre compared to the B/Wisconsin/1/2010 reference virus.



Haemagglutinin gene sequencing

A total of 184 HA genes from B viruses underwent sequence analysis and formed two distinct groups corresponding to the B/Brisbane/60/2008 and B/Wisconsin/1/2010 lineages (Figures 15 and 16). All of the viruses of B/Victoria lineage belonged to the same genetic clade as the

B/Brisbane/60/2008 reference virus. B/Yamagata lineage viruses fell into two genetic clades, one represented by B/Wisconsin/1/2010 and the other represented by B/Brisbane/3/2007 — viruses in both clades are antigenically closely related to the 2013 Southern Hemisphere vaccine strain B/Wisconsin/1/2010.

Figure 15. Phylogenetic tree of representative HA genes of B/Victoria viruses received by the Centre during 2012.

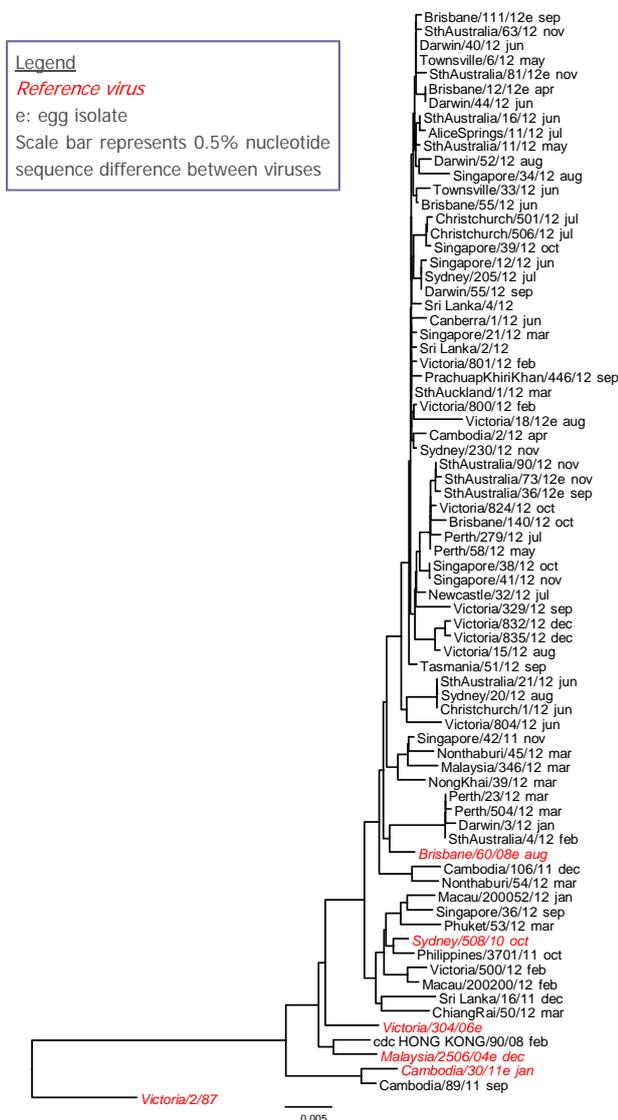
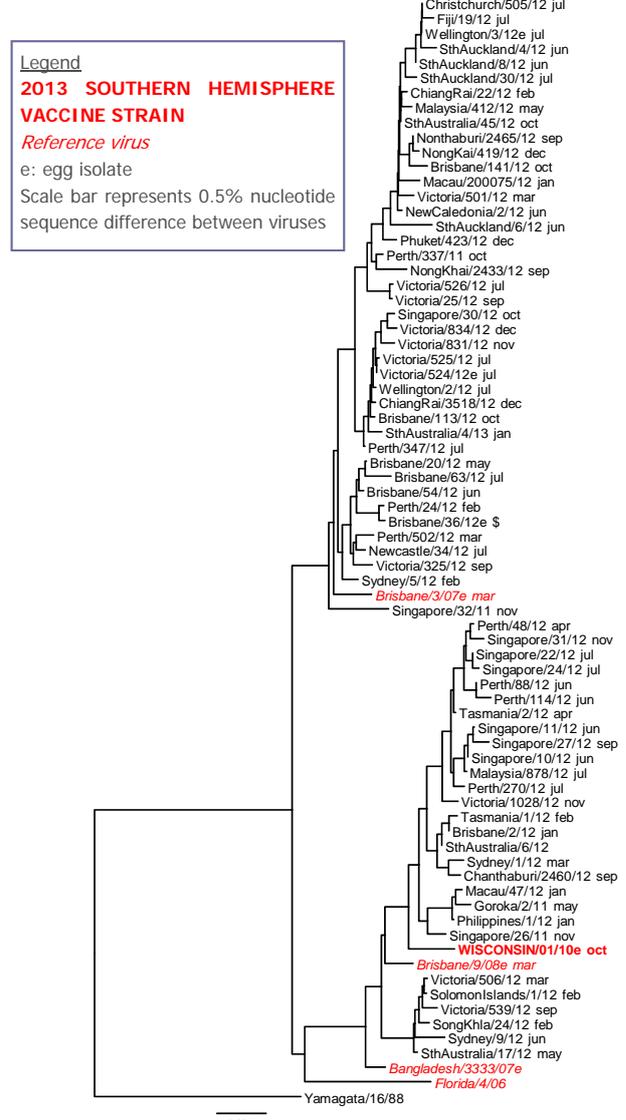


Figure 16. Phylogenetic tree of representative HA genes of B/Yamagata viruses received by the Centre during 2012.



Antiviral Drug Resistance Testing

Sensitivity to Oseltamivir and Zanamivir

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) and zanamivir (Relenza) using the neuraminidase inhibition (NAI) assay.

Antiviral resistance analyses 2012

NAI assays were used to analyse 2846 viruses assay for reduced sensitivity to oseltamivir and zanamivir. Two viruses were found to have reduced sensitivity to oseltamivir. One of these viruses, an A(H1N1)pdm09 virus from Perth, was confirmed to contain the mutation from histidine to tyrosine at position 275 (H275Y) that reduces sensitivity to oseltamivir. The other virus was an A(H3N2) virus from Victoria and was confirmed to contain the mutation from glutamic acid to valine at position 119 (E119V) that is known to reduce sensitivity to oseltamivir. A B virus from New Zealand was also found to have highly reduced sensitivity to zanamivir.

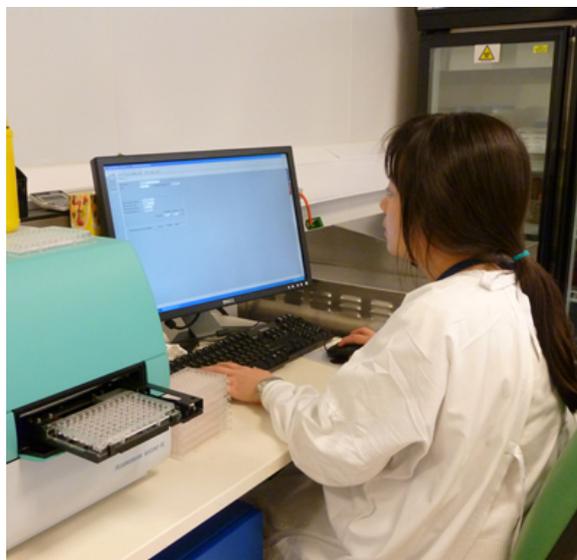


Table 6. Oseltamivir sensitivity of viruses received by the Centre in 2012.

Type/Subtype	A(H1N1)pdm09		A(H3N2)		B	
Country	No. tested	No. with highly reduced sensitivity (%)	No. tested	No. with highly reduced sensitivity (%)	No. tested	No. with highly reduced sensitivity (%)
Australasia						
Australia	65	1 (1.5%)	1276	1 (0.1%)	652	0
New Zealand	64	0	201	0	72	0
South Pacific						
Fiji	4	0	5	0	9	0
New Caledonia	1	0	6	0	3	0
Papua New Guinea	6	0	3	0	1	0
Solomon Islands	0	0	0	0	1	0
South East Asia						
Cambodia	26	0	44	0	57	0
Malaysia	2	0	3	0	14	0
Philippines	4	0	41	0	28	0
Singapore	30	0	43	0	43	0
Thailand	5	0	9	0	27	0
East Asia						
Macau	0	0	27	0	43	0
South Asia						
Sri Lanka	3	0	17	0	11	0
TOTAL	210	1 (0.5%)	1675	1 (0.1%)	961	0

Table 7. Zanamivir sensitivity of viruses received by the Centre in 2012.

Type/Subtype	A(H1N1)pdm09		A(H3N2)		B	
Country	No. tested	No. with highly reduced sensitivity	No. tested	No. with highly reduced sensitivity	No. tested	No. with highly reduced sensitivity
Australasia						
Australia	65	0	1276	0	652	0
New Zealand	64	0	201	0	72	1 (1.4%)
South Pacific						
Fiji	4	0	5	0	9	0
New Caledonia	1	0	6	0	3	0
Papua New Guinea	6	0	3	0	1	0
Solomon Islands	0	0	0	0	1	0
South East Asia						
Cambodia	26	0	44	0	57	0
Malaysia	2	0	3	0	14	0
Philippines	4	0	41	0	28	0
Singapore	30	0	43	0	43	0
Thailand	5	0	9	0	27	0
East Asia						
Macau	0	0	27	0	43	0
South Asia						
Sri Lanka	3	0	17	0	11	0
TOTAL	210	0	1675	0	961	1 (0.1%)

Resistance to Adamantanes

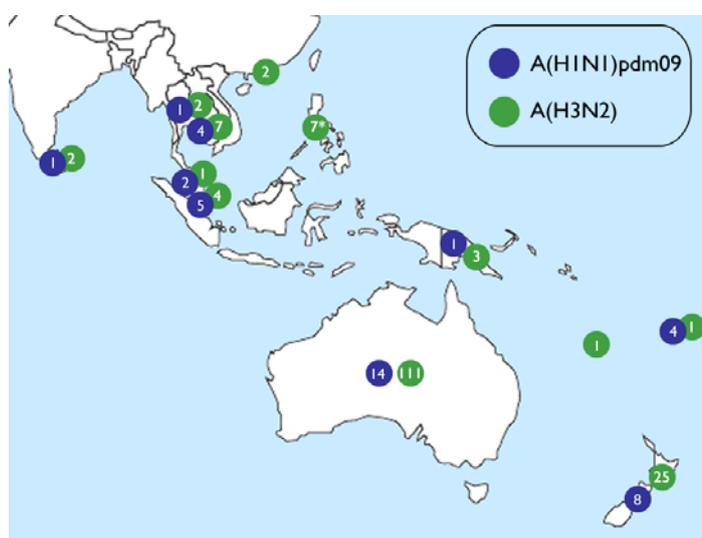
Background

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 2012

Real-time PCR or sequencing was used to analyse 206 influenza A viruses, selected as representative of those submitted to the Centre during 2012 (Figure 17). Based on S31N analysis all tested viruses were resistant to the adamantanes except for one A(H3N2) virus from the Philippines.

Figure 17. Geographic spread of viruses received at the Centre during 2012 and screened for adamantane resistance. One A(H3N2) virus from the Philippines (marked with *) was found not to contain the S31N mutation.



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 influenza vaccines. Twice a year the WHO Collaborating Centres and Essential Regulatory Laboratories in the WHO surveillance network exchange panels of sera taken 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. Serum panels from children, younger adults (20-64 years old) and older adults (≥ 65 years old) are assessed.

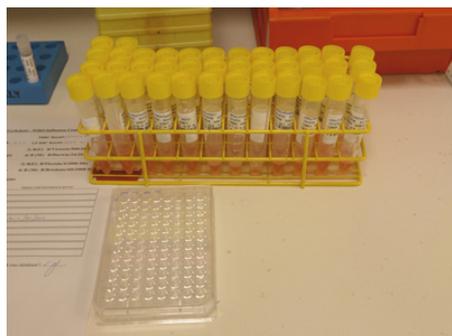
Serum panel analyses in 2012

In February the Centre analysed serum panels from recipients of seasonal trivalent influenza vaccines in Australia, China, Europe, USA and Japan. The combined data from all WHO Collaborating Centres and ERLs showed that, in general, vaccines containing A/California/7/2009-like antigens stimulated anti-HA antibodies of similar geometric mean titre to the vaccine virus and most recent representative A(H1N1) pdm09 isolates. However, vaccines containing A/Perth/16/2009-like antigens stimulated antibodies with geometric mean HI titres that were lower to the majority of recent viruses than to the vaccine virus. Current vaccines containing B/Brisbane/60/2008 antigens stimulated antibodies with similar titres against the vaccine viruses and recent viruses of the B/Victoria/2/87 lineage; however, titres were lower to recent B/Yamagata/16/88 lineage viruses.

In September, serum panels from Australia, Europe and Japan were analysed at the Centre. The combined data from all WHO Collaborating Centres and ERLs showed that, in general, vaccines containing A/California/7/2009-like, A/Victoria/361/2011-like, and B/Brisbane/60/2008-like or B/Wisconsin/1/2010-like antigens stimulated anti-HA antibodies of similar geometric mean titre to the relevant virus and most recent representative A(H1N1)pdm09, A(H3N2), B/Victoria/2/87 lineage and B/Yamagata/16/88 lineage isolates, respectively.

Table 8. Representative and vaccine candidate strains used for serological analyses during 2012.

A(H1N1)pdm09	
February	September
A/California/7/2009*	A/California/7/2009*
A/Wisconsin/26/2011	A/Victoria/537/2012
A/Cape Town/60/2011	A/Norway/418/2012
A/Perth/533/2011	
A(H3N2)	
February	September
A/Victoria/210/2009*	A/Perth/16/2009*
A/Kentucky/5/2011	A/Victoria/361/2011*
A/Utah/12/2011	A/Ohio/2/2012
A/Christchurch/28/2011	A/Victoria/527/2012
A/Victoria/361/2011	
A/Brisbane/299/2011	
B	
February	September
B/Brisbane/33/2008 ^{^*}	B/Brisbane/60/2009 [^]
B/Nevada/3/2011 [^]	B/Brisbane/12/2012 [^]
B/Dominican Republic/5486/2011 [^]	B/Wisconsin/1/2010 ⁺ *
B/Texas/6/2011 ⁺	B/Massachusetts/2/2012 ⁺
	B/Taiwan/1367/2012 ⁺
	B/Hubei-Wujiagang/158/2009 ⁺
* Vaccine strain [^] B/Victoria-lineage viruses ⁺ B/Yamagata-lineage viruses	



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.

Since 2009, the number of viruses isolated in eggs at the Centre has increased as a result of additional support received under a Letter of Agreement with the International Federation of Pharmaceutical Manufacturers and Associations (IFPMA).

Isolation of viruses in eggs in 2012

In 2012, 23 viruses were successfully isolated in eggs at the Centre, representing an overall isolation rate of 19.5% (Tables 9 and 10).

Table 9. Virus isolation in eggs at the Centre in 2012

Type/subtype	Isolates attempted	Isolates obtained	Success rate (%)
A(H1N1)pdm09	18	3	16.7
A(H3N2)	57	11	19.3
B	43	9	20.9
Total	118	23	19.5

Table 10. Potential candidate vaccine strains successfully isolated in eggs at the Centre in 2012

A(H1N1)pdm09	A(H3N2)	B
A/Victoria/523/2012	A/Victoria/361/2011	B/Brisbane/12/2012
A/Victoria/537/2012	A/Brisbane/1/2012	B/Brisbane/36/2012
A/Victoria/637/2012	A/Brisbane/6/2012	B/Victoria/524/2012
	A/Ohio/2/2012	B/Wellington/3/2012
	A/Virginia/5/2012	B/Victoria/18/2012
	A/Maryland/2/2012	B/Brisbane/111/2012
	A/South Australia/30/2012	B/South Australia/36/2012
	A/Victoria/527/2012	B/South Australia/81/2012
	A/Christchurch/512/2012	B/South Australia/73/2012
	A/Singapore/22/2012	
	A/Sydney/195/2012	

Preparation and Analysis of Vaccine Seed Viruses

The Centre exchanges candidate vaccine viruses that have been isolated in eggs, as well as post-infection ferret antisera raised against these and other reference viruses, with the other WHO Collaborating Centres to enable direct comparison of strains isolated in the five centres. During 2012, 14 candidate vaccine viruses were received from other WHO Collaborating Centres and laboratories and then passaged in eggs at the Centre (Table 11).

Selected egg-isolated candidate vaccine strains are made available to the three laboratories that undertake virus reassortment for WHO — CSL Limited (Australia), the National Institute for Biological Standards and Control (NIBSC, UK) and New York Medical College (NYMC, USA) — where they are reassorted with established egg-adapted strains to produce potential vaccine seed strains. The reassortant vaccine seed viruses are returned to the Centre, where they are analysed by HI assay and genetic sequencing to ensure that key antigenic and genetic properties of the vaccine virus have been retained.

The vaccine seed viruses are distributed to other WHO Collaborating Centres and vaccine manufacturers worldwide through Essential Regulatory Laboratories at the Therapeutic Goods Administration (Australia), NIBSC and the Centre for Biologics Evaluation and Research, Food and Drug Administration (USA).

Table 11. Potential candidate vaccine viruses received from other WHO Collaborating Centres during 2012.

Type/Subtype	Strain
A(H1N1)pdm09	A/Valparaiso/17275/2011 NIB-74XP (A/Christchurch/16/2010) A/Cape Town/60/2011 A/Washington/24/2012
A(H3N2)	X-213(A/Indiana/10/2011) NYMC X-217 (HY A/Victoria/361/2011) NYMC X-217A (HY A/Victoria/361/2011)
B	B/Dominican Republic/5486/2011 B/Stockholm/12/2011 NYMC BX-49 (HY B/Texas/6/2011) B/Texas/6/2011 B/Linares/34634/2012 B/New Jersey/01/2012 B/Massachusetts/02/2012

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, monoclonal antibodies and viral antigens for reference influenza strains. During 2012, 56 kits were sent to 27 laboratories in 16 countries. Each kit contained 10 mL each of the reference antigens A/Perth/16/2009, A/California/7/2009, B/Wisconsin/1/2010 and B/Brisbane/60/2008, and homologous antisera.

Recipients of the 2012 Kit			
Australia	Institute of Medical and Veterinary Science, Adelaide, South Australia Queensland Health Scientific Services, Coopers Plains, Queensland Westmead Hospital, Westmead, New South Wales University of Queensland, Queensland	New Zealand	Institute of Environmental Science and Research, Wellington University of Auckland, Auckland Auckland City Hospital, Auckland Canterbury Health Services, Christchurch
Cambodia	Institut Pasteur du Cambodge, Phnom Penh	Papua New Guinea	Papua New Guinea Institute of Medical Research, Goroka
Fiji	Fiji Centre for Communicable Disease Control, Suva	Philippines	College of Public Health, Manila
Hong Kong, China	University of Hong Kong	Romania	National Institute, Bucharest
India	Manipal University, Karnataka Haffkine Institute, Mumbai	Singapore	Singapore General Hospital National Public Health Laboratory Duke-NUS Graduate Medical School
Kenya	Center for Virus Research, Kenya Medical Research Institute, Nairobi	South Africa	National Institute for Communicable Diseases, Johannesburg
Macau, China	Public Health Laboratory	Sri Lanka	Medical Research Institute, Colombo
Malaysia	Institute for Medical Research, Kuala Lumpur University of Malaya, Kuala Lumpur	Taiwan	National Cheng Kung University, Tainan
		Thailand	National Institute of Health, Bangkok

Primer Sequences for Full Genome Sequencing

The Centre has developed primer sequences and protocols for full genome sequencing of both Type A and Type B viruses, and makes these available other influenza laboratories on request. Primer sequences and protocols were sent to the following institutions during 2012:

- DSO National Laboratories, Singapore
- Chinese National Influenza Center, National Institute for Viral Disease Control and Prevention, China CDC, Beijing, China
- Department of Virology, Norwegian Institute of Public Health (Norwegian National Influenza Centre), Oslo, Norway
- Hospital Universitari Vall d'Hebron, Barcelona, Spain

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 (isriv) Antiviral Group. In 2012 panel kits were composed of 2 vials (250 µL) of each the reference viruses listed in the table below. Kits based on the 2011 panel were also sent to a small number of laboratories.

Contents of the 2012 NAI assay panel

Reference virus	Amino acid residues of interest in NA protein	Sensitivity to antiviral drugs
A/Mississippi/3/01 (H1N1) wild-type (A/New Caledonia/20/99-like)	Histidine at position 275 (275H)	Susceptible to zanamivir Susceptible to oseltamivir carboxylate
A/Mississippi/3/01 (H1N1) variant (A/New Caledonia/20/99-like)	Tyrosine at position 275 (275Y)	Susceptible to zanamivir Reduced susceptibility to oseltamivir carboxylate
A/Fukui/20/04 (H3N2) wild-type (A/Fujian/411/2002-like)	Glutamic acid at position 119 (119E)	Susceptible to zanamivir Susceptible to oseltamivir carboxylate
A/Fukui/45/04 (H3N2) variant (A/Fujian/411/2002-like)	Valine acid at position 119 (119V)	Susceptible to zanamivir Reduced susceptibility to oseltamivir carboxylate
B/Perth/211/2009 wild-type (B/Sichuan/379/1999-like)	Aspartic acid at position 197 (197D)	Susceptible to zanamivir Susceptible to oseltamivir carboxylate
B/Perth/211/2009 variant (B/Sichuan/379/1999-like)	Glutamic acid at position 197 (197E)	Susceptible to zanamivir Reduced susceptibility to oseltamivir carboxylate
A/Perth/265/2009 (H1N1)pdm09 wild-type (A/California/7/2009)-like	Histidine at position 275 (275H)	Susceptible to zanamivir Susceptible to oseltamivir carboxylate
A/Perth/265/2009 (H1N1)pdm09 variant (A/California/7/2009)-like	Tyrosine at position 275 (275Y)	Susceptible to zanamivir Reduced susceptibility to oseltamivir carboxylate



Recipients of the 2011 NAI assay panel in 2012

University of Florida, NY, USA
Cellex Inc, Rockville MD, USA
Janssen Pharmaceutica, Belgium

Recipients of the 2012 NAI assay panel in 2012

Hoffmann-La Roche, Nutley, NJ USA
National Center for Epidemiology, Budapest, Hungary
Institut National d'Hygiène, Morocco
Biota Holdings Ltd, Melbourne, Australia
Federal Drug Agency (FDA), USA
Focus Diagnostics, Cypress, CA USA
Mechnikov Research Institute of Vaccines and Sera, Russia
National Institute of Hygiene and Epidemiology, Hanoi, Vietnam
Niigata University, Japan

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; 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 OFFLU, the University of Cambridge, several WHO National Influenza Centres and other relevant organisations from time to time. In 2012, WHO made the recommendations reported here.

WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2012–2013, Geneva, Switzerland, 20–23 February 2012

It is recommended that vaccines for use in the 2012–2013 influenza season (northern hemisphere winter) contain the following:

- an A/California/7/2009 (H1N1)pdm09-like virus;
- an A/Victoria/361/2011 (H3N2)-like virus*;
- a B/Wisconsin/1/2010-like virus.

WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2013, Beijing, China, 17-19 September 2012

It is recommended that vaccines for use in the 2013 influenza season (southern hemisphere winter) contain the following:

- an A/California/7/2009 (H1N1)-like virus;
- an A/Victoria/361/2011 (H3N2)-like virus*;
- a B/Wisconsin/1/2010-like virus.

It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Brisbane/60/2008-like virus*.

*This virus was originally isolated at the WHO Collaborating Centre in Melbourne.



Left: WHO officials, advisers from the WHO Collaborating Centres for Influenza and Essential Regulatory Laboratories, and observers from other centres in WHO GISRS attending the WHO Consultation on the Composition of Influenza Vaccines in February (image courtesy of Ivan Babovic, WHO).

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.

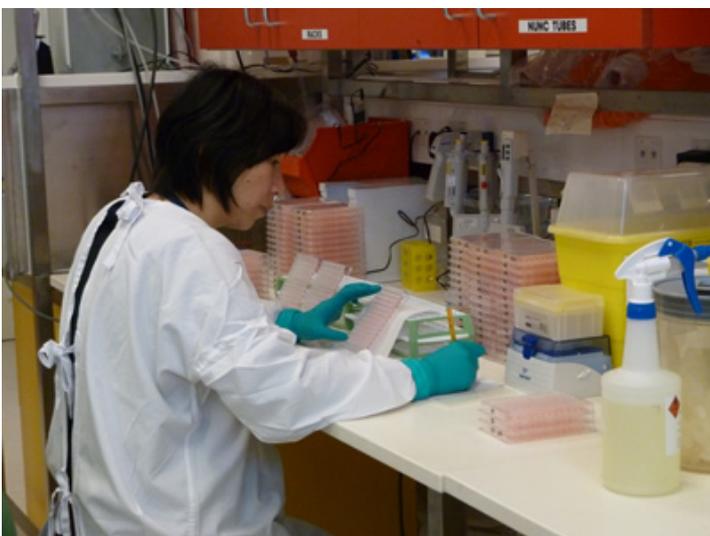
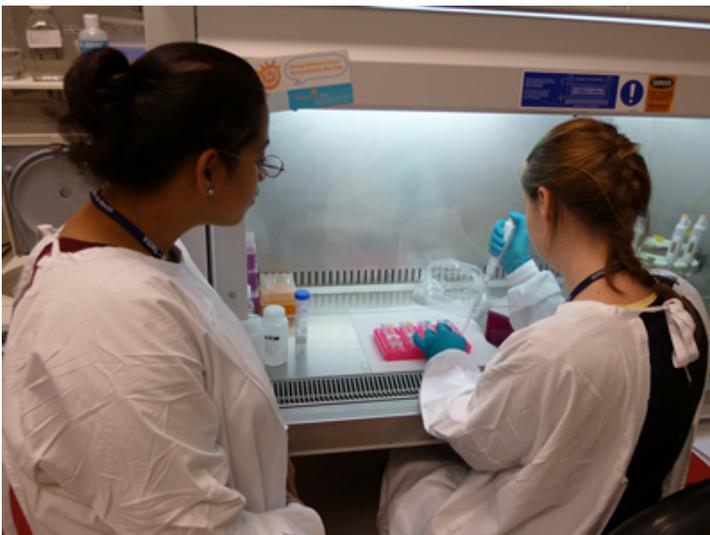
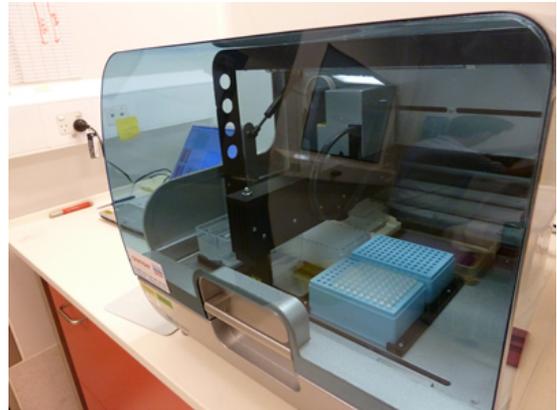
At its meeting on 3 October AIVC accepted the September WHO recommendation and decided that the Australian influenza vaccine for 2013 should contain the following:

- A(H1N1): an A/California/7/2009 (H1N1)-like strain, 15 µg HA per dose
- A(H3N2): an A/Victoria/361/2011 (H3N2)-like strain, 15 µg HA per dose
- B: a B/Wisconsin/1/2010 - like strain, 15 µg HA per dose

Analysis of Non-Human Influenza Viruses

Whilst not routinely conducting surveillance of non-human influenza viruses, the Centre maintains its relationships with the Australian Animal Health Laboratory (AAHL) and other groups to enable studies with low and highly pathogenic avian influenza viruses, as well as conducting studies to assess the pathogenicity and potential risk to humans in animal models.

During 2012, in response to separate farm outbreaks in Queensland and Western Australia, and in co-operation with the respective state health and agricultural departments and AAHL, the Centre isolated and performed genetic analysis of a number of swine and chicken influenza viruses. Full genome analysis was conducted on A(H3N2) and A(H1N2) swine viruses, as well as an A(H10N7) chicken virus isolated from a separate poultry outbreak in Queensland. One A(H3N2) swine virus underwent antigenic analysis.



Training

Training and Support of National Influenza Centres and Regional Laboratories

The Centre provides further support to the GISRS surveillance network by providing 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.

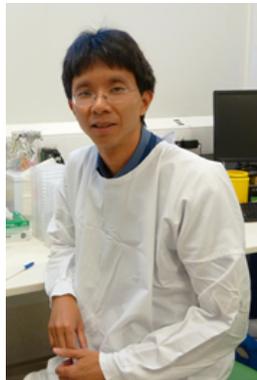
In-House Training

During 2012 the Centre hosted several scientists who visited for training in a range of techniques for analysis and characterisation of influenza isolates. The Centre was pleased to welcome scientists not only from diagnostic laboratories, but also collaborating research laboratories.

Associate Professor Jamal I-Ching Sam

NIC and Tropical Infectious Diseases Research and Education Centre at the University of Malaya, visited 3 February–1 June 2012.

Trained in serology techniques and NAI assays and then used these techniques to isolate and characterise influenza strains collected at University of Malaya 1995–2009. Conducted a study of influenza seroprevalence from pre- and post-pandemic Malaysian samples. A manuscript based on this work has been accepted for publication.



Ms Shirley Gek Kheng Seah

Defence Medical and Environmental Research Institute (DMERI), DSO National Laboratories, Singapore, visited 28 August.

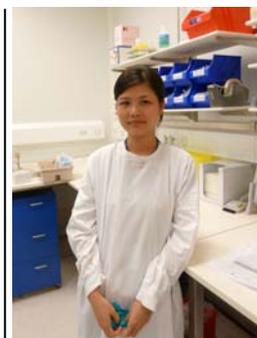
Trained in amniotic inoculations and harvesting of embryonated hens eggs.



Ms Vina Arguelles (left) and Ms Herma Base (right)

Research Institute for Tropical Medicine, Philippines, visited 17–28 September.

Trained in virus isolation, serology/HI assays, PCR-ID, sequencing and phylogenetic analysis and NAI assays.



Ms Le Thi Thanh

Virology Department, National Institute of Hygiene and Epidemiology (NIHE), Vietnam, visited 22 October–2 November.

Trained in high-throughput serology techniques.



(pictured left to right):

Ms Kiti Rehier (Majuro Hospital Laboratory, Majuro, Marshall Islands),

Mr Alan Mallari (Guam Public Health Laboratory, Guam),
Ms Shalini Pravin (Center for Communicable Disease Control, Suva, Fiji), visited 12–16 November.

Trained in real-time PCR, immunofluorescence for detection of influenza virus, cell culture and serology techniques relevant to influenza virus characterisation. The trainees were accompanied by **Ms Sala Elbourne** (far right) from the Secretariat of the Pacific Community.

Other Training

Dr Patrick Reading, the Centre's Educator, collaborated with WHO regional offices to improve the capabilities for influenza surveillance in regional laboratories through the organisation of laboratory renovations and purchase of relevant equipment. Dr Reading also visited the laboratories to assist in setting up equipment, training staff and formulating relevant standard operating procedures (SOPs).

Pohnpei State Hospital, Pohnpei, 9–17 February

Establishment of real-time PCR capabilities, in co-ordination with the WHO Office in the North Pacific.

A real-time PCR machine was purchased and staff members were trained in use of real-time PCR for influenza detection.

Fiji National Influenza Centre (Center for Communicable Disease Control), Suva, Fiji, 7–17 March

Establishment of cell culture and virus isolation capabilities, in coordination with WHO WPRO and WHO Office in the South Pacific.

Equipment and consumables for routine cell culture techniques were purchased. Laboratory staff were trained in cell culture, amplification of influenza virus in cell culture and in the use of virological/serological techniques to characterize cell culture grown viruses.

Dr Reading participated in the Improving Influenza Laboratory Management Practices Training Course, held in Bangkok Thailand, 27 February–3 March. He gave four talks at the workshop, which was attended by 35 participants from Asian, European and Pacific Island countries.

Dr Aeron Hurt visited the WHO National Influenza Center of National Institute for Hygiene and Epidemiology (NIC NIHE), in Hanoi, Vietnam, 17–28 September to improve influenza antiviral resistance diagnostic capabilities. He facilitated training of NIC NIHE staff on influenza antiviral diagnostic techniques, reviewed and develop SOPs, standardised and reviewed viral panels, designed primers for sequencing and reviewed and updated antiviral resistance testing strategies at NIC NIHE.

Staff Development

Anne Kelso attended the "Finance for Directors" course, run by the Australian Institute of Company Directors (AICD), in Melbourne on 2 February.

Karen Laurie attended "Statistics for Research Workers (using "R")", run by the Statistical Consulting Centre at The University of Melbourne, 15–22 February.

Naomi Komadina attended "Introduction to STATA", run by the School of Public Health and Preventive Medicine, Alfred Hospital in Melbourne on 15 March.

Aeron Hurt attended the Monash University Faculty of Science Research Supervision Level 1 Accreditation Workshop, in Melbourne on 15 June.



Research

Research interests at the Centre encompass a broad range of in-house and collaborative projects. During 2012, Centre staff were involved in the project areas described below.

Research Projects

Seroprevalence surveys to assess human population immunity to the 2011/2012 A(H3N2)v influenza outbreak

Centre staff	Karen Laurie, Louise Carolan, Ian Barr, Anne Kelso
Collaborators	Jodie McVernon (The University of Melbourne) Stephen Lambert (Queensland Children's Medical Research Institute, The University of Queensland, and Queensland Children's Health Services, Queensland Health Immunisation Program, Brisbane) Helen Faddy, Catherine Hyland and Hugh Capper (Australian Red Cross Blood Service) Brenda White and Amanda di Carlo (The Royal Children's Hospital, Melbourne)

Project overview

The level of antibodies to the novel A(H3N2)v virus detected in the USA during 2011 and 2012 was assessed in sera and plasma collected from Australian children and adults in late December 2011. This is the only serosurvey against this novel virus that has been performed in Australia.

Highlights and developments 2012

Results from this serosurvey indicated that the level of antibodies in the population is age biased, with those between the ages of 16-44 years most likely to have antibodies to the A(H3N2)v virus. A manuscript based on findings from this study is in preparation.

Comparison of serology microneutralisation assay protocols

Centre staff	Karen Laurie, Louise Carolan
Collaborators	Ralf Wagner (Paul-Ehrlich-Institut, Germany), Malik Peiris (University of Hong Kong), Katja Hoschler (Health Protection Agency, London (HPA)); Tian Bai (WHO CC for Influenza, China (CNIC)), Jackie Katz, Xiuhua Lu and Vic Veguilla (CDC, Atlanta, GA, USA), Emanuele Montomoli (University of Sienna, Italy), Maria Rita Castrucci (Istituto Superiore di Sanità, Italy), Noriko Kishida (National Institute of Infectious Diseases, Japan (NIID)); John Wood, Diane Major and Othmar Engelhardt (National Institute for Biological Standards and Control (NIBSC)), Olav Hungnes (Norwegian Institute of Public Health, Norway), Thedi Ziegler (National Institute for Health and Welfare, Finland) Nicholas Martin (Naval Medical Research Center, Silver Spring, USA); Gary Brice (Naval Health Research Center, San Diego, CA, USA)

Project overview

This project is being carried out as part of the Consortium for the Standardization of Influenza Seroepidemiology (CONSISE). The Consortium has been divided into two streams to consider a) Epidemiology and b) Laboratory Issues. The Centre is part of the Laboratory Issues group, which is comparing different methods for microneutralisation assays. Two main protocols, which take either 2 days or 3 days to perform, are used for microneutralisation assays in influenza seroepidemiology laboratories worldwide. This study is focused on comparing the 2-day and 3-day protocols. A protocol comparison of the haemagglutination inhibition (HI) assays is also underway.

Highlights and developments 2012

A total of 13 laboratories around the world performed both microneutralisation assays with A(H1N1)pdm09 virus multiple times using their own serum panels, then the data were collated and compared. The assays were found to be equally sensitive and gave comparable results. Centre staff co-ordinated the study and participated in the laboratory comparison. Further studies are now planned using seasonal A(H3N2) and A(H5N1) viruses. The Centre is also co-ordinating and participating in the studies for comparing HI assays.

Assessment of antigenic drift in a ferret model

Centre staff and student Karen Laurie, Louise Carolan, Teagan Guarnaccia, Patrick Reading, Ian Barr, Anne Kelso

Collaborators Jenny Mosse (Monash University)
James McCaw, Stephen Price and Jodie McVernon (The University of Melbourne)
Sebastian Maurer-Stroh and Raphael Lee (Bioinformatics Institute (BII), A*STAR, National University of Singapore)

Project overview

As a core part of its surveillance activities, the Centre traces the antigenic drift of influenza viruses to monitor how well the current influenza vaccine matches circulating influenza strains. Interestingly, to date the A(H1N1)pdm09 influenza virus has not shown significant drift. In this project a model of antigenic drift has been developed by passaging influenza virus through multiple cycles of infection in immunised and naïve ferrets. It is hoped that this model will provide insights into why and how antigenic drift occurs in influenza viruses circulating in the human population.

Highlights and developments 2012

Teagan Guarnaccia presented results from this project in both an oral presentation and a poster at the Influenza 2012 conference in Oxford, UK, in September. She also presented results in talks at three research institutes. A research paper based on the past three years' work on this project was submitted for publication. Data from this project has also been used in a mathematical modelling study, for which a paper has been submitted for publication.

Related publications 2012

Guarnaccia *et al.* Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection. (Poster, presented at Influenza 2012, 11–13 September)

Guarnaccia *et al.* Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2–6 December)

Assessment of cytokine responses in ferrets

Centre staff Karen Laurie, Louise Carolan, Aeron Hurt, Jeffrey Butler, Patrick Reading

Collaborators Steve Rockman (CSL Limited)

Project overview

Understanding the immune response following influenza virus infection can lead to improvements in treatment or prevention of virus infection. This study aims to assess cytokine responses in ferrets as markers of the early and late immune response.

Highlights and developments 2012

A TaqMan assay has now been established that allows identification of markers of the early and late immune response in samples from both the upper and lower respiratory tract of ferrets. This method will be used to assess the severity of influenza virus infection and the effect of antiviral drug treatment on reducing influenza virus pathogenesis and to assist in understanding various epidemiological findings. A manuscript based on results of this project is in preparation.

Related publications 2012

Carolan *et al.* Quantitation of cytokine mRNA levels in ferrets following influenza infection. (Poster, presented at 4th Australasian Vaccines and Immunotherapeutics Development Meeting, 2–4 May)

Carolan *et al.* Quantitation of cytokine mRNA levels in ferrets following influenza infection. (Poster, presented at Melbourne Health Research Week Symposium, 24–31 May)

Carolan *et al.* Cytokine profiling in ferrets: a real time RT-PCR assay to assess viral virulence and the immune response following influenza infection and immunisation. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2–6 December)

Viral fitness in ferret models

Centre staff Aeron Hurt, Jeffrey Butler, Ian Barr
Collaborators James McCaw and Jodie McVernon (The University of Melbourne)

Project overview

This project uses a competitive mixtures model in ferrets to investigate the relative fitness and transmissibility of different influenza viruses. Groups of ferrets are infected with a mixture of two influenza strains and the relative proportions of those viruses are monitored over time and over multiple cycles of transmission. The data are analysed by mathematical modelling to determine the relative fitness of one virus compared with another. The model has been applied to determining the fitness of neuraminidase inhibitor-resistant viruses and new antigenic variants. This project is supported by funding from the University of Melbourne Faculty Research Grant Support Scheme.

Highlights and developments 2012

Results from this project were presented in talks by Jeffrey Butler at the Melbourne Health Research Week Symposium, 24–31 May, and the 6th Orthomyxovirus Research Conference, 19–22 September, as well as by Aeron Hurt at the 2nd Antivirals Congress, 11–13 November.

Effectiveness of anti-viral treatments in a ferret model

Centre staff Aeron Hurt, Karen Laurie, Ian Barr, Anne Kelso
Collaborators Deborah Middleton and Sue Lowther (Australian Animal Health Laboratory)
James McCaw and Jodie McVernon (The University of Melbourne)

Project overview

This project investigates the effectiveness of oseltamivir as a treatment or prophylactic agent in reducing infectivity, transmissibility and growth of different viruses. To investigate the impact of different treatment strategies, ferrets are dosed with different concentrations of the drug at various time intervals either pre- or post-exposure to the virus. Virological, symptomatic and immunological variables are then measured over the course of infection and treatment.

Vaccine effectiveness

Centre staff Sheena Sullivan
Collaborators Heath Kelly and Kristina Grant (Epidemiology Unit, VIDRL)
James Fielding and Ee Laine Tay (VIDRL; National Centre for Epidemiology & Population Health, Australian National University)

Project overview

The WHO recommendations on the composition of influenza vaccines are issued 5–6 months prior to the release of the vaccine, leaving insufficient time to test vaccine efficacy and safety. The epidemiology group at VIDRL has a long-running influenza sentinel surveillance system that collects information on patients with influenza-like illness including their vaccination history and laboratory-confirmed influenza subtype. Vaccine effectiveness in the community can be estimated from the data using a test-negative design. This project is using data collected from 2007 to the present date to estimate vaccine effectiveness and will explore the validity of the epidemiological methods.

Highlights and developments 2012

In 2012 all influenza-positive samples from the sentinel system were forwarded to the Centre for antigenic analysis and a selection were sequenced to develop phylogenetic trees to aid interpretation of the vaccine effectiveness estimates. The Centre also began working more closely with the Australian Sentinel Practices Research Network (ASPREN) and with the sentinel practices network of WA (SPN(WA)) to develop vaccine effectiveness estimates and conduct antigenic and genetic analyses of samples collected by those networks

Related publications 2012 (see Centre publications p.42)

Reference no. 11

Understanding the representativeness of influenza virus samples sent to the Centre

Centre staff Sheena Sullivan

Project overview

It is important to understand whether the influenza viruses evaluated by the Centre are representative of the viruses circulating during a season. While it is infeasible to ensure equal representation of each virus strain, it is possible to estimate the proportion of samples received among laboratory-confirmed cases. This study will attempt to understand whether viruses received by the Centre are representative of those reported to National Notifiable Diseases Surveillance System in terms of type/subtype and the demographic, geographic and temporal distribution of infections.

Molecular analysis and structural modelling of influenza proteins

Centre staff Ian Barr, Yi-Mo Deng, Aeron Hurt, Naomi Komadina
Collaborators Sebastian Maurer-Stroh (Bioinformatics Institute (BII), A*STAR, National University of Singapore)
 Michael Parker (St Vincent's Institute of Medical Research)

Project overview

The Centre has several collaborations with external groups to use computer modelling to explore the structures of proteins of interest such as HA and NA, one of the key collaborations being with the group at the Bioinformatics Institute (A*STAR). This analysis allows a better understanding of the molecular basis for changes that affect the efficacy of influenza vaccines and antiviral drugs.

Highlights and developments 2012

Work has been performed with collaborators at the Bioinformatics Institute (A*STAR) in several areas during 2012. A better understanding of key mutations in the HA protein of A(H1N1)pdm09 viruses has been obtained with *in silico* analysis. Work is also continuing to better comprehend the role of permissive mutations and how they help to confer resistance to neuraminidase inhibitors in viruses.

Related publications 2012

Reference no. 8, 9

Antigenic cartography and molecular evolution of the influenza virus

Centre staff Ian Barr, Aeron Hurt, Naomi Komadina, Lumin Xue
Collaborators Derek Smith and Colin Russell (University of Cambridge, UK)
 Yoshihiro Kawaoka (University of Wisconsin-Madison, University of Tokyo)

Project overview

This project involves analysis of influenza viruses by antigenic cartography in combination with known amino acid changes in HA. The cartography system uses sophisticated computer algorithms to spatially plot each influenza virus in terms of its reactivity in an HI assay — analogous to a road map that interconnects towns and cities. Over the course of each year the virus strains form clusters that map differently over time, reflecting the changing nature of the influenza virus. Integration of these data with sequence data provides insight into the reasons for antigenic drift, with the ultimate goal of predicting the direction of antigenic drift before it occurs. This work has been partially funded by a grant from the HFSP (Human Frontier Science Program).

Highlights and developments 2012

A large “base-map” has been created for influenza A(H3) viruses and ferret antisera spanning several decades. This will be the basis of determining changes in reactivity as the A(H3) viruses have evolved and will also allow the comparison between antibody profiles from individual people in two cohorts in Vietnam who have been infected with seasonal influenza viruses over the last five years. A large series of recombinant influenza viruses has also been made to test the flexibility of amino acid changes in 7 key antigenic sites in contemporary A(H3) viruses. These will be tested using a modified “base map” of ferret antisera.

Assessment of a novel assay to detect ADCC antibodies to A(H1N1)pdm09 virus

Centre staff Karen Laurie, Louise Carolan, Ian Barr, Patrick Reading
Collaborators Stephen Kent and Sinthujan Jegaskanda (The University of Melbourne)

Project overview

Engagement of antibody-dependent cellular cytotoxicity (ADCC) antibodies by natural killer cells leads to killing of virus-infected cells and secretion of antiviral cytokines and chemokines. ADCC antibodies may target more conserved influenza virus antigens compared to neutralising antibodies. Novel assays have been developed to assess the specificity and function of influenza-specific ADCC antibodies. ADCC antibodies were found in healthy influenza seropositive young adults without detectable neutralising antibodies.

Highlights and developments 2012

A manuscript based on results of this study was accepted and a second was submitted for publication. Results were also presented in a talk at the 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2–6 December.

Related publications 2012

Jegaskanda S, Job ER, Kramski M, Laurie K, Isitman G, De Rose R, Winnall WR, Stratov I, Brooks AG, Reading PC and Kent SJ. Cross-reactive influenza-specific antibody-dependent cellular cytotoxicity (ADCC) antibodies in the absence of neutralizing antibodies. *J Immunol*, in press.

NHMRC Program Grant: Understanding and controlling influenza (2010 - 2014)

The Centre is a participant in a National Health and Medical Research Council Program Grant which commenced on 1 January 2010.

Centre staff Anne Kelso, Patrick Reading, Karen Laurie, Aeron Hurt
Chief Investigators Peter Doherty (The University of Melbourne)
David Jackson (The University of Melbourne)
Anne Kelso (WHO Collaborating Centre for Reference and Research on Influenza)
Weisan Chen (La Trobe University)
Stephen Turner (The University of Melbourne)
Lorena Brown (The University of Melbourne)

Program overview

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), the WHO Collaborating Centre, La Trobe University, the School of Population Health (UM) and the CSIRO Australian Animal Health Laboratory.

Highlights and developments 2012

Dr Bridie Day and Kim Charlton, working in the Department of Microbiology and Immunology under the supervision of Anne Kelso, have continued investigating the regulation of CD8 co-receptor expression in T lymphocytes during development in the thymus and following activation and effector differentiation of peripheral CD8 T cells. Unique patterns of DNA methylation at the CD8 α locus were identified at each of these stages, suggesting differential DNA modification in response to changing external signals. Even some effector cells with a heritable CD8^{low} phenotype retained both epigenetic and functional plasticity, responding to certain signals with demethylation and re-expression of the silenced CD8 α locus.

A Program retreat held on 27 August was attended by 64 people representing all of the research groups in the Program. Patrick Reading chaired a session and Karen Laurie was a facilitator of a group session workshop. Anne Kelso and Naomi Komadina also attended. A student seminar series meeting on influenza virus-specific T cells held on 20 June was attended by Aeron Hurt, Teagan Guarnaccia, Anne Kelso and Karen Laurie.

Related Publications 2012

Charlton *et al.* CD8⁺ T cell plasticity: Cytokines at the helm. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2–6 December)

Early recognition and response to influenza infection

Centre staff	Patrick Reading
Collaborators	Alberto Mantovani (Istituto Clinico Humanitas, IRCCS & State University of Milan, Italy) Erika Crouch (Washington University School of Medicine, St. Louis, MO, USA) Melinda Dean (Australian Red Cross Blood Service, Queensland) Stuart Turville (Westmead Millennium Institute, New South Wales) Nigel McMillan (Griffith University, Queensland) Andrew Brooks (The University of Melbourne) Stephen Kent (The University of Melbourne) Lorena Brown (The University of Melbourne)

Project overview

This project characterises how influenza virus is first recognised and destroyed by immune cells and soluble factors of the innate immune system. The innate immune system comprises pre-existing or rapidly induced defences that limit the spread of pathogens in the body during the first few days of infection prior to the emergence of more targeted adaptive immune responses. Many innate defences have been highly conserved throughout evolution and, as such, animal models of infection are widely used to investigate the role of innate defences and to gain insight as to how they might limit human disease.

Current studies in Dr Reading's laboratory at the University of Melbourne are focused on (i) understanding the role of soluble innate immune proteins of the collectin and pentraxin superfamilies in early host defence against influenza virus, (ii) understanding the role of membrane-associated C-type lectins expressed by macrophages and dendritic cells as receptors for influenza virus entry and destruction, and (iii) showing how short interfering (si)RNA can be used to induce innate immunity and prevent influenza disease. The research involves both *in vitro* studies using human proteins and cells and *in vivo* studies using mouse and ferret models of infection.

Highlights and developments 2012

This research resulted in 5 publications during 2012. Dr Reading presented several research talks at conferences and institutes during the year, including plenary talks at TLR OZ 2012 (Melbourne, 2-4 May) and the 9th Asia-Pacific Congress of Medical Virology (Adelaide, 6-8 June). He was also an invited speaker at the Australian Society for Microbiology Annual General Meeting (Brisbane, 1-4 July).

Two new collaborative projects were commenced during 2012, one with Stephen Kent (The University of Melbourne) and other Centre staff, examining antibody-dependent cell mediated cytotoxicity (ADCC) during influenza virus infections (see above p.32); and a second with Nigel McMillan (Griffith University), examining novel approaches to using siRNA to treat influenza virus infections. Additionally, 3 NHMRC Project Grants were awarded with Dr Reading listed as one of the chief investigators, with funding to commence in 2013. Dr Reading's PhD student Kirsty Short also submitted her thesis in November 2012.

Related publications 2012

Reference nos. 15, 18, 21, 22, 24

Macrophage galactose-type lectin: a new attachment and entry receptor for influenza virus on murine macrophages. (Poster, presented at Keystone Symposia: Cell Biology of Virus Entry, Replication and Pathogenesis, 26-31 March)

Ng *et al.* C-type lectin receptors for influenza virus entry. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2-6 December)

Londrigan *et al.* "Lending a helping hand": Macrophage galactose-type lectin (MGL) is involved in influenza A virus infection of macrophages. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2-6 December)

Job *et al.* Glycosylation on influenza A virus hemagglutinin modulates antibody-mediated recognition of H1N1 2009 pandemic viruses. (Poster, presented at 42nd Annual Scientific Meeting of the Australasian Society for Immunology, 2-6 December)

Spatial and temporal dynamics of human seasonal influenza B in Australia and New Zealand

Centre staff Ian Barr, Yi-Mo Deng, Naomi Komadina, Aeron Hurt
Collaborators Rebecca Halpin and David Wentworth (J. Craig Venter Institute, Rockville, MD, USA)
Vijaykrishna Dhanasekaran and Gavin Smith (Duke-NUS Graduate Medical School, Singapore)

Project overview

Whilst the evolutionary dynamics of seasonal A(H1N1) and A(H3N2) viruses have been extensively studied, the evolutionary dynamics of influenza B viruses are not well known and to date a large-scale population dynamic study has not been undertaken. Furthermore, the extent and consequences of reassortment between viruses of the B/Victoria and B/Yamagata lineages that have been co-circulating since the mid-1980's has not been well characterised.

This project aims to perform full genome sequencing on over 900 influenza B viruses collected in Australia and New Zealand from 2002 to 2011 and isolated at the Centre. Genomic data from this repository will be crucial to understanding the fundamental processes of molecular evolution of each gene segment including reassortment events and also to determine the processes of importation, regional distribution and diversity of influenza B viruses over the 10 years since 2002 when the B/Victoria lineage became re-established in Australia and New Zealand. The full genome characterisation of these viruses will also play an important role in global efforts to better understand the molecular epidemiology, evolution and adaptive potential of these viruses.

Highlights and developments 2012

Viruses from the Centre have been shipped to the J. Craig Venter Institute and sequencing is under way.

Additional research collaborations

Centre staff members have also been involved in the following collaborations in 2012:

Wild bird avian influenza sequencing

Centre staff: Aeron Hurt
Collaborators: Simone Warner, Edla Arzey (Victorian and NSW Government Department of Primary Industries)
Related publications 2012: Reference no. 2
Presentation 2012: Aeron Hurt, "Establishment of the North American H10 lineage viruses in Australian wild birds", Avian Influenza in Wild Birds group meeting, 12–13 September.

Genetic analysis of equine influenza viruses from the Australian outbreak

Centre staff: Aeron Hurt
Collaborators: Peter Kirkland (EMAI, New South Wales)

Role of permissive mutations in neuraminidase

Centre staff: Aeron Hurt, Jeffrey Butler
Collaborators: Jesse Bloom (Fred Hutchinson Cancer Research Center, Seattle, WA, USA)

Novel amino acid mutations confer neuraminidase inhibitor resistance

Centre staff: Aeron Hurt, Jeffrey Butler
Collaborators: Rod Daniels (National Institute for Medical Research, UK)

Influenza vaccination in kidney transplant patients

Centre staff: Aeron Hurt
Collaborators: William Mulley, Kumar Visvanathan (Monash Medical Centre, Victoria)
Related publications 2012: Reference no. 17

Assessment of trivalent live attenuated influenza-SIV vaccines in macaques

Centre staff: Karen Laurie, Louise Carolan, Aeron Hurt
Collaborators: Stephen Kent and Sinthujan Jegaskanda (The University of Melbourne)
Related publications 2012: Reece JC, Alcantara S, Gooneratne S, Jegaskanda S, Amaresena T, Fernandez CS, Laurie K, Hurt A, O'Connor SL, Harris M, Petravic J, Martyushev A, Grimm A, Davenport MP, Stambas J, De Rose R, Kent SJ. Trivalent live attenuated Influenza-SIV vaccines: efficacy and evolution of CTL escape in macaques. *J Virol*, *in press*.

Understanding the emergence of T cell escape variants in influenza in an immunocompromised patient

Centre staff: Karen Laurie, Anne Kelso, Naomi Komadina, Pina Iannello
Collaborators: Katherine Kedzierska (The University of Melbourne)

Research Funding and Awards

Centre staff are chief or associate investigators in three research projects that were awarded grants by the National Health and Medical Research Council (NHMRC) in 2012:

Mechanisms of respiratory virus induced pneumococcal infection in the middle ear.

\$623,997 awarded for the period 1 January 2013 – 31 December 2015. Chief Investigators Odilia Wijburg, **Patrick Reading** and Stephen O'Leary. The grant will be administered by The University of Melbourne and the work will be undertaken at the University.

Antibody-dependent cellular cytotoxicity based immunity to influenza.

\$651,295 awarded for the period 1 January 2013 – 31 December 2015. Chief Investigators Stephen Kent, **Patrick Reading** and Ivan Statov. **Ian Barr** and **Karen Laurie** are Associate Investigators. The grant will be administered by The University of Melbourne and the work will be undertaken at the University.

Stealth liposomes and siRNA for the treatment of respiratory viral infections.

\$509,374 awarded for the period 1 January 2013 – 31 December 2015. Chief Investigators Nigel McMillan, Paul Young and **Patrick Reading**. The grant will be administered by Griffith University, and the work will be undertaken at Griffith University, The University of Queensland and The University of Melbourne.

Aeron Hurt was runner-up in the Life Sciences and Biological Sciences category of the 2012 Scopus Young Researcher of the Year Awards. The awards are given to recognise outstanding young scientists and researchers in Australasia who have made significant contributions in their areas of research.

Collaborative Agreements

The Centre is party to two ongoing collaborative research and development agreements with industry bodies and in 2012 also undertook a research project in collaboration with CSL Limited. As with all potential collaborations with the commercial sector, these agreements have undergone review by the Australian Government Advisory Committee 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) (2012-2013)

Centre staff Chantal Baas, Jayde Galletti, Scott Reddiex, Robert Shaw, Anne Kelso, Ian Barr

Project 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 2012

A total of 23 egg isolates were obtained from 118 inoculations with original clinical specimens from various geographical locations. Isolation rates varied from 17% to 21% according to virus type/subtype. Suitable isolates were made available to other laboratories and industry for reassortment and assessment as vaccine candidates.

Collaborative agreements (continued)

Cooperative Research and Development Agreement (CRADA) with Novartis Vaccines & Diagnostics (Marburg, Germany): Development and provision of influenza virus strains isolated on MDCK 33016PF cells for vaccine production (2012–2013)

Centre staff Heidi Peck, Joelle Dharmakumara, Scott Reddiex, Robert Shaw, Anne Kelso, Ian Barr

Project overview

The suitability of a proprietary Novartis cell line for isolating and growing influenza viruses as a basis for cell-based vaccine manufacture is being evaluated. Some 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 2012

During 2012, 87 clinical specimens were cultured in MDCK 33016PF cells, of which 77 (88.5%) produced isolates. As in previous years, this was much higher than the rate of isolation in eggs. The isolates, which comprised A(H3N2) and B viruses, were sent to Novartis in Marburg for further evaluation as potential vaccine candidates produced by cell culture. No A(H1N1)pdm09 viruses were cultured.

Due to low isolation rates of influenza A(H3N2) viruses in eggs it was decided to determine if viruses isolated in the MDCK33016PF cell line could be grown and reassorted in embryonated hen's eggs. Of 20 A(H3N2) MDCK33016PF isolates that were attempted to be propagated in eggs, four were successfully grown and sent to CSL Limited for reassortment and evaluation of HA activity. Heidi Peck presented a poster based on this work and was awarded a poster prize at the 4th Australasian Vaccines and Immunotherapeutics Development Meeting.

Related publications 2012

Peck *et al.* Evaluation of H3N2 viruses isolated and passaged in the qualified MDCK suspension cell line (MDCK33016PF) and embryonated chicken eggs. (Poster, presented at the 4th Australasian Vaccines and Immunotherapeutics Development Meeting, Brisbane, 2–4 May.)

Agreement with CSL Limited: Analysis of human seasonal vaccine immunogenicity and efficacy in a ferret model (2012)

Centre staff Karen Laurie, Aeron Hurt, Louise Carolan, Heidi Peck, Ian Barr

Collaborators Steve Rockman, Martin Pearse (CSL Limited)

Project overview

This study had two aims:

- to evaluate influenza-specific antibody responses to different doses of human influenza trivalent vaccine in ferrets.
- to determine whether multiple vaccinations with human trivalent influenza vaccine, in the absence of adjuvant, can limit A(H1N1)pdm09 lung infection after intranasal or intra-tracheal dosing, in a ferret model.

Highlights and developments 2012

The experimental component of this study was completed and analysis of the data is continuing.



Research Students

PhD Candidate

Ms Teagan Guarnaccia, who commenced her PhD candidature at the Centre in 2010, has continued her project entitled "Analysis of the contribution of immune pressure on antigenic drift of influenza A viruses", under the supervision of Dr Karen Laurie and Ms Jenny Mosse (Monash University, Gippsland).

During 2012 Teagan gave oral presentations at two conferences, at the Melbourne Health Research Week Symposium (Melbourne, May 24–31) and Influenza 2012 (Oxford UK, September 11–13). She also gave talks whilst visiting collaborators at Duke-NUS Graduate Medical School (Singapore), Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR) (Singapore) and Erasmus Medical Centre (Rotterdam, The Netherlands) during September.

MSc Candidate

Ms Chantal Baas commenced her MSc candidature part-time at the Centre in March 2012 under the supervision of Dr Aeron Hurt, Dr Ian Barr and Ms Jenny Mosse (Monash University, Gippsland). Her project is entitled "Comparison of influenza transmission models in the domestic ferret". The major aims of the project are:

- 1) To develop a model to assess aerosol transmission of influenza virus between ferrets.
- 2) To compare contact transmission and aerosol transmission models of influenza with different swine, avian and human influenza virus types and subtypes by monitoring infection time, transmission efficiency, severity and pathology.

Honours Student

Ms Sophie Vitesnik, a BSc student from the University of Melbourne, completed her Honours research project at the Centre during 2012. She was supervised by Dr Aeron Hurt and achieved First Class Honours. In her project, titled "Characterisation of influenza virus variants selected *in vitro* in the presence of neuraminidase inhibitors", Sophie cultured contemporary circulating influenza strains in the presence of four different neuraminidase inhibitor drugs to select for drug resistant variants. The variants were characterised using various functional assays to assess antiviral susceptibility, NA enzyme activity, and replication kinetics. Sophie detected a number of drug resistant variants, some with novel NA mutations, which demonstrated differing viral fitness or replication efficiency.



Masters Students

Dr Muhammad Waliur Rahman, an MPH student from the University of Melbourne, undertook a research project, titled "Evaluation of alternative analytical approaches for estimating seasonal influenza vaccine effectiveness in the Victorian population from 2007 to 2011 using test negative controls", under the joint supervision of Dr Sheena Sullivan and Dr Heath Kelly (Epidemiology Unit, VIDRL) from March to May.

Mr Simon Kwok (*pictured at right*), a Masters in Laboratory Science candidate from RMIT University, conducted a research project at the Centre from 23 July to 9 November, under the supervision of Dr Aeron Hurt and Ms Sook-Kwang (Leah) Leang. He received a distinction for his project titled "Assessment of drug sensitivities against influenza viruses with plaque reduction assay and neuraminidase inhibition assay".



Undergraduate Students

Ms Jacqueline D'Souza, Bachelor of Biomedical Science student from Deakin University, completed a work placement at the Centre 9–20 January.

Mr Mitch Batty, a BSc(Biotech) student from Monash University (Gippsland), visited the Centre to undertake a research project supervised by Dr Karen Laurie for three weeks in June 2012, and once a week thereafter for the remainder of the semester. His project focused on developing a real time RT-PCR assay to distinguish between mRNA, cRNA and vRNA of influenza A viruses, with the eventual goal of quantifying the amount of RNA variants in lower respiratory bronchoalveolar lavage samples from A(H1N1)pdm09-infected ferrets.

Communications and Advisory Activities

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

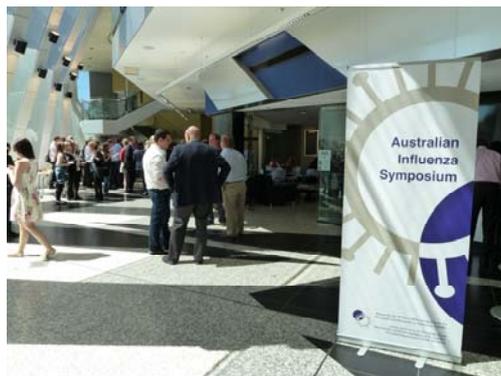
Australian Influenza Symposium

The 8th Australian Influenza Symposium, co-hosted by the Centre and the Therapeutic Goods Administration (TGA), was held at the John Curtin School of Medical Research, Australian National University, Canberra on 4–5 October 2012. The organising committee was Ian Barr, Anne Kelso, Jayde Simpson and Gary Grohmann (TGA).

The symposium was attended by 109 delegates from Australia, New Zealand, Cambodia, USA and Canada. A diverse range of presentations encompassed topics including influenza biology and clinical research, vaccine technologies, zoonotic viruses, epidemiology and population modelling, surveillance and public health strategies. Five invited international speakers attended and gave presentations:

- Dr Philippe Buchy (Institut Pasteur du Cambodge, Phnom Penh, Cambodia)
- Prof Edward Holmes (Pennsylvania State University USA/University of Sydney)
- Dr Danuta Skowronski (British Columbia Centre for Disease Control, Vancouver Canada)
- A/Prof S Mark Thompkins (Influenza Pathogenesis and Immunology Center, University of Georgia College of Veterinary Medicine, USA)
- Prof Ralph Tripp (University of Georgia College of Veterinary Medicine, USA)

Other highlights included a roundtable discussion on ethical issues surrounding manipulation of influenza viruses in life science research and a historical account of the Centre presented by its past and current Directors in recognition of its 20th anniversary as a WHO Collaborating Centre. The majority of staff members from the Centre attended the Symposium. Ian Barr opened the Symposium. Anne Kelso presented a plenary talk, chaired a session and was a panellist in the roundtable discussion. Karen Laurie and Jeffrey Butler co-chaired a session.



Engagement in WHO Activities

Event; Location, Date	Centre staff involved
Co-authorship on behalf of the WHO Consultation on Pandemic Influenza A(H1N1) 2009 Virus Resistance to Antivirals: Hurt AC et al. (2012) Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. <i>Lancet Infect Dis</i> , 12 (3): 240-8.	Aeron Hurt
WHO Technical Consultation on H5N1 Research Issues Geneva, Switzerland, 16–17 February	Anne Kelso
WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2012–2013 Geneva, Switzerland, 20–23 February	Ian Barr, Anne Kelso
WHO Meeting with Pandemic Influenza Preparedness (PIP) Advisory Group Geneva, Switzerland, 24 February	Anne Kelso
6th Meeting of National Influenza Centres in the Western Pacific and South-East Asia Regions Hanoi, Vietnam, 29–31 May	Ian Barr (session chair and speaker) Yi-Mo Deng Aeron Hurt (speaker) Anne Kelso (session chair and speaker) Patrick Reading Rob Shaw Sheena Sullivan (speaker)
WHO PCR Working Group Geneva, Switzerland, 21–28 June	Yi-Mo Deng (speaker)
2nd Meeting of the Expert Working Group for WHO GISRS on Surveillance of Antiviral Susceptibility Geneva, Switzerland, 28–29 June	Aeron Hurt (speaker)
WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2013 Beijing, China, 17–19 September	Ian Barr, Anne Kelso
WHO International Meeting on Influenza Vaccine Effectiveness Geneva, Switzerland, 3–4 December	Sheena Sullivan



Left: Members of the WHO PCR Working Group, including Yi-Mo Deng (front row, 3rd from right).

Committees and Advisory Groups

Centre staff members served on the following governing boards, committees and advisory groups during 2012.

Ian Barr

Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Australian Vaccine and Immunotherapeutics Development (AVID) Group, *Organising Committee*
Influenza Research and Treatment, *Editorial Board*
Influenza and Other Respiratory Viruses, *Editorial Board*
Public Health Laboratory Network (Department of Health and Ageing)

Yi-Mo Deng

WHO Working Group for GISRS PCR detection for influenza surveillance

Aeron Hurt

Antiviral Research, *Editorial Board*
International Society for Influenza and other Respiratory Virus Diseases, Antiviral Special Interest Group, *Committee member*
Influenza Specialist Group, *Scientific Committee*
Avian Influenza in Wild Birds, Australian Wildlife Health Network, *Steering Committee*
WHO Expert Group for GISRS Surveillance on Antiviral Susceptibility, *Chair*

Anne Kelso

Influenza and Other Respiratory Viruses, *Associate Editor*
International Immunology, *Associate Editor*
Australian Influenza Vaccine Committee (Therapeutic Goods Administration)
Florey Neuroscience Institutes, *Board and Council of Governors*
International Society for Influenza and other Respiratory Virus Diseases, *Board of Trustees*
BioMed Central Immunology, *Editorial Board*
Influenza Surveillance Strategy Working Group (Australian Government Department of Health and Ageing)
Burnet Institute, *Research Advisory Committee*
National Health and Medical Research Council, *Assigners Academy*
National Health and Medical Research Council, *Council*
Nossal Institute for Global Health (The University of Melbourne), *Advisory Council*
Australasian Society for Immunology Annual Scientific Meeting 2012, *Scientific Advisory Board*
Options for the Control of Influenza VIII, Cape Town, 2013, *Scientific Committee*
WHO/OIE/FAO H5N1 Evolution Working Group
Else Kröner-Fresenius-Stiftung Award selection committee
Peter Doherty Institute for Infection and Immunity: *Project Control Group, Collocation Group, Operational Management Committee (Chair), Research Strategy Advisory Group (Chair)*
Telethon Institute for Child Health Research: *Board, Scientific Advisory Committee (Chair)*
Queensland University of Technology: *Council, University Research and Innovation Committee, Corporate Review of the Institute of Health and Biomedical Innovation (Chair)*

Naomi Komadina

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

Karen Laurie

Global Influenza Seroepidemiology Standardisation Working Group
Peter Doherty Institute for Infection and Immunity: *Research Strategy Advisory Group*
Victorian Infectious Diseases Reference Laboratory, Safety Committee

Patrick Reading

Peter Doherty Institute for Infection and Immunity: *Education Strategy Advisory Group (Deputy Chair)*

Sheena Sullivan

Peter Doherty Institute for Infection and Immunity: *Public Health Strategy Advisory Group*
Influenza Surveillance Strategy Working Group (Australian Government Department of Health and Ageing)

Community Engagement

The Director and Deputy Director participated in requests from media representatives for interviews and comments throughout the year.

Anne Kelso

Catalyst, ABC TV, interview 14 March

"Changes to make flu vaccine safe for children", The World Today, ABC News, interview 20 June

"Jab fears in worst outbreak since swine flu", The Australian, newspaper interview 11 July 2012

Interview, Channel 9 News, 20 July 2012



Ian Barr

"Winter flu infects twice as many people as usual", ABC Radio AM, interview, 11 July

"Australians warned of killer flu season", ABC TV News, interview 11 July

"Dangerous flu season upon us", The Age, newspaper, interview 11 July

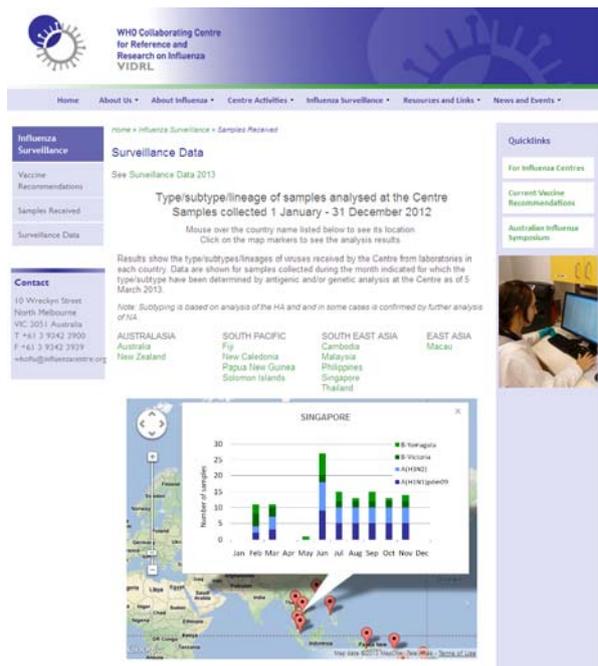
"Discovery of potentially dangerous swine flu strain prompts calls for better pig surveillance", ABC Radio PM, interview 11 September

"New swine flu virus identified in Korea", ABC Rural, interview 11 September

Centre Website

The Centre website continued to be maintained and updated throughout the year. During 2012, the website was viewed by 5,897 unique visitors from 118 different countries. The majority of visits to the website came from Australia, followed by the United States. Most of the traffic to the website came from search engines.

A new feature added to the website during 2012 was the inclusion of the types, subtypes and/or lineage of samples collected during the year and analysed at the Centre, updated on a monthly basis.

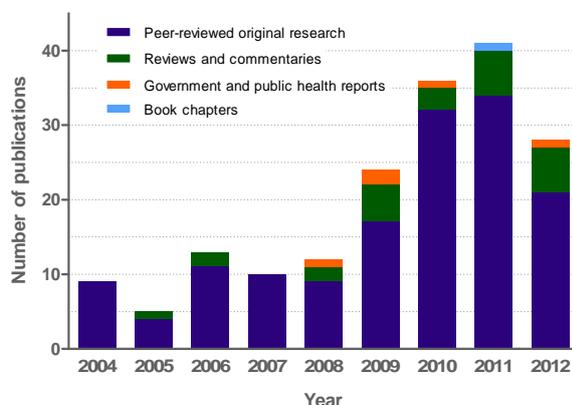


Publications and Reports

Publication Highlights

The Centre continued to build its research profile in 2012 with the publication of 28 original research papers, reviews and reports (Figure 18).

Figure 18. Centre publications 2004-2012.



Centre Publications 2012

1. Ampofo WK, Baylor N, Cobey S, Cox NJ, Daves S, Edwards S, Ferguson N, Grohmann G, Hay A, Katz J, Kullabutr K, Lambert L, Levandowski R, Mishra AC, Monto A, Siqueira M, Tashiro M, Waddell AL, Wairagkar N, Wood J, Zambon M, Zhang W and **WHO Working Group**. (2012) Improving influenza vaccine virus selection: report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, 14-16 June 2010. *Influenza Other Respi Viruses*, 6(2): 142-52, e1-5.
2. Arzey GG, Kirkland PD, Arzey KE, Frost M, Maywood P, Conaty S, **Hurt AC, Deng YM, Iannello P, Barr I**, Dwyer DE, Ratnamohan M, McPhie K and Selleck P. (2012) Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerg Infect Dis*, 18(5): 814-6.
3. **Barr IG** and **Jelley LL**. (2012) The Coming Era of Quadrivalent Human Influenza Vaccines: Who will Benefit? *Drugs*, 72(17): 2177-85.
4. **Deng YM, Iannello P**, Smith I, Watson J, **Barr IG**, Daniels P, **Komadina N**, Harrower B and Wong FY. (2012) Transmission of influenza A(H1N1) 2009 pandemic viruses in Australian swine. *Influenza Other Respi Viruses*, 6(3): e42-7.
5. Hsu AC, Parsons K, **Barr I**, Lowther S, Middleton D, Hansbro PM and Wark PA. (2012) Critical role of constitutive type I interferon response in bronchial epithelial cell to influenza infection. *PLoS One*, 7(3): e32947.
6. **Hurt AC, Butler J, Kelso A** and **Barr IG**. (2012) Influenza antivirals and resistance: the next 10 years? *Expert Rev Anti Infect Ther*, 10(11): 1221-3.
7. **Hurt AC**, Chotpitayasunondh T, Cox NJ, Daniels R, Fry AM, Gubareva LV, Hayden FG, Hui DS, Hungnes O, Lackenby A, Lim W, Meijer A, Penn C, Tashiro M, Uyeki TM and Zambon M. (2012) Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. *Lancet Infect Dis*, 12(3): 240-8.
8. **Hurt AC**, Hardie K, Wilson NJ, **Deng YM**, Osbourn M, **Leang SK**, Lee RT, **Iannello P**, Gehrig N, **Shaw R**, Wark P, **Caldwell N**, Givney RC, Xue L, Maurer-Stroh S, Dwyer DE, Wang B, Smith DW, Levy A, Booy R, Dixit R, Merritt T, **Kelso A**, Dalton C, Durrheim D and **Barr IG**. (2012) Characteristics of a widespread community cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza in Australia. *J Infect Dis*, 206(2): 148-57.
9. **Hurt AC, Leang SK**, Speers DJ, **Barr IG** and Maurer-Stroh S. (2012) Mutations I117V and I117M and oseltamivir sensitivity of pandemic (H1N1) 2009 viruses. *Emerg Infect Dis*, 18(1): 109-12.
10. **Hurt AC**, Okomo-Adhiambo M and Gubareva LV. (2012) The fluorescence neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol*, 865: 115-25.
11. Kelly HA, **Sullivan SG**, Grant KA and Fielding JE. (2012) Moderate influenza vaccine effectiveness with variable effectiveness by match between circulating and vaccine strains in Australian adults aged 20-64 years, 2007-2011. *Influenza Other Respi Viruses*: doi: 10.1111/irv.12018.

Centre Publications 2012 (continued)

12. **Kelso A.** (2012) CD4⁺ T cells limit the damage in influenza. *Nat Med*, 18(2): 200-2.
13. **Kelso A** and **Hurt AC.** (2012) The Ongoing Battle Against Influenza: Drug-resistant influenza viruses: why fitness matters. *Nat Med*, 18(10): 1470-1.
14. Klimov AI, Garten R, Russell C, **Barr IG**, Besselaar TG, Daniels R, Engelhardt OG, Grohmann G, Itamura S, **Kelso A**, McCauley J, Odagiri T, Smith D, Tashiro M, Xu X, Webby R, Wang D, Ye Z, Yuelong S, Zhang W and Cox N. (2012) WHO recommendations for the viruses to be used in the 2012 Southern Hemisphere Influenza Vaccine: epidemiology, antigenic and genetic characteristics of influenza A(H1N1)pdm09, A(H3N2) and B influenza viruses collected from February to September 2011. *Vaccine*, 30(45): 6461-71.
15. Londrigan SL, Tate MD, Brooks AG and **Reading PC.** (2012) Cell-surface receptors on macrophages and dendritic cells for attachment and entry of influenza virus. *J Leukoc Biol*, 92(1): 97-106.
16. Meijer A, Jonges M, van Beek P, Swaan C, Osterhaus A, Daniels R, **Hurt A** and Koopmans M. (2012) Oseltamivir-resistant influenza A(H1N1)pdm09 virus in Dutch travellers returning from Spain, August 2012. *Euro Surveill*, 17(36).
17. Mulley WR, Visvanathan K, **Hurt AC**, Brown FG, Polkinghorne KR, **Mastorakos T**, Lewicki MC, Stuart RL, Tan SJ, Chean R, Kerr PG and Kanellis J. (2012) Mycophenolate and lower graft function reduce the seroresponse of kidney transplant recipients to pandemic H1N1 vaccination. *Kidney Int*, 82(2): 212-9.
18. Ng WC, Tate MD, Brooks AG and **Reading PC.** (2012) Soluble host defense lectins in innate immunity to influenza virus. *J Biomed Biotechnol*, 2012: 732191.
19. Okomo-Adhiambo M, **Hurt AC** and Gubareva LV. (2012) The chemiluminescent neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol*, 865: 95-113.
20. Rockman S, Middleton DJ, Pearse MJ, **Barr IG**, Lowther S and Brown LE. (2012) Control of pandemic (H1N1) 2009 influenza virus infection of ferret lungs by non-adjuvant-containing pandemic and seasonal vaccines. *Vaccine*, 30(24): 3618-23.
21. Short KR, Brooks AG, **Reading PC** and Londrigan SL. (2012) The fate of influenza A virus after infection of human macrophages and dendritic cells. *J Gen Virol*, 93(Pt 11): 2315-25.
22. Short KR, **Reading PC**, Wang N, Diavatopoulos DA and Wijburg OL. (2012) Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of *Streptococcus pneumoniae*. *MBio*, 3(5).
23. Soh SE, Cook AR, Chen MI, Lee VJ, Cutter JL, Chow VT, Tee NW, Lin RT, Lim WY, **Barr IG**, Lin C, Phoon MC, Ang LW, Sethi SK, Chong CY, Goh LG, Goh DL, Tambyah PA, Thoon KC, Leo YS and Saw SM. (2012) Teacher led school-based surveillance can allow accurate tracking of emerging infectious diseases - evidence from serial cross-sectional surveys of febrile respiratory illness during the H1N1 2009 influenza pandemic in Singapore. *BMC Infect Dis*, 12: 336.
24. Tate MD, Brooks AG, **Reading PC** and Mintern JD. (2012) Neutrophils sustain effective CD8⁺ T-cell responses in the respiratory tract following influenza infection. *Immunol Cell Biol*, 90(2): 197-205.
25. Verity EE, Camuglia S, Agius CT, Ong C, **Shaw R**, **Barr I**, Middleton D and Rockman S. (2012) Rapid generation of pandemic influenza virus vaccine candidate strains using synthetic DNA. *Influenza Other Respi Viruses*, 6(2): 101-9.
26. Wang B, Taylor J, Ratnamohan M, McPhie K, Kesson A, Dixit R, Booy R, **Hurt A**, Saksena N and Dwyer D. (2012) Frequency of oseltamivir resistance in Sydney, during the Newcastle outbreak of community transmitted oseltamivir-resistant influenza A(H1N1)pdm09 virus, Australia, June to August 2011. *Euro Surveill*, 17(27): pii: 20210.
27. **WHO/OIE/FAO-H5N1WorkingGroup.** (2012) Continued evolution of highly pathogenic avian influenza A (H5N1): updated nomenclature. *Influenza Other Respi Viruses*, 6(1): 1-5.
28. **WPRO Members of the Western Pacific Region Global Influenza Surveillance and Response System.** (2012) Epidemiological and virological characteristics of influenza in the Western Pacific region of the World Health Organization, 2006-2010. *PLoS One*, 7(5): e37568.

Oral Presentations

Centre staff members gave oral presentations at numerous events during 2012, including national and international conferences, WHO meetings, government advisory meetings, educational lectures and research seminars.

Event Location, date	Speaker, Title
Immunology Summer Camp 2012 for Selected Students from Tsinghua University, Beijing, at Department of Microbiology and Immunology, University of Melbourne Melbourne, 1 February	Anne Kelso: <i>Immunology and the WHO's Global Influenza Programme.</i>
Influenza Specialist Group (ISG) Annual Scientific Conference Melbourne, 6 February	Aeron Hurt: <i>What's new in influenza antivirals and resistance.</i> Ian Barr: <i>Flu epidemiology update.</i>
A*STAR-Australian NHMRC Joint Symposium 2012 on Combating Emerging Infectious Diseases through Integrative Technology Approaches Singapore, 27–28 February	Ian Barr: <i>Influenza surveillance and how it impacts on current vaccine development and drug resistance awareness.</i>
Improving Influenza Laboratory Management Practices Training Course Bangkok, Thailand, 27 February – 3 March	Patrick Reading: <i>The role of National Influenza Centres in the Global Influenza Surveillance Network.</i> Patrick Reading: <i>The WHO EQA programme for PCR.</i> Patrick Reading: <i>Documents and document management.</i> Patrick Reading: <i>Data storage and archiving, including electronic databases.</i>
Lecture to 3rd year Medical Microbiology students at RMIT Melbourne, 12 April	Patrick Reading: <i>Laboratory-based surveillance of influenza virus in the Western Pacific Region.</i>
Research group meeting at the School of Population Health, The University of Melbourne Melbourne, 27 April	Aeron Hurt and Karen Laurie: <i>Virological and immunological experiments in ferrets.</i>
4th Australasian Vaccines and Immunotherapeutics Development Meeting Brisbane, 2–4 May	Karen Laurie: <i>Antigenic drift of the A(H1N1)pdm virus in the ferret model of vaccination.</i>
TLR OZ 2012 Melbourne, 2–4 May	Patrick Reading: <i>Glycans as a target for cell-associated and soluble lectins of the innate immune system.</i> (Plenary talk)
Lecture to 3rd year students, University of Melbourne Breadth Subject "Global health, security and sustainability" Melbourne, 9 May	Anne Kelso: <i>Influenza.</i>

Oral Presentations (continued)

Event Location, date	Speaker, <i>Title</i>
Melbourne Health Research Week Symposium Melbourne, 24–31 May	Jeffrey Butler: <i>Assessing the fitness of drug-resistant influenza viruses from a cluster of community cases in Newcastle, NSW, in 2011.</i>
	Sook Kwan Leang: <i>Influenza antiviral resistance in the Asia-Pacific region during 2011.</i>
	Teagan Guarnaccia: <i>Antigenic drift of the A(H1N1)pdm virus in the ferret model of vaccination.</i>
6th Meeting of National Influenza Centres in the Western Pacific and South-East Asia Regions Hanoi, Vietnam, 29–31 May	Ian Barr: <i>Update on seasonal influenza in the Southern Hemisphere in 2011-2.</i>
	Aeron Hurt: <i>A widespread community cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza in Australia.</i>
	Anne Kelso: <i>The future of GISRS - a Collaborating Centre perspective.</i>
	Sheena Sullivan: <i>Potential collaborative influenza strain circulation manuscript: example data from WHOCC, Melbourne.</i>
South Australian Branch of the Australian Society for Microbiology, Virology Symposium Adelaide, 5 June	Patrick Reading: <i>Laboratory-based influenza surveillance in the Western Pacific Region.</i>
9th Asia-Pacific Congress of Medical Virology Adelaide, 6–8 June	Patrick Reading: <i>Innate barriers to influenza virus infection.</i> (Plenary talk)
	Patrick Reading: <i>Pentraxins are sialylated inhibitors of influenza virus infection.</i>
WHO PCR Working Group Geneva, Switzerland, 21–28 June	Yi-Mo Deng: <i>Molecular surveillance at the WHO CC Melbourne, Australia.</i>
	Yi-Mo Deng: <i>New molecular developments at WHO CC, Melbourne.</i>
2nd Meeting of the Expert Working Group for WHO GISRS on Surveillance of Antiviral Susceptibility Geneva, 28–29 June	Aeron Hurt: <i>Use of standards for interpretation of in vitro sensitivity testing results.</i>
New Zealand Branch of the Australasian Society for Immunology Annual Scientific Meeting Dunedin, New Zealand, 28–29 June	Anne Kelso: <i>Will the next influenza pandemic be man-made?</i> (Keynote address)

Oral Presentations (continued)

Event Location, date	Speaker, Title
Australian Society for Microbiology (ASM) Annual General Meeting Brisbane, 1–4 July	Patrick Reading: <i>Glycosylation alters sensitivity of influenza virus to neutralizing antibodies in vitro and in vivo.</i>
9th Annual Australia-China Symposium on Healthy Ageing: New approaches from genomics, stem cells and smart technologies Canberra, 23 July	Anne Kelso: <i>Influenza pandemics in the 21st century.</i> (Plenary talk)
isriv International Conference on Seasonal and Pandemic Influenza: Incidence, Severity, and Impact 2012 Munich, Germany, 5–8 September	Anne Kelso: <i>What changes are needed to surveillance systems to better address the problems identified in this meeting?</i> (Plenary talk)
Bioinformatics Institute, Agency for Science, Technology and Research (A*STAR) Singapore, 6 September	Teagan Guarnaccia: <i>Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection.</i>
Duke-NUS Graduate Medical School Singapore, 6 September	Teagan Guarnaccia: <i>Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection.</i>
Oxford Glycobiology Institute, Department of Biochemistry Oxford, UK, 9 September	Patrick Reading: <i>Understanding interactions of influenza viruses with macrophages and dendritic cells of the innate immune system.</i>
Influenza 2012 Oxford, UK, 11–13 September	Teagan Guarnaccia: <i>Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection.</i>
	Patrick Reading: <i>Glycans on the hemagglutinin of influenza A virus are a target for recognition by soluble and cell-surface C-type lectins.</i>
Avian Influenza in Wild birds group meeting Sydney, 12–13 September	Aeron Hurt: <i>Establishment of the North American H10 lineage viruses in Australian wild birds.</i>
Erasmus Medical Centre Rotterdam, Netherlands, 17 September	Teagan Guarnaccia: <i>Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection.</i>
6th Orthomyxovirus Research Conference Quebec, Canada, 19–22 September	Jeffrey Butler: <i>Assessing the fitness of drug-resistant influenza viruses using a competitive mixtures model in ferrets.</i>
Mt Sinai School of Medicine, New York, USA New York, USA, 24 September	Jeffrey Butler: <i>Influenza in ferrets - Assessing the fitness of drug resistant viruses; Investigating the role of the PB2 E627K mutation up on the pathogenicity of H5N1.</i>

Oral Presentations (continued)

Event Location, date	Speaker, Title
8th Australian Influenza Symposium Canberra, 4–5 October	Anne Kelso: <i>People, pigs and politics: the WHO Collaborating Centre and WHO's global influenza program today.</i>
The first meeting on seasonal influenza vaccines in the Western Pacific Region Manila, Philippines, 22–23 October	Ian Barr: <i>Influenza viruses; Our constant companions.</i>
Severe Influenza: Burden, Pathogenesis and Management Hanoi, Vietnam, 29–31 October	Aeron Hurt: <i>Neuraminidase inhibitor resistant and sensitive reference viruses for use in susceptibility testing.</i>
Joint NHMRC-Gates Foundation forum: H5N1: are we prepared? Canberra, 1 November	Aeron Hurt: <i>Avian Influenza surveillance in wild birds.</i> Aeron Hurt: <i>A(H5N1) antiviral resistance studies.</i> Anne Kelso: <i>The role of WHO in global pandemic preparedness and its relevance in our region.</i>
One Health Program, run by the Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, 2 November	Aeron Hurt: <i>Establishment of the North American H10 lineage viruses in Australian wild birds.</i>
2nd Antivirals Congress Boston, MA, USA, 11–13 November	Aeron Hurt: <i>Using ferrets to assess the fitness of the oseltamivir-resistant A(H1N1)pdm09 viruses responsible for a cluster of community cases in Australia in 2011.</i>
5th HKU-Pasteur Immunology Course for Research Postgraduate Students Hong Kong SAR, China, 18–30 November	Patrick Reading: <i>Immune responses to influenza virus I: innate and adaptive immunity.</i> Patrick Reading: <i>Immune responses to influenza virus II: immune evasion, immunopathology and disease.</i>
Singapore International Conference on Dengue and Emerging Infections Singapore, 21–23 November	Ian Barr: <i>Detecting and responding appropriately to the next influenza pandemic.</i> Aeron Hurt: <i>Antiviral resistance studies in ferrets.</i>
Walter and Eliza Hall Institute Symposium for opening of the new building Melbourne, 22 November	Anne Kelso: <i>Chasing influenza.</i>

Poster and Oral Presentations by non-Centre collaborators

Centre staff contributed to the authorship and presentation of several posters and talks that were presented at conferences and meetings in 2012. Heidi Peck was awarded a poster prize at the 4th Australasian Vaccines and Immunotherapeutics Development Meeting (marked with asterisk)

Event Location, date	Title and authors (presentations are posters unless otherwise indicated, Centre authors are marked in bold, presenting author is underlined)
Keystone Symposia: Cell Biology of Virus Entry, Replication and Pathogenesis, Whistler, British Columbia, Canada, 26–31 March	Macrophage galactose-type lectin: a new attachment and entry receptor for influenza virus on murine macrophages. <u>Ng WC</u> , Londrigan SL, Liong S, Brooks AG and Reading PC .
4th Australasian Vaccines and Immunotherapeutics Development Meeting Brisbane, 2–4 May	* Evaluation of H3N2 viruses isolated and passaged in the qualified MDCK suspension cell line (MDCK33016PF) and embryonated chicken eggs. <u>Peck H</u> , Baas C , Reddiex S , Trusheim H, Barr I . Quantitation of cytokine mRNA levels in ferrets following influenza infection. <u>Carolán LA</u> , Butler J , Reading PC , Hurt AC , Kelso A , Barr IG , Laurie KL .
Melbourne Health Research Week Symposium Melbourne, 24–31 May	Quantitation of cytokine mRNA levels in ferrets following influenza infection. <u>Carolán LA</u> , Butler J , Reading PC , Hurt AC , Kelso A , Barr IG , Laurie KL .
9th Asia-Pacific Congress of Medical Virology Adelaide, 6–8 June	A novel pyrosequencing assay that rapidly determines the lineage of human influenza B viruses. <u>Iannello P</u> , Komadina N , Kelso A , Barr IG , Deng YM .
isirv International Conference on Seasonal and Pandemic Influenza: Incidence, Severity, and Impact 2012 Munich, Germany, 5–8 September	<i>Oral presentation by non-Centre collaborator:</i> Influenza serosurveillance in Australia, 2009–2011 – observations and implications for optimal study design. <u>McVernon J</u> , Lambert S, Owen R, Faddy H, Irving D, Laurie K , Barr I and Kelso A .
Influenza 2012 Oxford, UK, 11–13 September	Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection. <u>Guarnaccia T</u> , Carolán L , Job E, Reading PC , Petrie S, McCaw J, McVernon J, Maurer-Stroh S, Kelso A , Barr I , Mosse J, Laurie K .

Heidi Peck (second from right) and other poster prize awardees at the 4th Australasian Vaccines and Immunotherapeutics Development Meeting.



Poster and Oral Presentations by non-Centre collaborators (continued)

Event Location, date	Title and authors (presentations are posters unless otherwise indicated, Centre authors are marked in bold, presenting author is underlined)
42nd Annual Scientific Meeting of the Australasian Society for Immunology Melbourne, 2–6 December	<p>Cytokine profiling in ferrets: a real time RT-PCR assay to assess viral virulence and the immune response following influenza infection and immunisation. Carolan L, Butler J, Hurt AC, Rockman S, Kelso A, Barr I and <u>Laurie K</u>.</p> <p>Antigenic drift of the A(H1N1)pdm09 virus in the ferret model of vaccination and infection. Guarnaccia T, Carolan L, Job E, Reading PC, Petrie S, McCaw J, McVernon J, Maurer-Stroh S, Chuen R, Hurt A, Kelso A, Mosse J, Barr I and <u>Laurie K</u>.</p> <p>Interferon-γ exposure is critical for the development of optimal CD8⁺ T cell effector responses. <u>Apte SH</u>, Scharer CD, Groves PL, Roddick JS, Kelso A, Boss JM and Doolan DL.</p> <p>CD8⁺ T cell plasticity: Cytokines at the helm. <u>Charlton KL</u>, Day EB, Apte SH, Turner SJ and Kelso A.</p> <p>“Lending a helping hand”: Macrophage galactose-type lectin (MGL) is involved in influenza A virus infection of macrophages. <u>Londrigan SL</u>, Ng WC, Liong S, Tate MD, Brooks AG and Reading PC.</p> <p>C-type lectin receptors for influenza virus entry. <u>Ng WC</u>, Londrigan SL, Brooks AG and Reading PC.</p> <p>Oseltamivir and influenza: inflammation-induced morbidity versus generation of anti-viral T cell immunity. <u>Bird NL</u>, Hurt A, Doherty PC, Reading PC and Kedzierska K.</p> <p>Glycosylation on influenza A virus hemagglutinin modulates antibody-mediated recognition of H1N1 2009 pandemic viruses. <u>Job E, Deng Y-M, Barfod K, Tate M, Caldwell N, Reddiex S, Maurer-Stroh S, Brooks A and Reading P.</u></p> <p><i>Oral presentation by non-Centre collaborator:</i> DNA methylation contributes to IL-4-induced silencing of CD8 expression in CD8⁺ T cells. <u>Day EB</u>, Charlton KL, Russ BE, Apte SH, Doherty PC, Turner SJ and Kelso A.</p> <p><i>Oral presentation by non-Centre collaborator:</i> Increased nasopharyngeal bacterial titers and local inflammation facilitate transmission of Streptococcus pneumoniae. <u>Wijburg O</u>, Short K, Reading PC, Wang N and Diavatopoulos D.</p> <p><i>Oral presentation by non-Centre collaborator:</i> Influenza-induced inflammation drives bacterial otitis media. <u>Short KR</u>, Reading PC, Brown LE, Pedersen J, Gilbertson B, Job ER, Edenborough KM, Zomer A, Hermans PWM, Diavatopoulos DA and Wijburg OL.</p> <p><i>Oral presentation by non-Centre collaborator:</i> Cross-reactive ADCC antibodies to 2009-pandemic influenza in the absence of neutralising antibodies. <u>Jegaskanda S, Laurie K, Kramski M, Winnall W, Job ER, De Rose R, Brooks AG, Barr IG</u>,</p>

Other Conference and Meeting Participation

In addition to oral and poster presentations listed, Centre staff members also participated in the following conferences as attendees and/or in other roles.

Event; Location, date	Centre staff involvement
Influenza Specialist Group (ISG) Annual Scientific Conference; Melbourne, 6 February 2012	Anne Kelso, Patrick Reading
Telethon Institute for Child Health Research: Festschrift for Fiona Stanley (Symposium); Perth, 30 April 2012	Anne Kelso
4th Australasian Vaccines and Immunotherapeutics Development Meeting; Brisbane, 2–4 May 2012	Ian Barr (session chair)
Melbourne Health Research Week Symposium; Melbourne, 24–31 May 2012	Aeron Hurt, Karen Laurie
9th Asia Pacific Congress of Medical Virology; Adelaide, 6–8 June 2012	Ian Barr
Global Virus Network Meeting, Naples, Italy; 7–9 June 2012	Anne Kelso
13th National Immunisation Conference; Darwin, 19–21 June 2012	Naomi Komadina, Sheena Sullivan
NHMRC Program on Understanding and Controlling Influenza Student Seminar Series: Influenza Virus Specific T-Cells; Melbourne, 20 June 2012	Anne Kelso (chair), Aeron Hurt, Karen Laurie, Teagan Guarnaccia attended.
Australian Society for Microbiology (ASM) Annual General Meeting; Brisbane, 1–4 July	Patrick Reading (session chair)
Ken Shortman's 75th Birthday Celebration Symposium at the Walter and Eliza Hall Institute of Medical Research; Melbourne, 19 July	Anne Kelso
Introduction to phylogenetic analysis Workshop; Sydney, 24–25 July	Natalie Caldwell, Yi-Mo Deng, Naomi Komadina, Heidi Peck
National Health and Medical Research Council (NHMRC) Program on Understanding and Controlling Influenza Annual Retreat; Melbourne, 27 August	Patrick Reading (session chair), Karen Laurie (workshop facilitator), Anne Kelso, Naomi Komadina
Defence Science Institute: Medical Counter Measures Workshop; Melbourne, 16 October	Anne Kelso
Joint NHMRC-Gates Foundation Forum: H5N1: are we prepared?; Canberra, 1 November	Ian Barr
Closed meeting of the National Health and Medical Research Council (NHMRC) of Australia on H5N1 issues; Canberra, 2 November	Anne Kelso
Understanding Infectious Disease Models Workshop, run by Public Health, Epidemiology & Health Services Research Domain at the University of Melbourne; Melbourne, 16 November	Karen Laurie, Sheena Sullivan
42nd Annual Scientific Meeting of the Australasian Society for Immunology; Melbourne, 2–6 December	Anne Kelso (scientific advisory committee, plenary session chair), Karen Laurie (session co-chair), Patrick Reading (discussion panellist in the Post Graduate Workshop)

Visitors to the Centre

The Centre welcomed the following visitors during 2012:

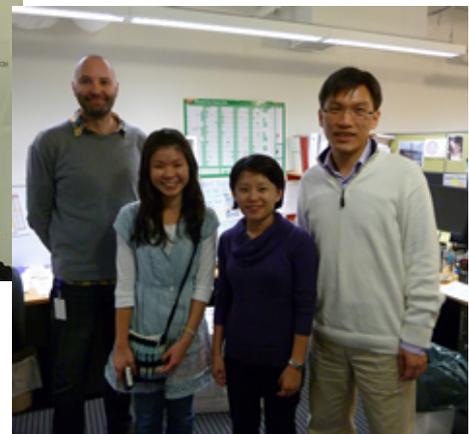
- 16–20 January Dr Vijay Dhanasekaran, Program of Emerging Infectious Diseases, Duke-NUS Graduate Medical School in Singapore, Singapore. *Research collaboration*
- 26 March Dr Fahimeh Rahnama, Senior Scientist, Auckland District Health Board, Auckland, New Zealand
- 11 April Dr Geethani Wickramasinghe, Former Director NIC, Medical Research Institute (Sri Lanka NIC), Colombo
- 4 June Ms Jacqui Ralston, Institute of Environmental Science and Research (ESR), Wellington, New Zealand
- 25–27 June Dr Ip Peng Kei, Director, Public Health Laboratory, Health Bureau, Macau SAR, China
- 1 August Delegation from the Defence, Medical and Environmental Research Institute (DMERI), Singapore:
Dr William Lau (Director)
A/Prof Eric Yap (Program Director of Biological Defence Program)
A/Prof Tan Boon Huan (Head of Detection and Diagnostic Lab) Ms Shirley Seah (Scientist)
- 2 August Dr Janice Lo, Director NIC, Centre for Health Protection, Hong Kong SAR, China
- 28 August Dr Nadine Holmes, Sydney Emerging Infectious Diseases and Biosecurity Institute (SEIB), Sydney, Australia. *Dr Holmes presented a seminar.*
- 1 October Dr Philippe Buchy, Director NIC, Institut Pasteur du Cambodge, Phnom Penh, Cambodia
- 1–3 October Dr Danuta Skowronski, Epidemiology Lead for Influenza and Emerging Respiratory Pathogens (Medical epidemiologist), British Columbia Centre for Disease Control, Vancouver, Canada
- 26 October Delegation from DSO National Laboratories, Singapore:
Dr Gary Yuk Fai Lau (Senior member of technical staff, Medical Countermeasures (Biological) Laboratory,)
Ms Lynn Lay Hoon Tang (Research scientist)
Ms Li Fang Kuah (Research scientist)



Dr Geethani Wickramasinghe (centre) with Aeron Hurt (left) and Anne Kelso (right)

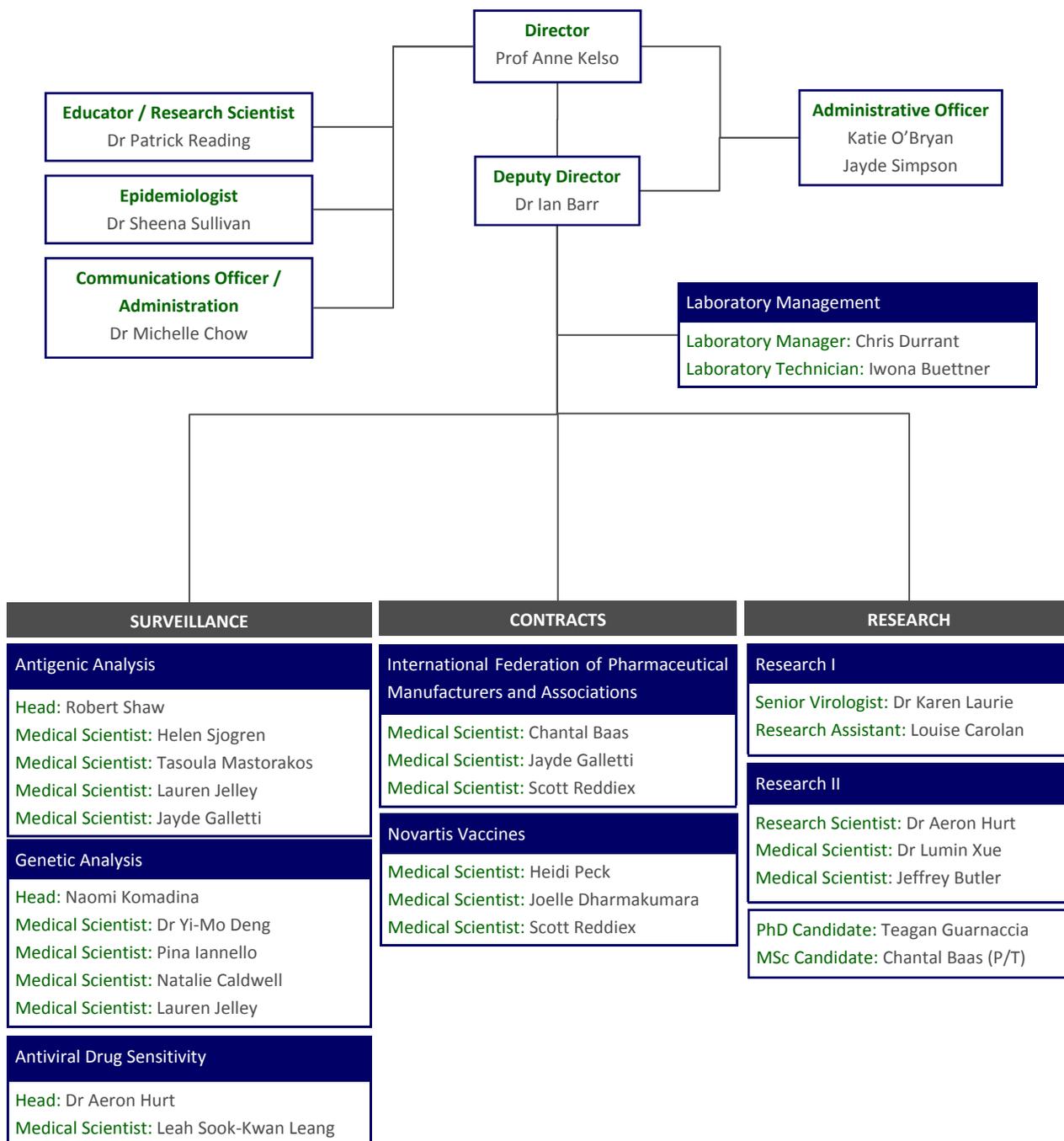


Dr Ip Peng Kei



Delegation visit from DSO National Laboratories, Singapore. From L to R: Aeron Hurt, Ms Li Fang Kuah, Ms Lynn Lay Hoon Tang, Dr Gary Yuk Fai Lau.

Management and Staff



Staff Changes 2012

Mrs Lauren Jelley joined the Centre in January with her time divided between the Antigenic Analysis and Genetic Analysis divisions of surveillance activities.

Mr Scott Reddiex, who completed his BBiomedSci(Hons) project at the Centre in 2010, left the Centre in March to commence his PhD at Monash University. Mr Reddiex was replaced in May by Mrs Joelle Dharmakumara, who returned from maternity leave, and Ms Jayde Galletti. In November Ms Galletti took on further responsibilities to replace Ms Tasoula Mastorakos who went on maternity leave.

Ms Jayde Simpson was appointed in June to replace Ms Katie O'Bryan who went on maternity leave.